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ORIGINAL ARTICLE

Pediatric Obesity

Circulating levels of urocortin neuropeptides are impaired in children with overweight

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Abstract

Objective: The corticotropin-releasing factor neuropeptides (corticotropin-releasing hormone [CRH] and urocortin [UCN]-1,2,3) and spexin contribute to the regulation of energy balance and inhibit food intake in mammals. However, the status of these neuropeptides in children with overweight has yet to be elucidated. This study investigated the effect of increased body weight on the circulating levels of these neuropeptides.

Methods: A total of 120 children with a mean age of 12 years were enrolled in the study. Blood samples were collected to assess the circulating levels of neuropeptides and were correlated with various anthropometric, clinical, and metabolic markers.

Results: Plasma levels of UCNs were altered in children with overweight but less so in those with obesity. Furthermore, the expression pattern of UCN1 was opposite to that of UCN2 and UCN3, which suggests a compensatory effect. However, no significant effect of overweight and obesity was observed on CRH and spexin levels. Finally, UCN3 independently associated with circulating zinc-alpha-2-glycoprotein and UCN2 levels, whereas UCN1 was strongly predicted by TNF α levels.

Conclusions: Significant changes in neuropeptide levels were primarily observed in children with overweight and were attenuated with increased obesity. This suggests the presence of a compensatory mechanism for neuropeptides to curb the progression of obesity.

INTRODUCTION

The rise in the prevalence of childhood obesity is an increasing public health problem worldwide, particularly in societies that have witnessed a profound change in lifestyle associated with higher food intake and decreased physical activity during the past few

decades (1). The number of children aged 5 to 19 years old living with obesity in 2019 is estimated to be 158 million worldwide and is expected to reach approximately 254 million by 2030 (2). These changes are accompanied by a rise in obesity-related metabolic disorders, including insulin resistance, dyslipidemia, and hormonal dysregulation (3). More alarmingly, nearly 80% of children and

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adolescents with obesity are expected to continue to have obesity in adulthood (1). Therefore, the impact of these dysregulations on morbidity, including cardiovascular disease and reduced life-span in future generations, emphasizes the importance of understanding the pathophysiological mechanisms that occur in children with obesity (4).

Obesity induces dysregulated energy metabolism and a disturbance of hormonal system function, as reflected by insulin resistance and increased levels of leptin and adipokines in an attempt to control the energy balance by the brain and other organs such as the liver and adipose tissue (3). The corticotropin-releasing factor (CRF) family of neuropeptides (corticotropin-releasing hormone [CRH] and 3 urocortins [(UCN)-1,2,3]) and their receptors (CRHR1 and CRHR2) contribute to the regulation of energy balance and inhibit food intake in mammals (5). They are concomitantly expressed in the brain, pancreas, heart, adipose tissue, and skeletal muscle (6-8). Despite the different roles for each of these peptides and their receptors in metabolic pathways (9), previous studies have indicated beneficial effects of overexpression or treatment with some of these neuropeptides, such as the administration of UCN1-protected cardiomyocytes (10). Similar cardioprotective effects were reported for UCN2 and UCN3 (11). Moreover, UCN3 overexpression protected rodents from metabolic dysregulation induced by a high-fat diet (HFD) (6). UCN2-knockout mice displayed insulin sensitivity and resistance against HFD-related effects (12), whereas UCN3 knockout increased food intake and reduced insulin sensitivity (13). The overexpression of UCN3 in the brain increased energy expenditure but reduced insulin sensitivity (14), whereas injection of UCN3 into the hypothalamus reduced food intake (15). Furthermore, we and others have reported that UCN3 expression is dysregulated in obesity, type 2 diabetes (T2D), and polycystic ovary syndrome (8,16,17). In an independent study, UCN3 and its receptor, CRHR2, were suggested as targets for obesity treatment because of their colocalization with quantitative trait loci for obesity (18). Recently, we reported decreased levels of UCN3 in the plasma of nondiabetic individuals with obesity, whereas increased levels were observed in subcutaneous adipose tissue from these people (16).

Spexin is another neuropeptide involved in energy homeostasis. Following injection into goldfish or a diet-induced obesity rodent model, spexin decreases food intake (19,20). Nevertheless, the impact of spexin levels on human obesity remains controversial, as one study reported decreased levels in both adults and children with obesity and diabetes (21), whereas another study showed no differences in serum spexin levels between adolescents with normal weight, obesity, and obesity with diabetes (22). We recently reported decreased plasma levels of spexin in adults with obesity, which were increased in response to regular moderate physical exercise (23).

Considering the available data on CRF, UCNs, and spexin and their expected protective role in metabolic disorders, we hypothesized that their expression and plasma levels are dysregulated in

Study Importance

What is already known?

- The corticotropin-releasing factor (CRF) family is known to play a role in energy balance, food intake, inflammation, and stress response. However, its role and expression levels in children with overweight and obesity have yet to be investigated.

What does this study add?

- Our study is the first, to our knowledge, to report an impaired expression of these neuropeptides in plasma from children with overweight and obesity.

How might these results change the direction of research or the focus of clinical practice?

- The observed differential dysregulation in the levels of circulating CRF neuropeptides is indicative of early dimorphic metabolic disturbance and suggests possible compensatory mechanisms to blunt the increase in body weight.

children with obesity, thereby affecting the cross talk between the brain and peripheral organs. Therefore, this study evaluated CRF, UCN1, UCN2, UCN3, and spexin levels in the plasma of children with normal weight, children with overweight, and children with obesity and their association with anthropometric and biochemical markers.

METHODS

Study cohort characteristics

A total of 120 boys and girls (21 with normal weight, 14 with overweight, and 85 with obesity) with a mean age of 12 years were recruited for the study (Table 1). Written informed consent was obtained from the parent(s) of all children before their enrollment, and the study was approved by the Review Board of the Dasman Diabetes Institute (Kuwait City, Kuwait) and was conducted in line with the principles of the Declaration of Helsinki. All children visited the obesity clinics at the Dasman Diabetes Institute in the Farwaniya Hospital or in the Al-Adan Hospital between January 2014 and December 2016. The inclusion criterion was age = 6 to 17 years, whereas exclusion criteria were any chronic illnesses or endocrine diseases unrelated to diabetes. Children were divided into groups with normal weight (BMI percentile < 85th), overweight (85th ≤ BMI percentile < 95th), and obesity (BMI percentile ≥ 95th) (24).

TABLE 1 Characteristics of the study population

	NW (n = 21)	OW (n = 14)	OB (n = 85)	Bonferroni post hoc p value			Overall p value
				NW vs. OW	NW vs. OB	OW vs. OB	All children
Physical and clinical characteristics							
Sex (M/F)	13/8	4/10	50/35	0.829	0.054	0.032	0.085
Age (y)	11.9 ± 4.0	13.1 ± 3.0	13.0 ± 2.9	0.766	0.368	0.983	0.288
Weight (kg)	45.4 ± 19.1	61.7 ± 13.4	87.9 ± 26.5	0.04	<0.001	0.001	<0.001
Height (m)	1.50 ± 0.21	1.57 ± 0.12	1.60 ± 0.15	0.720	0.060	0.857	0.065
BMI (kg/m ²)	18.9 ± 3.5	24.7 ± 2.8	33.8 ± 6.4	0.012	<0.0001	<0.0001	<0.001
Percentile	54.9 ± 19.8	92.0 ± 1.6	98.5 ± 1.1 ^a	<0.0001	<0.0001	0.045	<0.0001
Body fat (%)	7.3 ± 6.0	18.4 ± 6.7	32.8 ± 13.0	0.018	<0.001	<0.001	<0.001
Metabolic and hormonal markers							
Cholesterol (mmol/L)	3.9 ± 0.5	4.3 ± 0.6	4.3 ± 0.7	0.507	0.170	1.000	0.150
HDL (mmol/L)	1.48 ± 0.27	1.35 ± 0.24	1.20 ± 0.45	0.453	0.016	0.709	0.017
LDL (mmol/L)	2.20 ± 0.49	2.60 ± 0.61	2.65 ± 0.72	0.286	0.022	1.000	0.026
TGL (mmol/L)	0.65 ± 0.30	0.92 ± 0.45	1.00 ± 0.43	0.172	0.002	1.000	0.003
GLU (mmol/L)	4.8 ± 0.4	5.4 ± 1.7	5.1 ± 0.4	0.040	0.463	0.240	0.046
Total protein (mmol/L)	72.7 ± 3.5	74.4 ± 3.4	74.3 ± 3.3	0.588	0.282	1.000	0.221
Albumin (mmol/L)	41.7 ± 3.2	41.8 ± 3.2	40.7 ± 2.8	1.000	0.600	0.728	0.283
Total bilirubin (mmol/L)	10.4 ± 5.8	10.8 ± 3.9	8.1 ± 2.9	1.000	0.078	0.086	0.017
ALT (mmol/L)	26.1 ± 6.8	23.1 ± 6.6	37.2 ± 22.3	0.240	0.080	0.060	0.014
AST (mmol/L)	27.5 ± 7.2	21.0 ± 5.8	25.2 ± 8.8	0.011	0.901	0.328	0.111
Alkaline phosphatase (mmol/L)	161.8 ± 78.1	170.7 ± 115.3	169.7 ± 77.4	0.807	0.702	0.977	0.931
GGT (mmol/L)	21.0 ± 4.4	25.5 ± 8.5	30.5 ± 17.5	0.168	0.085	0.500	0.182
HbA _{1c} (%)	5.2 ± 0.3	5.56 ± 1.7	5.62 ± 2.3	0.443	0.141	0.932	0.712
Insulin (mIU/L)	5.5 ± 3.0	9.3 ± 3.9	18.2 ± 13.7	0.003	<0.001	0.037	<0.001
HOMA-IR	1.23 ± 0.74	1.95 ± 0.75	4.32 ± 3.42	0.016	<0.001	0.014	<0.001
Adiponectin (µg/mL)	9.8 ± 1.4	8.9 ± 1.4	8.1 ± 2.0	0.065	0.001	0.411	0.001
TNFα (ng/mL)	4.3 ± 12.8	32.8 ± 8.8	35.0 ± 33.0	0.014	<0.001	1.000	<0.001
Other markers							
Leptin (ng/mL)	7.5 ± 2.6	13.3 ± 7.0	16.1 ± 7.5	0.049	<0.001	0.466	<0.001
C-pep (ng/mL)	0.26 ± 0.17	0.18 ± 0.08	0.27 ± 1.06	0.120	1.000	1.000	0.787
Glucagon (ng/mL)	3.59 ± 1.53	1.92 ± 1.20	2.35 ± 0.98	<0.001	<0.001	0.576	<0.001
NGAL (ng/mL)	22.9 ± 6.1	35.7 ± 15.1	33.5 ± 13.9	0.018	0.005	1.000	0.004
RBP4 (µg/mL)	20.2 ± 5.5	55.30 ± 25.1	63.76 ± 103.2	0.011	0.152	1.000	0.147
sICAM-1 (ng/mL)	128.3 ± 78.8	255.2 ± 199.4	217.3 ± 252.2	0.015	0.019	1.000	0.204
ZAG (µg/mL)	1.6 ± 0.2	4.9 ± 0.6	3.3 ± 2.0	<0.001	0.001	0.005	<0.001
Resistin (ng/mL)	4.9 ± 2.7	4.6 ± 1.4	5.4 ± 2.2	0.706	0.507	0.280	0.487

Note: Data are presented as mean ± SD. One-way ANOVA followed by Bonferroni post hoc test.

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; C-pep, connecting peptide; F, female; GGT, gamma-glutamyltransferase; GLU, fasting plasma glucose; HbA_{1c}, hemoglobin A_{1c}; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment for insulin resistance; LDL, low-density lipoprotein; M, male; NGAL, neutrophil gelatinase-associated lipocalin; NW, normal weight; OB, obesity; OW, overweight; TGL, triglyceride; TNFα, tumor necrosis factor alpha; RBP4, retinol-binding protein 4; sICAM-1, soluble intercellular adhesion molecule-1; ZAG, zinc-alpha-2-glycoprotein.

^aOnly percentiles between 95 and 99 were included for the children with obesity.

Anthropometric measurements

Children underwent a standard physical examination by a pediatrician endocrinologist, and anthropometric measurements were obtained based on World Health Organization (WHO) recommendations. Body weight and percentage of body fat were measured by Pediatric Body Composition Analyzer/Segmental (GAIA KIKO Jawon Medical) while children were wearing light clothes without footwear. Height was measured using a stadiometer. BMI percentile was calculated according to the Centers for Disease Control and Prevention (CDC) growth chart, and the measured BMI was used to classify children as having normal weight (BMI percentile < 85th), overweight (85th \geq BMI percentile < 95th), and obesity (\geq 95th percentile) (24). Children were further grouped by BMI percentile based on cutoff points, as defined by Cole et al. (25).

Sample collection and blood analysis

Venous blood was collected in EDTA tubes after overnight fasting. Peripheral blood mononuclear cells were isolated using ready-to-use, sterile Ficoll-Hypaque density gradient medium and a centrifugation method. Plasma was aliquoted and stored at -80°C . Lipid and glucose profiles were measured using a Siemens Dimension RXL chemistry analyzer (Diamond Diagnostics). Hemoglobin A_{1c} was measured with a Variant device (Bio-Rad Laboratories). Insulin concentration and liver function enzymes were measured using Access 2 and AU480 Systems (Beckman Coulter) respectively. The homeostatic model assessment of insulin resistance (HOMA-IR) index was calculated using the following formula: $\text{HOMA-IR} = (\text{glucose} \times \text{insulin})/22.5$.

Plasma levels of UCN1 (#LS-F6155, Lifespan Biosciences, Inc.), UCN2 (#LS-F39013, Lifespan Biosciences), UCN3 (#LS-F12902, Lifespan Biosciences), CRH (#LS-F5352, Lifespan Biosciences), spexin (#EK-023-81, Phoenix Pharmaceuticals), insulin, and ultrasensitive connecting (C)-peptide (Merckodia AB) were measured with enzyme-linked immunosorbent assay (ELISA) kits. Sample dilutions were determined through optimization, and ELISA was performed according to the manufacturer's instructions. Absorbance was measured using a Synergy H4 plate reader (BioTek Instruments).

Obesity and diabetes markers were measured using the following panels and the bead-based multiplexing technology Bio-Plex 200 system: obesity (Metabolism/Obesity 5-Plex Human ProcartaPlex Panel 1 [EPX09A-15804-901], Metabolism/Obesity 9-Plex Human ProcartaPlex Panel 2 [EPX09A-15804-901]) and diabetes (MILLIPIXEL MAP Human Diabetes Panel, Premixed 5-Plex Assay, Millipore HDIAB-34K-PMX5). Fluorescence intensities were measured using Bio-Plex manager software version 6 (Bio-Rad Laboratories).

Statistical analysis

All statistical analyses were performed using SPSS Statistics software version 25.0 (IBM Corp.). Descriptive statistics were used to report the mean and standard deviation for continuous variables, and frequency statistics were used to calculate the number and percentage for categorical variables. Categorical parameters are described as number and percentage. The neuropeptide variables were log transformed using SPSS. Post log-transformation variables were checked for normality. To evaluate the effects of groups, we conducted a 1 way ANOVA test with post hoc Bonferroni correction that included the entire study population. Bonferroni post hoc *p* values based on ANOVA for each outcome, as well as the entire study population combined, are included in Table 1. Partial correlation was used for all population analyses adjusted for age and gender, and Spearman rank correlation was performed for gender-specific analysis. Univariate and stepwise multivariate linear regression analyses were performed to examine the predictive effect of selected factors, with and without adjustment for age and sex. *P* values <0.05 were considered statistically significant.

RESULTS

Characteristics of study population

The study population consisted of 120 children (21 with normal weight, 14 with overweight, and 85 with obesity; 67 male individuals and 53 female individuals) with a mean age of 12 years. Blood samples were collected and processed for plasma and peripheral blood mononuclear cells. Table 1 summarizes the anthropometric, clinical, and biochemical characteristics of the cohort.

When compared with children with normal weight, the group with overweight exhibited a higher body fat percentage ($p < 0.05$). The glycemic markers insulin, fasting plasma glucose (GLU), and HOMA-IR were also markedly increased, whereas glucagon levels were decreased in this group ($p < 0.05$). Circulating levels of the inflammatory marker tumor necrosis factor α (TNF α) were significantly higher in the children with overweight and were associated with increased levels of the obesity markers leptin, retinol-binding protein 4 (RBP4), neutrophil gelatinase-associated lipocalin (NGAL), soluble intercellular adhesion molecule-1 (sICAM-1), and zinc-alpha-2-glycoprotein (ZAG; $p < 0.05$) (Table 1).

In the group with obesity, there was a marked increase in insulin and HOMA-IR levels ($p < 0.001$) compared with children with normal weight. Similarly, lipid profiles were impaired with significantly reduced high-density lipoprotein (HDL) and increased low-density lipoprotein (LDL) and triglyceride levels ($p < 0.05$). In addition, a significant increase ($p < 0.05$) in adiposity-related markers (leptin, TNF α , NGAL, sICAM-1, and ZAG) was also observed, whereas adiponectin and glucagon levels were decreased ($p < 0.05$). When

compared with the group with overweight, the group with obesity exhibited persistent increased levels of insulin and HOMA-IR ($p < 0.05$). The children with obesity were further stratified into metabolically healthy obesity (MHO) and metabolically unhealthy obesity (MUO) groups based on classification by HOMA-IR levels, with HOMA-IR < 3.16 for MHO and ≥ 3.16 for MUO (26). In addition to the glycemic parameters (GLU, insulin, and HOMA-IR), there was a significant increase in BMI and body fat percentage ($p < 0.05$) in the MUO group compared with the MHO group, but not with other tested markers (Supporting Information Table S1).

Plasma UCN1, UCN2, and UCN3 levels are associated with obesity and sex

We assessed the circulating levels of the neuropeptides UCN1, UCN2, UCN3, CRH, and spexin in the plasma of our study population and stratified children according to body weight groups (Supporting Information Figure S1). The results showed that UCN1 levels were significantly decreased ($p < 0.05$) in the plasma of the group with overweight (15.6 [6.4] pg/mL) compared with the group with normal weight (37.7 [7.6] pg/mL) but increased in the group with obesity to a similar level to that of the group with normal weight (43.1 [9.5] pg/mL; Supporting Information Figure S1A). Circulating UCN3 levels

were significantly increased with increased body weight ($p < 0.001$), with a marked increase in the group with overweight compared with the group with obesity (Supporting Information Figure S1C). A similar trend was observed for UCN2, in which UCN2 levels were significantly increased in the group with overweight (1,789 [103] ng/mL) compared with the group with normal weight (1,477 [101] ng/mL; $p < 0.043$), but only a statistically nonsignificant increase was observed in the group with obesity (Supporting Information Figure S1B). However, the circulating levels of CRH and spexin were not significantly affected by increased body weight, as shown in Supporting Information Figure S1D,E.

Considering the observed differential profiles of UCN1, UCN2, and UCN3 with body weight, we further evaluated these neuropeptide levels with respect to gender (Figure 1). UCN1 levels were globally lower in girls than boys regardless of their body weight, particularly in children with normal weight (19.3 [25.7] pg/mL and 49.1 [34.8] pg/mL, respectively; $p < 0.05$ Figure 1A). On the contrary, significantly higher levels of UCN2 and UCN3 were observed in girls compared with boys with overweight and obesity. In children with obesity, the levels of UCN2 in girls and boys were 1,838 (351) ng/mL and 1,498 (39) ng/mL, respectively, and the levels of UCN3 were 16.5 (22.4) ng/mL and 6.2 (8.0) ng/mL, respectively ($p < 0.01$; Figure 1B,C). Although not statistically significant, trends of higher levels of CRH were observed in all girls than boys, irrespective of

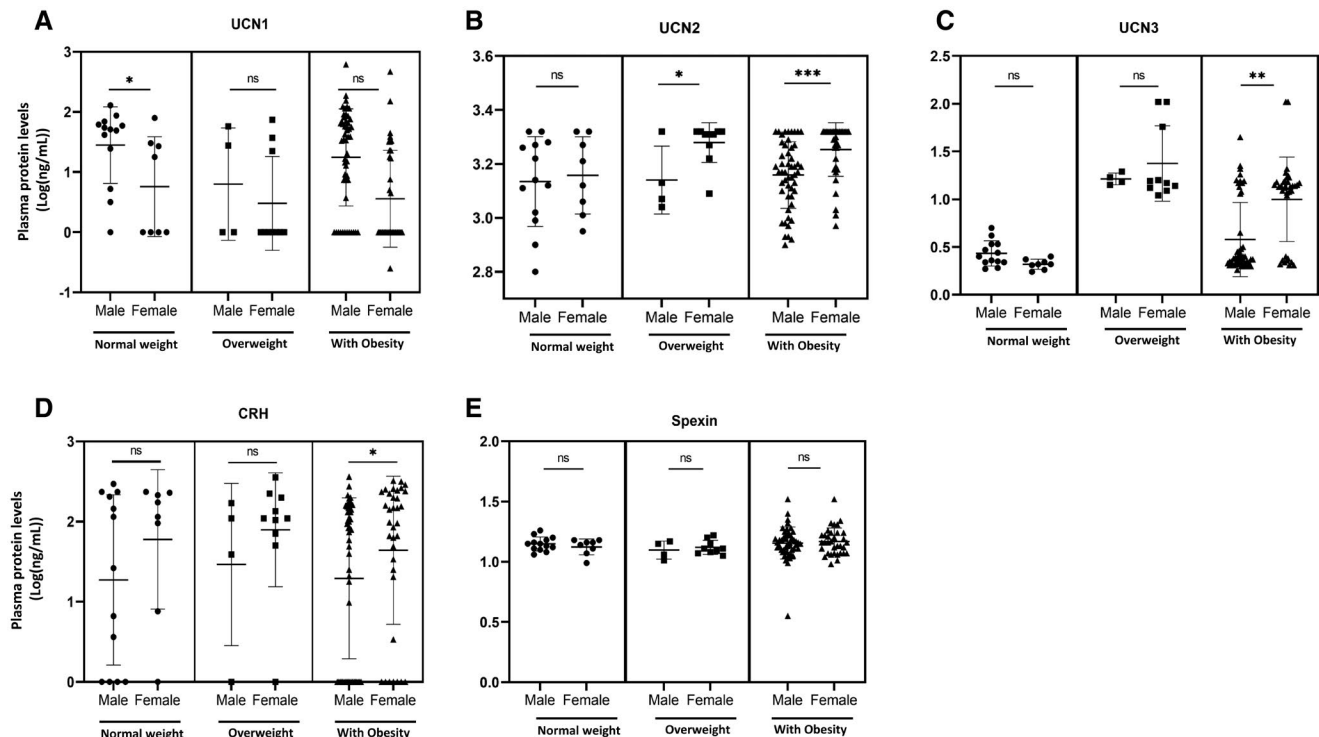


FIGURE 1 Urocortin (UCN) and spexin levels in plasma based on body weight groups and sex. Circulating levels of (A) UCN1, (B) UCN2, (C) UCN3, (D) CRH, and (E) spexin were measured by ELISA using plasma samples from boys and girls in the normal weight group ($n = 23$ and $n = 8$, respectively), the overweight group ($n = 4$ and $n = 10$, respectively), and the obesity group ($n = 50$ and $n = 35$, respectively). A 1-way ANOVA test was used to determine significance of the difference in means between the groups, and data were log transformed. Statistical significances are as follows: * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. CRH, corticotropin-releasing hormone; ns, not significant

TABLE 2 Partial correlation ranking of neuropeptides with selected study characteristics

All children	UCN1	UCN2	UCN3	CRH	Spexin
Height (m)	0.75*	-0.11	0.26	-0.11	0.45
Percentiles	-0.06	0.23	0.56*	0.44	-0.17
Total protein (mmol/L)	0.36*	-0.61	-0.06	-0.35	0.06
Albumin (mmol/L)	0.88**	-0.25	-0.01	-0.35	-0.39
AST (mmol/L)	0.45	-0.53	-0.17	-0.62*	-0.05
TNF α (ng/mL)	-0.33	0.33	0.87**	0.06	0.21
C-pep (ng/mL)	0.11	-0.20	-0.57*	-0.07	0.04
RBP4 (μ g/mL)	-0.19	0.00	0.70*	-0.20	0.02
ZAG (μ g/mL)	-0.21	0.24	0.96**	0.09	0.22
CRH (pg/mL)	-0.01	0.68*	-0.01	-	-0.10
UCN1 (pg/mL)	-	-0.12	-0.10	-0.01	0.27
UCN2 (ng/mL)	-0.12	-	0.16	0.68*	0.16
UCN3 (ng/mL)	-0.10	0.16	-	-0.01	0.20
Spexin (ng/mL)	0.27	0.16	0.20	-0.10	-

Note: Adjusted for age and sex. Data are presented as the *R* values.

Abbreviations: AST, aspartate aminotransferase; C-pep, connecting peptide; CRH, corticotropin-releasing hormone; RBP4, retinol-binding protein 4; TNF α , tumor necrosis factor alpha; UCN, urocortin; ZAG, zinc-alpha-2-glycoprotein.

* $p < 0.05$; ** $p < 0.01$.

body weight (Figure 1D). However, no differences were observed by sex in spexin levels (Figure 1E). In children with obesity, no significant differences existed between the MHO and MUO groups in the levels of all studied neuropeptides (Supporting Information Table S1).

Correlation analysis

Partial correlation analysis was performed on the entire cohort to evaluate the association of circulating UCN1, UCN2, UCN3, CRF, and spexin with various clinical parameters (Table 2). Overall, UCN1 and UCN3 were correlated with more variables compared with UCN2, CRH, and spexin. UCN1 levels were positively correlated with height, albumin, and total protein. UCN3 correlated positively and significantly with body percentile, TNF α , RBP4, and ZAG concentrations but was negatively correlated with C-peptide (Table 2). In this adjusted analysis, UCN2 correlated only with CRH, whereas the latter marker only displayed a negative correlation with aspartate aminotransferase (AST) in all children.

When separating all children into male and female subgroups, additional correlations were evident despite the relatively low number of children in each subgroup (Table 3). Accordingly, strong negative correlations were observed between UCN1 with albumin in boys and with HDL, TNF α , and ZAG in girls. However, UCN2 levels correlated negatively with alkaline phosphatase and positively with C-peptide and glucagon levels in boys (Table 3). In girls, UCN2 only correlated negatively with leptin levels. Furthermore, UCN3 correlated positively with TNF α , leptin, RBP4, and ZAG in boys and with percentile in the female group but correlated negatively with glucagon in the latter

group. Nevertheless, CRH displayed a negative correlation with albumin and spexin displayed a positive correlation with AST in girls, respectively. No correlation was observed in boys for these markers.

An univariate linear regression analysis (Supporting Information Table S2), followed by a multivariable stepwise linear regression analysis (Table 4), was performed, with each neuropeptide marker as a dependent variable, in the whole study population before and after adjustment for age and sex. In both unadjusted and adjusted models, independent associations were observed for UCN1 levels with TNF α and UCN3 ($p < 0.05$), whereas UCN3 and CRH were the only predictors for UCN2 (Table 4). However, the UCN2 and ZAG markers were independently associated with circulating UCN3 levels ($p < 0.05$). CRH was independently predicted by UCN2 and spexin ($p < 0.05$).

DISCUSSION

This study is the first one, to our knowledge, assessing the status of the main neuropeptides of the CRF family in children. The results showed a disturbed plasma profile of UCN neuropeptides with increased body weight in children. The main findings of this study are as follows: 1) major changes in UCN levels occurred in children with overweight, whereas these changes were less significant in children with obesity; 2) the variation in the pattern of UCN1 expression was opposite to that of UCN2 and UCN3, suggesting that the increase in UCN2 and UCN3 in children with overweight may be a compensatory effect; 3) no significant effect of overweight and obesity was observed on CRH and spexin levels; and 4) UCN3 was independently associated with circulating UCN1 and UCN2, whereas UCN1 was strongly predicted by TNF α levels.

TABLE 3 Spearman correlation coefficient ranking of neuropeptides with all study variables by sex

	Boys					Girls				
	UCN1	UCN2	UCN3	CRH	Spexin	UCN1	UCN2	UCN3	CRH	Spexin
Height (m)	0.83*	-0.076	0.145	-0.066	0.723	-0.811	0.730	0.98*	0.129	-0.397
Percentiles	0.006	0.163	0.255	0.623	-0.046	-0.779	0.553	0.98*	-0.050	-0.625
HDL (mmol/L)	0.150	0.180	-0.006	-0.561	0.645	-0.94*	0.387	0.715	0.792	0.014
LDL (mmol/L)	-0.110	-0.305	0.310	0.335	-0.524	0.94*	-0.051	-0.748	-0.504	0.431
Albumin (mmol/L)	-0.95**	-0.438	-0.046	-0.403	-0.411	0.382	-0.340	-0.053	-0.96*	-0.751
Total bilirubin (mmol/L)	0.67*	-0.191	-0.113	0.139	0.003	-0.843	0.094	0.859	0.065	-0.768
AST (mmol/L)	0.334	-0.145	0.280	-0.563	0.080	0.493	0.191	-0.614	0.365	0.98*
Alkaline phosphatase (mmol/L)	-0.268	-0.74*	0.348	-0.128	-0.219	-0.029	0.896	0.181	0.239	0.602
TNF α (ng/mL)	-0.166	-0.160	0.92**	-0.256	0.119	-0.96*	0.196	0.879	0.345	-0.551
Leptin (ng/mL)	-0.092	-0.365	0.88*	-0.089	-0.012	0.357	-0.97*	-0.479	-0.369	-0.430
C-pep (ng/mL)	-0.099	0.77*	0.002	0.279	0.009	0.652	-0.781	-0.923	0.067	0.414
Glucagon (ng/mL)	0.173	0.82*	-0.258	0.873	-0.065	0.937	-0.651	-0.97*	-0.390	0.287
RBP4 (μ g/mL)	-0.221	0.016	0.84*	-0.196	0.144	-0.249	0.224	0.624	-0.669	-0.863
sICAM-1 (ng/mL)	-0.381	0.150	0.268	-0.083	0.049	-0.480	0.437	0.183	0.95*	0.686
ZAG (μ g/mL)	-0.066	-0.160	0.97**	-0.136	0.197	-0.96*	0.600	0.956	0.439	-0.284
CRH (pg/mL)	0.160	0.610	-0.241	0.000	-0.333	-0.547	0.187	0.157	1.000	0.559
UCN1 (pg/mL)	1.000	0.215	0.047	0.160	0.482	1.000	-0.378	-0.882	-0.547	0.305
UCN2 (ng/mL)	0.215	1.000	-0.249	0.610	-0.133	-0.378	10.000	0.591	0.187	0.230
UCN3 (ng/mL)	0.047	-0.249	1.000	-0.241	0.308	-0.882	0.591	1.000	0.157	-0.499
Spexin (ng/mL)	0.482	-0.133	0.308	-0.333	1.000	0.305	0.230	-0.499	0.559	1.000

Note: Data are presented as the *R* values.

Abbreviations: AST, aspartate aminotransferase; C-pep, connecting peptide; CRH, corticotropin-releasing hormone; HDL, high-density lipoprotein; LDL, low-density lipoprotein; RBP4, retinol-binding protein 4; sICAM-1, soluble intercellular adhesion molecule-1; TNF α , tumor necrosis factor alpha; UCN, urocortin; ZAG, zinc-alpha-2-glycoprotein.

p* < 0.05; *p* < 0.01.

TABLE 4 Multivariable linear regression analysis in all children, with neuropeptides as the dependent variables^a

Neuropeptide	Independent variables	β coefficient	<i>p</i> value
UCN1	TNF α	-0.349	0.005
	UCN3	0.316	0.035
UCN2	UCN3	0.426	0.042
	CRH	0.373	0.029
UCN3	ZAG	0.292	0.030
	UCN2	0.121	0.03
CRH	UCN2	0.547	0.002
	Spexin	-0.245	0.018
Spexin	CRH	-0.242	0.027

Abbreviations: CRH, corticotropin-releasing hormone; TNF α , tumor necrosis factor alpha; UCN, urocortin; ZAG, zinc-alpha-2-glycoprotein.

^aAdjusted for age and sex.

In addition to the expected trends of dysregulated glycemic and lipid profiles, we observed that there were increased levels of leptin and other adipokines, as well as inflammatory markers, in children

with overweight and children with obesity compared with children with normal weight, which is in line with previous studies (27,28). This included TNF α , NGAL, RBP4, ZAG, and sICAM1, along with a significant decrease in adiponectin levels.

These expected observations suggest disturbed food intake and energy balance, glucose and fat metabolism, and metabolic stress response in these children. Moreover, we observed elevated alanine transferase (ALT) and an increased trend of γ -glutamyl transferase (γ GT) with increased weight. Both markers are surrogate markers for liver dysfunction and nonalcoholic fatty liver, as well as factors of metabolic syndrome. Increased ALT and γ GT are considered indicators of the development of prediabetes and T2D in apparently healthy individuals (28). ZAG is involved in lipolysis stimulation and reduction in body fat in mice, whereas its levels rise with the onset of T2D (29). Our data showed a strong increase in ZAG levels concomitant with increased BMI.

CRH family members are involved in the regulation of various physiological processes through autocrine/paracrine mechanisms (8,16), including food intake and energy balance (5,6,13-15), inflammation (9,30), and stress response (5). The present study demonstrated an altered pattern of UCNs in children associated with children's body weight. Interestingly, the major changes were

observed in children who had overweight compared with children with normal weight and children with obesity. Indeed, plasma levels of UCN1 levels decreased to half, whereas UCN3 levels increased nearly fivefold in children with overweight compared with the normal-weight control children.

These observations suggest a critical role for UCNs at the early stages of increased body weight and metabolic syndrome development and may indicate that UCNs could function as early sensors or be involved in an early response to excessive body weight increases and insulin resistance. Consistent with this hypothesis, UCN3-transgenic mice demonstrated a metabolically favorable phenotype that was resistant to obesity and hyperglycemia under controlled conditions following an obesogenic HFD challenge (9). Also, agonizing CRHR2 with UCN3 and UCN2 was shown to have beneficial effects not only on heart function, but also on glucose control, weight loss, and reduction in liver fat (5). In contrast, mice deficient in UCN3 and UCN3-null mice exhibited increased feeding, weight gain, and lower circulating insulin, likely resulting from white adipose tissue accumulation, along with reduced free fatty acid and glycerol levels (13). We recently reported that UCN3, the most recently discovered member of the CRF family, was differentially expressed in the circulation and subcutaneous adipose tissue of people with normal weight and those with overweight, although this pattern was opposite to that observed here, in children (16). In these adults, UCN3 levels were more affected in adults with overweight with a higher insulin secretion than in people with diabetes. This suggests that UCN3 is involved in a feedback loop linking insulin secretion and glucose that seems to be disrupted, not only in established diabetes, but also in conditions in which glucose utilization and energy homeostasis are partially impaired, such as in nondiabetic individuals with obesity. Interestingly, circulating UCN3 was increased in people with early overweight with T2D compared with healthy control individuals (31). Even with an increased duration of T2D and with increased BMI, we observed augmented plasma UCN3 levels (16). These results suggest that circulating UCN3 critically regulates feeding, energy homeostasis, and is potentially involved in metaflammation status modulation in obesity. The latter assumption is supported by the positive correlation that we observed in our study between UCN3 and the TNF α , RBP4, and ZAG proteins. Therefore, the increased levels of UCN2, and particularly UCN3, observed in our study may reflect a compensatory mechanism to suppress further food intake in individuals with overweight. In support for this hypothesis, CRHR2 knockout mice were found to eat larger meals as opposed to smaller meals consumed in CRHR2 agonist-treated rats (32). Also, UCN2 peptide decreased nocturnal feeding and caused rats to consume smaller meals less frequently (32). Accordingly, an increase in UCN2 and UCN3 may be beneficial in regulating gastric emptying and meal satiation.

In previous studies, UCN1 has been associated with inflammatory and hypertensive conditions. For instance, circulating maternal and fetal UCN1 levels were increased, along with gestational hypertension, preeclampsia, and other hypertensive disorders, during pregnancy (33), whereas decreased plasma UCN1 levels were observed

in intrahepatic cholestasis pregnancy, potentially leading to fatal outcomes (34). As UCN1 has vasoactive properties, its increased levels may represent an adaptive stress response (33). UCN1 also increases neuronal activation related to the reduction of ghrelin secretion and food intake (35). Furthermore, increased plasma UCN2 following UCN2 gene transfer improved total body glucose disposal in HFD-fed mice with associated insulin resistance (36). However, nonischemic cardiomyopathy exhibited an 8-fold-increased plasma UCN2 level compared with healthy controls (37). Also, serum UCN2 levels in hypertensive patients were significantly higher when compared with non-hypertensive patients (38). In summary, these studies demonstrate a context-related status for UCNs.

In contrast to the UCNs, reduced circulating levels of CRH were reported in people with T2D, and its secretion was not stimulated by glucose (39). Shortly after inducing stress, increased plasma CRH may be partly responsible for stress-induced adrenocorticotrophic hormone (ACTH) secretion (40). Consistent with this, a reduction in plasma CRH levels was observed with decreased neurotoxicity in an antioxidant-treated Alzheimer's disease rat model (41). Therefore, reduced CRH levels are mostly associated with dysregulated homeostasis and related disorders. In our study, circulating CRH levels were not affected with increased BMI in children. This may result from the relatively lower cellular and metabolic stress in this age group compared with adult individuals who frequently exhibit insulin resistance and other metabolic disorders. Notably, our data revealed a strong positive correlation between CRH and UCN2, but not with UCN1 and UCN3, across the weight groups. These data are interesting because UCN2 and UCN3 are homologous to one another and bind to the same receptor (CRHR2), whereas CRH binds to the CRFR1 receptor in the same family.


Our data revealed striking sex dimorphic differences in the levels of UCNs between male and female children. Although UCN1 was lower in girls, UCN2 and UCN3 were higher in girls than boys. Comparable observations have been previously reported in CRHR2- and CRFR1-deficient mice that exhibited sexually dichotomous anxiety-like behavior (42). CRHR2 knockout mice developed impaired glucose tolerance in males fed with a chow diet, but not in females. Therefore, CRFR dysregulation is a sexually dimorphic factor associated with the development of diabetes and other metabolic syndromes (43). As the hypothalamic-pituitary-adrenal axis interacts with many other physiological pathways, the changes in endocrine function are also sex-specific and age-dependent. However, the implication of such observations on the role of UCNs and their receptors in sex-related vulnerability or resistance to metabolic diseases requires further investigation. Furthermore, the low number of participants in the groups with normal weight and overweight should be taken into consideration before generalizing this conclusion.

Spexin is another neuropeptide that has similar functionalities to the CRH neuropeptide family. In previous studies, chronic subcutaneous injection with spexin reduced food intake and led to weight loss in diet-induced-obesity mice and rats (20). Consistent with these findings, we recently reported that circulating levels of spexin were decreased with obesity and diabetes in adults and

inversely correlated with adiposity indicators (BMI, waist circumference, and hip circumference), blood pressure, and lipid markers (LDL, triglycerides, and total cholesterol), but positively correlated with HDL levels (23). However, regular moderate physical exercise for 3 months was able to normalize spexin in people with obesity. No change in BMI was observed, which highlights the reversibility of the spexin secretion activity (23). Taken together, these data support a role for spexin in energy metabolism and weight regulation, with a potential link to obesity and diabetes (21). Despite these previous studies, including ours, that have reported decreased spexin levels with obesity and T2D in adults, we did not find significant changes in children with overweight and obesity when compared with matched control children with normal weight. Consistent with this finding, Hodges et al. [22] did not find any variation in spexin levels in adolescents with obesity or diabetes and there was no correlation with body composition or blood measurements, indicating that spexin may not act as a metabolic regulator in adolescents. These findings suggest that spexin may have different functions in obesity and diabetes depending on age, as both disorders are associated with age.

To our knowledge, the present study is the first to evaluate the status of circulating levels of the main neuropeptides of the CRF family in children with overweight and obesity. However, the study had some limitations. First, the cross-sectional study design did not allow us to determine whether the attenuated neuropeptide levels contributed to the development of obesity. Second, the low number of participants in the groups with normal weight and overweight limited the power of correlations between neuropeptide levels and other clinical parameters. On the other hand, owing to multiple comparisons, the statistical significances must be interpreted with caution. Furthermore, no data regarding diet or physical activity of the participants was collected. Also, the family history or psychological status of these children were not included and were beyond the scope of this study. In addition, the measured levels of the neuropeptides in the plasma may not reflect their central and peripheral bioavailability.

CONCLUSION

Our study showed a disturbed plasma profile of UCN neuropeptides with increased body weight and obesity in children. The significant changes in neuropeptide levels were primarily observed in participants with overweight and were attenuated with increasing body weight reaching the level of obesity. This suggests that a compensatory mechanism may be involved in neuropeptides to curb the development and progression of obesity and its comorbidities. 

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

AUTHOR CONTRIBUTIONS

SK, AK, and AT designed the study. SW, HA, and MA enrolled the children and collected data and blood samples. SK and DM performed the experiments. SK, SD, and AT analyzed the data. SK and AT wrote and revised the manuscript. AK, HK, and JT contributed to the discussion and the revision of the manuscript. FA and JA revised the manuscript.

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SUPPORTING INFORMATION

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