

Serum Levels of Spexin and Kisspeptin Negatively Correlate With Obesity and Insulin Resistance in Women

P. A. KOŁODZIEJSKI¹, E. PRUSZYŃSKA-OSZMALEK¹, E. KOREK⁴, M. SASSEK¹, D. SZCZEPANKIEWICZ¹, P. KACZMAREK¹, L. NOGOWSKI¹, P. MAĆKOWIAK¹, K. W. NOWAK¹, H. KRAUSS⁴, M. Z. STROWSKI^{2,3}

¹Department of Animal Physiology and Biochemistry, Poznan University of Life Sciences, Poznan, Poland, ²Department of Hepatology and Gastroenterology & The Interdisciplinary Centre of Metabolism: Endocrinology, Diabetes and Metabolism, Charité-University Medicine Berlin, Berlin, Germany, ³Department of Internal Medicine, Park-Klinik Weissensee, Berlin, Germany, ⁴Department of Physiology, Karol Marcinkowski University of Medical Science, Poznan, Poland

Received August 18, 2016

Accepted June 19, 2017

On-line November 10, 2017

Summary

Spexin (SPX) and kisspeptin (KISS) are novel peptides relevant in the context of regulation of metabolism, food intake, puberty and reproduction. Here, we studied changes of serum SPX and KISS levels in female non-obese volunteers (BMI<25 kg/m²) and obese patients (BMI>35 kg/m²). Correlations between SPX or KISS with BMI, McAuley index, QUICKI, HOMA IR, serum levels of insulin, glucagon, leptin, adiponectin, orexin-A, obestatin, ghrelin and GLP-1 were assessed. Obese patients had lower SPX and KISS levels as compared to non-obese volunteers (SPX: 4.48±0.19 ng/ml vs. 6.63±0.29 ng/ml; p<0.001, KISS: 1.357±0.15 nmol/l vs. 2.165±0.174 nmol/l; p<0.01). SPX negatively correlated with BMI, HOMA-IR, insulin, glucagon, active ghrelin and leptin. Positive correlations were found between SPX and QUICKI index, McAuley index, serum levels of obestatin, GLP-1 and adiponectin and orexin-A. Serum KISS negatively correlated with BMI, HOMA-IR, serum levels of insulin, glucagon, active ghrelin and leptin. KISS positively correlated with QUICKI index, McAuley index and adiponectin. In summary, SPX and KISS show negative correlations with obesity, insulin resistance indices, and hormones known to affect insulin sensitivity in females. Both, SPX and KISS could be therefore relevant in the pathophysiology of obesity and insulin resistance.

Key words

Spexin • Kisspeptin • Obesity • Insulin resistance

Corresponding author

P. A. Kolodziejski, Department of Animal Physiology and Biochemistry, Poznan University of Life Sciences, Wolynska Street 28, 60-637 Poznan, Poland. E-mail: pawelbigi@o2.pl

Introduction

Kisspeptin (KISS) and spexin (SPX) are peptides involved in regulation of body weight, metabolism and sexual functions. In 2014, Kim and coworkers showed that *SPX* gene resides in the near vicinity of galanin (*GAL*) and *KISS* gene family. Moreover, a sequence comparison of mature SPX, GAL and KISS peptides revealed that they share similarities (Kim *et al.* 2014). SPX, also known as NPQ is a highly conserved peptide consisting of 14 amino acids, which belongs to *SPX/GAL/KISS* gene family (Mirabeau *et al.* 2007, Sonmez *et al.* 2009). It has been shown that SPX is a ligand for galanin receptor 2 (GALR2) and galanin receptor 3 (GALR3) (Kim *et al.* 2014). Porzionato described SPX expression in various rat tissues, such as liver, kidney, brain, hypothalamus, thyroid, ovary, testis, adrenal, skeletal muscle, heart, lung, pancreas and gastrointestinal tract (Porzionato *et al.* 2010). Expression of SPX in these tissues indicates its involvement in numerous physiological processes (Porzionato *et al.* 2012, Wong *et al.* 2013). Still little is known about physiological role of spexin in mammals. It is

known that spexin regulates food intake in mice as well as in fish, whereas it inhibits long chain fatty acid uptake in adipocytes and hepatocytes (Walewski *et al.* 2014, Wu *et al.* 2015, Jasmine *et al.* 2016). Moreover, Jasmine *et al.* showed that spexin downregulates liver lipid content in mice (Jasmine *et al.* 2016). A recent research published in 2017 indicates that insulin is able to regulate spexin secretion in goldfish (Ma *et al.* 2017).

The product of the KISS-1 gene is a precursor peptide termed preprokisspeptin, which consists of 144 amino acids. Its proteolysis yields a 54 amino acid peptide – kisspetide-54 (KISS54), formerly known as metastin. Three biologically active elimination products are known KISS14, KISS13 and KISS10. All three isoforms bind to GPR54 receptor (Messenger *et al.* 2005). GPR54 and KISS-1 are produced in the brain, spinal cord, placenta, liver, pancreas, adipose tissue, small intestine, heart, liver, muscle, spinal cord, ovary and kidney (Kotani *et al.* 2001, Ohtaki *et al.* 2001). The initially described role of KISS consists of stimulation of the secretion of sex hormones and the initiation of the maturation process (De Roux *et al.* 2003, Seminara *et al.* 2003). Impaired KISS signaling reduces energy expenditure and promotes glucose intolerance, and obesity (Tolson *et al.* 2014). Moreover, KISS is able to regulate insulin secretion in rodents (Bowe *et al.* 2009).

KISS affects insulin secretion, energy expenditure and modulates reproductive functions (Bowe *et al.* 2009, Tolson *et al.* 2014). SPX and KISS appear to regulate glucose and/or fat metabolism and may be relevant in the context of obesity or type 2 diabetes (Song *et al.* 2014, Walewski *et al.* 2014, Gu *et al.* 2015). Hepatic KISS expression is regulated by glucagon, and KISS suppresses glucose-stimulated insulin secretion (GSIS) from β cells (Song *et al.* 2014). Recently it was shown that SPX and KISS decrease body weight, reduce caloric intake, and enhance bowel movement in mice (Stengel *et al.* 2011, Walewski *et al.* 2014, Lin *et al.* 2015). *In vitro* studies showed that SPX inhibits stimulated fatty acid (FA) uptake in primary adipocytes isolated from obese mice. In obese humans, SPX and KISS mRNA are strongly downregulated in omental and subcutaneous fat (Brown *et al.* 2008, Walewski *et al.* 2014). Serum SPX levels are low in type 2 diabetes patients and negatively correlate with fasting blood glucose levels, HbA1c, triglycerides and LDL cholesterol (Gu *et al.* 2015).

Taking into account that the expression and roles of KISS and SPX are still not well understood, we measured SPX and KISS serum concentrations in

healthy, non-obese volunteers and obese patients. Moreover, we analyzed the relationships between serum levels of both peptides and body composition (fat mass, fat free mass, % body fat) defined by bioelectrical impedance analysis (BIA), body weight, insulin sensitivity, serum levels of insulin, glucagon, ghrelin, obestatin, adiponectin, orexin-A and leptin.

Methods

Study participants

The study participants were healthy female (n=15, BMI 22.50±0.58) and obese patients (n=15, BMI 40.23±1.31). Age, metabolic and hormonal profiles of study participants are shown in Table 1. The study was conducted according to the principles stated in the Declaration of Helsinki. All study participants were informed about the study objectives and the methodology. Each study participant gave a written consent. The participants were considered non-obese if their body mass index (BMI) was less than 25 kg/m². Obese patients (BMI above 35 kg/m²) were tested prior inclusion in the study for the presence of diabetes by oral glucose tolerance test (OGTT) using 75 g glucose. Diabetic individuals according to ADA criteria (Diabetes 2016) were excluded from the study.

Body composition

Body composition was analyzed by bioelectrical impedance analysis (BIA) method using segmental body composition analyzer Tanita BC-418 MA (Tanita, Japan).

HOMA-IR, McAuley, Quicki indices calculation

Homeostatic model assessment of insulin resistance (HOMA-IR) (Matthews *et al.* 1985), insulin sensitivity check index (QUICKI) (Katz *et al.* 2000), McAuley's index (McA) (McAuley *et al.* 2001) were calculated based on fasting glucose (G0), insulin (I0) and triglycerides (TG0) levels using the following formula:

$$\text{HOMA-IR} = (\text{G0} \times \text{I0}) / 22.5;$$

$$\text{QUICKI} = 1 / [\log(\text{G0}) + \log(\text{I0})];$$

$$\text{McA} = \exp [2.63 - 0.28 \ln(\text{I0}) - 0.31 \ln(\text{TG0})].$$

Metabolic profile

Triglycerides, NEFA, cholesterol and glucose levels in serum were determined by colorimetric assays (Pointe Scientific, USA). Optical density of samples was measured using a microplate reader Synergy 2 (Biotek, USA).

Table 1. Metabolic and hormonal profiles of study participants.

| Parameter | Non-obese group (n=15) | Obese Group (n=15) |
|--------------------------|------------------------|--------------------|
| Age (years) | 42.90 ± 5.26 | 42.21 ± 3.31NS |
| BMI (kg/m ²) | 22.26 ± 0.54 | 39.79 ± 1.01*** |
| Body fat (%) | 23.25 ± 1.95 | 40.76 ± 1.16*** |
| FM (kg) | 14.8 ± 1.34 | 45.5 ± 2.36*** |
| FFM (kg) | 46.89 ± 3.62 | 61.14 ± 4.06* |
| HOMA IR | 1.45 ± 0.10 | 2.26 ± 0.21* |
| QUICKI | 0.359 ± 0.006 | 0.341 ± 0.004* |
| McA index | 7.833 ± 0.20 | 6.311 ± 0.15*** |
| Glucose (mg/dl) | 97.13 ± 2.06 | 106.4 ± 3.14* |
| Triglycerides (mg/ml) | 107.1 ± 4.78 | 173.3 ± 9.12*** |
| NEFA (mmol/l) | 0.539 ± 0.019 | 0.657 ± 0.027** |
| Cholesterol (mg/dl) | 187.0 ± 5.32 | 214.9 ± 6.83** |
| Insulin (μM/ml) | 6.53 ± 0.66 | 8.55 ± 0.66* |
| Glucagon (pg/ml) | 70.44 ± 6.45 | 152.9 ± 6.83** |
| Adiponectin (μg/ml) | 14.06 ± 1.34 | 7.78±0.88** |
| Ghrelin active (pg/ml) | 24.69 ± 3.45 | 36.87 ± 2.63** |
| Ghrelin total (pg/ml) | 1,349 ± 126.0 | 1,674 ± 106.7NS |
| Obestatin (ng/ml) | 1.933 ± 0.17 | 1.216 ± 0.23* |
| Orexin-A (pg/ml) | 27.51 ± 5.38 | 14.48 ± 3.13NS |
| GLP-1 (ng/ml) | 6.47 ± 0.57 | 5.43 ± 0.27NS |
| Leptin (ng/ml) | 7.04 ± 1.34 | 37.21 ± 2.19*** |
| SPX (ng/ml) | 6.63 ± 0.29 | 4.48 ± 0.19*** |
| KISS (nmol/l) | 2.165 ± 0.174 | 1.357 ± 0.15** |

Statistically significant differences between means for non-obese and obese subjects are marked where * p<0.05, ** p<0.01 and *** p<0.001. FM – fat mass, FFM – fat free mass, HOMA-IR – homeostatic model assessment of insulin resistance, QUICKI – Quantitative insulin sensitivity check index, McA – McAuley index.

Determination of hormones concentrations

Hormone concentrations in serum were analyzed using human-specific ELISA or RIA kits. For RIA measurements, quantifications of gamma radiation were performed by Wallac Wizard 1470 Gamma Counter (Perkin Elmer, USA). ELISA measurements were performed using a microplate reader Synergy 2 Biotek. Detailed descriptions of ELISA/RIA kits are given in Table 2. Serum samples for determination of active ghrelin were collected according to the manufacturer's instruction and acidified with 1 N HCl and phenylmethylsulfonyl fluoride (PMSF).

Statistical analysis

Statistical analyses were performed using unpaired Student's t test (two-tailed distribution), and statistical significance was accepted at p<0.05 (*),

p<0.01 (**), and p<0.001 (***). Correlations between serum concentrations of KISS, SPX and all tested parameters were analyzed by Pearson's correlation model and linear regression.

Results

Serum KISS and SPX concentrations were lower in obese subjects than in non-obese volunteers (KISS in non-obese: 2.165±0.174 nmol/l, obese: 1.357±0.15 nmol/l, p<0.01; SPX in non-obese: 6.63±0.29 ng/ml, obese: 4.48±0.19 ng/ml, p<0.001; Fig. 1). There were negative correlations between KISS and BMI (r=-0.617; p<0.001; Fig. 2B), KISS and HOMA-IR (r=-0.509; p<0.01; Fig. 2D), SPX and BMI (r=-0.659; p<0.001; Fig. 2A), SPX and HOMA-IR (r=-0.509; p<0.05; Fig. 2C). Whereas positive

correlations were found between serum SPX and KISS with QUICKI, and McAuley indices: KISS vs. QUICKI ($r=0.561$; $p<0.01$; Fig. 2F), SPX vs. QUICKI ($r=0.568$; $p<0.01$; Fig. 2E), KISS vs. McAuley ($r=0.716$; $p<0.001$; Fig. 2H), SPX vs. McAuley ($r=0.673$; $p<0.01$; Fig. 2G). In addition, serum concentrations of insulin and glucagon

correlated negatively with SPX and KISS: insulin vs. SPX ($r=-0.487$; $p<0.05$; Fig. 3A), glucagon vs. SPX ($r=-0.754$; $p<0.001$; Fig. 3C), insulin vs. KISS ($r=-0.425$; $p<0.05$; Fig. 3B), glucagon vs. KISS ($r=-0.623$; $p<0.01$; Fig. 3D).

Table 2. The list of test used for characteristic of hormonal profile.

| Target | Kit name | Sensitivity | Cat. No. | Manufacturer |
|------------------|--|----------------------|-------------|------------------------------|
| Glucagon | Glucagon RIA kit | 20-400 pg/ml | GL-32K | Merck Millipore, USA |
| Insulin | Human Insulin-Specific RIA | 2-200 μ U/ml | HI-14K | Merck Millipore, USA |
| Spexin | Spexin / NPQ (Human, Mouse, Bovine) – EIA Kit | 0-100 ng/ml | EK-023-81 | Phoenix Pharmaceuticals, USA |
| Kisspeptin | Human Kisspeptin 1 (KISS-1) ELISA Kit | 50-800 pg/ml | 201-12-4106 | Sunred, Shanghai, China |
| Obestatin | Obestatin (Human, Monkey) - RIA Kit | 50-6,400 pg/ml | RK-031-92 | Phoenix Pharmaceuticals, USA |
| Ghrelin (active) | Human Ghrelin (ACTIVE) RIA | 10-2,000 pg/ml | GHRA-88HK | Merck Millipore, USA |
| Ghrelin (total) | Human Ghrelin (TOTAL) RIA | 100-10,000 pg/ml | GHRA-88HK | Merck Millipore, USA |
| GLP-1 | Glucagon-Like Peptide-1 (GLP-1) (7-36) Amide – EIA kit | 0-100 ng/ml | EK-028-11 | Phoenix Pharmaceuticals, USA |
| Adiponectin | Adiponectin Elisa | 0.6-31,000 μ g/l | E09 | Mediagnost, Germany |
| Leptin | Multi-Species Leptin RIA | 1-50 ng/ml | XL-85K | Merck Millipore, USA |
| Orexin-A | Orexin A (Human, Rat, Mouse) Extraction Free EIA Kit | 0-100 ng/ml | EKE-003-30 | Phoenix Pharmaceuticals, USA |

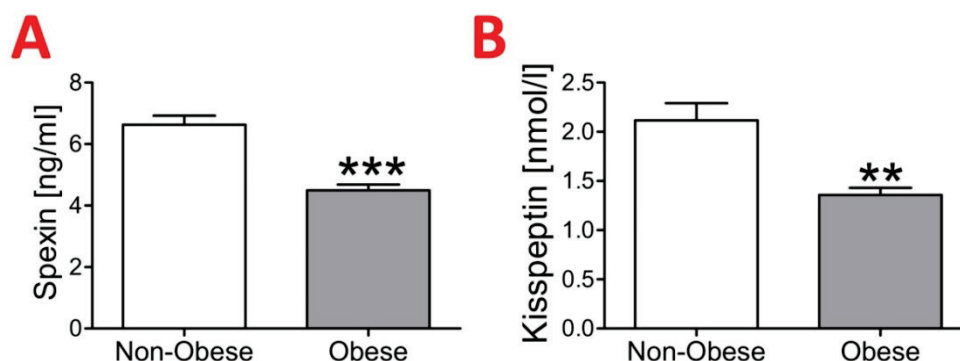


Fig. 1. Serum levels of SPX and KISS in non-obese and obese subjects. Values presented are means \pm SEM ($n=12$). Statistically significant differences between means for non-obese and obese subjects are marked where $p<0.05$ *, $p<0.01$ ** and $p<0.001$ ***.

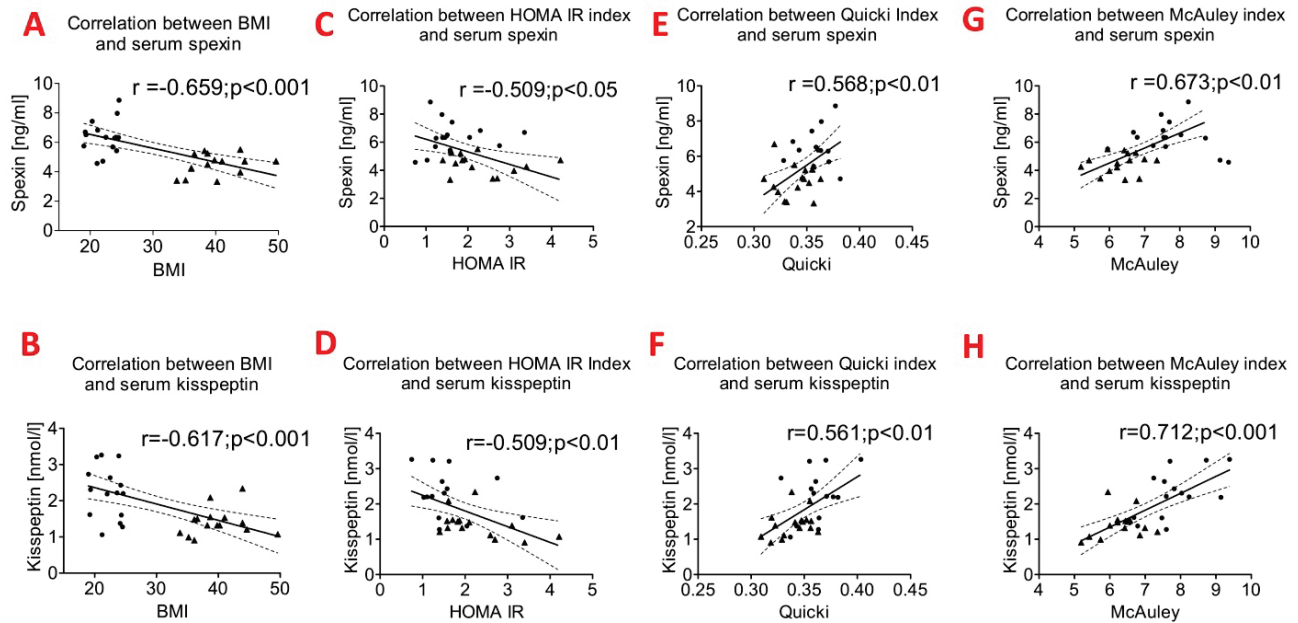


Fig. 2. Correlations between circulating spexin and kisspeptin concentrations, and BMI (**A, B**), HOMA IR (**C, D**), QUICKI index (**E, F**), McAuley index (**G, H**). Solid and dashed lines show the mean and 95 % confidence intervals, respectively, following linear regression analysis; symbols show r -Pearson and p -value. r -Pearson, shows the correlation; p -value shows significance of the correlation.

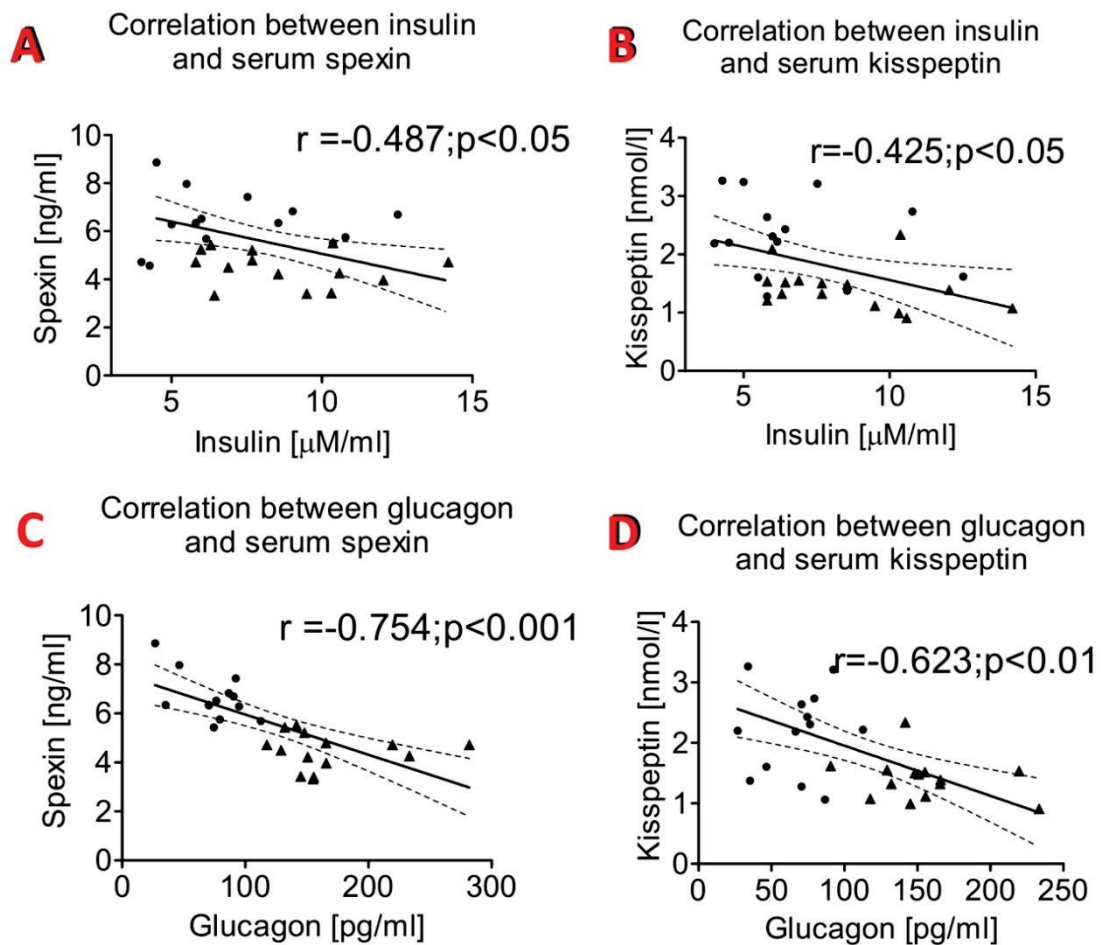


Fig. 3. Correlations between circulating spexin and kisspeptin concentrations and insulin (**A, B**), glucagon (**C, D**). Values for r and p are indicated in each graph. Solid and dashed lines show the mean and 95 % confidence intervals, respectively, following linear regression analysis; symbols show r -Pearson and p -value. r -Pearson, shows the correlation; p -value shows significance of the correlation.

Next, the correlations between gastrointestinal hormones obestatin, active ghrelin, total ghrelin, GLP-1 and SPX or KISS were tested. Positive or negative correlations were found between SPX and obestatin ($r=0.559$; $p<0.01$; Fig. 4A), active ghrelin ($r=-0.502$;

$p<0.01$; Fig. 4C) and GLP-1 ($r=0.496$; $p<0.01$; Fig. 4G). Serum concentration of KISS negatively correlated with active ghrelin, only ($r=-0.585$; $p<0.01$; Fig. 4D). There were no significant correlations between total ghrelin and SPX or KISS and total ghrelin, GLP-1 or obestatin.

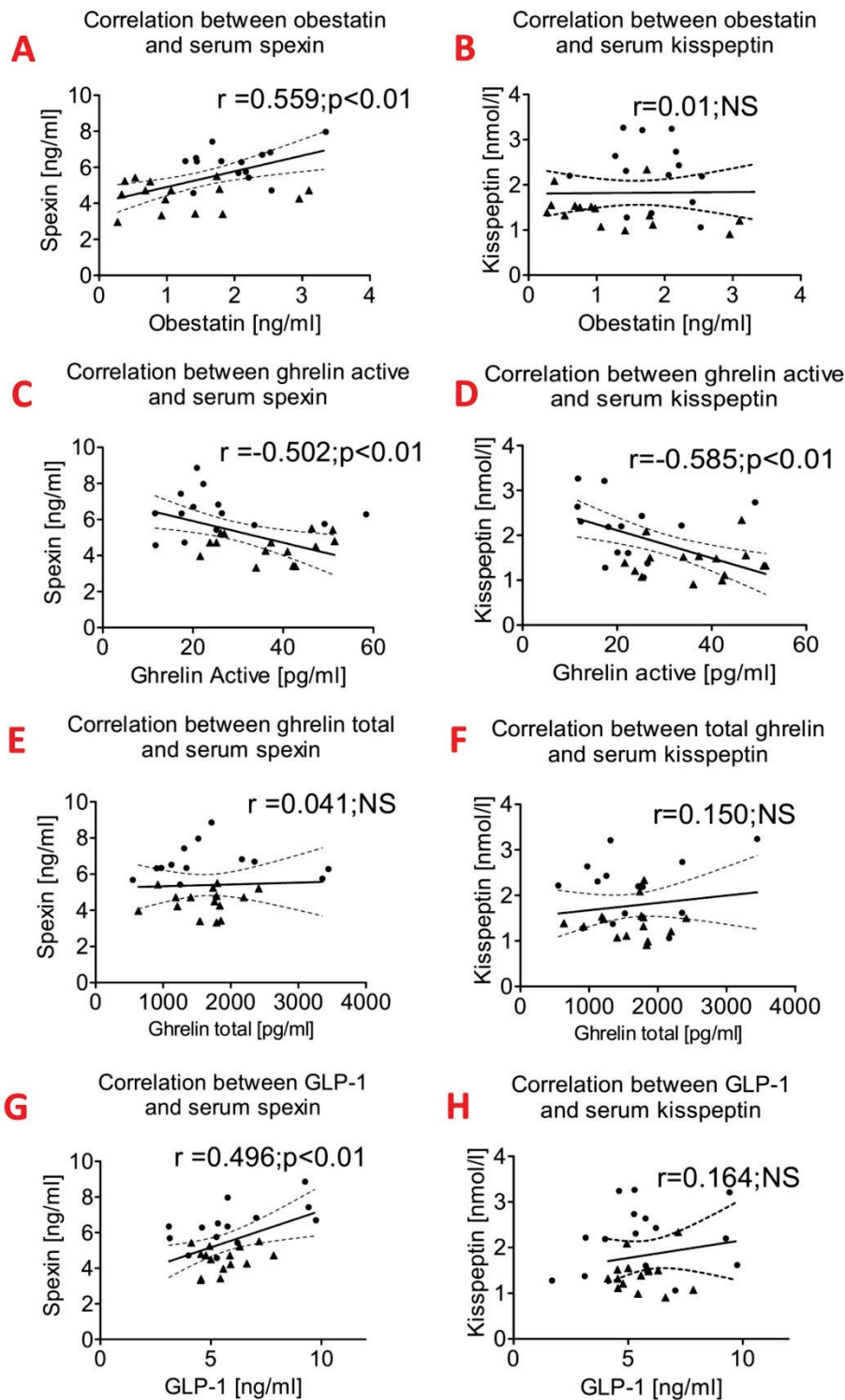


Fig. 4. Correlations between circulating spexin and kisspeptin concentrations and obestatin (A, B), active ghrelin (C, D), total ghrelin (E, F) and GLP-1 (G, H). Values for r and p are indicated in each graph. Solid and dashed lines show the mean and 95 % confidence intervals, respectively, following linear regression analysis; symbols show r -Pearson and p -value. r -Pearson, shows the correlation; p -value shows significance of the correlation.

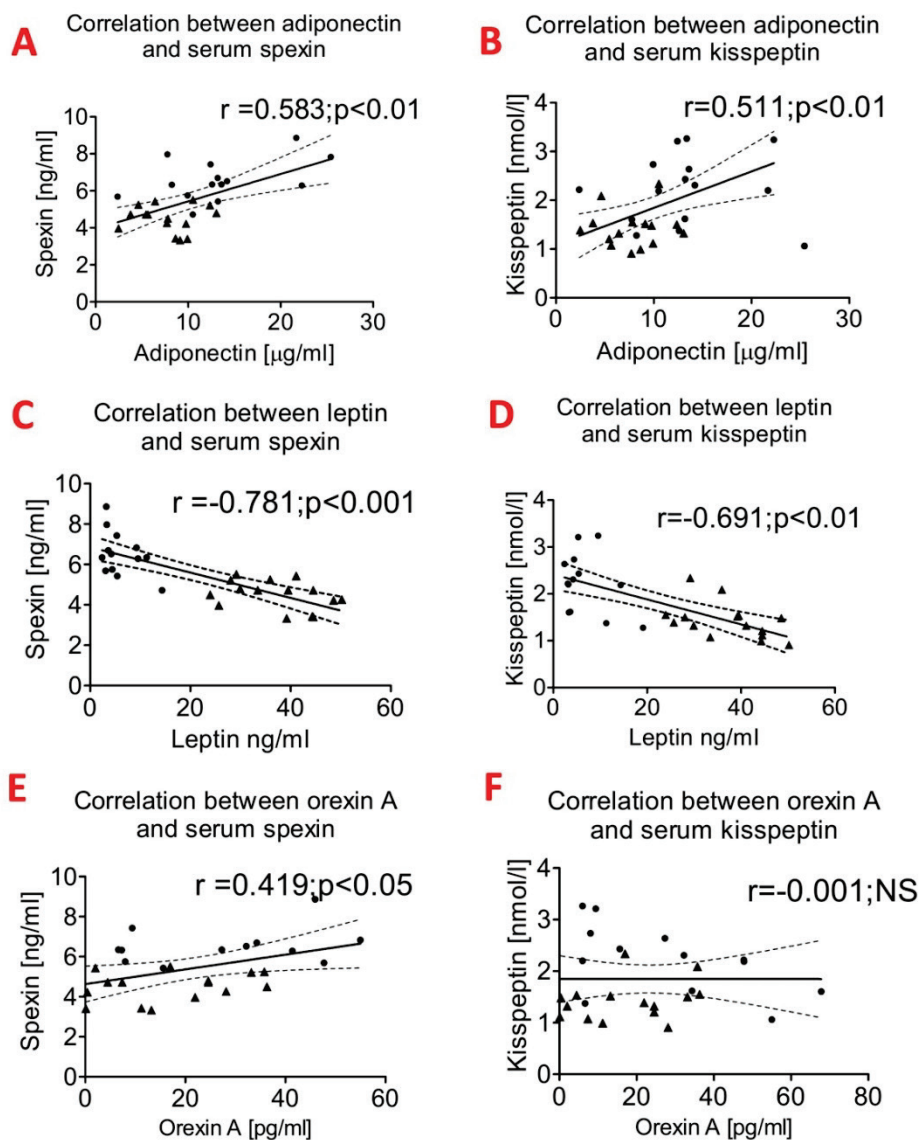


Fig. 5. Correlations between serum concentrations of spexin and kisspeptin and adiponectin (**A, B**), leptin (**C, D**), and orexin-A (**E, F**) Solid and dashed lines show the mean and 95 % confidence intervals, respectively, following linear regression analysis; symbols show r-Pearson and p-value. r-Pearson, shows the correlation; p-value shows significance of the correlation.

Serum adiponectin levels positively correlated with both SPX ($r=0.583$; $p<0.01$; Fig. 5A) as well as KISS ($r=0.511$; $p<0.01$; Fig. 5B). Serum leptin levels negatively correlated with SPX and KISS: SPX vs. leptin, ($r=-0.781$; $p<0.001$; Fig. 5C), KISS vs. leptin ($r=-0.691$; $p<0.01$; Fig. 5D). Serum concentration of SPX correlated positively with orexin-A ($r=0.419$; $p<0.05$; Fig. 5E). No significant correlation was observed between KISS and orexin-A.

Discussion

In the present study, we show that serum levels of SPX and KISS are low in obese female patients. We also report that serum SPX negatively correlate with

HOMA-IR, serum insulin, glucagon, active ghrelin, leptin an orexin-A. SPX positively correlates with QUICKI index, McAuley index, and serum levels of obestatin, GLP-1 and adiponectin. KISS as well as SPX, negatively correlate with HOMA-IR, insulin, glucagon, active ghrelin and leptin. Our analyses also show positive correlations between KISS and QUICKI index, McAuley index and adiponectin. Our findings with regard to decreased serum SPX in obese individuals and a correlation between SPX and leptin and are consistent with the results published by others (Walewski *et al.* 2014).

Nevertheless, we show here for the first time correlations between SPX and insulin resistance indices, as well as serum insulin, glucagon, active ghrelin,

adiponectin, orexin-A, obestatin and GLP-1 levels. Previously, it was demonstrated that high levels of plasma triglycerides are associated with low levels of KISS in obese rats (Overgaard *et al.* 2015). High BMI is linked to high serum triglycerides level in obese patients (Shamai *et al.* 2011). We decided to investigate correlation between BMI and KISS. We found lower KISS concentrations in obese female participants and negative correlations between KISS and BMI. In addition, we found correlations between KISS and HOMA-IR, QUICKI index, McAuley index, insulin, glucagon, active-ghrelin, leptin, and adiponectin.

In recent years, particular attention was paid to adipokines (leptin, adiponectin, resistin) and hormones regulating food intake (ghrelin, obestatin, GLP-1, orexin-A) as determinants of insulin resistance and obesity. Leptin plasma concentration is directly related to obesity and leptin resistance may be a direct cause of obesity (Maffei *et al.* 1995). As previously reported by others, adiponectin levels are low in obesity and show negative correlation with insulin resistance (Matsubara *et al.* 2002, Stefan *et al.* 2002, Yamauchi *et al.* 2002). Both adipokines leptin and adiponectin can enhance insulin sensitivity *via* activation of AMPK, an effect well known for the antidiabetic drug metformin. We found negative correlation between both investigated peptides and leptin and a positive one with adiponectin. The strong positive correlation between spexin, kisspeptin and adiponectin suggests that these peptides may be agonistic in regulating metabolic processes, e.g. improving insulin sensitivity of peripheral tissues. Research conducted in rodents confirmed that spexin administration in obese mice resulted in an attenuation of insulin resistance (Jasmine *et al.* 2016). On the other hand, negative correlation between KISS, SPX and leptin suggests that these biologically active substances play antagonistic roles in regulating food intake and/or metabolism. However, previous research showed that SPX as well as KISS inhibit food intake and, like leptin, decrease body weight. This activity contradicts our assumption which tempts us to perform a follow-up study to answer this question.

Ghrelin, obestatin, orexin-A and GLP-1 are relevant in the context of pathophysiology of adiposity (Suzuki *et al.* 2012). It was shown that these hormones regulate adipose tissue metabolism, such as lipolysis, lipogenesis, or glucose uptake by adipocytes. Furthermore, these hormones affect insulin resistance (Villanueva-Penacarrillo *et al.* 2001, Baragli *et al.* 2011,

Skrzypski *et al.* 2011, Pruszyńska-Oszmalek *et al.* 2013, Kolodziejcki *et al.* 2017). Moreover, all these peptides are involved in food intake and regulation of body weight. It was shown that ghrelin and orexin A stimulate food intake and increase body weight (Edwards *et al.* 1999, Wren *et al.* 2001), while GLP-1 and obestatin play opposite roles, as they inhibit food consumption and decrease body mass (Turton *et al.* 1996, Zhang *et al.* 2005). The positive correlations between obestatin, GLP-1 and SPX as well as between KISS and GLP-1 suggests a potential interplay of these peptides with each other, and partially confirms previously reported findings regarding an inhibitory effect of KISS and SPX on the food intake (Stengel *et al.* 2011, Walewski *et al.* 2014). On the other hand GLP-1 decreases intestinal motility, while SPX enhances bowel movements (Lin *et al.* 2015), which suggests, that despite anorexigenic role of both peptides, the body weight regulation may be achieved by different metabolic pathways. Obestatin was also demonstrated as an inhibitor of food intake (Zhang *et al.* 2005). Positive correlations between SPX and obestatin also indicate that SPX may play a role in regulating food intake directly *via* GALR2, GALR3 or by regulation of other anorectigenic peptides/hormones. By demonstrating the correlation between SPX, KISS and other metabolically-relevant hormones, our results suggest that both SPX and KISS can directly or indirectly regulate the secretion of these hormones, however *in vitro* and/or *in vivo* studies are needed to confirm this hypothesis. This relationship may be relevant in the context of obesity and/or insulin resistance.

Currently it is known that SPX concentration negatively correlates with blood glucose levels in patients with type 2 diabetes (Gu *et al.* 2015). In obese patients, low SPX concentration in serum and a negative correlation between the levels of SPX and leptin were reported (Walewski *et al.* 2014). SPX as well as galanin are able to activate galanin receptors 2 and 3 (Kim *et al.* 2014). However, decrease of SPX concentration in obesity is not accompanied by changes of galanin concentrations, which levels are increased in obesity (Baranowska *et al.* 1997). As compared to SPX, galanin has opposite effects on body metabolism. Galanin increases body weight, promotes obesity, stimulates the expression and membrane translocation of GLUT4 (Guo *et al.* 2011). SPX activates GALR2 and GALR3, however, thereby reducing food intake, facilitating body weight loss, and reducing the uptake of long chain fatty acids by adipocytes (Walewski *et al.* 2014). In goldfish,

SPX can inhibit basal, NPY- or orexin-stimulated food consumption and decrease the expression of orexigenic genes like NPY, AgRP and apelin, while simultaneously rising the expression of CCK, CART, POMC, MCH and CRH (Wong *et al.* 2013). Gastrointestinal hormones like ghrelin, obestatin or GLP-1 can regulate food intake *via* NPY- and AgRP-neurons (Kamegai *et al.* 1997, Kohno *et al.* 2003). Given the above, we tested whether the concentrations of ghrelin, obestatin and GLP-1 correlate with the concentration of SPX. We found a positive correlation between GLP-1 and obestatin levels, and SPX. These results suggest an interaction between SPX and other peptides controlling food intake and appetite.

It was earlier demonstrated that SPX suppresses LH secretion in goldfish (Liu *et al.* 2013). In 2015 two different studies showed that SPX is able to enhance bowel movement in mice and that serum levels of SPX decrease after oral glucose tolerance test in patients with type 2 diabetes (Gu *et al.* 2015, Wu *et al.* 2015). These results suggest that SPX is a potential ligand for GALR2 and GALR3, and could act contrary as compared to galanin in obesity.

Decreased KISS concentrations in obese patients are probably indirectly or directly related to disorders associated with changes of sex hormones, regulating obesity and puberty. In favor of this data it was shown that changes of serum KISS are detectable only after cessation of puberty in obese patients (Logie *et al.* 2012). Our results also show that there is a relationship between leptin, adiponectin and KISS in obese patients. Furthermore, the latest research indicates that glucagon is responsible for regulation of hepatic *Kiss-1* gene expression, while KISS stimulates insulin secretion from pancreatic islets in mice and rats (Bowe *et al.* 2009, Schwetz *et al.* 2014, Song *et al.* 2014). We found that serum KISS levels correlate with both insulin and

glucagon levels in obese patients. These results may indicate that KISS play an important role in regulation of pancreatic hormones secretion, and may affect insulin resistance/sensitivity in peripheral tissues.

Ghrelin and obestatin also play important roles in controlling puberty and gonadotropic axis (Tena-Sempere 2007). Our results show also that KISS concentration in serum correlates with the level of active ghrelin in obese patients, which may indicate that interactions between these peptides may be important in puberty processes.

In summary, our results show that obesity is associated with decreased serum levels of SPX and KISS and confirms that SPX correlates with serum leptin levels. Our results show for the first time correlations between SPX, KISS, insulin resistance indices, serum levels of glucagon, obestatin, ghrelin, GLP-1, leptin and adiponectin in patients with obesity. These results indicate that SPX and KISS could be involved in pathophysiology of human obesity or insulin resistance.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

This study was supported by the National Science Centre, Poland 2015/19/N/NZ4/00572 PRELUDIUM grant. Contribution Statement: PAK, designed the study, obtained the data and wrote the manuscript. KWN, EPO, LN, HK, MZS and PM contributed to the study design, edited, supported and critically revised the manuscript and contributed to the discussion. MS, EK, DSZ, obtained the data, collected and analyzed the data and revised the manuscript. All authors have given final approval to the current version to be published.

References

- BARAGLI A, GHÈ C, ARNOLETTI E, GRANATA R, GHIGO E, MUCCIOLI G: Acylated and unacylated ghrelin attenuate isoproterenol-induced lipolysis in isolated rat visceral adipocytes through activation of phosphoinositide 3-kinase γ and phosphodiesterase 3B. *Biochim Biophys Acta* **1811**: 386-396, 2011.
- BARANOWSKA B, WASILEWSKA-DZIUBINSKA E, RADZIKOWSKA M, PLONOWSKI A, ROGUSKI K: Neuropeptide Y, galanin, and leptin release in obese women and in women with anorexia nervosa. *Metabolism* **46**: 1384-1389, 1997.
- BOWE JE, KING AJ, KINSEY-JONES JS, FOOT VL, LI XF, O'BYRNE KT, PERSAUD SJ, JONES PM: Kisspeptin stimulation of insulin secretion: Mechanisms of action in mouse islets and rats. *Diabetologia* **52**: 855-862, 2009.

- BROWN RE, IMRAN SA, UR E, WILKINSON M: KiSS-1 mRNA in adipose tissue is regulated by sex hormones and food intake. *Mol Cell Endocrinol* **281**: 64-72, 2008.
- DE ROUX N, GENIN E, CAREL JC, MATSUDA F, CHAUSSAIN JL, MILGROM E: Hypogonadotropic hypogonadism due to loss of function of the KiSS1-derived peptide receptor GPR54. *Proc Natl Acad Sci U S A* **100**: 10972-10976, 2003.
- DIABETES AA: Standards of medical care in diabetes - 2016. *Am Diabetes Assoc* **37**: 14-80, 2016.
- EDWARDS CM, ABUSNANA S, SUNTER D, MURPHY KG, GHATEI MA, BLOOM SR: The effect of the orexins on food intake: Comparison with neuropeptide Y, melanin-concentrating hormone and galanin. *J Endocrinol* **160**: R7-R12, 1999.
- GU L, MA Y, GU M, ZHANG Y, YAN S, LI N, WANG Y, DING X, YIN J, FAN N, PENG Y: Spexin peptide is expressed in human endocrine and epithelial tissues and reduced after glucose load in type 2 diabetes. *Peptides* **71**: 232-239, 2015.
- GUO L, SHI M, ZHANG L, LI G, ZHANG L, SHAO H, FANG P, MA Y, LI J, SHI Q, SUI Y: Galanin antagonist increases insulin resistance by reducing glucose transporter 4 effect in adipocytes of rats. *Gen Comp Endocrinol* **173**: 159-163, 2011.
- JASMINE G, WALEWSKI J, ANGLADE D, BERK P: Regulation of hepatocellular fatty acid uptake in mouse models of fatty liver disease with and without functional leptin signaling: roles of NfKB and SREBP-1C and the effects of spexin. *Semin Liver Dis* **36**: 360-372, 2016.
- KAMEGAI J, TAMURA H, SHIMIZU T, ISHII S, SUGIHARA H, WAKABAYASHI I: Central effect of ghrelin, an endogenous growth hormone secretagogue, on hypothalamic peptide gene expression. *Endocrinol* **138**: 5079-5082, 1997.
- KATZ A, NAMBI SS, MATHER K, BARON A, FOLLMANN D, SULLIVAN G, QUON MJ: Quantitative insulin sensitivity check index: A simple, accurate method for assessing insulin sensitivity in humans. *J Clin Endocrinol Metab* **85**: 2402-2410, 2000.
- KIM DK, YUN S, SON GH, HWANG JI, PARK CR, KIM JI, KIM K, VAUDRY H, SEONG JY: Coevolution of the spexin/galanin/kisspeptin family: Spexin activates galanin receptor type II and III. *Endocrinology* **155**: 1864-1873, 2014.
- KOHNO D, GAO HZ, MUROYA S, KIKUYAMA S, YADA T: Ghrelin directly interacts with neuropeptide-y-containing neurons in the rat arcuate nucleus ca²⁺ signaling via protein kinase a and n-type channel-dependent mechanisms and cross-talk with leptin and orexin. *Diabetes* **52**: 948-956, 2003.
- KOŁODZIEJSKI PA, PRUSZYŃSKA-OSZMALEK E, SASSEK M, KACZMAREK P, SZCZEPANKIEWICZ D, BILLERT M, MAĆKOWIAK P, STROWSKI MZ, NOWAK KW: Changes in obestatin gene and receptor-GPR39 expression in peripheral tissues of rat models of obesity, type 1 and type 2 diabetes. *J Diabetes* **9**: 353-361, 2017.
- KOTANI M, DETHEUX M, VANDENBOGAERDE A, COMMUNI D, VANDERWINDEN JM, LE POUL E, BRÉZILLON S, TYLDESLEY R, SUAREZ-HUERTA N, VANDEPUT F, ET AL.: The metastasis suppressor gene KiSS-1 encodes kisspeptins, the natural ligands of the orphan G protein-coupled receptor GPR54. *J Biol Chem* **276**: 34631-34636, 2001.
- LIN C, ZHANG M, HUANG T, YANG L, FU H, ZHAO L, ZHONG LL, MU H, SHI X, LEUNG CF, FAN B, JIANG M, LU A, ZHU L, BIAN Z: Spexin enhances bowel movement through activating L-type voltage-dependent calcium channel via galanin receptor 2 in mice. *Sci Rep* **5**: 12095, 2015.
- LIU Y, LI S, QI X, ZHOU W, LIU X, LIN H, ZHANG Y, CHENG CH: A novel neuropeptide in suppressing luteinizing hormone release in goldfish, *Carassius auratus*. *Mol Cell Endocrinol* **374**: 65-72, 2013.
- LOGIE JJ, DENISON FC, RILEY SC, RAMAESH T, FORBES S, NORMAN JE, REYNOLDS RM: Evaluation of kisspeptin levels in obese pregnancy as a biomarker for pre-eclampsia. *Clin Endocrinol (Oxf)* **76**: 887-893, 2012.
- MA A, HE M, BAI J, WONG MK, KO WK, WONG AO: Dual role of insulin in spexin regulation: functional link between food intake and spexin expression in a fish model. *Endocrinology* **158**: 560-577, 2017.

- MAFFEI M, HALAAS J, RAVUSSIN E, PRATLEY RE, LEE GH, ZHANG Y, FEI H, KIM S, LALLONE R, RANGANATHAN S: Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. *Nat Med* **1**: 1155-1161, 1995.
- MATSUBARA M, MARUOKA S, KATAYOSE S: Inverse relationship between plasma adiponectin and leptin concentrations in normal-weight and obese women. *Eur J Endocrinol* **147**: 173-180, 2002.
- MATTHEWS DR, HOSKER JP, RUDENSKI AS, NAYLOR BA, TREACHER DF, TURNER RC: Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* **28**: 412-419, 1985.
- MCAULEY KA, WILLIAMS SM, MANN JI, WALKER RJ, LEWIS-BARNED NJ, TEMPLE LA, DUNCAN AW: Diagnosing insulin resistance in the general population. *Diabetes Care* **24**: 460-464, 2001.
- MESSAGER S, CHATZIDAKI EE, MA D, HENDRICK AG, ZAHN D, DIXON J, THRESHER RR, MALINGE I, LOMET D, CARLTON MB, ET AL.: Kisspeptin directly stimulates gonadotropin-releasing hormone release via G protein-coupled receptor 54. *Proc Natl Acad Sci U S A* **102**: 1761-1766, 2005.
- MIRABEAU O, PERLAS E, SEVERINI C, AUDERO E, GASCUEL O, POSSENTI R, BIRNEY E, ROSENTHAL N, GROSS C: Identification of novel peptide hormones in the human proteome by hidden Markov model screening. *Genome Res* **17**: 320-327, 2007.
- OHTAKI E, SHINTANI Y, HONDA S, MATSUMOTO H, HORI A, KANEHASHI K, TERAO Y, KUMANO S, TAKATSU Y, MASUDA Y, ET AL.: Metastasis suppressor gene KiSS-1 encodes peptide ligand of a G-protein-coupled receptor. *Nature* **411**: 613-617, 2001.
- OVERGAARD A, AXEL A, LIE M, HANSEN H, MIKKELSEN J: High plasma triglyceride levels strongly correlate with low kisspeptin in the arcuate nucleus of male rats. *Endocr Regul* **49**: 51-57, 2015.
- PORZIONATO A, RUCINSKI M, MACCHI V, STECCO C, MALENDOWICZ LK, DE CARO R: Spexin expression in normal rat tissues. *J Histochem Cytochem* **58**: 825-837, 2010.
- PORZIONATO A, RUCINSKI M, MACCHI V, STECCO C, SARASIN G, SFRISO MM, DI GIULIO C, MALENDOWICZ LK, DE CARO R: Spexin is expressed in the carotid body and is upregulated by postnatal hyperoxia exposure. *Adv Exp Med Biol* **758**: 207-213, 2012.
- PRUSZYNSKA-OSZMALEK E, SZCZEPANKIEWICZ D, HERTIG I, SKRZYPSKI M, SASSEK M, KACZMAREK P, KOLODZIEJSKI PA, MACKOWIAK P, NOWAK KW, STROWSKI MZ, WOJCIECHOWICZ T: Obestatin inhibits lipogenesis and glucose uptake in isolated primary rat adipocytes. *J Biol Regul Homeost Agents* **27**: 23-33, 2013.
- SCHWETZ TA, REISSAUS CA, PISTON DW: Differential stimulation of insulin secretion by glp-1 and kisspeptin-10. *PLoS One* **9**: 1-10, 2014.
- SEMINARA SB, MESSAGER S, CHATZIDAKI EE, THRESHER RR, ACIERNO JS JR, SHAGOURY JK, BO-ABBAS Y, KUOHUNG W, SCHWINOF KM, HENDRICK AG, ET AL.: The GPR54 gene as a regulator of puberty. *N Engl J Med* **349**: 1614-1627, 2003.
- SHAMAI L, LURIX E, SHEN M, NOVARO GM, SZOMSTEIN S, ROSENTHAL R, HERNANDEZ AV, ASHER CR: Association of body mass index and lipid profiles: Evaluation of a broad spectrum of body mass index patients including the morbidly obese. *Obes Surg* **21**: 42-47, 2011.
- SKRZYPSKI M, T LE T, KACZMAREK P, PRUSZYNSKA-OSZMALEK E, PIETRZAK P, SZCZEPANKIEWICZ D, KOLODZIEJSKI PA, SASSEK M, ARAFAT A, WIEDENMANN B, ET AL.: Orexin A stimulates glucose uptake, lipid accumulation and adiponectin secretion from 3T3-L1 adipocytes and isolated primary rat adipocytes. *Diabetologia* **54**: 1841-1852, 2011.
- SONG WJ, MONDAL P, WOLFE A, ALONSO LC, STAMATERIS R, ONG BW, LIM OC, YANG KS, RADOVICK S, NOVAIRA HJ, FARBER EA, FARBER CR, TURNER SD, HUSSAIN MA: Glucagon regulates hepatic kisspeptin to impair insulin secretion. *Cell Metab* **19**: 667-681, 2014.
- SONMEZ K, ZAVERI NT, KERMAN IA, BURKE S, NEAL CR, XIE X, WATSON SJ, TOLL L: Evolutionary sequence modeling for discovery of peptide hormones. *PLoS Comput Biol* **5**: e1000258, 2009.
- STEFAN N, BUNT JC, SALBE AD, FUNAHASHI T, MATSUZAWA Y, TATARANNI PA: Plasma adiponectin concentrations in children: relationships with obesity and insulinemia. *J Clin Endocrinol Metab* **87**: 4652-4656, 2002.

-
- STENGEL A, WANG L, GOEBEL-STENGEL M, TACHÉ Y: Centrally injected kisspeptin reduces food intake by increasing meal intervals in mice. *Neuroreport* **22**: 253-257, 2011.
- SUZUKI K, JAYASENA CN, BLOOM SR: Obesity and appetite control. *Exp Diabetes Res* **2012**: 824305, 2012.
- TENA-SEMPERE M: Ghrelin and reproduction: ghrelin as novel regulator of the gonadotropic axis. *Vitam Horm* **77**: 285-300, 2007.
- TOLSON KP, GARCIA C, YEN S, SIMONDS S, STEFANIDIS A, LAWRENCE A, SMITH JT, KAUFFMAN AS: Impaired kisspeptin signaling decreases metabolism and promotes glucose intolerance and obesity. *J Clin Invest* **124**: 3075-3079, 2014.
- TURTON M, O'SHEA D, GUNN I, BEAK S, EDWARDS C, MEERAN K, CHOI S, TAYLOR G, HEATH M, LAMBERT P, ET AL.: A role for glucagon-like peptide-1 in the central regulation of feeding. *Nature* **379**: 69-72, 1996.
- VILLANUEVA-PENACARRILLO ML, MARQUEZ L, GONZALEZ N, DIAZ-MIGUEL M, VALVERDE I: Effect of GLP-1 on lipid metabolism in human adipocytes 1. *Horm Metab Res* **33**: 73-77, 2001.
- WALEWSKI JL, GE F, LOBDELL H, LEVIN N, SCHWARTZ GJ, VASSELLI JR, POMP A, DAKIN G, BERK PD: Spexin is a novel human peptide that reduces adipocyte uptake of long chain fatty acids and causes weight loss in rodents with diet-induced obesity. *Obesity* **22**: 1643-1652, 2014.
- WONG MK, SZE KH, CHEN T, CHO CK, LAW HC, CHU IK, WONG AO: Goldfish spexin: solution structure and novel function as a satiety factor in feeding control. *Am J Physiol Endocrinol Metab* **305**: E348-E366, 2013.
- WREN A, SEAL L, COHEN M, BRYNES A, FROST G, MURPHY K, DHILLO W, GHATEI M, BLOOM S: Ghrelin enhances appetite and increases food intake in humans. *J Clin Endocrinol Metab* **86**: 5992-5995, 2001.
- WU H, LIN F, CHEN H, LIU J, GAO Y, ZHANG X, HAO J, CHEN D, YUAN D, WANG T, LI Z: Ya-fish (*Schizothorax prenanti*) spexin: identification, tissue distribution and mRNA expression responses to periprandial and fasting. *Fish Physiol Biochem* **42**: 39-49, 2015.
- YAMAUCHI T, KAMON J, MINOKOSHI Y, ITO Y, WAKI H, UCHIDA S, YAMASHITA S, NODA M, KITA S, UEKI K, ET AL.: Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. *Nat Med* **8**: 1288-1295, 2002.
- ZHANG J, REN P, AVSIAN-KRETCHMER O, LUO C, RAUCH R: Obestatin, a peptide encoded by the ghrelin gene, opposes ghrelin's effects on food intake. *Science* **310**: 996-999, 2005.
-