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Serum levels of spexin and kisspeptin negatively correlate with obesity and insulin resistance in women

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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 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.

Introduction

Kisspeptin (KISS) and spexin (SPX) are peptides involved in regulation of body weight, metabolism and sexual functions. In 2014, Dong-Kyu 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 (Messager *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 2. 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/m^2. Obese patients (BMI above 35 kg/m^2) 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: HOMA-IR= (G0 x I0)/22.5; QUICKI=1/[log(G0)+ log(I0)]; McA=exp[2,63-0.28 In (I0)-0.31 ln(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).

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).

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.

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, 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 acitivies , 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; Pruszynska-Oszmalek *et al.* 2013; Kolodziejski *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 interplayof 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 ma by 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, GLAR3 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, a 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 concentration 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.

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Conflicts of interest: none

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Table 1. Metabolic and hormonal profiles of study participants

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.

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*
Triglicerydes [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]	1349±126.0	1674±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**

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 μU/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(KISS1)ELISA Kit	50-800 pg/ml	201-12-4106	Sunred, Shanghai, China
Obestatin	Obestatin (Human, Monkey) - RIA Kit	50-6400 pg/ml	RK-031-92	Phoenix Pharmaceuticals, USA
Ghrelin (active)	Human Ghrelin (ACTIVE) RIA	10–2000 pg/mL	GHRA-88HK	Merck Millipore, USA
Ghrelin (total)	Human Ghrelin (TOTAL) RIA	100–10000 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 - 31000μg/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

Figure legends

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

Figure 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.

Figure 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.

Figure 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.

Figure 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.

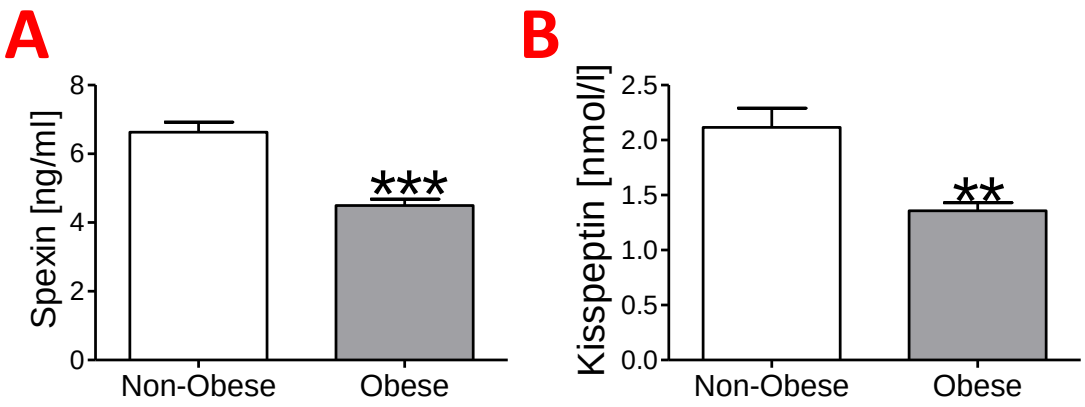
A
Spexin [ng/ml]
8
6
4
2
0
***
Non-Obese
Obese
B
Kisspeptin [nmol/l]
2.5
2.0
1.5
1.0
0.5
0.0
**
Non-Obese
Obese

Figure 1

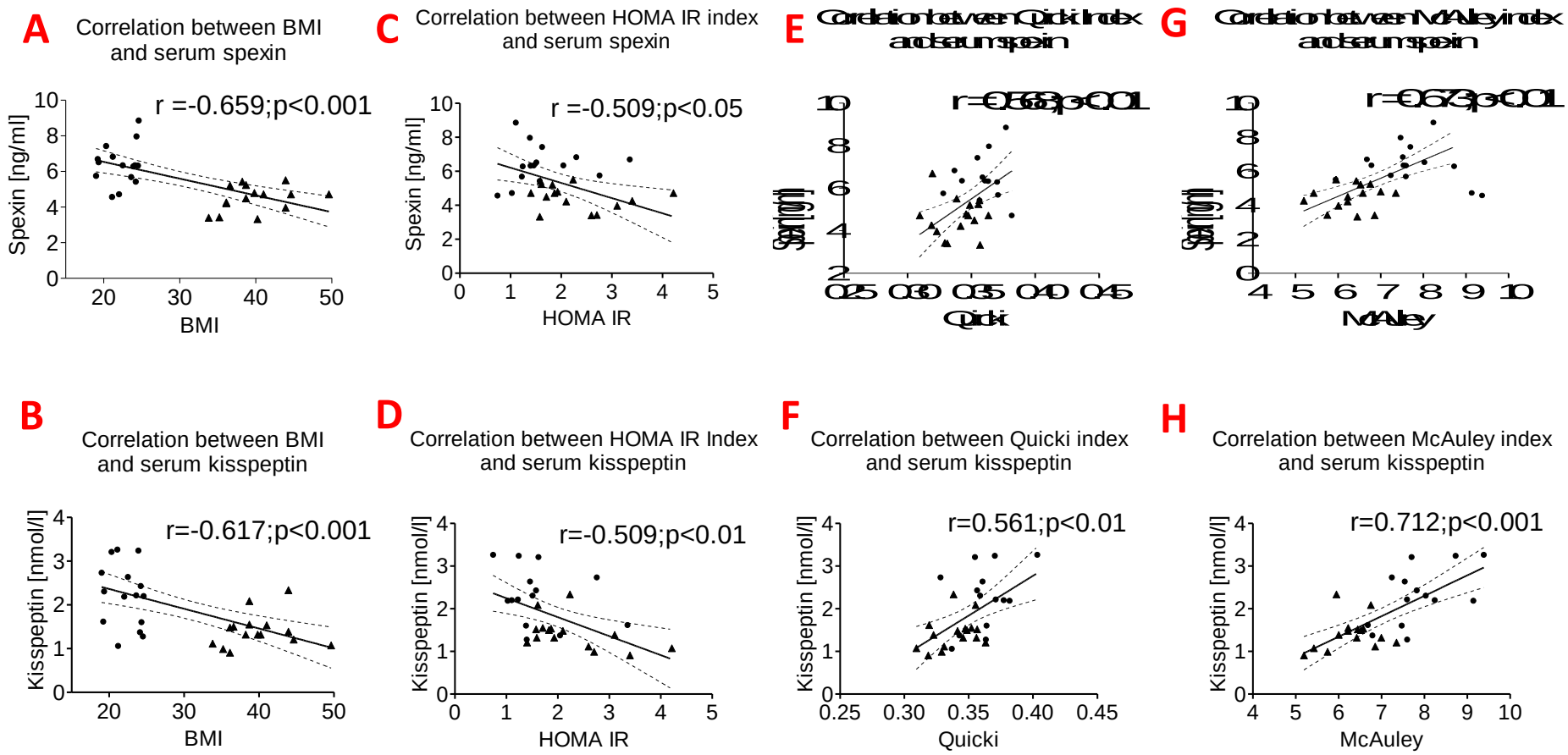
A
Correlation between BMI and serum spexin
r =-0.659;p<0.001
Spexin [ng/ml]
BMI
C
Correlation between HOMA IR index and serum spexin
r =-0.509;p<0.05
Spexin [ng/ml]
HOMA IR
E
G
B
Correlation between BMI and serum kisspeptin
r=-0.617;p<0.001
Kisspeptin [nmol/l]
BMI
D
Correlation between HOMA IR Index and serum kisspeptin
r=-0.509;p<0.01
Kisspeptin [nmol/l]
HOMA IR
F
Correlation between Quicki index and serum kisspeptin
r=0.561;p<0.01
Kisspeptin [nmol/l]
Quicki
H
Correlation between McAuley index and serum kisspeptin
r=0.712;p<0.001
Kisspeptin [nmol/l]
McAuley

Figure 2

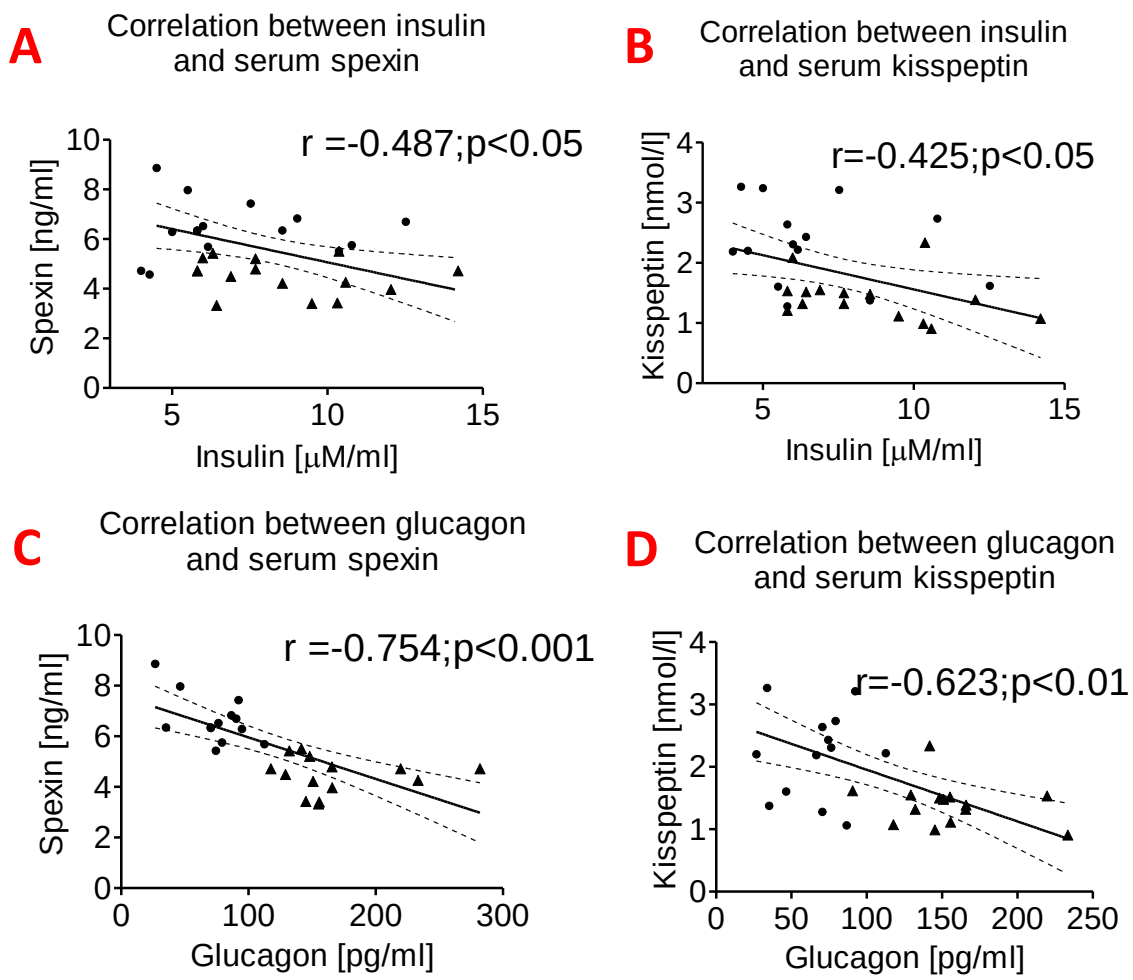
A
Correlation between insulin and serum spexin
r =-0.487;p<0.05
Spexin [ng/ml]
Insulin [μM/ml]
B
Correlation between insulin and serum kisspeptin
r=-0.425;p<0.05
Kisspeptin [nmol/l]
Insulin [μM/ml]
C
Correlation between glucagon and serum spexin
r =-0.754;p<0.001
Spexin [ng/ml]
Glucagon [pg/ml]
D
Correlation between glucagon and serum kisspeptin
r=-0.623;p<0.01
Kisspeptin [nmol/l]
Glucagon [pg/ml]

Figure 3

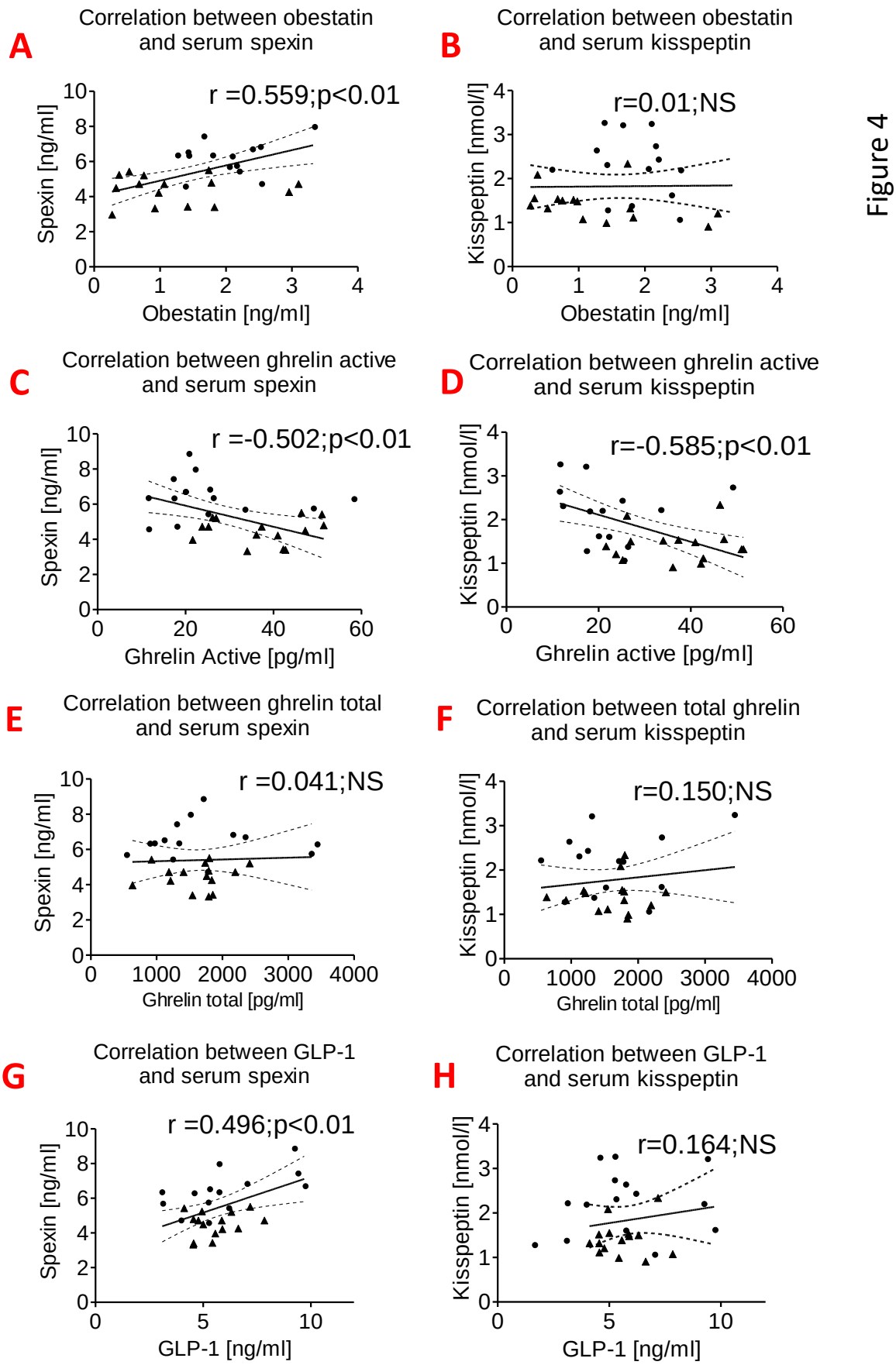
A
Correlation between obestatin and serum spexin
r =0.559;p<0.01
Spexin [ng/ml]
Obestatin [ng/ml]
B
Correlation between obestatin and serum kisspeptin
r=0.01;NS
Kisspeptin [nmol/l]
Obestatin [ng/ml]
C
Correlation between ghrelin active and serum spexin
r =-0.502;p<0.01
Spexin [ng/ml]
Ghrelin Active [pg/ml]
D
Correlation between ghrelin active and serum kisspeptin
r=-0.585;p<0.01
Kisspeptin [nmol/l]
Ghrelin active [pg/ml]
E
Correlation between ghrelin total and serum spexin
r =0.041;NS
Spexin [ng/ml]
Ghrelin total [pg/ml]
F
Correlation between total ghrelin and serum kisspeptin
r=0.150;NS
Kisspeptin [nmol/l]
Ghrelin total [pg/ml]
G
Correlation between GLP-1 and serum spexin
r =0.496;p<0.01
Spexin [ng/ml]
GLP-1 [ng/ml]
H
Correlation between GLP-1 and serum kisspeptin
r=0.164;NS
Kisspeptin [nmol/l]
GLP-1 [ng/ml]

Figure 4

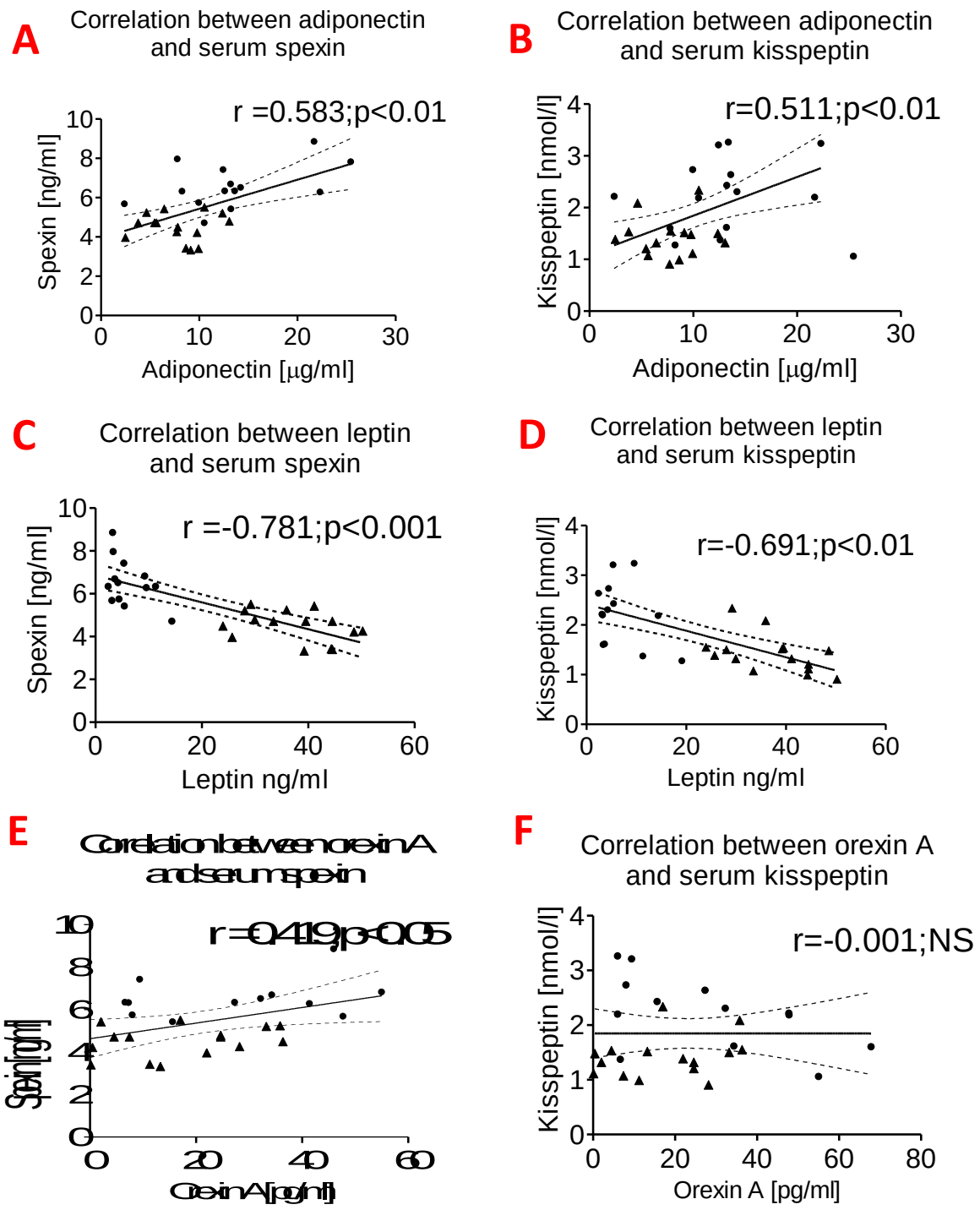
A
Correlation between adiponectin and serum spexin
r =0.583;p<0.01
Spexin [ng/ml]
Adiponectin [μg/ml]
B
Correlation between adiponectin and serum kisspeptin
r=0.511;p<0.01
Kisspeptin [nmol/l]
Adiponectin [μg/ml]
C
Correlation between leptin and serum spexin
r =-0.781;p<0.001
Spexin [ng/ml]
Leptin ng/ml
D
Correlation between leptin and serum kisspeptin
r=-0.691;p<0.01
Kisspeptin [nmol/l]
Leptin ng/ml
E
r=0.419;p<0.05
F
Correlation between orexin A and serum kisspeptin
r=-0.001;NS
Kisspeptin [nmol/l]
Orexin A [pg/ml]

Figure 5