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Cord Blood Levels of Spexin, Leptin, and Visfatin in Term Infants Born Small, Appropriate, and Large for Gestational Age and Their Association with Newborn Anthropometric Measurements

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What is already known on this topic?

Birthweight is associated with an increased risk of obesity and cardiovascular disease later in life. Umbilical cord blood adipokines serving as a measure of adipose tissue activity are associated with birth outcomes.

What this study adds?

Cord blood spexin (SPX) levels were found to be associated with neonatal anthropometric measurements. The lowest SPX levels were found in SGA babies.

Abstract

Objective: Children born small for gestational age (SGA) are at risk of future obesity and associated comorbidities. Therefore the identification of risk factors and novel biomarkers which are associated with this risk are needed for early detection and to improve preventive strategies. Spexin (SPX), a novel neuropeptide that is involved in the regulation of obesity and fat metabolism, is a candidate biomarker for predicting obesity and related comorbidities at an early age. The aim of this study was to investigate serum levels of SPX in term infants born small, appropriate, and large for gestational age (LGA) and its association with newborn anthropometric measurements.

Methods: One hundred and twenty term newborn babies classified as SGA, appropriate for gestational age (AGA), or LGA and their mothers were included. SPX, leptin and visfatin were measured in cord blood and maternal serum by enzyme-linked immunosorbent assay.

Results: Fifty-six (46.7%) neonates were girls and 64 (53.3%) were boys. The mean birth weight was 3170.70 ± 663 g, birth length was 48.9 ± 2.79 cm, and head circumference was 34.5 ± 1.67 cm. Birth weights, lengths, and head circumferences of the neonates in the SGA, AGA, and LGA groups were significantly different. Cord blood SPX and leptin levels in the SGA groups were significantly lower than those of both the LGA and AGA groups. Cord blood visfatin levels were significantly lower in the AGA group than the LGA and SGA groups. Maternal SPX levels of SGA babies were significantly lower than those of the mothers in both the LGA and AGA groups, but no significant difference was observed between the SGA and LGA groups. Maternal visfatin levels of the AGA babies were significantly higher than the maternal levels of SGA and LGA groups. There was no difference in terms of maternal leptin levels. Cord blood SPX and leptin levels were positively correlated with birth weight, length and head circumference. Birth weight increased significantly in line with maternal pregestational body mass index.

Conclusion: The lowest SPX levels were found in the SGA babies and cord SPX level was significantly correlated with newborn length, weight, and head circumference.

Keywords: Adipokine, speksin, antropometry, newborn, umblical cord

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Introduction

Intrauterine growth is under control of genetic, growth and nutritional factors related to the fetus, mother, and placenta (1). Birth weight, length, and head circumference values according to gestational age and gender in neonates are evaluated on the basis of growth curves, and newborns are classified as small for gestational age (SGA), appropriate for gestational age (AGA), or large for gestational age (LGA) (2).

Infant birth weight is one of the determinants of perinatal mortality and morbidity. Placental insufficiency and adverse intrauterine conditions are also associated with numerous long- and short-term sequelae in postnatal life, with an increase in perinatal morbidity and mortality, by affecting the development of the fetus. Obesity, insulin resistance, metabolic syndrome, chronic kidney diseases, and cardiovascular diseases are thought to be associated with epigenetic changes occurring in fetal metabolic programming (Barker's hypothesis) affected by adverse conditions during the intrauterine period (3). Studies of control mechanisms in natal and postnatal growth have shown that adipose tissue behaves like an active endocrine organ (4) and regulates numerous physiological functions in the body, such as insulin sensitivity, inflammation, growth, puberty, and cardiovascular functions by secreting messenger molecules known as 'adipokines' (5). Adipokines are circulating factors that mediate the cross-talk between metabolic systems and different organs (5).

The most frequently studied adipokine is leptin. Leptin and its receptors are widely produced and expressed in fetal and placental tissues (6). It is predominantly released by white adipose tissue and affects appetite and food intake, gonadotropin release, immune modulation, and lipogenesis (7). Low serum and placental leptin levels have been demonstrated in newborns with intrauterine growth retardation (IUGR), and high concentrations in the macrosomic babies of diabetic mothers (6). Visfatin is specific to visceral adipose tissue and is a marker of insulin resistance-associated fat deposition (8). Studies have suggested that high visfatin levels may be a prognostic marker of the probable future development of metabolic syndrome (9,10,11). Spexin (SPX), also known as neuropeptide Q, is a novel, 14 amino acid peptide first described by Mirabeau et al. (12) in 2007. SPX binds to and activates galanin receptor 2/3 (GALR2/3) (13,14). SPX mRNA is expressed in different parts of the human body, particularly in white adipose tissue, but also in the brain, heart, thyroid, gonads, gastrointestinal system, and pancreas. Studies of SPX have shown that it is involved in energy homeostasis, glucose and lipid metabolism, obesity, gastrointestinal functions, pain, regeneration and neuron renewal, Alzheimer's disease, cerebral ischemia and stroke, epilepsy, anxiety disorders, and cardiovascular and renal functions (15). The few studies of SPX in the pediatric age group have investigated its association with obesity and metabolic diseases (16). Two of these studies were performed in the neonatal period and only one of them investigated the levels of SPX in umbilical cord blood from term LGA, SGA, and AGA infants and the association with anthropometric measurements. This study reported no significant difference in terms of SPX levels (17,18).

Anthropometric measurements in newborns have long been known as a risk factor for conditions such as obesity, cardiovascular disease and type 2 diabetes in adulthood (19). Changes in adaptation to life in the early period, known as "early life programming", may become maladaptive with advancing age and increase the risk of obesity associated cardiometabolic diseases. It is well established that children born SGA are at risk of future obesity and associated comorbidities. Therefore the identification of risk factors and novel biomarkers which are associated with this risk are needed for early detection and to improve preventive strategies. SPX is a novel neuropeptide that has an effect in many systems and has been shown to be involved in the regulation of obesity, and fat metabolism. It is a candidate biomarker for predicting obesity and related comorbidities at an early age. The aim of the present study was to investigate serum levels of SPX in term SGA, AGA and LGA infants and its association with newborn anthropometric measurements. SPX levels, together with those of the well-known and frequently studied leptin and visfatin, measured in umbilical cord serum specimens obtained from SGA, AGA and LGA infants were investigated. The relationship between the results and neonate anthropometric measurements was then examined. Potential relationships between mother-infant adipokine levels and anthropometric measurements were evaluated by analyzing the serum leptin, visfatin, and SPX levels of the mothers in blood samples collected during labor simultaneously with those of the babies.

Methods

SGA, AGA and LGA neonates born in the Pamukkale Medical Faculty Hospital, Turkey, between September 2019 and April 2020, and their mothers, were eligible for the study. Written informed consent was obtained from all parents and mothers prior to their participation in the study. The research was approved by the Pamukkale University Ethics Committee (approval number: 10.09.2019/21, date: 10.12.2019) and was performed in accordance with the Declaration of Helsinki.

Infants' gestational ages were determined from the date of the last menstrual cycle based on the New Ballard scoring system. Term neonates born between 37 and 42 weeks were divided into three groups using Lubchenko's intrauterine development curves - SGA, consisting of babies under the 10th percentile, AGA, babies with birth weights for gestational age between the 10th and 90th percentiles, and LGA, babies above the 90th percentile (2). One hundred and twenty newborn babies, 40 in each group, and their mothers were included in the study. Babies with congenital malformations, syndromic babies, and those with chromosomal disease, severe infection, hypothyroidism, and similar disorders capable of causing growth and development retardation, and the babies of mothers with histories of maternal hypothyroidism, early membrane rupture, pre-eclampsia, and gestational/pregestational diabetes were excluded. The blood specimens required for the study were collected from the umbilical cord blood supply together with blood samples (blood gas, blood group) collected routinely in order to evaluate the baby's condition in the delivery room, and from the mothers of the babies before they left the delivery room.

Blood samples for SPX, leptin, and visfatin measurement were collected from the umbilical vein on the placental side of the umbilical cord, and venous blood samples collected from the antecubital veins of the mothers were placed into pre-prepared biochemistry tubes containing a gel separator. Following a maximum waiting period of 30 minutes, the sera were separated by centrifugation at 3000xg for 10 minutes. These were then placed into Eppendorf tubes and stored at -80 °C until analysis. The birth weights, lengths, and head circumferences of all the babies in the study were measured by the same individual. Body weight was measured using electronic scales sensitive to 5 g, with the baby wearing no clothing or nappy. Length was measured with the baby lying in a supine position, and head circumference using a non-elastic tape measure, and passing this over the most protruberant point on the back of the head, the parietal region on the side, and the glabella on the front. Serum SPX levels were measured using an Enzyme Amplified Sensitivity Immunoassay (EASIA) method on an Lbiont Human SPX (C12orf39) Enzyme-linked Immunosorbent Assay (ELISA) (Catalog no. YLA1034HU) test kit. The sensitivity was 4.95 pg/mL and detection range was 10 pg/mL-4000 pg/mL. Serum leptin levels were measured using EASIA on a Boster Picokine Human Lep pre-coated ELISA (catalog no. EK0437) test kit. The sensitivity was 4.95 pg/mL and detection range was 10 pg/mL-4000 pg/mL respectively. Serum visfatin levels were calculated using the ELISA method on a CUSABIO Human Visfatin ELISA (Catalog no. CSB-E08940h) test kit. The sensitivity and detection ranges were 0.156 ng/mL and 0.625 ng/mL-40 ng/mL, respectively. No data out of read limits

Statistical Analysis

Statistical analysis of the study findings was performed on Statistical Package for Social Sciences for Windows, version 22.0 (IBM INC., Armonk, NY, USA). In addition to descriptive methods, the Kruskal-Wallis and Mann-Whitney U tests were used in the comparison of numerical data since the parameters were all non-parametric, and Bonferroni correction was applied. The chi-square test was employed for the comparison of qualitative data. Since the parameters were not normally distributed, Spearman's correlation test was applied to examine relationships between parameters, and the partial correlation test was performed in case of the presence of potentially confounding factors. Robust regression analysis was performed, again due to non-normal distribution. A multiple regression model was employed in order to examine the effect of each adipokine on newborn anthropometric measurements. Cord blood SPX, leptin, and visfatin were used as dependent variables in this model, and length, weight, and head circumference as independent variables. A $p < 0.05$ was regarded as statistically significant.

Results

One hundred and twenty neonates and their mothers who meet the study criteria were included in the research. Fifty-six (46.7%) neonates were girls and 64 (53.3%) were boys. Across the whole cohort the mean birth weight was 3170.70 ± 663 g, mean birth length was 48.90 ± 2.79 cm and mean head circumference was 34.50 ± 1.67 cm. The mean age of the mothers was 30.38 ± 5.83 years, and the mean gestational week was 38.05 ± 0.77. The mean weight of the mothers prior to delivery was 62.46 ± 6.32 kg, their mean height was 159.10 ± 4.17 cm, and their mean body mass index (BMI) was 24.7 ± 2.8. Mean weight gain during pregnancy was 11.60 ± 1.43 kg (range 9 to 15 kg). Based on their pre-pregnancy BMI values, 40.83% of mothers were overweight or obese. Birth weights (2407.7 ± 296.9 vs. 3219.2 ± 245.9 vs. 3885.1 ± 264.6), lengths (46.2 ± 2.6 vs. 49.2 ± 1.3 vs. 51.1 ± 1.6), and head circumferences of the neonates (32.8 ± 1.3 vs. 34.6 ± 0.7 vs. 36 ± 1.1) in the SGA, AGA, and LGA groups, exhibited statistically significant differences, as expected. The mean age of the mothers in the SGA group was significantly higher than that of the mothers in the AGA group ($p < 0.05$).

Across the whole study cohort neonatal median cord blood SPX concentration was 304.88 pg/mL (12.564-1739.22), median leptin concentration was 6.50 ng/mL (0.58-41.60), and median visfatin concentration was 3.28 ng/mL (0.39-

8.50). Similarly, for the mothers, median plasma SPX concentration was 336.09 pg/mL (15.62-1216.45), median leptin concentration was 19.59 ng/mL (1.73-53.94), and median visfatin concentration was 3.28 ng/mL (0.39-8.56).

SPX, leptin, and visfatin concentrations of the neonates in the SGA, AGA, and LGA group were measured and compared togethere with those values obtsined from their mothers (Table 1). Cord blood SPX concentrations of SGA neonates were significantly lower than those of both the LGA and AGA groups. The venous blood SPX concentrations of mothers of SGA babies were significantly lower than those of the mothers in both the LGA and AGA groups. The SPX concentration of mothers in the AGA and LGA groups did not differ.

Comparison of leptin concentrations in cord blood samples showed that neonates from the SGA group had significantly lower median leptin concentrations than those found in either the LGA or AGA groups. Furthermore, median cord blood leptin levels were also significantly lower in AGA group neonates than in LGA group neonates (Table 1). No statistically significant difference in maternal venous blood leptin concentrations was observed between the groups.

Cord blood visfatin concentrations were significantly lower in the neonates from the AGA group compared to those from both the LGA and SGA groups. However, median concentration values of visfatin obtained from babies in the SGA and LGA groups did not differ (Table 1). Similarly, maternal visfatin levels were significantly higher in the mothers of neonates from the AGA group compared to those of neonates from both the SGA and LGA groups (Table 1).

In correlation analysis, cord blood SPX and leptin levels were positively correlated with birth weight, length and head circumference (Table 2). Birth weight increased significantly in line with maternal pregestational BMI ($r = 0.505$, $p < 0.001$). Correlation analysis of the adipokine concentration of all the neonates and their mothers showed strong correlation between infant and maternal SPX levels ($r = 0.818$, $p < 0.001$) but a weak correlation between infant and maternal leptin levels ($r = 0.214$, $p = 0.019$) (Table 2).

Regression analysis showed cord blood leptin concentrtion had a significant positive association with birth weight and head circumference [Odds ratio (OR): 0.584, 95% confidence interval (CI): 0.003-0.011, $p = 0.002$ and OR: -0.334, 95% CI: -3.00-0.142, $p = 0.032$], but that SPX and visfatin levels exerted no significant effects on birth indices.

Discussion

This study investigated the relationships between anthropometric measurements and levels of the novel adipocytokine SPX and of leptin and visfatin in the cord blood of term SGA, LGA, and AGA babies and in plasma specimens from their respective mothers. Cord blood SPX and leptin levels were lowest in the SGA neonates, while visfatin levels were similar between babies from the SGA and LGA groups but higher than those in babies from the AGA group. Significant correlation was found between neonatal cord blood SPX and leptin levels and newborn length, weight, and head circumference.

The birth weight of the newborn baby is the most important anthropometric measurement used in the evaluation of pregnancy, and also one of the most important predictors of perinatal mortality and morbidity (20). The understanding that numerous common chronic diseases are caused by adaptations developed to combat adverse intrauterine conditions in the early period of life has significantly

Table 1. Serum and cord blood levels of spexin, leptin, and visfatin in SGA, AGA, LGA newborns and mothers

	SGA (1) (n = 40)	AGA (2) (n = 40)	LGA (3) (n = 40)	p
Cord blood spexin (pg/mL)	191.2 (12.6-1739.2)	6.34 (1.8-25.1)	9.75 (1.2-35.4)	**0.0001 (1-2)[§] (1-3)** **0.413 (2-3)**
Cord blood leptin (ng/mL)	3.54 (0.4-34.7)	6.34 (1.8-25.1)	9.75 (1.2-35.4)	**0.046 (1-2)** **0.0001 (1-3)[®]** **0.035 (2-3)**
Cord blood visfatin (ng/mL)	5.84 (0.4-34.7)	3.20 (0.3-8.6)	5.69 (2.8-17.8)	**0.0001 (1-2/2-3)** 1.000 (1-3)
Maternal spexin (pg/mL)	196.28 (15.6-1216.4)	366.047 (129.6-837.3)	392.34 (24.56-1148.96)	**0.0001 (1-2/1-3)**
Maternal leptin (ng/mL)	19.18 ± 8.39	16.73 ± 9.78	22.03 ± 11.94	0.069*
Maternal visfatin (ng/mL)	1.55 (0.39-6.85)	4.43 (0.42-7.38)	1.66 (0.41-8.56)	**0.0001 (1-2) (2-3)[¶]** 1.000 (1-3)

Values are expressed as mean ± standard deviation, median (minimum-maximum). Comparison between groups: Kruskal-Wallis test, *Anova.
SGA: small for gestational age, AGA: appropriate for gestational age, LGA: large for gestational age
[§](1-2) Comparison between SGA and AGA
[®](1-3) Comparison between SGA and LGA
[¶](2-3) Comparison between AGA and LGA

increased interest in endocrine programming. Following the initial observation of an association between the risk of mortality due to ischemic heart disease and the individual's birth weight, similar relationships were found in other diseases, such as stroke, type 2 diabetes mellitus, and dyslipidemia (21).

Obesity is a global health problem and a risk factor for chronic diseases (22). There is a known link between maternal obesity and health problems in newborn babies, and the increase in obesity rates among women of reproductive age is worrying. The effect on the fetus of adverse intrauterine conditions is known to affect the risk of development of disease in subsequent periods of life, and the process, known as "early life programming", in which the fetus adapts to the adverse intrauterine environment, increases the likelihood of its survival (23). An adverse intrauterine environment can affect the amount and function of adipose tissue, extending into adulthood (24,25). Changes due to early life programming may become maladaptive with advancing age and exacerbate the risk of several chronic diseases, such as obesity, diabetes, and hypertension (26).

The placenta, and the hormones and adipokines produced by it, are of considerable importance in providing the nutritional resources essential for growth and development of the fetus and in maternal metabolic adaptation to pregnancy (27). The relationship between neonatal cord blood adipokine levels and anthropometric measurements has been the subject of frequent investigation, and efforts have been made to predict the risk of obesity, metabolic syndrome, and cardiovascular disease development in at-risk newborn babies (25,28,29). In this respect, one of the most studied adipokines in the neonatal period is leptin. Placental leptin production is the main maternal source of leptin. High molecular weight leptin of maternal origin is unable to cross the placenta, and measurement of cord blood leptin levels therefore show fetal adipose tissue and placental production. Umbilical cord blood leptin levels have been found to be associated with placental weight, and the infantile body weight, length, head circumference, ponderal index, adiposity, and bone mineral density. These studies have shown higher cord blood leptin values in LGA neonates compared to those born AGA and SGA (30), and lower cord blood leptin levels and decreased leptin gene expression in those with IUGR (30,31,32). In their meta-analysis examining the relationship between cord blood leptin levels and anthropometric measurements, Karakosta et al. (33) reported a correlation between leptin levels and birth weight (r = 0.46). A significant difference was also determined in cord blood leptin levels between the groups in the present study. As expected, the lowest leptin level was observed in the SGA group, and the highest in the LGA group. Leptin level elevation at birth may result in adverse programing of the hypothalamus (via an impaired leptin surge), the effects of which usually manifest after the third year of life. The initial negative correlation between cord leptin and adiposity may be due to the anorexigenic effect of leptin, with subsequent leptin resistance, in turn leading to hyperphagia and increased adiposity (25,34,35,36). Studies investigating the relationship between maternal leptin levels and neonatal anthropometric measurements have reported positive, negative, and even no correlation (37,38,39). There are also studies showing a direct association between maternal obesity markers and neonatal leptin levels (40,41). These inconsistent observations in pregnant women suggest that most of the leptin levels measured in mothers originate from the placenta, and that other factors or complications of pregnancy may affect leptin levels (42). In the present study, individual evaluation of the groups revealed weak correlation between maternal leptin and cord blood leptin levels, but only between the babies and their mothers in the AGA group. Positive correlation was observed between cord

Table 2. Correlation analysis of birth weight, length, head circumference, maternal BMI, spexin, leptin, and visfatin levels

	Cord spexin (n = 120)		Cord leptin (n = 120)		Cord visfatin (n = 120)	
	r	p	r	p	r	p
Birth weight	**0.417**	**0.0001**	0.404	**0.0001**	0.045	0.628
Birth length	**0.363**	**0.0001**	0.293	**0.001**	0.074	0.421
Birth head circumference	**0.375**	**0.0001**	0.314	**0.0001**	-0.124	0.178
Maternal BMI	**0.21**	**0.028**	0.207	**0.023**	0.047	0.613
Maternal spexin	0.818	**0.000**	0.05	0.958	0.44	0.630
Maternal leptin	-0.076	0.409	0.214	**0.019**	-0.90	0.326
Maternal visfatin	0.20	0.831	-0.107	0.247	0.09	0.329
Cord spexin			0.155	0.091	-0.43	0.637
Cord leptin	0.155	0.091			-0.175	0.055
Cord visfatin	-0.043	0.637	-0.175	0.055		

BMI: body mass index

blood leptin levels and maternal BMI, but no correlation was found between maternal BMI or weight gain in pregnancy and maternal leptin levels.

Visfatin is an adipocytokine mostly produced in visceral adipose tissue, and is also secreted by the placenta and amniotic membranes, and that results in increased synthesis of proinflammatory cytokines (43). The pathophysiological role of visfatin has frequently been investigated in conditions such as chronic inflammatory and rheumatological diseases, cardiovascular diseases, diabetes mellitus, and obesity in which glucose metabolism is affected (43). Insulin resistance increases the expression of visfatin. Levels of visfatin also rise in normal pregnancies in which physiological insulin resistance is observed, peaking between the nineteenth and twentyfifth weeks (44). This increase becomes more pronounced when accompanied by obesity and related diseases. Changes in visfatin levels have been linked to pre-eclampsia, IUGR, premature birth, and gestational diabetes (GDM) (45). It is not always easy to ascertain whether these differences derive from the mother, placenta, or fetal metabolism alone, or from a collective interaction. In research on this subject, that may be regarded as pioneering, Malamitsi-Puchner et al. (11) compared two newborn groups, AGA and IUGR, and reported no significant difference in umbilical cord visfatin levels, but observed significantly higher serum visfatin levels in mothers who gave birth to babies with IUGR than those giving birth to AGA babies. Similarly, Estrada-Zúñiga et al. (46) investigated cord blood visfatin levels in 128 newborns divided into groups according to birth weight and reported no difference in cord blood levels, while Mazaki-Tovi et al. (47) found no difference in cord blood visfatin concentrations in normotensive mothers of babies born AGA and SGA. In addition, in a study of mothers and children with no morbidity, Meral et al. (10) reported higher visfatin concentrations in LGA neonates compared to SGA neonates, and in SGA neonates compared to AGA babies. Shang et al. (48) reported higher visfatin levels in neonates with IUGR compared to a macrosomic neonate control group, while Cekmez et al. (49) reported slightly higher cord visfatin levels in neonates in an SGA group compared to an AGA group. Similarly, in the present study, cord blood visfatin levels were significantly higher in the LGA and SGA group neonates compared to the AGA group. Visfatin is produced in visceral adipose tissue, and we hypothesize that increased visceral adipose tissue in LGA and SGA neonates may have resulted in this elevation. Visfatin may be an early marker of the development of insulin resistance capable of emerging later in life, and it has also been suggested that it possesses prognostic value in terms of the future development of metabolic syndrome in neonates with IUGR (10).

Information concerning the physiological functions of SPX has increased as studies have revealed the presence of SPX in a wide range of tissues and organ systems including the central nervous system (hypothalamus, pons, cerebral cortex, and anterior pituitary gland), white adipose tissue, kidneys, ovaries, thyroid, stomach, adrenal glands, pancreas and many other human tissues (50). These studies have shown that SPX is associated with feeding behavior, body weight, obesity, diabetes mellitus, gastrointestinal motility, mental illness, and reproductive and cardiovascular functions (51). Studies of adult and pediatric obese patients have shown lower SPX levels than those of normal-weight controls, with SPX levels being reported to exhibit inverse correlation with such metabolic parameters as BMI, waist circumference, low-density lipoprotein, triglycerides, leptin, ghrelin, insulin, and HOMA-IR (52,53,54,55,56). However, there are also studies reporting no difference in SPX levels between adolescent obese, normal, and type 2 DM groups (57). While some studies have reported decreased serum SPX levels in patients with type 1 and type 2 DM compared to a control group and that serum SPX levels are not associated with glycemic parameters, lipids, or BMI, and that the decreased SPX levels in patients with Type 2 DM are related to fasting glucose and HbA1c, others have concluded that SPX is positively associated with blood lipid levels and that SPX levels increase in patients whose blood sugars improve following intervention (58).

Studies have sought to identify the facilitating or preventive role of different adipokines in the emergence of GDM, which can develop as a result of insulin resistance during the natural course of pregnancy and that have adverse effects on maternal and infant health (59). There has been an increase in the numbers of studies investigating the physiological role of SPX during pregnancy. Akbas et al. (60) compared pregnant women with and without GDM in the third trimester of pregnancy and observed higher serum SPX levels in women with GDM compared to controls with no GDM. In that study, serum SPX levels were directly correlated only with HOMA-IR ($r = 0.234$, $p = 0.04$), while no correlation was observed with patient age, glucose, insulin, BMI, or fetal weight. Al-Daghri et al. (61) followed-up 102 pregnant women, 63 non-GDM and 39 with GDM and reported that serum SPX levels in pregnant women with GDM increased significantly in line with glucose levels, while serum SPX levels decreased in the non-GDM patients, although this decrease was not associated with glucose levels. Yavuzkir et al. (62) investigated subfatin and SPX levels in the serum of pregnant women with and without GDM, before and during an oral glucose tolerenace test performed between weeks 24 and 28 weeks of pregnancy, and in baby cord blood at birth. These authors reported significantly higher SPX and

subfatin levels in women with GDM during pregnancy and during delivery, while cord blood SPX and subfatin levels in babies born to mothers with GDM were significantly higher than those of babies born to mothers without GDM. Serum levels of these adipokines were positively correlated with the lipid, glucose, and HOMA-IR levels of mothers with GDM ($p < 0.05$). The common conclusion of all these studies is that the SPX levels of pregnant women with GDM were significantly higher than in the controls, and this has led to SPX being proposed as a potential marker in the diagnosis and follow-up of GDM. SPX levels in mothers with and without GDM and in babies' umbilical cord blood were examined in only one of these studies to date. Sanli et al. (17) investigated the effect of SPX, leptin, ghrelin, free 25(OH) vitamin D3, glucose, and insulin levels in umbilical cord blood from term LGA, SGA, and AGA infants on anthropometric measurements. They observed that only leptin levels were higher in the LGA group than in the SGA and AGA groups, while no significant difference was observed in terms of SPX, ghrelin, free 25(OH) vitamin D3, or insulin levels. To the best of our knowledge, the present study is the first to investigate SPX levels measured in healthy pregnant women and cord blood from newborns classified as LGA, SGA, and AGA, with the lowest SPX levels being found in the SGA babies and mothers. No statistically significant difference was observed between AGA and LGA babies and mothers' SPX levels. Cord blood SPX levels were positively correlated with neonatal birth weight, birth length, and head circumference, and maternal BMI, and maternal SPX levels. We speculate that the presence of this correlation suggests that adverse conditions affecting fetal development in that period cause a decrease in SPX synthesis or SPX levels exert a positive effect on growth in the intrauterine period. Compared with the research of Sanli et al. (17) which is the only other study to report results at least partly similar to our own findings, cord blood leptin levels exhibited a positive effect on anthropometric measurements, but in contrast to their findings, we found that cord blood SPX levels were associated with neonatal anthropometric measurements. These differences in results may be due to the difference between the two study populations. Sanli et al. (17) conducted their study in Istanbul, which is home to different ethnic groups and that has received considerable immigration in recent years, and is the world's 14th largest metropolis. In contrast, our study was conducted in a small city with a relatively homogenous population. The variation in SPX levels may be attributable to the difference in the numbers of patients, and to that study being conducted in a city such as Istanbul. Being born SGA is now well established as a risk factor for type 2 DM, obesity, hypertension, and cardiovascular diseases in which insulin resistance is a common feature (18). The most interesting finding of the present study was that SPX levels, which has been shown to be negatively correlated with insulin resistance parameters in insulin resistance and associated metabolic disorders, were also low in SGA neonates (18). Although maternal SPX levels correlated with maternal BMI and cord blood SPX levels, no difference was observed between the mothers of SGA and AGA babies in terms of BMI values. We speculate that placental production of SPX might contribute to the levels of both fetal and maternal SPX levels. We think that this hypothesis can be tested by studying the placental weights and SPX expressions of pregnant women with GDM in order to explain the high SPX levels in pregnant women with GDM, in contrast to the low SPX levels in type 2 DM in particular.

Study Limitations

There are a number of limitations to this study. First, neonatal and maternal insulin resistance parameters were not investigated. Second, placental weights were not measured and SPX expression in placental tissue was not evaluated. Third, the neonates were not followed-up long term. Thus, more extensive studies including the measurement of cord levels at birth and long-term follow-up of these patients will clarify whether SPX may be a clinially useful predictive marker for metabolic disorders and cardiovascular diseases that may not present until adolescence or adulthood.

Conclusion

SPX level, which has previously been shown to be negatively correlated with insulin resistance parameters in insulin resistance and associated metabolic disorders, was also found to be low in SGA neonates in this study. Furthermore, cord SPX concentration significantly correlated with newborn length, weight, and head circumference. Given the clinical association between SGA infants and the later risk of diseases associated with insulin resistance, we believe that this association warrants further extensive research.

Ethics

Ethics Committee Approval: The research was approved by the Pamukkale University Ethics Committee (approval number: 10.09.2019/21, date: 10.12.2019) and was performed in accordance with the Declaration of Helsinki.

Informed Consent: Written informed consent was obtained from all parents and mothers prior to their participation in the study.

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Authorship Contributions

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