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BASAL AND POSTPRANDIAL PLASMA LEVELS OF PYY, GHRELIN, CHOLECYSTOKININ, GASTRIN AND INSULIN IN WOMEN WITH MODERATE AND MORBID OBESITY AND METABOLIC SYNDROME

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Metabolic syndrome (MS), defined as central obesity, hyperinsulinemia, insulin resistance, hypertension, dyslipidemia and glucose intolerance, has been associated with inflammatory biomarkers and cardiovascular diseases. This study was carried out on three groups of women; lean controls, moderately obese with MS (OB-MS) and morbidly obese with MS (MOB-MS). The main objectives were: 1. to analyze the plasma levels of total and acylated ghrelin, peptide YY_{3-36} (PYY_{3-36}), cholecystokinin (CCK), gastrin and insulin levels under basal conditions and in response to a standard mixed meal, and 2. to elucidate the relationship between the plasma levels of these gut peptides and metabolic syndrome parameters. Plasma levels of the gut hormones were measured by radioimmunoassays at time 0 just before the meal and at 30, 60 and 120 min after a meal ingestion. Traditional lipid profile and high-sensitivity C reactive protein (hs-CRP), the strongest biomarker of inflammation were also determined in OB-MS and MOB-MS. When compared to OB-MS, MOB-MS exhibited much higher antropometric parameters such as waist circumference, higher fat mass and higher plasma levels of low density lipoprotein-cholesterol (LDL-C) and hs-CRP. Both these obese groups revealed significantly higher values of body mass index (BMI), fat mass, total cholesterol (TC), LDL-C, fasting glucose, fasting insulin, insulin resistance (IR) calculated from homeostatic model assessment (HOMA) and hs-CRP compared to the values recorded in lean subjects. Fasting PYY_{3-36} level was lower, while fasting acylated ghrelin was higher in MOB-MS than in OB-MS. Plasma total and acylated ghrelin levels were significantly lower in OB-MS compared to lean women. In MOB-MS women the fasting PYY_{3-36} levels were lower compared to lean controls and OB-MS, whilst

postprandially in both OB-MS and MOB-MS, it was much lower than in lean women. The fasting plasma levels of total and acylated ghrelin and their postprandial decrease were significantly smaller in both obese groups compared to lean subjects. Plasma hs-CRP levels correlated positively with BMI, waist circumference, fat mass, fasting glucose, HOMA IR and fasting active ghrelin, whilst it negatively correlated with plasma fasting and total ghrelin. Moreover, plasma fasting acylated ghrelin correlated positively with fat mass. Fasting total ghrelin correlated positively with BMI, HDL-C and negatively with HOMA IR. We conclude that MS features of obesity are closely related to fasting and postprandial alterations of concentrations of PYY_{3-36}, CCK and ghrelin, suggesting that determination of gut hormones controlling food intake might be considered as a valuable tool to assess the progression of MS to comorbidities of obesity.

Key words: *obesity, PYY, ghrelin, cholecystokinin, gastrin, insulin, metabolic syndrome*

INTRODUCTION

Obesity is a major healthcare priority of the present century. The World Health Organization has estimated that worldwide, over one billion adults exhibit overweight, with at least 300 million of them being obese. Overweight is defined by body mass index (BMI) of 25.0-29.9 kg/m^2 and obesity is defined as having a BMI of 30 kg/m^2 or greater. Obesity has reached epidemic proportions not only in USA but also in European-developed countries, including Poland. According to National Health and Nutritional Examination Survey from 1988-1994 (NHANES III) to NHANES 1999-2000, the prevalence of overweight in adults increased from 55.9% to 64.5% and obesity from 22.9% to 30.5% (1). According to Polish studies (NATPOL PLUS) in 2003, the prevalence of obesity was; overweight by 56% and obesity by 30% (2). In Poland obese subjects with BMI above 40 constitute about 1% (287.000 obese), and those with BMI above 35 constitute 5.1% (1.5 million obese) of adult subjects. All above obese subjects are candidates for surgical weigh-loss treatments because conservative weight-reduction programs based on dieting and exercise are usually ineffective (3). Over the past two decades, the number of people with the metabolic syndrome (MS) has greatly increased around the globe. Obesity may be associated with MS and type 2 diabetes (T2D), which refers to a constellation of risk factors for cardiovascular disease, including hyperinsulinemia, glucose intolerance, increased plasma low level of high-density lipoproteins (HDL) and hypertension, in which the underlying pathophysiological mechanism is believed to be insulin resistance (4-6). Recently, compelling evidence was obtained that the complications of obesity appear to be primarily induced by adipokines and other factors secreted by adipose tissue (7). It has been also pointed out that obesity may be considered as a state of low-grade systemic permanent inflammation (8, 9). Well-documented clinical studies indicate that C-reactive protein (CRP) is increased in obese individuals concomitantly to the

number of existing components of the MS (10, 11). Furthermore, prospective supportive studies have demonstrated the predictive role of inflammatory markers, such as CRP, in the subsequent development of glucose intolerance and cardiovascular events (12, 13).

Much less is known whether the MS in obese individuals affects the release of gastrointestinal (GI) hormones. Hormonal peptides are present and released not only in the GI mucosa but also in other tissues including peripheral and central nervous system (CNS), and they may exert different metabolic effects (14, 15). GI hormones have been associated with the regulation of feeding behavior and energy balance.

PYY exists in two forms; one is 36-aminoacid peptide, PYY_{1-36}, released by endocrine L-cells in distal small bowel and colon, serving as a precursor of peptide that is more bioactive, PYY_{3-36}. Because of the rapidity of PYY_{1-36} conversion to PYY_{3-36}, the main circulating form of PYY in postprandial plasma is PYY_{3-36}. Both forms of PYY bind to the Y2 receptors (Y2R) and both forms inhibit gastric acid and pancreatic enzyme secretion and suppress gastrointestinal motility. Similar to neuropeptide Y (NPY), both forms injected to cerebral ventricles are potent stimuli for feeding in the CNS. In contrast to the central effects, the circulating PYY_{3-36} acts as major endogenous mediator of satiation, reducing the body weight gain through the action on Y2R. PYY_{3-36} has received special attention in clinical studies after Batterham *et al.* demonstrated that infusions of the PYY_{3-36} in doses mimicking the postprandial increments in plasma levels of this peptide in both normal-weight and obese subjects, reduced appetite and food intake for 12-24 h (17, 18). Obese subjects have been reported to exhibit lower fasting and postprandial plasma PYY levels, so its deficiency might contribute to the increase in food intake and obesity. PYY is co-secreted with GLP-1, another anorexigenic peptide, from the same endocrine L-cells in the distal segment of the small bowel and its truncated 34-aminoacid form (PYY_{3-36}) is created from PYY_{1-36} by separation from the N terminus of Tyr-Pro residues by dipeptydyl peptidase IV (DPPIV). PYY_{3-36} inhibits food intake directly due to its high affinity for the presynaptic inhibitory Y2R of neurons in the hypothalamic arcuate center (ARC), and also for Y2R present in afferent vagal fibers. Plasma concentrations of total PYY are lower during fasting, increasing subsequently after food ingestion to reach plateau after 1-2 hours, with peaking dependent upon meal composition and caloric content (12). PYY_{3-36} is known as “meal terminator” opposed to ghrelin considered as “meal initiator” in the digestive behavior (11). Grandt *et al.* found that PYY_{3-36} accounts for only 37% of the total PYY circulated in fasting conditions but becomes more predominant (54%) after a standard meal (19). In another study this proportion of plasma PYY_{3-36} to PYY_{1-36} after a meal was found to be 3.6 in lean subjects and 3.2 in obese persons (20). The glucose, lipids and CCK stimulate, while gastrin and GLP-1 inhibit PYY release in rodents and humans (21). In plasma of normal subjects, PYY_{3-36} rises immediately after meals, suggesting that this peptide may be involved in short-term feeding control (22).

Higher PYY serum concentrations were found in diseases such as celiac disease, hepatic cirrhosis, T2D, and following gastrectomy and gastric-bypass surgery (23). In humans, peripherally-administered PYY_{3-36} lowers circulating ghrelin levels. The distribution of PYY is gradually increasing, while opposing ghrelin is decreasing along the gut from the stomach to the colon (24).

Ghrelin is a 28-amino acid peptide isolated from human and rat stomachs as an endogenous ligand for the growth hormone secretagogue receptor (GHS-R). There are two major forms of ghrelin in plasma: acylated-ghrelin with an n-octanoyl group on the N-terminal third amino acid and desacyl-ghrelin (24 - 27). Desacyl-ghrelin is a predominant form in blood, represents 79-86% of total ghrelin, and has physiological functions distinct from acylated-ghrelin (11). More recent studies showed the anorectic properties of desacyl-ghrelin in rats and mice. This effect appears to be exerted by expression of *fos* in the PVN nucleus, presumably involving corticotrophin-releasing hormone neurons. Desacyl-ghrelin is also synthesized in the rat hypothalamus, playing an important role in feeding regulation. Apart from stimulating GH secretion, ghrelin stimulates prolactin and ACTH secretion increases gastric motility and acid secretion, promoting pancreatic peptide synthesis (28). Ghrelin does not affect basal pancreatic exocrine excretion, but eliminates the stimulating effect of CCK on pancreatic protein production (29).

Ghrelin powerfully increases food intake in diverse species including humans and is the only hormone known to do so. Contrary to satiation GI peptides (PYY, CCK, leptin, GLP-1), ghrelin increases GI motility and decreases insulin secretion. Also in contrast to satiation peptides, circulating ghrelin levels surge shortly before meals and this preprandial rise of plasma ghrelin seems to be a cephalic response, probably involving the sympathetic nervous system, that participates in the anticipatory processes enabling animals to prepare for food intake and nutrient disposition. It is of interest that fasting plasma ghrelin are suppressed by ingestion of nutrients (with carbohydrates being more effective than proteins, which are more effective than lipids). This postprandial suppression of plasma ghrelin does not require exposure to luminal nutrient of the stomach where the majority of ghrelin production takes place (15), but probably results from neurally transmitted (nonvagal) intestinal signals, augmented by insulin. The pattern of ghrelin suppression by nutrients is broadly consistent with the observation that while the satiation hormones (induced by ingested nutrients) such as PYY, GLP-1 or CCK, rise postprandially, the circulating orexigenic hormone, ghrelin, falls, while simultaneously an insulin secretion increases (16). In healthy humans, acylated- and desacylated-ghrelin levels increase before every meal and decrease almost immediately after eating. Lean subjects have average fasting ghrelin concentrations higher than obese subjects, particularly at night. Ghrelin levels are decreased in response to high doses of PYY_{3-36} in the premeal period (15). Batterham *et al.* have proved that infusion of PYY_{3-36} markedly decreases circulating ghrelin levels attenuating the preprandial rise of this peptide

both in lean and obese subjects (17, 18). Circulating total ghrelin shows a pulsating pattern in normal subjects. In obese subjects, there is no day and night ghrelin rhythm and no post-meal decrease (15). Postprandial decrease of ghrelin concentration is associated with insulin and glucose increase.

In addition to its role in short-term feeding control, ghrelin appears also to contribute to long-term body weight regulation. Circulating levels of ghrelin respond in compensatory manner to bi-directional body-weight changes achieved by diverse means, increasing with weight loss and *vice versa*. Ghrelin influences neuronal activity through its receptors in several areas in the brain, particularly in the hypothalamus, to govern long-term energy homeostasis and adiposity. Chronic administration of ghrelin increases body weight and adipose tissue content due to numerous anabolic effects on food intake, energy expenditure and fuel utilization. Despite of the above mentioned finding, the importance of the ghrelin in energy homeostasis remains unclear, partly due to the fact that this peptide exists in two molecular forms, one is 28-amino acid peptide with an n-octanoyl modification at the N-terminal third amino acid which is indispensable for its biological activity and another which is desacyl-ghrelin. In this study we decided to determine the changes in both acylated and desacylated ghrelin levels in healthy controls and in moderately and morbidly obese women with MS.

Cholecystokinin (CCK) has been known as a short-term satiety hormone produced by endocrine I cells in proximal intestines. Predominant forms of CCK in plasma are CCK8, CCK22, CCK33 and CCK58. CCK release is stimulated largely by the absorption of fatty acids or amino acids through the intestinal mucosa. In normal, healthy, lean volunteers postprandial plasma CCK concentrations reaches a maximum at 15 min (30). There are gender differences in CCK plasma concentrations; women have higher levels because of estrogens influence (31). CCK transmits satiety signals to the brain through CCK receptor 1 (CCK1R, formely CCK-A for alimentary) localized on vagal afferent fibers, acting as “meal terminator” in short-term feeding control. CCK satiety signal is enhanced by leptin due to co-localization of CCK1R and leptin receptors (Ob-R) on the same vagal-sensory fibers (32). Chronic administration of CCK reduces the meal-size but increases meal frequency. CCK may stimulate ghrelin release by mucosal cells of gastric fundus. Recent data reported that bulimia, obesity, T2D, gallbladder disease, and functional dyspepsia may be associated with CCK/CCK1R alterations (23, 33).

Gastrin is structurally homologous to CCK, produced and released in several molecular forms by the G-cells in the antral and duodenal mucosa. It does not appear to affect feeding behavior and is the principal mediator of meal-induced gastric phase of acid secretion acting *via* paracrine stimulation of histamine release from gastric ECL-cells. It also plays a role in the cephalic and intestinal phases of gastric acid secretion (34). Gastrin acts on the parietal cells through high affinity CCK2 receptor (CCK2R, formely known as CCK-B, for ‘brain”). Both sulfated and non-sulfated forms of 34-amino acid (gastrin34) and 17-amino acid (gastrin17) are

present in plasma, being equipotent stimulants of gastric acid secretion. Circulating gastrin concentration is ten to twenty folds higher than CCK. Gastrin also acts as growth/differentiation factor in the gastrointestinal tract. Increased gastrin levels may be the first sign of such disorders such as *H. pylori* infection, short-bowel syndrome, antral G-cell hyperfunction-hyperplasia, *gastrinoma,* ECL hyperplasia and acid-peptic disorders (11). Gastrin is an important mediator of gastric mucosal growth, which may progress to the formation of gastric carcinoids (35). Gastrin secretion is suppressed by somatostatin, therefore, some inflammatory agents which suppress somatostatin release may lead to hypergastrinemia and higher acid secretion (36). Peripheral CCK administration, alone or with gastrin, stimulates ghrelin expression in peptic cells of gastric fundic glands (37).

Insulin, secreted by beta-pancreatic islet cells, and leptin, produced by adipocytes and peptic cells in oxyntic mucosa, belong to long-term adiposity signals that influence eating behavior at individual meals (16). They act in the brain, especially the hypothalamus, by enhancing central sensitivity to input from short-acting peripheral satiation signals, such as CCK (16). Analogous synergism between long- and short-acting signals occurs in the gut at the level of L cells, where activation of CCK-receptors augments the secretion of GPL-1, another satiety gut peptide. In this study, we decided to monitor the release of insulin and correlate it with the blood levels of glucose as well as other gut hormones affecting food intake.

The aim of this study was to examine the plasma concentrations of PYY_{3-36}, total ghrelin and acylated ghrelin, CCK, gastrin and insulin under fasting conditions and after a mixed meal and to answer the question whether and relationship exists between these peptide levels and symptoms of metabolic syndrome in obese women.

METHODS

Subjects

Eight lean, healthy women served as control group. Twelve moderately obese women with MS (OB-MS), and 17 morbidly obese women with MS (MOB-MS), qualified for weight-loss surgery, who had demonstrated a stable body weight for three months, were enrolled into this study. MOB-MS women reported earlier onset of obesity in adolescence (at ages of 11-19 years) in contrast to older moderately obese women (OB-MS), who became obese in adulthood (age above 20 years) (*Table 1*). All obese subjects were recruited from the endocrine outpatient clinics of Medical University of Silesia. The lean, healthy volunteers were recruited from medical and laboratory staff. The study was approved by the local Ethics Committee of the Medical University. All subjects agreed to participate in this study after oral and written information. To be eligible for this study, all volunteers declared to be free of major medical illnesses, such as renal or hepatic diseases, congestive heart failure, malignancy, or coronary artery disease and T2D. The criteria to enter the experimental design of the study also included the negative status of *H. pylori* infections, lack of hypothyroidism or any endocrinological disorders. In addition, they remained free of medications

known to alter body weight or cause the insulin resistance. MS was defined by 3 or more criteria; fasting plasma glucose >100 mg/dL (6.1 mmol/L), plasma triacylglycerides ≥ 150 mg/dL (1.7 mmol/L), plasma HDL-C <50 mg/dL (1.29 mmol/L), systolic blood pressure ≥ 140 mmHg systolic and diastolic pressure ≥ 90 mmHg or on antihypertensive treatment, abdominal obesity waist circumference >88 cm (derived from the National Cholesterol Education Panel, World Health Organization and International Diabetes Federation). We excluded participants with elevated hs-CRP levels above 10 mg/L, possibly indicating other clinically relevant, inflammatory conditions. Healthy, lean controls participated in the initial laboratory screening to exclude any feature of MS and hs-CRP levels above 1 mg/mL as signs of low grade inflammation. None of the controls were taking any medication or had a history of food dietary restrictions.

Study protocol

On the test day, at 08.00, after an overnight fast (at least 12 h), the subjects were admitted to the laboratory at the Department of Physiology. Anthropometric variables such as height, weight and waist circumference (WC) were measured. BMI, defined as weight (kg) per height squared (m^2), was calculated and used as an index for obesity. Body composition was estimated using bioelectrical impedance analyzer - B.I.A. 2000 M (Data Input GmbH, Frankfurt, Germany). Studies were conducted at an ambient temperature of 22±1°C. Following baseline blood sample collection (*T* 0 min), subjects consumed a standard mixed breakfast of about 527 kcal during 15 min. The meal consisted of 50 g white bread, 33 g black bread, 18 g margarine, 30 g cheese, 9 g jam and 200 mL of 0.5% fat milk (24.1% fat, 54.4% carbohydrate, 21.5% protein). Blood samples were collected at 30, 60 and 120 min after the meal ingestion.

Plasma hormone determination

The samples were collected into chilled tubes containing 1.2 mg EDTA and aprotinin (500 KIU/mL; Trasylol; Bayer Corp., Leverkusen, Germany) for hormone analyses. All samples were kept in an ice bath until centrifugation at 2000 rpm for 15 min at 4°C. Additionally, plasma for both total and acylated ghrelin and PYY_{3-36} was collected according to laboratory recommendations using HCl, PMSF and DPPIV inhibitor, respectively. The separated plasma samples were stored at –70°C until the time of the assay. All samples of one subject were run in duplicate in the same RIA performed partly in the laboratory of professor J. Rehfeld, Copenhagen, Denmark (except insulin which was assayed in Isotopic Laboratory of Department of Physiology, Cracow, Poland). Plasma PYY_{3-36}, total ghrelin and acylated ghrelin were measured using a commercial RIA kits (Linco Research, Missouri, USA); inter-assay and intra-assay coefficients for total ghrelin, acylated ghrelin, PYY_{3-36} were 7.0% and 14.3%, 3.3% and 17.8%, 11 and 15%, respectively. Plasma CCK concentrations were measured by CCK specific RIA. The lowest detectable concentration was 0.3 pmol/L. The intra-assay and inter-assay precisions were 4.4 % and 4.2 %, respectively. One volume of plasma (usually 1.0 mL) was mixed twice with 960 mL/L and stirred using mixer for 10 s. The mixture was then centrifuged for 30 min at 1200 x g, and the supernatant was decanted and evaporated at 37 °C in a Speed-Vac concentrator (SVC 200 H, Savant Instruments). The dried extracts were then reconstituted to the original volume with assay buffer and assayed. The measurements of CCK in plasma were performed with RIA using a panel of eight high titer CCK antisera specific for the C-terminal-amidated sequence of CCK as well as an antiserum specific for the N-terminus of human CCK22 and an antiserum specific for CCK glycine-extended at the C-terminus (37). Measurements of plasma gastrin were performed by RIA using antiserum 2604, which binds all carboxyamidated gastrins (gastrin-71, -34, -17, and -14) with equimolar potency regardless of their degree of sulfation. Antiserum 2604 does not bind any CCK peptide. The routine

detection limit of the assay, as employed in the present study, was 5 pg of synthetic human gastrin (SHG) per mL of plasma. Insulin was assayed using RIA kits purchased from Polatom, Otwock-Swierk in accordance with the manufacturer's recommendations.

Other investigations

In addition, glucose, total cholesterol (TC), high density lipoprotein cholesterol (HDL-C) and triacylglyceroles (TG) levels were measured using spectrophotometrical method on reagents (Diagnostic System GmbH, Holzheim, Germany). Low-density lipoprotein cholesterol (LDL-C) was calculated by the Friedwald formula (38) and high specific C-reactive protein (hs-CRP) using commercial kit (COBASA INTEGRA, C-Reactive Protein Latex). Insulin resistance was calculated by homeostatic model assessment (HOMA-IR) and was estimated using Matthews formula: [fasting insulin (μU/mL) x fasting glucose (mM)]/22.5 (39).

Statistical analysis

All results are expressed as means ± SEM. Comparisons between groups were made using Mann-Whitney's U-test. Differences between means were considered statistically significant at $P < 0.05$. All calculations were performed with STATISTICA 7.0.

RESULTS

Baseline characteristics of lean and obese subjects

As shown in *Table 1* for two groups of obese women (OB-MS and MOB-MS) with the onset of obesity 19-21 years ealier, the anthropometric features and some aspects of MS differed significantly. The MOB-MS women were approximately five years younger, had significantly higher waist circumference (WC), BMI and higher fat body mass (FBM) with simultaneously lower lean body mass (LBM) ($P<0.05$). In MOB-MS women, the low density lipoprotein-cholesterol (LDL-C) concentrations were slightly lower, whereas hs-CRP was almost 2-folds higher than those found in the OB-MS group. Other components of MS such as total cholesterol (TC), plasma glucose and insulin levels, HOMA-IR and also frequency of hypertension were comparable in both groups of obese women groups. In the lean, control group, the women's anthropometric features and all biochemical tests were within the normal range for our laboratory.

Hormonal responses to the test meal

PYY_{3-36}. As shown in *Fig. 1* and *Table 2,* there were no differences in fasting plasma PYY_{3-36} concentrations between OB-MS and lean control women, whereas MOB-MS women had significantly lower fasting PYY_{3-36} than lean controls and OB-MS women. After a test meal, mean plasma PYY_{3-36} concentration in lean controls increased by about 40% of fasting level at 60 min and remained elevated over fasting level to the end of postprandial observation period (120 min). In obese groups, the PYY_{3-36} responses to meal differed from those in lean controls.

Table 1. Patient characteristics and laboratory data in studied groups.

Characteristics	Control n = 8	OB-MS n = 12	MOB-MS n = 17
Age (y)	33.9 ± 3.7	37.1 ± 2.2	32.3 ± 1.7
Duration of obesity (y)	-	21.4 ± 1.8	19 ± 3.2
Waist circumference (cm)	76.4 ± 2.1	106.2 ± 1.9 *	123.3 ± 4.4 * #
BMI (kg/m^2)	23.2 ± 0.7	34.9 ± 0.9 *	46.9 ± 1.6 * #
Fat mass (%)	15.5 ± 1.1	34.3 ± 2.0 *	45.3 ± 1.3 * #
Lean body mass (%)	84.4 ± 2.8	65.7 ± 2.0 *	54.7 ± 1.3 * #
TC (mmol/L)	4.63± 0.75	5.42 ± 0.97	4.6 ± 0.67
HDL-C (mmol/L)	1.47± 0.12	1.44 ± 0.19	1.29 ± 0.42
TG (mmol/L)	1.01 ± 0.17	1.8 ± 0.32*	1.69 ± 0.2*
LDL-C (mmol/L)	2.55 ± 0.17	3.35 ± 0.53*	2.66 ± 0.24#
Fasting glucose (mmol/L)	5.5 ± 0.2	5.7 ± 0.4 *	6.1 ± 0.3 *
Fasting insulin (µU/mL)	16.1 ± 1.5	21.18 ± 4.8	26.8 ± 2.2 *
HOMA IR	1.9 ± 0.4	6.2 ± 2.4 *	6.6 ± 2.6 *
hs-CRP (mg/L)	0.7 ± 0.2	4.3 ± 0.6 *	8.6 ± 1.7 * #
Hypertension n (%)	0 (0)	6 (50)	8 (47)

The values are presented as mean±SEM; $^{*}P < 0.05$ *vs.* control group; $^{\#}P < 0.05$ *vs.* OB-MS group. Abbreviations: OB-MS – obese women with metabolic syndrome, MOB-MS – morbidly obese women with metabolic syndrome, BMI – body mass index, TC – total cholesterol, HDL-C – high density lipoprotein cholesterol, TG – triacylglicerydes, LDL-C – low density lipoprotein cholesterol, HOMA IR – homeostasis model assessment, hs-CRP – high specific C reactive protein.

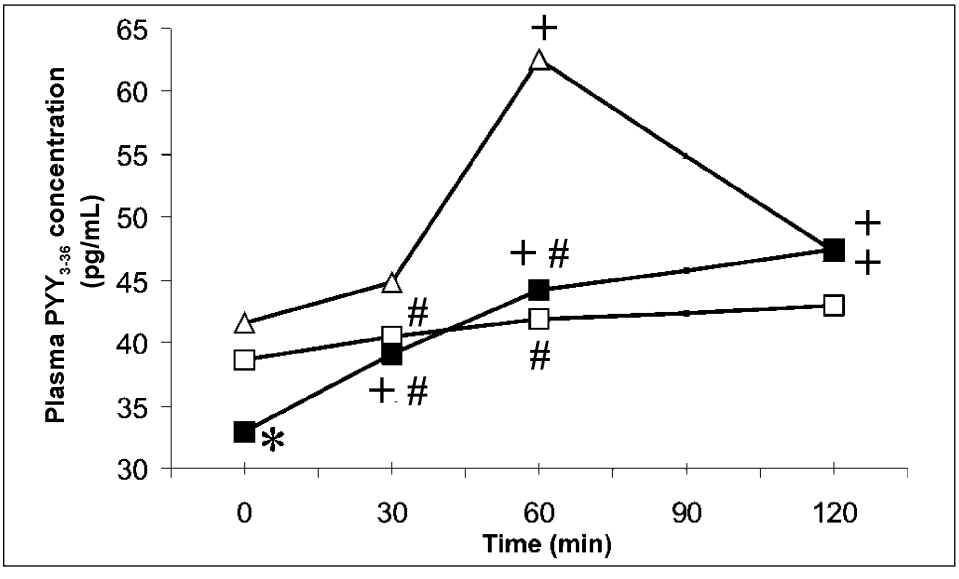

Fig. 1. Fasting and postprandial concentrations of PYY_{3-36} after 30, 60 and 120 min after meal in lean control women (Δ) and in those with moderate obesity (OB-MS) (□) and with morbid obesity (MOB-MS) (■). Mean ± SEM. Asterisk indicates significant ($P < 0.05$) decrease as compared to the fasting values recorded in lean control women. Cross indicates significant increase as compared to the fasting values. Hash indicates significant decrease below the value recorded in lean controls.

Table 2. Fasting plasma and postprandial responses for PYY_{3-36}, total ghrelin, active ghrelin, CCK, gastrin, glucose and insulin.

	Control n = 8	OB-MS n = 12	MOB-MS n = 17
PYY (3-36) Fasting values (pg/mL)	41.5 ± 2.3	38.6 ± 1.1	32.9 ± 1.2*
Total ghrelin Fasting values (pg/mL)	850 ± 86	555 ± 67*	701 ± 78
Acylated ghrelin Fasting values (pg/mL)	199 ± 23	108 ± 12*	194 ± 27#
CCK Fasting values (pmol/L)	1.27 ± 0.08	1.11 ± 0.05	0.35 ± 0.02*
Gastrin Fasting values (pmol/L)	7.2 ± 1.27	8.2 ± 1.81	7.9 ± 1.04
Glucose Fasting values (mmol/L)	5.6 ± 0.3	5.1 ± 0.2	6.6 ± 0.4*
Insulin Fasting values (μU/mL)	16.0 ± 1.3	20.8 ± 3.4	25.3 ± 4.3

The values are presented as mean±SEM; *P<0.05 *vs.* control group; #P<0.05 *vs.* OB-MS group. Abbreviations: OB-MS – obese women with metabolic syndrome; MOB-MS – morbidly obese women with metabolic syndrome.

Postprandial plasma PYY_{3-36} concentrations in MOB-MS women showed small but significant increase at 30 and 60 min after meal, but these increments were significantly lower than in lean controls. Then, the hormone level slightly increased to achieve its highest values at 120 min, which was not different from that observed in lean controls. In contrast, the standard meal failed to affect the plasma release of PYY_{3-36} in OB-MS women at all time periods tested (*Fig. 1*). Thus, in OB-MS women, the plasma PYY_{3-36} level after a meal was not significantly increased when compared to that measured in lean controls and MOB-MS women. Both obese groups showed postprandial (measured at 30 and 60 min after meal) plasma PYY_{3-36} levels significantly lower than those observed in lean controls.

Total ghrelin. *Fig. 2* and *Table 2* show the plasma fasting total ghrelin levels that were significantly lower in the OB-MS and MOB-MS compared with those in lean controls (P<0.05). Only in the lean controls, total plasma ghrelin fell significantly by about 30% at 30 min after the meal and remained lowered throughout the observation period (60 and 120 min). In both obese groups, the meal ingestion failed to cause any significant alterations in total ghrelin levels. Thus, there were no significant effects of the meal on total ghrelin response in MOB-MS patients. In OB-MS women the total plasma levels of ghrelin showed only a minor and not significant decrease after a meal and the ghrelin concentrations were significantly lower than those in healthy lean controls and MOB-MS women *(Fig. 2).*

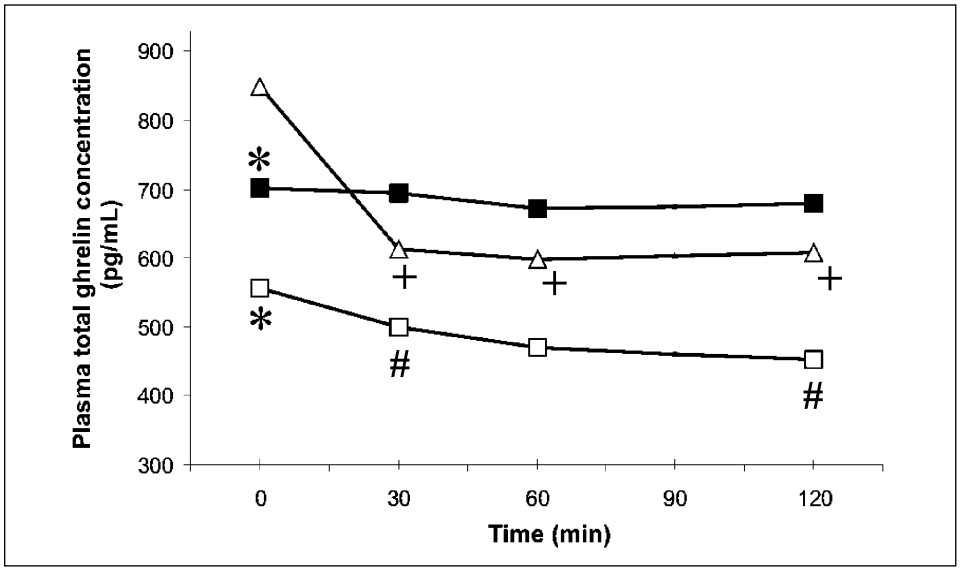

Fig. 2. Fasting and postprandial concentrations of total ghrelin measured after 30, 60 and 120 min after meal in lean control women (Δ) and in those with moderate obesity (OB-MS) (□) and with morbid obesity (MOB-MS) (■). Mean ± SEM. Asterisk indicates significant decrease ($P < 0.05$) as compared to the values recorded under fasting conditions in lean control women. Cross indicates significant decrease as compared to the fasting values. Hash indicates significant decrease below the value recorded in lean controls.

Acylated ghrelin. As shown in *Fig. 3 and Table 2,* there were no differences in fasting acylated plasma ghrelin concentrations between lean controls and MOB-MS women, while fasting acylated ghrelin levels were significantly decreased by about 50% in OB-MS women. Similarly as total ghrelin, also acylated ghrelin levels in lean controls dropped significantly following meal ingestion by about 60% at 30 min ($P<0.05$) and remained decreased till the end of 2 h observation period. Neither OB-MS nor MOB-MS women showed any significant alteration in the acylated ghrelin levels after ingestion of a meal, though these levels in OB-MS were significantly lower compared to those observed in MOB-MS women.

Cholecystokinin (CCK). As shown in *Fig. 4* and *Table 2,* fasting plasma CCK concentrations were markedly lower in MOB-MS women when compared to those observed in lean controls and OB-MS women ($P<0.05$). In lean control women, after ingestion of meal, CCK concentrations increased by about 3-folds at 30 min, slightly decreasing at 60 min and increasing again at 120 min following meal. The plasma CCK responses to a meal in OB-MS and MOB-MS subjects showed a steady increase, reaching peak values at 60 min (*Fig. 4*). The postprandial CCK increase in MOB-MS was significantly lower than those in lean controls and in OB-MS women (except for 60 min after a meal).

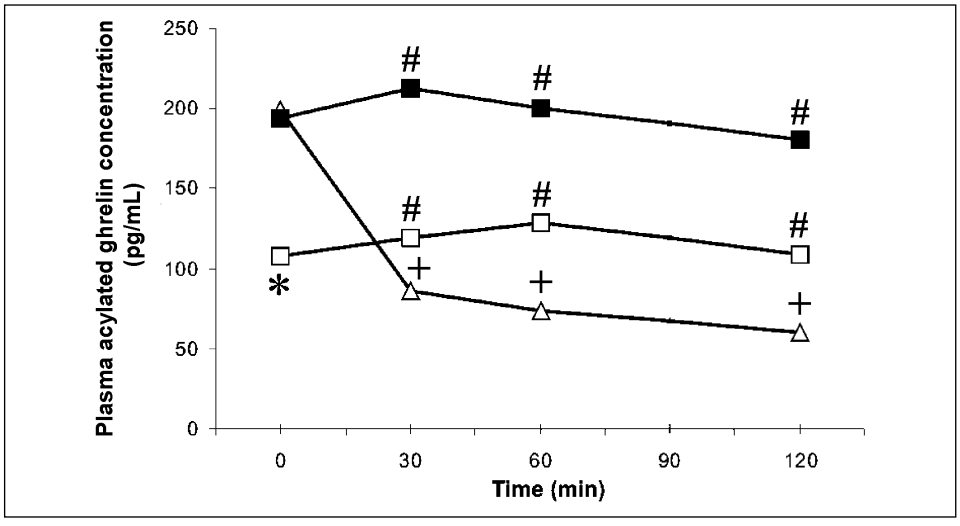

Fig. 3. Fasting and postprandial concentrations of acylated ghrelin measured after 30, 60 and 120 min after meal in lean control women (Δ) and in those with moderate obesity (OB-MS) (□) and with morbid obesity (MOB-MS) (■). Mean ± SEM. Asterisk indicates significant decrease ($P < 0.05$) as compared to the values recorded under fasting conditions in lean control women. Cross indicates significant decrease as compared to the fasting values. Hash indicates significant increase below the value recorded in lean controls.

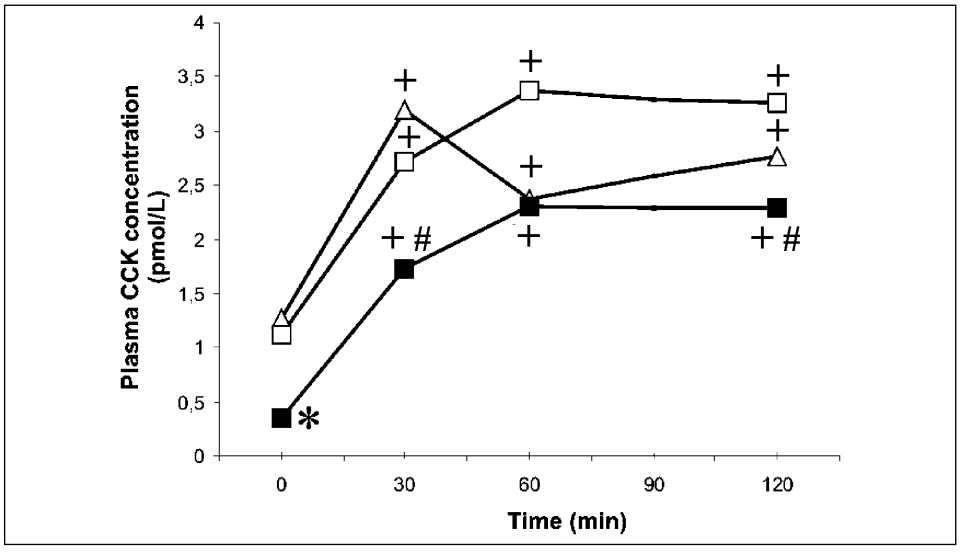

Fig. 4. Fasting and postprandial concentrations of cholecystokinin measured after 30, 60 and 120 min after meal in lean control women (Δ) and in those with moderate obesity (OB-MS) (□) and with morbid obesity (MOB-MS) (■). Mean ± SEM. Asterisk indicates significant decrease ($P < 0.05$) as compared to the values recorded under fasting conditions in lean control women. Cross indicates significant increase as compared to the fasting values. Hash indicates significant decrease below the value recorded in lean controls.

Gastrin. As shown in *Table 2* and *Fig. 5,* in lean controls and both obese groups, fasting plasma gastrin levels and incremental rise after meal displayed similar patterns. Fasting gastrin concentrations were similar in all three groups tested. Postprandially, gastrin concentrations peaked by nearly 5-folds in lean women and almost 4-folds in OB-MS, at 60 min and 30 min, respectively, whereas in MOB-MS group the postprandial peak increase in plasma gastrin release was significantly diminished when compared to that observed in lean controls and OB-MS group.

Insulin. *Fig. 6* and *Table 2* show fasting plasma insulin concentrations, which were similar both in lean controls and obese groups. Postprandially, plasma insulin levels in lean controls reached the peak at 30 min after meal and then declined gradually towards the fasting levels at the end of 2 h period. In both obese groups (OB-MS and MOB-MS) the meal-induced increments in plasma insulin levels were significantly higher than those observed in lean controls. The peaks of plasma insulin in both obese groups occurred 60 min after meal, reaching the values several folds higher than in lean controls. At the end of postprandial period (2 h) plasma insulin levels in both obese groups were still significantly higher than those recorded under basal conditions and about 3-folds higher as compared to those in the lean controls.

Glucose. Fasting plasma glucose in MOB-MS was significantly higher compared to lean controls (5.4 *vs.* 6.1 mmol/L) but not to OB-MS women (*Fig. 7* and *Table 2*). Postprandial increments in glucose level observed at 30 min after

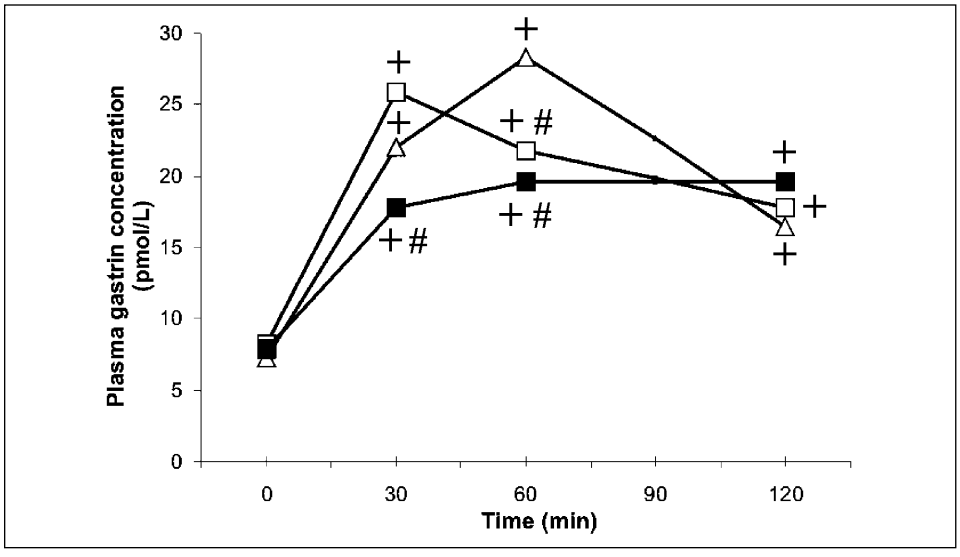

Fig. 5. Fasting and postprandial concentrations of gastrin measured after 30, 60 and 120 min after meal in lean control women (Δ) and in those with moderate obesity (OB-MS) (□) and with morbid obesity (MOB-MS) (■). Mean ± SEM. Cross indicates significant ($P < 0.05$ increase as compared to the fasting values. Hash indicates significant decrease below the value recorded in lean controls.

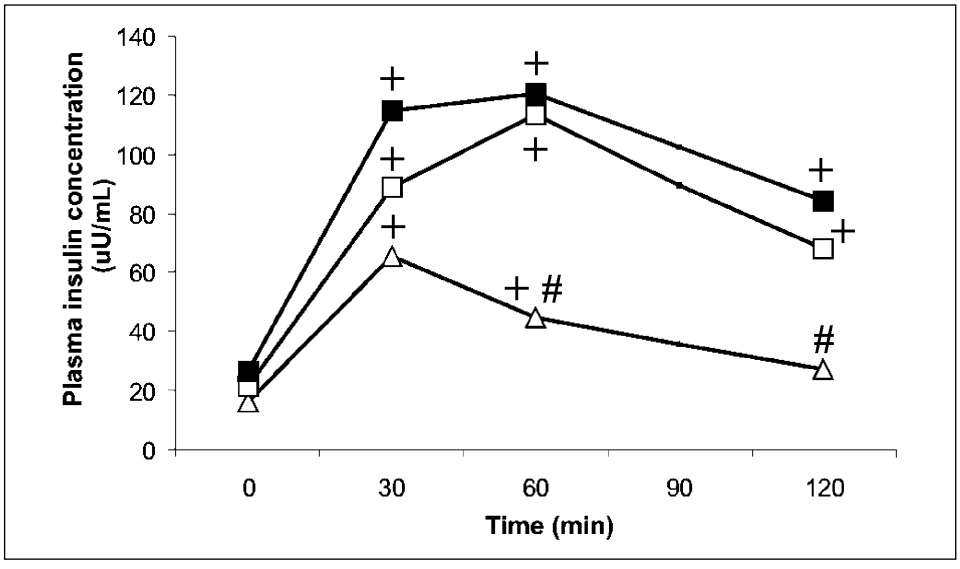

Fig. 6. Fasting and postprandial concentrations of plasma insulin measured after 30, 60 and 120 min after meal in lean control women (Δ) and in those with moderate obesity (OB-MS) (□) and with morbid obesity (MOB-MS) (■). Mean ± SEM. Cross indicates significant ($P < 0.05$) increase as compared to the fasting values. Hash indicates significant decrease below the value recorded in obese patients.

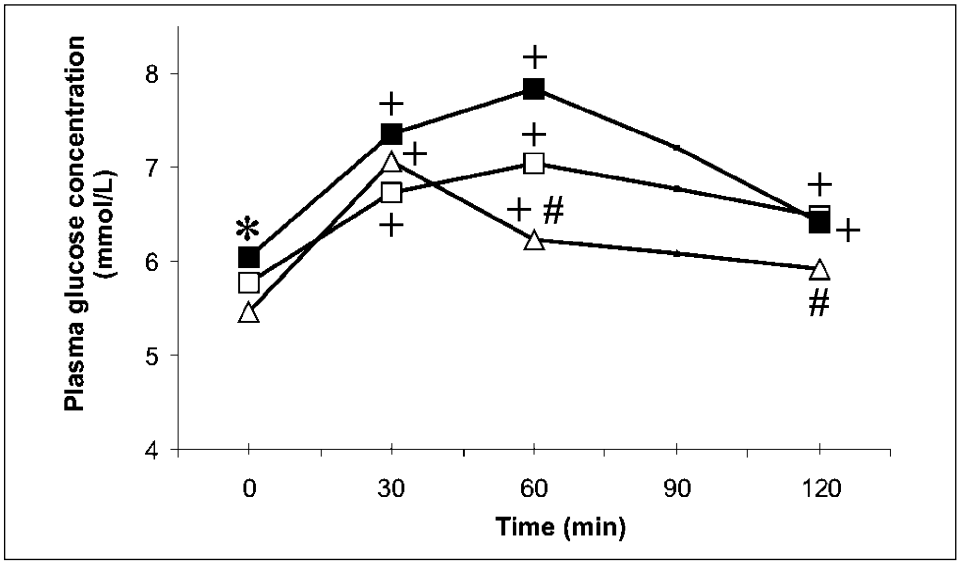

Fig. 7. Fasting and postprandial concentrations of plasma glucose measured after 30, 60 and 120 min after meal in lean control women (Δ) and in those with moderate obesity (OB-MS) (□) and with morbid obesity (MOB-MS) (■). Mean ± SEM. Asterisk indicates significant increase ($P < 0.05$) as compared to the values recorded under fasting conditions in lean control women. Cross indicates significant decrease as compared to the fasting values. Hash indicates significant decrease below the value recorded in obese patients.

Table 3. Spearman's correlation coefficients of the relation between different variables in obese patients.

	Hs-CRP	Fasting PYY_{3-36}	Fasting active ghrelin	Fasting total ghrelin
BMI	0.71 (< 0.001)	NS	NS	- 0.35 (0.02)
Waist circumference	0.62 (< 0.001)	NS	NS	NS
Fat mass percentage	0.49 (< 0.001)	NS	0.50 (0.04)	0.43 (0.04)
Fasting glucose	0.32 (0.009)	NS	NS	NS
HOMA-IR	0.47 (< 0.001)	NS	NS	- 0.44 (0.004)
TC	NS	0.41 (0.002)	- 0.41 (0.009)	NS
LDL-C	NS	0.28 (0.002)	- 0.48 (0.003)	NS
HDL-C	NS	NS	NS	0.31 (0.04)
Fasting total ghrelin	- 0.31 (0.03)	NS	NS	-
Fasting active ghrelin	0.45 (0.003)	NS	-	NS

All values are mean correlation coefficients (r), *P* values in parentheses; NS – no significant. Abbreviations: BMI – body mass index; Hs-CRP – high specific C-reactive protein; TC – total cholesterol; LDL-C – low density lipoprotein cholesterol; HDL-C – high density lipoprotein cholesterol.

meal were similar in all three groups, but then at 60 and 120 min, the peak glucose levels in OB-MS and MOB-MS were significantly higher than in lean controls.

Relationship between hs-CRP, metabolic syndrome features and gut hormones

As shown in *Table 3* there was a highly significant correlation between plasma concentrations of hs-CRP and metabolic features of MS such as BMI, waist circumference, percentage of fat mass, fasting glucose and HOMA IR. In contrast, there were negative correlations between hs-CRP and fasting acylated or total ghrelin. We found also a positive correlation between fasting PYY_{3-36} concentrations, whilst negative between fasting acylated ghrelin and TC or LDL-C. In addition, there was a positive correlation between fasting acylated or total ghrelin and percentage of fat mass. Furthermore, there were negative correlations between total fasting ghrelin and BMI or HOMA-IR. Moreover, there was a significant correlation between fasting total ghrelin and HDL-C. No other clinically relevant correlations were found among PYY_{3-36}, ghrelin, CCK, gastrin, hs-CRP data, and features of metabolic syndromes.

DISCUSSION

The major finding of this study is the demonstration of the fasting and postprandial changes in plasma levels of gut peptides involved in the control of food intake such as total ghrelin, acylated ghrelin, PYY_{3-36}, CCK and insulin in

lean controls as well as two subcategories of obesity with MS, one concerning older women with moderate obesity, smaller BMI and fat mass (OB-MS) and another including younger women with morbid obesity (MOB-MS) (41-49).

Total and acylated ghrelin, which represents the most powerful orexigenic hormone, failed to show a decrease in plasma levels in our obese women following ingestion of mixed meal that is typically observed in lean controls. Instead, elevated plasma levels of total and acylated ghrelin were sustained after a meal, especially in MOB-MS women, which exhibited also significantly higher BMI and fat mass and lower lean body mass. Our finding that the plasma levels of both total and acylated plasma ghrelin are significantly elevated, particularly in MOB-MS, suggests that the constant feeding drive observed in these patients may originate from relatively high plasma level of this potent orexigenic peptide. Our study confirms that endogenous ghrelin exerts a potent appetitive behavior, possibly through influencing the activity of vagal afferent nerves and receptors localized in the hypothalamic NPY/AGRP neurons of the arcuate nucleus and in feeding center. This notion is supported by the observations in experimental animals that chronic ghrelin administration increases body weight through numerous effects such as stimulation of food intake, reduction in energy expenditure and fuel utilization. It is worthy to emphasize that the most characteristic feature of the obese-related increase in plasma ghrelin is the lack of the decrease in plasma hormone after a meal ingestion. Our extremely obese women with a stable body weight did not demonstrate any suppression of total or acylated ghrelin after a meal, the phenomenon observed in all lean controls. These data are consistent, in part, with reports showing that circulating total fasting ghrelin level is usually lower in obese subjects exhibiting an insulin resistance (40). This is also consistent with other observations that nutrients induced insulin secretion fails to suppress ghrelin in obese subjects (50). We also measured acylated ghrelin, which in postprandial conditions was highest in extremely obese subjects and constituted about 30% of total ghrelin, whereas in non-obese lean controls - only 15% of total ghrelin. We confirmed that total ghrelin concentration does not reflect the changes in the concentrations of acylated ghrelin in obese subjects with metabolic syndrome as proposed previously (51, 52). In our study paradoxically ‘’hyperghrelinemia’’of acylated form occurred mainly in severely obese subjects. It is likely that clinical consequences of such permanent hyperphagia, so characteristic for exceptionally obese individuals, seems to be understandable because active ghrelin accelerates gastric emptying and gastrointestinal transit by activating excitatory, cholinergic pathways in the vagal nerves. Furthermore, our results agree with paradoxically high basal ghrelin level and its responses recorded by Beck *et al.* in heavy, obese Zucker rats as compared to slightly obese animals (53). Considering the metabolic features associated with morbid obesity, the present study shows that total ghrelinemia is inversely correlated with higher BMI, insulin resistance and CRP and fat mass percentage, while acylated ghrelinemia was positively correlated with the percentage of fat mass and also with CRP levels.

Furthermore, it was also found that circulating acylated ghrelin levels are negatively correlated with TC and LDL-C levels. On the basis of the cohort study of lipid profile in 527 severe obese patients before gastric surgery, Dixon & O'Brien reported that fasting TC and LDL-C concentrations were usually normal and also shown that increasing BMI is associated with lower TC in severely obese patients (54). Such lipids profile in extremely obese subjects remains unclear. Our study has confirmed that TC and LDL-C concentrations, without significantly decreased HDL-C, were also usually normal in morbidly obese individuals. Among many other functions the active ghrelin have beneficial metabolic effects by participating in lipid metabolism at least by two mechanisms: active ghrelin co-purify with HDL, destroys lipid peroxides on LDL and may down-regulate CD36 scavenger macrophage receptor (55, 56). The current studies suggest that circulating acylated ghrelin is also closely related to paraoxonase-1 (PON1) that is associated with high-density lipoproteins and capable of hydrolyzing oxidized lipids as well as preventing the oxidation of low-density lipoproteins. Octanoyl group of ghrelin may be bound with an ester to paraoxonase suggesting a possible role of this enzyme in the conversion of acylated- to desacylated-ghrelin form (57). Among positive effects of acylated ghrelin that need to be mentioned is its anti-inflammatory, as well as indirect effect preventing hypertension in obesity. Unfortunately, in our study we were unable to show the relationship between the concentration of ghrelin and the presence of hypertension in obese subjects. On the other hand, recently, ghrelin has been shown to exert an vasoconstrictive action and also its positive association with carotid artery atheroclerosis in cohort study in males (58). We hypothesise that the results of the present study indicate the compensatory upregulation of active ghrelin concentrations particularly in severely obese patients.

As indicated in the introduction, PYY belongs to PP family of 36 amino acid peptides co-released together with GLP-1 from endocrine distal-intestinal L cells in response to caloric load with micronutrient potency of lipids being greater than that of carbohydrates, which is greater than that of protein. PYY_{1-36} released from L cells into the circulation is immediately proteolyzed by DPP-IV to bioactive PYY_{3-36} that exerts a potent anorexigenic influence observed in lean control subjects. Since fasting levels of PYY_{3-36} was significantly reduced in obese, particularly in morbidly obese patients, compared to lean controls, it may be reasonable to assume that the enhanced appetite in these obese subjects originates, at least in part, from the deficiency of plasma circulating level of PYY_{3-36} (20, 41). Moreover, obese patients, unlike lean controls, failed to show any major increase in plasma levels of PYY_{3-36}, explaining the lack of usual postprandial reduction in food intake and in appetitive drive. In our study, the plasma levels of PYY_{3-36}, which in lean control women showed a marked increment 60 min following mixed meal, exhibited a marked suppression observed in obese women throughout the postprandial observation period. In contrast to other studies on PYY_{3-36}, a lower fasting concentration of PYY_{3-36} was

discovered only in massively obese patients (20, 41). As presented in the introduction, PYY_{3-36} constitutes the dominant form of total PYY in circulation; nevertheless, due to the lack of corresponding data on the secretion of PYY_{3-36} in available literature, it is not possible to compare our results with the data related to total peptide.

According to le Roux *et al*. (41), the ratio of plasma concentrations between PYY_{1-36} to PYY_{3-36} was similar in normal-weight and obese subjects (41). Other authors, however, demonstrated that the secretion of PYY_{3-36} constitutes slightly above 50% of the portion of the total circulating PYY_{1-36} (19). In our study, we failed to explored the total PYY, therefore, the results we obtained for PYY_{3-36} cannot be compared to the total PYY. Many earlier reports show that the total PYY is suppressed in obese subjects as a consequence of insulin resistance and higher glucose concentration (20, 41). In our study, two examined obese women groups differed from lean controls by showing lower plasma PYY_{3-56} both under basal conditions and after meal and this was accompanied by higher postprandial blood glucose and insulin levels. Combined with this fact, the differences in the levels of PYY_{3-36} concentration in both obese groups, in our opinion, may be connected with increased blood glucose and augmented postprandial levels of insulin and possibly other hormonal and metabolic disturbances, originating from factors released form adipose tissue (7).

However, considering the smaller postprandial secretion of PYY_{3-36} in obese patients, it should be noted that exposure to the anorectic effects of this gut-brain peptide over the postprandial period of 2 h in severely obese persons was much smaller than that in healthy lean subjects, suggesting that in patients with massive obesity, functional damage to the hypothalamus may occur, resulting in decrease of satiety and relative increase of food intake. Thus, obese subjects may have PYY deficiency that would reduce satiety and thus could reinforce their obesity (43). The PYY_{3-36}, a most potent endogenous Y2R agonist may, modulate insulin action *via* Y2R. On the other hand, hypothalamic overexpression of NPY and its receptors during stress, induced obesity and insulin resistance in mice and may mask the anorectic properties of PYY_{3-36}. Results conducted on animals show that the Y2R appears in arteries of spontaneous hypertensive rats (SHR). The activation of this receptor may result in vasoconstrictors effects suggesting that Y2R plays a role in the regulation of blood pressure (44). Hoek *et al.* also demonstrated that PYY_{3-36} augmented insulin activity in glucose disposal in mice fed a high–fat diet through a mechanism independent of food intake and body weight (45). Taking into account metabolic effects demonstrated in the empirical model of PYY_{3-36} participation in insulin sensitivity, it can be assumed that low concentrations of PYY_{3-36} in obese subjects may partly contribute to their insulin resistance. Our results demonstrating higher levels of circulating ghrelin, that oppose and counteract a PYY-acting hormone, may actually be involved in the suppression of PYY_{3-36} but only in particularly obese individuals. More explanations come from the studies concerning visceral banded gastroplasty and

jejuno-ileal-bypass, where plasma level of total PYY is significantly increased compared to non-operated controls. These studies suggest that the number of PYY-containing cells is decreased in obese patients and increased following operation. Operations or diseases, which hinder absorption, stimulate basic and meal-induced PYY levels (46). Thus, the combination of increased PYY levels and elevated activity of DPP-IV, resulting in increased PYY_{3-36} observed after bariatric operations, may lead to reduction in appetite and decrease in food intake.

In human studies with DPP-4's chemical inhibitors preventing the inactivation of both glucagon-like peptide-1 (GLP-1) and gastric inhibitory peptide (GIP) and lowering blood glucose; enhanced levels of incretin hormones, such as GLP-1, GIP, and insulin occurred, preventing weight gain (42, 47). Batterham *et al*. has shown the inhibitory effects of an infusion of PYY_{3-36} on appetite and food intake for obese patients, but so far the attempts of using PYY_{3-36} in humans have not contributed significantly to substantial reduction in body mass, and, moreover, its use is limited by side effects (17, 18, 48, 49).

We also attempted to determine the patterns of secretion of other gut hormones such as CCK and gastrin in lean controls and obese women. In our study we found the lowest fasting CCK plasma levels and lower plasma CCK response to a meal in morbidly obese women as compared to lean controls. Our results are in agreement with data of French *et al.* who also found differences in CCK postprandial profile between massive obese patients and healthy subjects (59). Low fasting plasma CCK levels and reduced postprandial peak of this anorexigenic gut hormone may be an insufficient signal for the satiety center at the level of hypothalamus, leading to increased food intake and subsequent obesity. Recent studies using CCK receptor agonist or antagonist showed a significant effect of physiologically released CCK on gastrointestinal motility, postprandial contraction, inhibition of gastric contractions and inhibition of colon transit (38, 60). In our obese patients we demonstrated the reduced and delayed meal-stimulated CCK release similar to that shown in bulimia nervosa also accompanied by deficiency of CCK (61). We suggest that not only low postprandial concentrations of CCK but also altered patterns of CCK release by meal may contribute to obesity. Moreover, CCK interacts with PYY and also with ghrelin. CCK stimulates release PYY from L-cells and antagonizes orexigenic ghrelin effects on vagal afferent fibres (62). Kobelt *et al.* showed that peripherally administered CCK together with ghrelin inhibited ghrelin-induced feeding behavior and ghrelin-induced Fos expression in the hypothalamic arcuate nucleus (63). CCK acting *via* CCK-1 receptors on antral D cells that stimulate somatostatin release, which has an inhibitory effect on gastrin secretion from G cells. Since both fasting and postprandial release of CCK in severely obese individuals were much lower than in moderately obese women (OB-MS) and lean controls, it is possible that the attenuation of the satiating action of this hormone, similarly to that of PYY_{3-36}, could contribute to the increase in food intake and to increased appetitive behavior observed in obese patients.

We conclude that: 1. metabolic syndrome features accompanying obesity are closely related to plasma alterations of major orexigenic peptides such as ghrelin and of anorexigenic hormones such as PYY_{3-36} and CCK both under fasting and postprandial conditions; and 2. determination of plasma levels of hormones controlling food intake could be considered as a valuable tool to assess progression of metabolic syndrome to comorbidities in obesity.

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