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Original article

Associations of Spexin and cardiometabolic parameters among women with and without gestational diabetes mellitus

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ABSTRACT

Spexin (SPX) is a novel biomarker abundantly expressed in several animal and human tissues implicated in food intake and glucose control, respectively. As new roles for SPX are emerging, the present study explored for the first time, the associations of SPX to several cardiometabolic indices and inflammatory markers in pregnant women, a demographic not yet investigated with respect to SPX. A total of 117 Saudi women subdivided to those with gestational diabetes mellitus (GDM) (N = 63) and those without (N = 54) were included in this cross-sectional study. Anthropometry, glycemic, lipid, vitamin D, adipocytokines and inflammatory markers were measured consecutively at baseline and after the 2nd and 3rd trimesters. Age- and BMI adjusted comparisons revealed that levels of SPX were not significantly different in pregnant women with and without GDM. In all subjects, circulating levels of SPX showed modest associations with glucose ($R = 0.18$; $p = .08$) and HOMA β ($R = -0.19$; $p = .09$) as well as significant positive associations with total cholesterol ($R = 0.25$; $p = .02$), LDL-cholesterol ($R = 0.25$; $p = .02$), 25(OH)D ($R = 0.22$; $p = .04$), albumin ($R = 0.30$; $p < .01$) and IL1β ($R = 0.41$; $p < .01$). Stepwise regression analysis also suggested that IL1β, leptin and albumin were the significant predictors of SPX. In summary, SPX levels modestly affect glucose and insulin sensitivity in pregnant women but is not associated with GDM and obesity. The significant association of SPX to ILβ warrants further investigation as to the role of SPX in immune modulation.

1. Introduction

The novel peptide spexin (SPX) was recently discovered in 2007 (Mirabeau et al., 2007; Sonmez et al., 2009). In humans, SPX is a product of the Ch12orf39 gene consisting of 14 amino acids (116 amino acids as pre-peptides) and widely expressed in endocrine and epithelial tissues, among others (Gu et al., 2015; Mirabeau et al., 2007; Porzionato et al., 2010; Sonmez et al., 2009). The abundant expression of SPX in human tissues suggests its potential involvement in many physiological functions that are yet to be established. Studies performed in animals so far offered clues. In goldfish (*Carassius auratus*), SPX injection inhibited basal and neuropeptide Y (NPY)-induced feeding behavior and consumption (Wong et al., 2013). Consequently, Walewski et al. (2014) demonstrated anti-obesity activity of SPX on mice with diet-induced obesity (DIO), seen as reduced energy intake (~32%) and an inverse correlation between circulating SPX and leptin. Both *in vivo* animal studies hint that SPX modulates satiety. Early investigations performed among children and adolescents seem to support this premise, with SPX observed to be down regulated in obese adolescents, and, similar to animal models, associated inversely with leptin (Kumar et al., 2017). Among the limited studies performed in adult humans so far, Gu et al. (2015) demonstrated that SPX is inversely correlated to glycemic indices and lipids, with reduced SPX levels reported in patients with type 2 diabetes mellitus (T2DM) as compared to non-diabetic subjects, suggesting that SPX may have a role in glucose and lipid metabolism. These

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preliminary findings however were not replicated in adolescents (Hodges et al., 2017), suggesting possible differences in SPX functions according to age groups. Other populations also remain under investigated, including pregnant women.

Gestational Diabetes Mellitus (GDM) is defined as impaired tolerance of glucose diagnosed at the 24–28th week of pregnancy (Rani and Begum, 2016). Worldwide the prevalence of GDM varies 1.0–14% depending on the definition used (Chen et al., 2016). The absence of universal definition prevalence of GDM is on the rise worldwide according to WHO (4). In Saudi Arabia, the over-all prevalence of GDM was ~37% (Al-Rubeaan et al., 2014) with regional variations: Medina (Western region) at 51% (Alfadhli et al., 2015) and Riyadh (Central region) at 24% (Wahabi et al., 2017). As pregnancy is considered an insulinogenic state (Barbour et al., 2007), GDM as a complication of pregnancy may potentially provide interesting insights about SPX's evolving role in glucose metabolism as this type of investigation has never been carried out in this population. Furthermore, no study has been undertaken ascertaining the associations of SPX to a multitude of biomarkers including adipocytokines and inflammatory markers, aside from glycemic, lipid and anthropometric indices combined in the same population. In this cross-sectional study, we assessed circulating SPX levels and their correlations with the different parameters mentioned in participants with and without GDM.

2. Methods

2.1. Subjects

A total of 117 Saudi pregnant women identified to be at high risk of GDM (personal history of GDM or polycystic ovarian syndrome, glycosuria, family history of T2DM, severe obesity, macrosomia), recruited at various hospitals in Riyadh, Saudi Arabia were included in this study. In brief, only pregnant Saudi women aged 18–35 were included and were excluded to have a relatively homogenous cohort. Additional criteria for inclusion/exclusion have been described previously (Al-Ajlan et al., 2015). Written informed consent was obtained from each participant prior to inclusion. The study was approved by the Ethics Committee of the College of Science, King Saud University, Riyadh, Kingdom of Saudi Arabia (KSA).

2.2. Anthropometry and blood collection

Pregnant women in their first trimester pre-natal visit were subjected to anthropometric and blood withdrawal procedures as described previously (Al-Ajlan et al., 2015).

2.3. Sample collection and analyses

Blood samples collected from the subjects during their first visit were analyzed for 25(OH)D and various biochemical parameters. Serum glucose, lipid profile, albumin and calcium were measured using a chemical analyzer (Konelab, Espoo, Finland). Serum-free insulin concentration was determined by electrochemiluminescence method (Cobas e411; Roche Diagnostics, Mannheim, Germany). Serum TNF-α, leptin, IL1β and IL6 (human bone magnetic bead panel) [intra-assay variation was 1.4–7.9% and inter-assay variation of <21%. Minimum detectable concentrations (MDC) were as follows: Leptin, 85.4 pg/ml IL-6, 0.4 pg/ml and TNFα, 0.14 pg/ml] as well as adiponectin and resistin (human adipokine magnetic bead panel) [intra-assay variation was 1.4%-7.9% and inter-assay variation of <21%. Minimum detectable concentrations (MDC) for adiponectin was adiponectin was 145.4 pg/ml and 6.7 pg/ml for resistin] were measured using Milliplex Map® (Millipore, Billerica, MA, USA) multiple assays by Luminex® xMAP® (Luminex Corp, Austin, TX, USA). Serum 25(OH)D was determined as described before (Al-Ajlan et al., 2015) with a Roche Elecsys modular analytics (Cobas e411) using an electrochemiluminescence immunoassay (Roche Diagnostics, GmbH, Mannheim, Germany) and commercially available IDS kits (IDS Ltd, Boldon Colliery, Tyne & Wear, UK). Variation for the 25(OH)D ELISA were 5.3 % and 4.6 %, respectively, with 100% cross-reactivity to 25(OH) D3 and 75% cross-reactivity to 25(OH)D2. Circulating SPX measurements were carried out using an enzyme-linked immunoassay (ELISA) following the manufacturer protocol (Phoenix Pharmaceuticals, Inc., Burlingame, CA) with a linear range of 0.11–1.07 ng/ml, intra assay variation of <10% and inter-assay variation of <15%.

2.4. Oral glucose tolerance test (OGTT)

OGTT was conducted with ingestion of seventy-five gram glucose and GDM was diagnosed according to International Association for Diabetes in Pregnancy Society Group (IADPSG) guidelines (Agarwal et al., 2015), if one of the following applies: fasting glucose ≥5.1 mmol/l, 1 h glucose ≥10 mmol/l or 2 h glucose ≥8.5 mmol/l.

2.5. Calculations done

Waist-hip ratio was calculated as waist (cm) circumference divided by hips (cm). BMI was calculated as weight in kg/height in m^2. LDL-cholesterol was calculated using the Friedwald formula = [Total-cholesterol − HDL-cholesterol − (Triglycerides/2.2)] where all concentrations are given in mmol/L. HOMA-β was calculated using the formula HOMA-β = (20 ∗ fasting insulin)/(fasting glucose − 3.5). HOMA-IR was calculated using the formula HOMAIR = (fasting insulin ∗ fasting glucose)/22.5. For both HOMA-IR and HOMA-B, glucose was measured in mmol/l and insulin was measured in lU/ml.

2.6. Statistical analysis

Data was entered and analyzed using SPSS version 21 (SPSS Inc., Chicago, IL). Results were presented as mean ± SD for normal variables and median (1st–3rd quartile) for non-normal variables. Differences between groups (Non-GDM and GDM) were tested using Student *t* test for normal variables and Mann–Whitney U-test for non-normal variables. Analysis of covariance (ANCOVA) was used to adjust for age and BMI. Differences across three visits were tested using repeated measures ANOVA for normal variables and Friedman test for non-normal variables. Spearman rank correlation coefficient (R) was used to test correlation between continuous variables. A scatter graph using the linear model was used to display the correlation. Stepwise regression analysis was used to identify the significant predictors for SPX. The results obtained from stepwise regression analysis achieved the power of more than 90% with the effect size of 0.53 at 95% confidence interval. Significance was set at $p < .05$.

3. Results

Baseline characteristics of all participants according to the presence of GDM is shown in Table 1, the mean ages of GDM and non-GDM participants were (29.1 ± 4.8 and 29.9 ± 5.8) and the mean gestational ages were (26.4 ± 3.0 and 26.1 ± 3.6) respectively. As expected, participants under the GDM group had a significantly higher glucose level than the non-GDM group (p = .02). The non-GDM group on the other hand had a significantly higher adiponectin (p < .001) and resistin levels (p = .001) than the GDM group. Anthropometrics, other glycemic parameters such as HbA1c, insu-

Table 1
Baseline characteristics and differences between GDM and non GDM participants.

Parameters	Non GDM	GDM	P-value*
N	54 (46.2)	63 (53.8)	–
Age (years)	29.1 ± 4.8	29.9 ± 5.8	–
BMI (kg/m^2)	26.7 ± 5.7	29.7 ± 6.6	–
Waist-Hip Ratio	0.8 ± 0.1	0.8 ± 0.1	0.17
Gestational Age (weeks)	26.4 ± 3.0	26.1 ± 3.6	0.48
Total Gestational Weight Gain#	7.3 (4.9–9.3)	6.2 (4.2–8.3)	0.21
Parity#	2.0 (1.0–4.0)	2.0 (1.0–4.0)	0.63
Systolic Blood Pressure (mmHg)	110.0 ± 12.3	112.1 ± 13.8	0.76
Diastolic Blood Pressure (mmHg)	65.2 ± 8.2	67.4 ± 10.0	0.46
Glucose (mmol/l)	4.7 ± 0.7	5.2 ± 1.0	0.02
HbA1c	5.0 ± 0.4	5.1 ± 0.5	0.86
Insulin (uU/ml)#	7.9 (4.0–13.5)	7.9 (4.7–15.4)	0.70
HOMA-IR#	1.7 (0.8–2.9)	1.8 (1.0–3.2)	0.71
HOMA-β#	141.7 (83–225.4)	148.2 (74–232.8)	0.50
SPX (ng/ml) #	0.5 (0.3–0.9)	0.3 (0.2–0.8)	0.82
Total Cholesterol (mmol/l)	4.8 ± 1.1	5.1 ± 1.0	0.53
HDL-Cholesterol (mmol/l)	1.2 ± 0.3	1.3 ± 0.4	0.24
LDL-Cholesterol (mmol/l)	3.1 ± 0.8	3.2 ± 0.8	0.88
Triglycerides (mmol/l)	1.2 ± 0.6	1.4 ± 0.6	0.21
Vitamin D (nmol/l) #	25.5 (18.0–38.3)	28.7 (19.1–39.9)	0.27
Calcium (mmol/l)	2.3 ± 0.2	2.2 ± 0.2	0.32
Albumin (g/L)	37.0 ± 3.3	37.2 ± 4.4	0.48
Leptin (pg/ml)	739.9 (63.2–3190.2)	769.8 (55.6–3774.8)	0.55
Adiponectin (ug/ml) #	210.0 (130.7–271)	91.2 (32–181.0)	<0.001
Resistin (ng/ml) #	288 (154.8–437.7)	82.9 (35–303.4)	0.001
TNF-α (pg/ml) #	0.3 (0.2–0.8)	0.2 (0.2–0.6)	0.47
IL6 (pg/ml) #	1.1 (0.5–8.5)	0.8 (0.6–1.9)	0.14
IL1β (pg/ml) #	0.3 (0.1–0.5)	0.3 (0.1–0.5)	0.90

Note: Data presented as Mean ± SD for normal variable while non-normal variables are presented as median (Quartile 1–Quartile 3); # indicates non-normal variables; P-value < 0.05 considered significant.
* Indicates p-value adjusted for age and BMI.

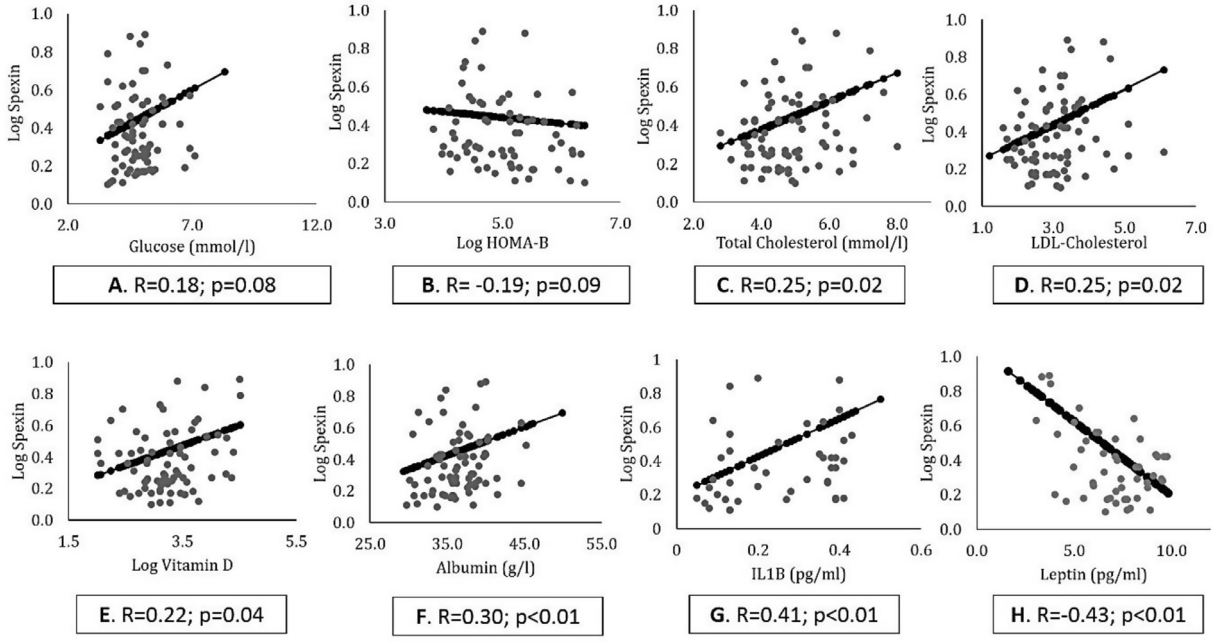

Fig. 1. Modest and significant associations of SPX in (A) glucose, (B) log HOMAβ, (C) total cholesterol, (D) LDL-cholesterol, (E) log vitamin D, (F) albumin, (G) IL1β and (H) leptin (all subjects N = 117).

lin, HOMA-IR, HOMA β, lipids, adipocytokines and inflammatory markers were not significantly different from one another. Furthermore, there was no significant difference between baseline SPX levels in both GDM and non-GDM groups. All comparisons were age- and BMI-adjusted.

In all participants, circulating levels of SPX was modestly associated with glucose (R = 0.18; p = .08) (Fig. 1A) and HOMA β (R = −0.19; p = .09) (Fig. 1B). SPX levels were also significantly and positively associated with total cholesterol (R = 0.25; p = .02) (Fig. 1C), LDL-cholesterol (R = 0.25; p = .02) (Fig. 1D), 25(OH)D (R = 0.22; p = .04) (Fig. 1E), albumin (R = 0.30; p < .01) (Fig. 1F), IL1β (R = 0.41; p < .01) (Fig. 1G) and leptin (R = −0.43; p < .01). When categorized according to the presence of GDM, SPX levels of the non-GDM group elicited significant positive associations with

Table 2
Baseline Associations between SPX and Cardiometabolic Parameters Measured.

Parameters	Non-GDM		GDM	
	R	P-value	R	P-value
Age (years)	−0.08	0.61	−0.22	0.17
BMI (kg/m^2)	**0.40**	**<0.01**	−0.09	0.58
Waist-hip Ratio	0.12	0.43	**−0.33**	**0.04**
Systolic Blood Pressure (mmHg)	−0.01	0.93	−0.10	0.53
Diastolic Blood Pressure (mmHg)	0.09	0.57	−0.12	0.46
Glucose (mmol/l)	0.24	0.11	0.21	0.19
HbA1c	−0.06	0.70	−0.22	0.16
Insulin (uU/ml) #	0.23	0.14	−0.07	0.67
HOMA-IR #	0.25	0.11	−0.06	0.73
HOMA-β #	−0.19	0.22	−0.15	0.36
Total Cholesterol (mmol/l)	**0.49**	**<0.01**	0.09	0.56
HDL-Cholesterol (mmol/l)	0.09	0.53	0.27	0.08
LDL-Cholesterol (mmol/l)	**0.48**	**<0.01**	0.08	0.61
Triglycerides (mmol/l)	**0.36**	**0.01**	−0.26	0.10
Vitamin D (nmol/l) #	0.13	0.40	**0.36**	**0.02**
Calcium (mmol/l)	−0.20	0.19	−0.16	0.34
Albumin (g/L)	**0.30**	**0.04**	0.26	0.11
Leptin (pg/ml)	**−0.48**	**<0.01**	−0.30	0.16
Adiponectin (ng/ml) #	−0.10	0.50	−0.02	0.92
Resistin (ng/ml) #	−0.08	0.58	−0.04	0.81
TNF-α (pg/ml) #	−0.30	0.09	−0.15	0.53
IL6 (pg/ml) #	−0.10	0.60	0.13	0.57
IL1β (pg/ml) #	**0.68**	**<0.01**	0.07	0.73

Note: Data presented as Spearman correlation coefficient (R). # indicates non-normal variables. Significant correlations highlighted in bold. Significant at $p < .05$.

Table 3
Significant Predictors of SPX.

Parameters	Beta ± Standard error	P-value
IL1β (pg/ml)	0.90 ± 0.24	0.005
Leptin (pg/ml)	−0.08 ± 0.02	<0.001
Albumin (g/L)	0.03 ± 0.01	0.027

Note: Data presented as Beta ± Standard errors. SPX was used as dependent variable and IL1β, leptin, albumin, total-cholesterol, LDL-cholesterol, vitamin D, glucose, and Homa-β as independent variables. Significant at $p < .05$.

BMI ($R = 0.40$; $p < .01$), total cholesterol ($R = 0.49$; $p < .01$), LDL-cholesterol ($R = 0.48$; $p < .01$), triglycerides ($R = 0.36$; $p = .01$), albumin ($R = 0.30$; $p = .04$), and IL1β ($R = 0.68$; $p < .01$). Leptin was also inversely associated with SPX in the non-GDM group ($R = -0.48$; $p < .01$). In the GDM group, SPX levels were inversely and significantly associated with waist-hip ratio ($R = -0.33$; $p = .04$) and 25(OH)D ($R = 0.36$; $p = .02$). The rest of the associations were not significant (Table 2). Furthermore, stepwise regression was conducted using predictors, indicating significant associations with SPX in correlation analysis, to identify the best predictors. Results of the stepwise regression analysis suggested that IL1B, leptin and albumin were the best predictors of SPX (see Table 3).

4. Discussion

The present cross-sectional study identified for the first time, associations of the novel peptide SPX to several cardiometabolic, adipocytokine and inflammatory parameters among pregnant women with and without the presence of GDM. Studies done so far with respect to SPX involve mostly animal models with few involving human children and adults but none in the pregnant population. Among the present findings include the significant inverse association of waist-hip ratio, a gross measure of visceral obesity, to circulating levels of SPX in the GDM group, confirming available evidence that SPX expression in humans is downregulated by omental fat (Walewski et al., 2014; Mirabeau et al., 2007). The absence of expression in abdominal fat and, probably, the lack of significant associations of SPX to several adipocytokines (with the exception of leptin) linked to central obesity, supports the theory that SPX may play a role in gut metabolism (Mirabeau et al., 2007). The inverse association of SPX to leptin in all subjects as well as the non-GDM also supports the theory of SPX's function in satiety (Mirabeau et al., 2007, Kumar et al., 2017; Koloziejskii et al., 2017).

Circulating SPX was also associated modestly with glucose and inversely with HOMAβ in the present study, with significant positive associations with lipid parameters (with the exception of HDL-cholesterol), albumin and ILβ in all subjects, suggesting that SPX is a promising candidate biomarker for the identification of cardiometabolic risk in the pregnant population. The role of SPX in humans is still emerging, and while preliminary observations indicate a possible role in glucose control, results from several research groups are less consistent and more conflicting. Gu and colleagues in their recent study involving 226 adults [N = 121 with type 2 diabetes mellitus (T2DM) and N = 105 controls] documented lower levels of SPX among T2DM patients and significant inverse associations of SPX with lipids and glycemic parameters (Gu et al., 2015). These observations were replicated by a more recent study by Koloziejskii et al. (2017) involving 30 women with varying degrees of obesity. Their observations however contradict the present findings as the associations elicited in the present study were significantly positive in terms of lipids and modest to no significant association in glycemic indices. These major differences may be due to sample size and more importantly, the choice of kits used (Cusabio versus Phoenix). The choice of ELISA kit used is a legitimate reason for difference, as the lack of discernible association with glycemic parameters and lipids in the present study majorly support studies done in children and adolescents (Hodges et al., 2017; Kumar et al., 2017, 2016) where the choice of kit was similar to the present study. As SPX is a relatively new biomarker, evaluation and validation of techniques is extremely crucial for the consistency of results, as methodological differences largely account for errors in biomedical experiments (Tighe et al., 2015). Other significant associations elicited in the study such as the significant but weak association of SPX to vitamin D levels and WHR in the GDM group are preliminary and may need further clarification using larger cohorts.

The significant association of SPX to IL1β is worthy of highlight since IL1βs are known for their autoimmune and inflammatory functions (Zhang, 2013). This significant association however may only be an indirect connection due to SPX's inverse association with leptin, a more established cytokine known to modulate not only the adaptive immune system but also inflammatory response and progression of certain autoimmune conditions (Cojocaru et al., 2013; Procaccini et al., 2015). As such, modulation of SPX may also have implications in autoimmune diseases and infections, and, while the associations elicited in the present study are at most, suggestive, it merits further investigation as to whether the mechanisms of action of SPX also encompasses immunoregulation aside from its leaning role in glucose metabolism.

The authors acknowledge several limitations. The small sample size may not have been adequate enough to elicit significant associations between other parameters of interest and SPX. Furthermore, the present study is cross-sectional and causality cannot be determined. Nevertheless, this is the first study to demonstrate associations of SPX to known cardiometabolic, adipocytokine and inflammatory markers among pregnant women with and without GDM. Findings of the present study shed light on the different factors that modulate SPX expression in different populations. Further studies involving larger sample sizes may confirm present findings.

5. Conclusions

SPX levels modestly affect glucose and insulin sensitivity in pregnant women but is not associated with GDM. SPX is not associated with indices of obesity including adiponectin and resistin, but is positively associated with lipids and inversely with leptin, highlighting its role in satiety and gut metabolism. The significant association of SPX to ILβ warrants further investigation as to the role of SPX in immune modulation. Evaluation and validation of techniques assessing qualitative determination of SPX is crucial for confirmation of present findings.

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