Decreased Circulating Levels of Spexin in Obese Children

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Context: Spexin is a novel peptide that is implicated in obesity and related energy homeostasis in animals and adult humans. Little is known about its role in children.

Objective: The aim of the current study was to determine the potential role of Spexin in obese children and explore its relationships with various cardiometabolic risk factors.

Design and Participants: This was a cross-sectional study composed of 69 children (51 obese and 18 normal weight; age 15.3 $\pm$ 0.26 y).

Outcome Measures: Spexin was measured using a specific enzyme-linked immunosorbent assay. Leptin, total and high-molecular-weight adiponectin, IL-6, high-sensitivity C-reactive protein, glucose, and insulin were also measured. Mann-Whitney *U* test, Pearson and Spearman rank correlations, logistic regression, and cluster analysis were used for the analysis and interpretation of the data.

Results: Spexin levels were significantly lower in obese vs normal-weight children, median(IQR) (0.33 ng/mL [0.27–0.44] vs 0.42 ng/mL [0.33–0.55]; $P = .024$), but did not correlate with other adipokines and/or insulin and glucose levels. Ordinal categorical variables of Spexin showed a strictly reverse association of obesity with the level of Spexin. Cluster analysis of Spexin and body mass index *z* score resulted in splitting the participants into normal-weight and obese-weight groups with high accuracy.

Conclusions: Lower circulating levels of Spexin in obese children compared with their normal-weight counterparts and the ability to discriminate obese and normal-weight groups based on Spexin concentration enabled us to suggest a potential role for this novel peptide in childhood obesity. The clinical significance of these findings needs additional investigation. **(*J Clin Endocrinol Metab* 101: 2931–2936, 2016)**

The prevalence of obesity has tripled in the last three decades, and currently 17% of children in the United States are obese and 35% are either overweight or obese (1). Childhood obesity is associated with a number of adverse health consequences including type 2 diabetes (T2DM), dyslipidemia, and hypertension, all which lead to premature cardiovascular disease (2, 3).

Adipose tissue in addition to serving as a site for energy storage has been found to have an endocrine function with secretion of a whole variety of adipokines (4–6). Some of these adipokine influence weight by their effect on satiety, hunger, and glucose and lipid metabolism (7–10). Spexin is a peptide initially identified using Markov modeling (11). Its biological activity was subsequently recognized

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Abbreviations: BMI, body mass index; HMW, high molecular weight; IQR, interquartile range; T2DM, type 2 diabetes mellitus.

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by Walewski et al (12). They reported that Ch12:orf39, the gene that encoded *Spexin*, was the most down-regulated gene in obese omental and sc human fat (13). The same group also showed significantly lower Spexin concentrations in obese compared with lean adults and proposed a potential satiety-inducing role for it in humans (12). Spexin also reduced adipocyte uptake of long-chain fatty acids (12). In addition, Spexin administration in rodents with diet-induced obesity provokes weight loss by decreasing caloric intake and increasing locomotion (12). Circulating Spexin levels are low in patients with T2DM and inversely related to blood glucose and lipids, suggesting that the peptide may play a role in glucose and lipid metabolism in T2DM (14). Taken together, it seems that Spexin plays an important role in obesity and related comorbidities. However, there are no reports on Spexin concentrations in children. The aim of the study was to determine Spexin concentrations in obese vs normal-weight children in relation to various cardiometabolic risk factors.

Materials and Methods

A total of 69 children (51 obese and 18 normal weight; age 15.3 ± 0.26 y) were studied. The study participants were euthyroid, nondiabetic, obese or normal-weight adolescents between the ages of 12 and 18 years who had participated in a clinical trial assessing the effect of vitamin D_3 supplementation on 25 hydroxy vitamin D levels or cardiometabolic risk markers (www.clinicaltrials.gov, NCT00858247 and NCT01058720). Exclusion criteria included current cancer; hepatic or renal disorders; and use of metformin, oral hypoglycemic medications, or glucocorticoids in the previous 6 months; and any medical or psychiatric condition that would interfere with study participation.

Participants were considered obese if body mass index (BMI) was at or greater than 95th percentile for age and sex and nonobese if BMI was between the fifth and 85th percentile for age and sex (15). The protocol for the study was approved by Institutional Review Board of Mayo Clinic. Informed consent and assent were obtained from participants and parents. The subjects' BMI *z* scores were determined using the age-specific and sex-specific median BMI, generalized coefficient of variation (S), and the power of the Box-Cox transformation (L) based on U.S. Centers for Disease Control and Prevention growth curves (15).

Biochemical measurements

Spexin was measured using a specific ELISA from Phoenix Pharmaceuticals, Inc. Total and high-molecular-weight (HMW) adiponectin, leptin, and IL-6 were also measured by ELISA (R&D Systems Inc.).Serum insulin was measured using commercial electrochemiluminescence immunoassay kits (Roche E Modular, Roche Diagnostics). Glucose was measured using Analox GM7 Glucose analyzer.

Statistical analysis

Demographics, clinical characteristics, and Spexin values were summarized by normal-weight and obese subjects. Quantitative variables were summarized by mean (SD) or median (interquartile range [IQR]), whichever was appropriate. A two-sample *t* test or a Mann-Whitney *U* test was used to compare the mean or median of the quantitative variables. Categorical variables were summarized by number and percentage of the patients. A χ^2 test was used to compare the distribution of sex between normal-weight and obese-weight groups. A Pearson correlation or Spearman rank correlation was performed to examine the association of Spexin with age and quantitative clinical characteristics. A Mann-Whitney *U* test was used to compare the median Spexin between two groups because there were some outliers in both groups. To further examine the association of Spexin with obesity, we categorized children in ordinal groups based on the levels of Spexin. We formed three group variables using median split, tertiles and quartiles of Spexin. Then, we examined the distributions of obese and normal-weight patients over the groups of these three categorical variables. In addition, we performed logistic regression to examine the association of obesity with the ordinal categories of Spexin. We also performed a cluster analysis of Spexin and BMI *z* score to group children. Three distinct groups of children were identified based on the correlation of Spexin and BMI *z* score. Distribution of obese and normal-weight children was also examined over three groups. BMI *z* score as well as other clinical and demographic variables were examined by three cluster groups.

Model or test assumptions were checked and an appropriate transformation or an equivalent nonparametric test was used. All tests were two tailed at the level of significance of $P = 0.05$. The statistical software R version 3.2 and SPSS version 22 were used for the analysis.

Results

Clinical characteristics

Fifty-one obese adolescents (29 females, 22 males) recruited to participate in a study to examine the effect of vitamin D_3 on 25(OH)D levels and cardiovascular risk markers had their laboratory studies drawn between September 18, 2008 and July 9, 2010 (16). Eighteen normal-weight adolescents (8 females, 10 males) were recruited as part of a study to examine the effect of obesity on increment in 25(OH)D levels following vitamin D_3 administration had laboratory tests drawn between November 13, 2009 and October 6, 2010 (17).

Demographics, clinical characteristics, and Spexin concentration of the participants are outlined in Table 1. The mean (±SD) age was 15.34 (2.21) years and 14.62 (2.29) years in the normal-weight and obese-weight group, respectively. As expected, the mean (SD) BMI was 20.25 (1.89) and 31.95 (4.49) and median (IQR) BMI percentile was 46.92 (40.98–55.16) and 98.47 (98.00–98.87). They were significantly different between the normal-weight and obese-weight groups. All patients in the normal-

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Table 1. Baseline Anthropometric and Laboratory Characteristics of Study Subjects by Normal-Weight and Obese-Weight Status

	Normal Weight			Obese			
Characteristics	**Median**	**Q1s**	**Q3s**	**Median**	**Q1s**	**Q3s**	***P* Value**
Age, y	14.84	13.64	17.16	13.93	13.31	15.74	.86
BMI, kg/m^2	19.98	18.95	21.01	32.03	30.56	32.55	<.001
BMI *z* score	−0.08	−0.23	0.13	2.16	2.06	2.28	<.001
Spexin, ng/mL	0.44	0.33	0.62	0.32	0.28	0.36	.02
Leptin, ng/mL	9.22	2.48	14.07	31.51	15.40	47.91	<.001
Total adiponectin, μg/mL	9.25	7.21	12.65	6.73	5.78	10.44	.05
HMW adiponectin, (μg/mL	5.54	3.31	6.00	2.56	2.14	5.21	<.001
Total/HMW adiponectin	1.94	1.77	2.33	2.39	2.02	2.93	<.001
CRP, mg/L	0.18	0.08	0.26	1.44	0.82	2.04	<.001
Glucose, mg/dL	81.17	78.90	87.89	81.92	77.33	86.47	.55
Insulin, mcIU/mL	6.89	5.52	9.19	11.03	10.30	14.45	<.001
IL-6, pg/mL	0.71	0.53	0.96	1.91	0.84	2.56	<.001

Abbreviation: CRP, C-reactive protein.

weight category and all but two in the obese category were Caucasian.

Spexin levels and weight status

Median (IQR) Spexin levels were significantly lower in obese vs normal-weight children, 0.33 ng/mL (0.27–0.44) vs 0.44 ng/mL (0.33–0.62); $P = .025$ (Table 1 and Figure 1). Further, age and sex did not show correlations with Spexin levels (data not shown).

Table 2 presents the distributions of normal-weight and obese patients by Spexin level based ordinal categorical groups. In each of the three ordinal variables (quartiles, tertiles, and median splits), as the Spexin level increased, proportion of obese children strictly decreased. Compared with the group of children with the maximum level of Spexin in each of three variables, there were strictly increasing trends of the odds ratios of obese children with decreasing levels of Spexin. For example, odds of being obese in this group of children with the minimum level of Spexin (Q1) is 5.25 times as high as it is in the group of children with the maximum level of Spexin (Q4).

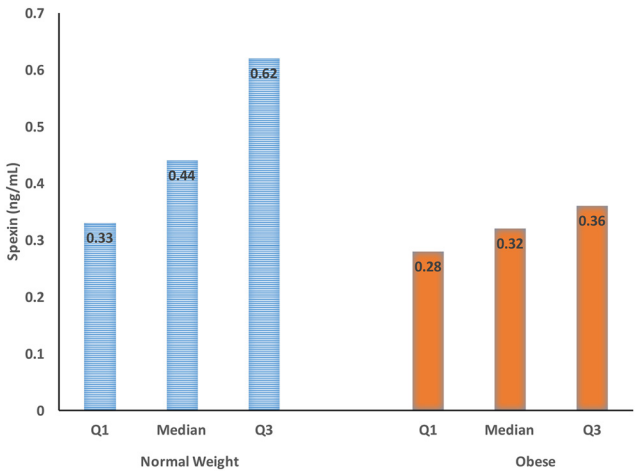

Figure 1. Circulating serum spexin levels (in ng/mL) among normal-weight (n = 18) and obese children (n = 51). Q1, first quartile; Q3, third quartile.

Cluster analysis of Spexin and BMI *z* score categorized children in three distinct groups based on the correlation between these two variables. The analysis was actually able to classify 62 children of 69 in distinct groups of normal weight and obese indicating a very strong reverse association of Spexin levels and obesity. Group 2 contains 16 patients, all of whom were normal weight. Children in this group, has a moderate level of Spexin. Group 3 contains 46 patients, all of whom were obese. This group of children contains the lowest level of Spexin. Group 1 contains seven children, of whom five were obese. In this group, level of Spexin is very high: median (IQR) was 0.91 (0.88–1.51).

In this dataset, we did not observe significant correlation of Spexin levels with fasting insulin, glucose levels, or with any of the adipokines and C-reactive protein.

Discussion

In the current study, we found significantly lower circulating levels of Spexin in obese children compared with normal-weight children. The lower concentration of Spexin observed in children is similar to that reported in severely obese adults (12). Intriguingly, Spexin did not show significant relationship with cardiometabolic risk factors in children, contrary to the data in adults. To our knowledge, this is the first study on Spexin levels and its potential relation with adipokines and other cardiometabolic risk markers in children.

Spexin was initially identified using Markov modeling as a novel peptide hormone whose biological significance

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Table 2. Distribution of Normal Weight and Obese Children by Ordinal Categorical Groups of Serum Spexin Concentrations

Ordinal Variables and Groups (Spexin)[a] ng/mL	Normal Weight, N (%)	Obese, N(%)	OR (95% CI)	*P* Value
Quartiles				
Q1 (0.09–0.28)	2 (11.8)	15 (88.2)	5.25 (0.9–30)	.07
Q2 (0.29–0.34)	3 (17.6)	14 (82.4)	3.27 (0.68–15.82)	.14
Q3 (0.34–0.45)	6 (33.3)	12 (66.7)	1.4 (0.35–5.54)	.63
Q4 (0.47–2.2)	7 (41.2)	10 (58.8)	1	—
Tertiles				
T1 (0.09–0.3)	3 (13)	20 (87)	5.13 (1.18–22.24)	.03
T2 (0.3–0.4)	5 (21.7)	18 (78.3)	2.77 (0.76–10.05)	.12
T_3 (0.41–2.2)	10 (43.5)	13 (56.5)	1	—
Median Splits				
M1 (0.09–0.34)	5 (14.7)	29 (85.3)	3.43 (1.06–11.05)	.04
M2 (0.34–2.2)	13 (37.1)	22 (62.9)	1	—

Abbreviation: CI, confidence ratio; M, median split; OR, odds ratio; Q, quartile; T, tertile.

OR denotes odds ratio of obese children compared with the group of children with the highest ranges of concentration of Spexin in each of three variables; the likelihood of obesity decreases as the level of serum concentration of Spexin increases.

[a] Spexin: Range of serum concentration of Spexin (ng/mL) in categories of ordinal variables.

was not clear (11). Tissue distribution studies in rat and goldfish revealed that this peptide is widely expressed in a variety tissues including skin and digestive, nervous, and endocrine systems (18, 19). Microarray studies on surgical fat biopsies subsequently identified the Spexin gene as the most down-regulated gene in obese human fat compared with nonobese human fat (13). The significantly lower Spexin concentration in obese children in the current study is consistent with that reported by Walewski (12) and colleagues in adults with severe obesity. Remarkably, cluster analysis of the data allowed us to split the participants into two major groups (normal weight and obese) and a small (outlier) subgroup based on the concentration of Spexin in relation to BMI *z* score (Table 3.). The high Spexin level of the five children in the small outlier group was clearly a contrast with the trend we observed in most obese children. This analysis is robust and the data suggest an important role of Spexin in childhood obesity. However, its proposed role in energy homeostasis and body weight regulation from adult studies could not be explored and confirmed in the current study. But Spexin administration reduces caloric intake in rats and increase locomotion in mice (12). In addition, Spexin seems to have a role in the normal regulation of adipose tissue function including uptake of long chain fatty acids, into adipocytes (12).

In the present study, Spexin levels did not show the expected correlations with various cardiometabolic risk factors, including leptin. This is in contrast with the study in severely obese adults (12). We are not aware of any previous study that has examined the relationship between circulating Spexin levels and cardiometabolic markers in children. Spexin levels have been noted to be inversely correlated with fasting blood glucose in adults with type 2 diabetes (14), but such associations could not be found in

Table 3. Characterization of Study Variables by Cluster Groups

Variables	Group 1	Group 2	Group 3	*P*-Value
n	7	16	46	
Obesity				<.001
Normal weight	2 (11.1)	16 (88.9)	0 (0)	
Obese	5 (9.8)	0 (0)	46 (90.2)	
Sex				.43
Male	5 (15.2)	7 (21.2)	21 (63.6)	
Female	2 (7.4)	9 (33.3)	16 (59.3)	
Spexin(ng/mL)				
Median (IQR)	0.91 (0.88–1.51)	0.40 (0.32–0.52)	0.32 (0.27–0.39)	<.001
BMI *z* score				
Mean (SE)	1.53 (0.41)	0.07 (0.16)	2.27 (0.04)	<.001

Cluster analysis of Spexin and BMI *z* score categorized children into two main groups (obese and normal weight) and mixed/outlier subgroup based on the correlation between these two variables. Group 1 (mixed/outlier subgroup) contains seven children, five of whom were obese. In this group, level of Spexin is very high, median (IQR) = 0.91(0.88- 1.51). Group 2 contains 16 patients, all of whom were normal weight. Children in this group have a moderate level of Spexin. Group 3 contains 46 patients, all of whom were obese. The children in this group have the lowest Spexin levels.

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the current study in children. Due to the criteria used for exclusion of participants in the trials (16, 17) we did not have patients with high blood glucose in the study, which could have contributed to the lack of association between Spexin and fasting plasma glucose levels. In children, although the likelihood of obesity decreases as the level of serum concentration of Spexin increases, the disparities in the results between the previous studies in adults and our study could be related to several other factors. Foremost, children are not really little adults and studies have shown fundamental differences in the mechanistic regulation of various factors in children vs adults. Although the participants in our study were adolescents with uncomplicated obesity, the adult subjects in one study were severely obese undergoing bariatric surgical procedures (12) and in the other study had type 2 diabetes (14). The duration and severity of obesity are important factors that contribute to cardiometabolic risk later in adulthood (20). Therefore, the obesity-related cardiometabolic abnormalities are more likely to be present in the adults, especially with severely obese adults. This could have contributed to the observed relationship between levels of Spexin and various risk factors for cardiometabolic disease. Because the participants in the current study are school-age children, their activity levels are likely to be quite different from the severely obese adults or those with T2DM. So it is likely that the differences in the data are related to the overall better health of the obese children in our study compared with the severely obese adults or diabetic patients in the adult study.

The data must be interpreted based on the various strengths and limitations of the study. Important strengths of our study include the relatively large sample size, the ethnic homogeneity of the participants, and the absence of obesity-related comorbidities. Limitations include cross-sectional nature of the study and lack of longitudinal data. We were not able to determine whether Spexin levels were influenced by regional fat distribution, pubertal status, physical activity patterns, and/or the duration of obesity as these data are not available in the current study. We acknowledge the possibility that the lower Spexin levels in obese children may simply reflect a mere association and does not demonstrate its causative role in obesity or obesity-related comorbidities such as diabetes and cardiovascular disease.

In conclusion, circulating levels of Spexin are low in obese children compared with their normal-weight counterparts. The fact that Spexin concentration in participants in the current study enabled the discrimination of children into obese and normal weight with high accuracy is remarkable. This observation must be explored further to validate a potential role for Spexin as biomarker in discriminating children into obese and normal-weight groups with accuracy. The exact function of this peptide is currently under investigation and additional studies in larger populations are required to validate the observations in the current study and determine the immediate and long-term clinical significance of these findings.

Acknowledgments

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