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Circulating spexin levels are influenced by the presence or absence of gestational diabetes

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ABSTRACT

The study aimed to determine whether circulating spexin (SPX) is modified during the course of pregnancy and whether it is affected by the presence of glucose intolerance, i.e., Gestational Diabetes Mellitus (GDM). This prospective study included 102 pregnant women (63 non-GDM and 39 GDM; mean age 29.4 ± 5.1 years; mean BMI 28.0 ± 6.1 kg/m^2). Anthropometrics, glycemic and lipid profiles, as well measurements of circulating adipocytokines and SPX were measured at baseline and after 3 and 6 months. In GDM patients, SPX levels increased significantly after 6-months, in parallel with a borderline significant increase in glucose ($p = 0.07$). In non-GDM patients, however, median SPX level decreased from baseline to 6-months ($p < 0.01$), and this change was not associated with changes in glucose levels. Change in glucose from baseline to 6-months was positively associated with change in SPX in GDM patients only ($R = 0.37$; $p < 0.05$). SPX levels are positively influenced by glucose intolerance in pregnant women with GDM, while they decrease in control women without GDM.

1. Introduction

Spexin (SPX) is a relatively novel protein discovered using cutting-edge bioinformatics [1,2]. Majority of the studies on SPX have so far involved animal models. SPX immunoreactivity is cytoplasmic and the protein is abundantly expressed in various organs in rat models, suggesting pleotropic functions, perhaps including neuroendocrine roles [3]. These initial observations led to further investigations suggesting that SPX may be a potent central modulator of cardiovascular and renal functions [4]. In fish models, SPX is abundantly expressed in brain and ovary, and was shown to suppress luteinizing hormone, suggesting a potential role in the regulation of the hypothalamic-pituitary–gonadal axis [5]. In addition, also in fish models, SPX was suggested to have a role in the regulation of several metabolic functions, including satiety control [6,7].

Human studies involving SPX have slowly started to gain momentum but still in its infancy. Preliminary observations showed that circulating SPX levels were significantly lower in obese than in normal weight children, but were not associated with biomarkers of insulin resistance [8]. This lack of association with glucose control and metabolism extended to adolescents [9], however, a link with obesity was observed, with a low SPX/high leptin ratio in obese individuals [10]. While data about SPX in adult populations are also limited, select investigations contradict findings in children and adolescents, suggesting a significant inverse association between SPX and glucose and lipid metabolism in obesity [11,12]. This discrepancy might be explained by the influence of developmental status and age in circulating SPX levels. More observational data may clarify the etiology of this difference.

To date, no prospective study of circulating SPX has been conducted in pregnant women over the course of gestation. At a cross-sectional level however we have previously demonstrated that human circulating SPX modestly affects glucose and insulin sensitivity during the first trimester of pregnancy [13], but whether such associations persist as gestation develops remain uninvestigated. Pregnancy may offer insights on the role of SPX, granted that the metabolic status of expectant mothers undergoes radical changes. This study aims to determine the levels of SPX and other biomarkers of obesity and insulin resistance prospectively in a group of pregnant women with varying levels of glucose tolerance, namely with or without gestational diabetes mellitus (GDM).

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Table 1
Clinical profile of all participants at all 3 visits.

Parameters	Baseline	3-Months later	6-Months later	P^A	P^B	P^{A*}	P^{B*}
N	102						
Age (years)	29.4 ± 5.1						
Gestational Age (weeks)	10.3 ± 4.9						
Matsuda Index	5.7 (2.5–11.5)						
BMI (kg/m^2)	27.8 ± 5.8	30.8 ± 6.2	30.8 ± 6.2	< 0.001	< 0.001		
Systolic Blood Pressure (mmHg)	112.0 ± 14.7	110.7 ± 12.0	111.2 ± 12.7	0.92	1.00	1.00	1.00
Diastolic Blood Pressure (mmHg)	66.5 ± 9.5	64.9 ± 9.7	66.3 ± 9.8	0.23	1.00	0.75	1.00
Glucose (mmol/l)	4.9 ± 1.0	4.9 ± 1.1	5.1 ± 1.3	1.00	0.31	0.52	0.24
HbA1c (%)	5.1 ± 0.5	5.1 ± 0.6	5.0 ± 0.6	1.00	1.00	0.31	1.00
Insulin (uU/ml) #	9.1 (5.5–16.4)	6.6 (4.2–9.5)	7.2 (4.5–15.3)	0.036	0.49	0.21	1.00
HOMA-IR #	1.8 (1.0–3.7)	1.4 (0.8–2.4)	1.6 (0.9–3.5)	0.037	0.69	0.32	1.00
HOMA-β #	153.0 (88.5–252.7)	136.0 (70.3–186.0)	110.5 (55.0–198.4)	0.24	0.035	0.20	0.056
Triglycerides (mmol/l)	1.3 ± 0.6	2.1 ± 0.8	2.3 ± 1.0	< 0.001	< 0.001	< 0.001	< 0.001
Total Cholesterol (mmol/l)	5.1 ± 1.0	6.7 ± 1.2	6.5 ± 1.3	< 0.001	< 0.001	< 0.001	< 0.001
HDL-Cholesterol (mmol/l)	1.3 ± 0.3	1.6 ± 0.4	1.4 ± 0.4	< 0.001	0.09	< 0.001	0.24
LDL-Cholesterol (mmol/l)	3.2 ± 0.8	4.1 ± 1.1	4.0 ± 1.1	< 0.001	< 0.001	< 0.001	< 0.001
Adiponectin (ug/ml) #	241.6 (174.4–273.7)	985.6 (38.7–1979.9)	38.0 (35.0–42.5)	1.00	< 0.001	1.00	< 0.001
Leptin (ng/ml) #	2.0 (0.5–4.1)	1.3 (0.2–7.8)	8.3 (3.1–13.4)	1.00	0.47	1.00	1.00
SPX (ng/ml) #	0.28 (0.17–0.58)	0.25 (0.16–0.53)	0.27 (0.16–0.56)	1.000	1.00	1.00	0.89

Note: Data presented as Mean ± SD for normal variables and Median (1st Quartile – 3rd Quartile) for non-normal variables. # indicates non-normal variables; $P < 0.05$ is considered significant; P^a indicates significance between baseline & 3-months; P^b indicates significance between baseline & 6-months; * indicates p-values adjusted for age, BMI and GDM status.

2. Methods

2.1. Subjects

A total of 102 Saudi pregnant women (63 non-GDM and 39 GDM) were randomly selected from a larger cohort to ascertain novel risk factors for GDM involving more than 500 pregnant patients attending 3 major tertiary recruited hospitals in Riyadh, Saudi Arabia [14,15]. In brief, only pregnant Saudi women aged 18–35 years old attending the maternity clinics were recruited from January 2014 until December 2015. Women with known DM or with a history of renal and liver diseases were excluded. A full description of the study population has been previously provided [14–16]. 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

Participants in their first trimester pre-natal visit (< 14 weeks of gestation) were requested to visit their respective hospital at a fasted state (8–10 h) and to fill-in a standardized questionnaire detailing demographic and medical history. Anthropometrics were collected and included height (m), weight (kg) and blood pressure (mmHg). Body mass index (BMI) was calculated as weight in kg/height in m^2. These measurements were repeated during subsequent follow-ups after 3 months and 6 months. Anthropometric data were obtained by trained research nurses.

2.3. Sample collection and analyses

Blood samples collected from the participants at baseline were analyzed for various biochemical parameters. Serum glucose and lipid profile 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 leptin and adiponectin were measured were measured using Milliplex Map® (Millipore, Billerica, MA, USA) multiple assays by Luminex® xMAP® (Luminex Corp, Austin, TX, USA). Leptin (human bone magnetic bead panel), intra-assay variation 1.4%-7.9%, inter-assay variation < 21%, minimum detectable concentrations (MDC) 85.4 pg/ml]. Adiponectin (human adipokine magnetic bead panel, intra-assay variation 1.4–7.9%, inter-assay variation < 21%, minimum detectable concentrations (MDC) 145.4 pg/ml] 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%. LDL-cholesterol was calculated using the Friedewald formula = [Total-cholesterol − HDL-cholesterol − (Triglycerides/2.2)] where all concentrations are given in mmol/L [17]. HOMA-β was calculated using the formula HOMA-β = (20 * fasting insulin)/(fasting glucose − 3.5) [18]. HOMA-IR was calculated using the formula HOMA-IR = (fasting insulin * fasting glucose)/22.5 [18]. For both HOMA-IR and HOMAβ, glucose was measured in mmol/l and insulin in lU/ml. Blood collection and analysis of metabolic parameters were repeated after 3 months and 6 months.

2.4. Oral glucose tolerance test (OGTT)

GDM screening was done for all participants. Oral glucose tolerance test (OGTT) was performed with the ingestion of 75 g glucose and GDM was diagnosed according to International Association for Diabetes in Pregnancy Society Group (IADPSG) guidelines [19], 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. Matsuda index was calculated as previously described [20].

2.5. Statistical analysis

Data were entered and analyzed using SPSS version 21.0 (SPSS Inc., Chicago, IL). Results are presented as mean ± SD for normal variables and median (1st quartile – 3rd quartile) for non-normal variables. Differences over time were tested by using repeated measures ANOVA for normal variables and Friedman test for non-normal variables. Repeated measures analysis of covariance was used to adjust for age, BMI and GDM status. Spearman Rank correlation coefficient (R) was used to test correlation between continuous variables. A scatter graph using the linear model was used to display a correlation. Significance was set at $p < 0.05$.

3. Results

A total of 102 pregnant women (mean age 29.4 ± 5.1 years; mean BMI 28.0 ± 6.1 kg/m^2) participated in this study (Table 1). BMI increased from baseline at the 3 and 6 month follow-ups. Among the glycemic parameters measured fasting glucose remained stable, while insulin and HOMA-IR showed significant reductions compared to baseline. Circulating insulin in particular was significantly lower than baseline ($p = 0.036$) at the 3 month follow-up; it became insignificant after adjustment for covariates. Mean HOMA IR also decreased compared to baseline however it became insignificant after adjustment. No significant changes were observed in circulating levels of HbA1c. The change in lipid profile over time was statistically significant for total cholesterol (TC), HDL-C, LDL-C and triglycerides. Circulating levels of these parameters increased significantly from baseline levels at the 3 month (p-values < 0.001) and 6 month ($p < 0.001$) visits. However, HDL-C become insignificant across time points after adjustment for covariates. Changes in TC, LDL-C and triglycerides remained consistent after adjustment for age, BMI and GDM status. Modest changes were observed in adipocytokines over time, with a sharp and significant rise in median adiponectin after 3 months and a sharp decline after 6 months. Leptin levels also showed an insignificant decline after 3 months and an insignificant rise in median leptin after 6 months. No changes in circulating SPX levels were observed but a slight but insignificant reduction in p-value after adjustment for covariates suggested that changes in SPX might be influenced by BMI or GDM status.

Table 2 shows SPX and covariates of interest (BMI and glucose) measured overtime comparatively in GDM and non-GDM women. In non-GDM women, median SPX level decreased significantly from baseline to 6-months ($p < 0.01$), along with a significant increase in BMI but not in circulating glucose. In GDM women, SPX levels increased significantly after 6-months ($p = 0.015$), along with an increase in glucose after 3 ($p < 0.01$) and 6-months ($p = 0.07$). Correlation analysis revealed that change in glucose from baseline to 6-months was significantly and positively associated with SPX ($R = 0.21$; $p < 0.05$) in all women (Fig. 1). Further analysis showed that the association between glucose and SPX was only significant in GDM women ($R = 0.37$; $p < 0.05$) (Fig. 2).

4. Discussion

The main finding of this study is that changes in SPX levels are positively associated with changes in glucose levels among pregnant women during the course of pregnancy. However, after stratification, this association is limited only to participants with GDM. Furthermore, GDM altered this association, with decreasing or increasing SPX levels in non-GDM and GDM women respectively. This finding suggests that SPX may function differently in normal *vs*. GDM pregnancies. Given that the difference in SPX levels were apparent in GDM and non-GDM only after progression from the first to the second trimester, we hypothesize that maternal glycemic changes may significantly influence SPX expression during the course of pregnancy. The first trimester of pregnancy corresponds to rapid placental growth, and GDM patients experience more intense placental angiogenesis secondary to hyperglycemia, resulting in elevated levels of placental growth factors [21] and inflammatory markers such as IL-6 [22]. These early trimester markers of GDM, although not measured in the present study, may explain the late-onset but significant difference in SPX expression in GDM and non-GDM patients, as these biomarkers, including SPX, are all influenced by the presence of insulin resistance and altered glucose metabolism. This observation is further confirmed in a more recent study involving rat endocrine pancreatic cells, where insulin secretion were observed to be downregulated after insulin secretion and increased pancreatic cell viability after SPX treatment. Further studies are needed to confirm this theory [23].

As the most common pregnancy-related metabolic disorder, GDM as a form of glucose intolerance, alters the expression of adipocytokines known to influence glucose metabolism, such as leptin and adiponectin. Circulating median leptin levels were higher in the present cohort after 6 months of monitoring, as expected. Inversely, lower adiponectin levels were observed in all patients at the 6 month follow-up visit. Of note, above normal levels of leptin and decreased levels of adiponectin in the 2nd or 3rd trimester of pregnancy are an independent risk factors for GDM [24,25].

In an earlier cross-sectional study, we demonstrated a borderline significant association between circulating glucose and SPX in GDM women [13]. The adipocytokine findings, especially leptin, are noteworthy, as the low SPX – high leptin ratio theory suggested in children and adolescents seems to be true in pregnant women with and without GDM as well. The lower expression of SPX overall in pregnant patients may be partially explained by elevated estrogen, as sex hormones are inhibitory to SPX expression, at least in fish models [26].

Other findings in the study, such as the decreasing insulin sensitivity (HOMA-β) with the progression of pregnancy, even in the absence of significant changes in glycemic parameters, confirm that pregnancy alone is a state of insulin resistance [27,28]. This metabolic bias expands to changes in lipids, which also increase in pregnancy even in the absence of GDM [14,29].

The study acknowledges several limitations. The sample size is relatively small, and as such, generalizations cannot be made. Furthermore, dietary information and other potentially important confounders were not included. Lastly, fetal outcomes such as birthweight were not documented as it would have provided substantial information if such parameters could be linked to SPX independently of other maternal variables. Nevertheless, the study is the first to define changes in SPX levels in pregnant participants over the course of gestation and the first to observe a positive association between glucose levels and SPX in women with GDM, a finding that was opposite to the observations in non-GDM controls and in contrast to associations elicited in children and adolescents. Further studies using a larger sample size are needed to confirm the present findings.

Table 2
BMI, glucose and SPX levels of Non-GDM and GDM participants at all 3 visits.

Parameters	Non-GDM (N = 63)			GDM (N = 39)		
	Baseline	3-Months	6-Months	Baseline	3-Months	6-Months
Age (years)	29.0 ± 4.4			30.5 ± 5.9		
Gestational age (weeks)	11.0 ± 4.3			9.4 ± 5.5^{A}		
Matsuda Index	8.3 (4.9–16.4)			2.5 (1.4 – 4.1)^{A}		
BMI (kg/m^2)	27.1 ± 5.7	29.7 ± 6.6^{A}	30.4 ± 6.1^{AB}	28.7 ± 5.8	29.9 ± 6.0	31.4 ± 6.5
Glucose (mmol/l)	4.7 ± 1.0	4.2 ± 0.4^{A}	4.9 ± 1.2^{B}	5.1 ± 1.0	5.9 ± 0.9^{A}	5.6 ± 1.3^{A}
SPX (ng/ml) #	0.28 (0.13–0.58)	0.25 (0.15–0.51)	0.25 (0.15–0.44)^{A}	0.29 (0.18–0.63)	0.23 (0.16–0.68)	0.35 (0.19–1.18)^{A}

Note: Data presented as Mean ± SD for normal variables and Median (1st Quartile – 3rd Quartile) for non-normal variables. # indicates non-normal variables; Superscripts ^{A} & ^{B} indicates significance from baseline and 3-months respectively; $p < 0.05$ is considered significant.

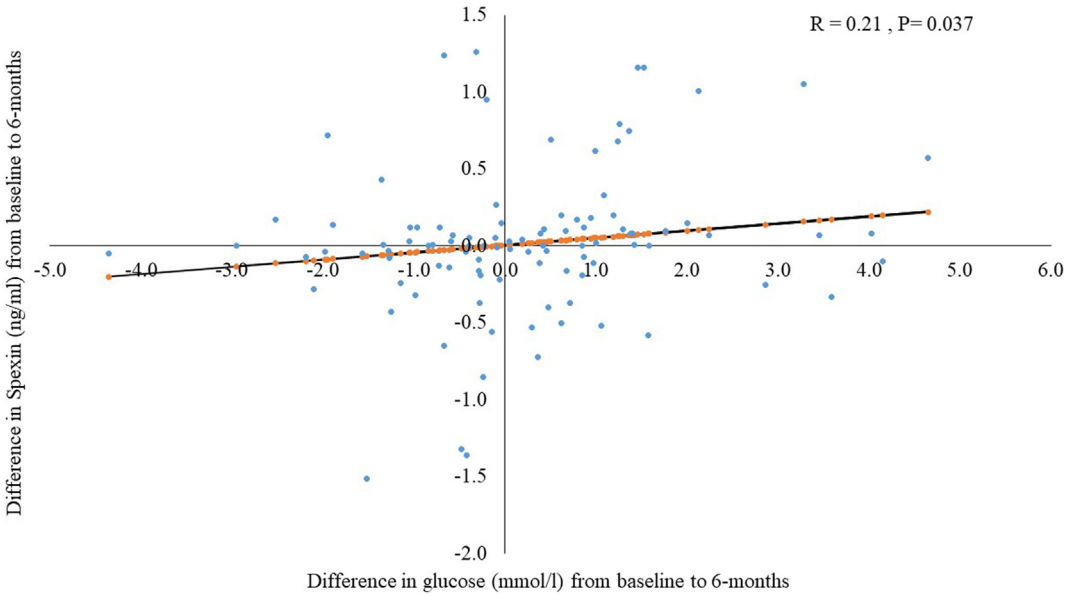

Fig. 1. Relations between changes in SPX *vs.* Glucose in all patients.

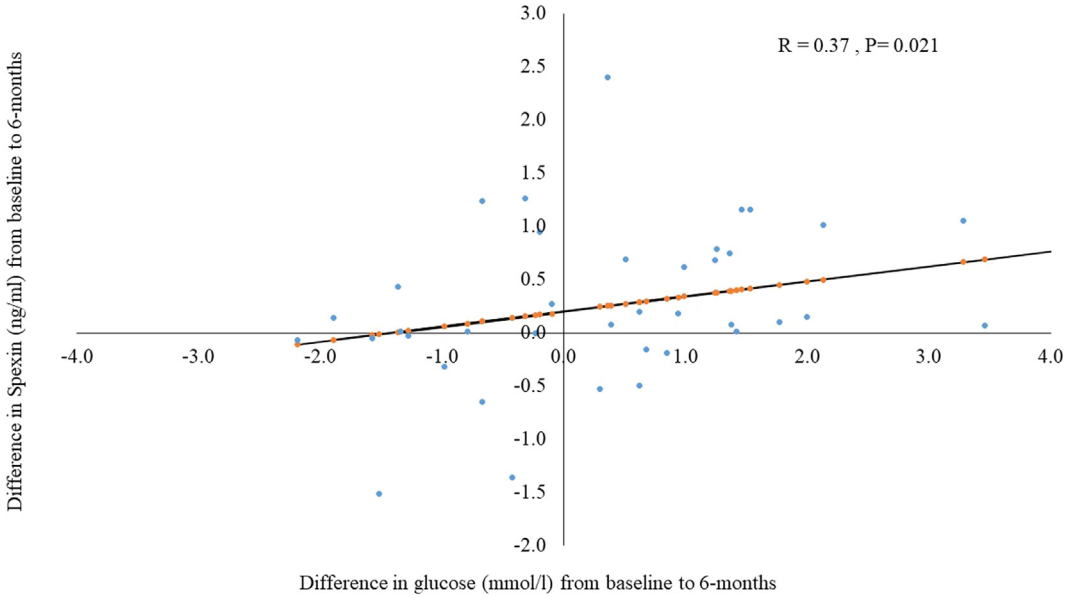

Fig. 2. Relations between changes in SPX *vs.* Glucose in GDM patients.

5. Conclusion

Changes in SPX levels seem to be influenced by the presence of GDM in pregnant women, with decreased levels in those without GDM, and increased in those with GDM.

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Conflict of Interest

None.

Author's contribution

NMA, SS, HA and MSA contributed in the design, subject recruitments and data collection. HA, AMA, AA, and AG carried out sample analysis, interpretation, and preparation of draft manuscript. SDH performed data analysis and results interpretation. SS, and GPC drafted and edited the final version of the manuscript. All authors read and approved the final manuscript.

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