



# Measurement of Serum Chemerin, Oxidized LDL, and Vitamin D Levels in Prader–Willi Syndrome: A Cross-Sectional Study in Pediatric Egyptian Patients

Manal M. Thomas<sup>1</sup> Moushira E. Zaki<sup>2</sup> Eman Youness<sup>2</sup> Khaled Hamed<sup>1</sup> Azzah A. Khedr<sup>3</sup>  
Phoebe M. Abd El-Massieh<sup>4</sup> Sara M. Abdo<sup>5</sup> Hala T. El-Bassyouni<sup>1</sup>

<sup>1</sup>Clinical Genetics Department, Center of Scientific Excellence, National Research Centre, Cairo, Egypt

<sup>2</sup>Department of Biological Anthropology, National Research Centre, Cairo, Egypt

<sup>3</sup>Human Genetics and Genome Research Division, Human Cytogenetics Department, National Research Centre, Cairo, Egypt

<sup>4</sup>Human Genetics and Genome Research Division, Oro-dental Genetics Department, National Research Centre, Cairo, Egypt

<sup>5</sup>Biochemistry Division, Chemistry Department, Faculty of Science, Helwan University, Cairo, Egypt

Address for correspondence Manal M. Thomas, MD, PhD, Human Genetics and Genome Research Division, Clinical Genetics Department, National Research Centre, 33<sup>rd</sup> El Bohouth St, Former El Tahrir St, Dokki, Giza 12622, Egypt (e-mail: nula\_m@hotmail.com).

J Child Sci 2020;10:e187–e195.

## Abstract

Prader–Willi syndrome (PWS) is the commonest genetic cause of obesity. Oxidative stress and chronic low-grade inflammation play a crucial role in the pathogenesis of obesity. Alterations of vitamin D (25-OHD) levels are commonly encountered with obesity. The aim of this study was to analyze serum chemerin, oxidized low-density lipoprotein (ox-LDL), and 25-OHD values in pediatric PWS patients in comparison with obese healthy children and nonobese control groups, highlighting possible correlations with body mass index (BMI) and obesity. Twenty-six PWS Egyptian patients and 26 obese healthy individuals referred to the outpatient clinic of the Clinical Genetics Department, National Research Centre, Cairo, Egypt, and 20 control patients with matching age and sex were enrolled in the study. Patients were clinically diagnosed and confirmed by routine cytogenetic and fluorescence in-situ hybridization analysis. Anthropometric measurements were performed, and BMI was calculated by weight/height<sup>2</sup> (kg/m<sup>2</sup>), and BMI z score was also determined. Serum chemerin, ox-LDL, and vitamin D were determined by enzyme-linked immunosorbent assay. Chemerin levels, which reflected chronic inflammation, were significantly elevated as compared with obese and nonobese controls ( $p \leq 0.0001$ ). Concerning oxidative damage, children with PWS showed higher Ox-LDL levels compared with obese and nonobese controls ( $p < 0.0001$ ). Vitamin D levels were significantly lower in PWS patients compared with obese and nonobese controls ( $p \leq 0.0001$ ). Our data showed that obesity in PWS is associated with oxidative stress and chronic low-grade inflammation. Ox-LDL is a good indicator of oxidative stress, and chemerin could be used as a biomarker for the chronic inflammatory state. Furthermore, vitamin D supplementation is recommended in PWS patients

## Keywords

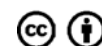
- ▶ PWS
- ▶ chemerin
- ▶ oxidized LDL
- ▶ 25 hydroxy vitamin D
- ▶ BMI

received  
July 10, 2020  
accepted after revision  
September 16, 2020

DOI <https://doi.org/10.1055/s-0040-1718896>.  
ISSN 2474-5871.

Copyright © 2020 Georg Thieme Verlag  
KG Stuttgart · New York

License terms



## Introduction

Prader–Willi syndrome (PWS; OMIM 176270)<sup>1</sup> is a genetic disorder with multisystem involvement. Its prevalence is ~1/15000 live births<sup>2</sup> and is reported to be more in Caucasians.<sup>3</sup> PWS is due to loss of paternally expressed genes from a de-novo paternal 15q11–q13 deletion (65–75% of cases), maternal uniparental disomy 15 (20–30% of cases), or imprinting defect (1–3%).<sup>4</sup>

PWS is characterized by infantile hypotonia and feeding difficulty leading to failure to thrive, but they develop hyperphagia and excessive weight gain with subsequent obesity in early childhood.<sup>5</sup> PWS patients have increased total body fat mass and decreased resting energy expenditure, which promote more gain of weight and abnormal body composition.<sup>6</sup> PWS is considered to be the commonest genetic cause of life-threatening obesity.<sup>7</sup> Other features of PWS include short stature, small hands and feet, hypogonadism, developmental and intellectual disability (ID), and behavioral problems.<sup>4,8–12</sup> Patients with PWS have distinctive facial features<sup>13</sup> and commonly encounter endocrinal disturbances in the form of insulin resistance and diabetes, hypothyroidism, adrenal insufficiency,<sup>12,14</sup> and growth hormone (GH) deficiency.<sup>13,15</sup>

Oxidative stress is the imbalance between the production of reactive oxygen species and the antioxidant defense system with disruption of redox signaling causing damage to biomolecules, including proteins, nucleic acids, and lipids. Dyslipidemia and increased lipid oxidation were found to be associated with obesity. Oxidized low-density lipoprotein (ox-LDL) is considered as one of the major and early lipid peroxidation products and, therefore, is used as one of the oxidative stress biomarkers.<sup>16</sup> Ox-LDL was found to be elevated in obesity,<sup>17</sup> insulin resistance,<sup>18</sup> metabolic syndromes,<sup>19</sup> and cardiovascular disease.<sup>20</sup>

The adipose tissue is a metabolically active organ, secreting various adipokines, and pro-inflammatory cytokines, which play an important role in the inflammatory and atherosclerotic processes.<sup>21</sup> Obesity is a pro-inflammatory state, usually associated with low-grade chronic inflammation and elevated levels of pro-inflammatory adipokines that are positively correlated to the body mass index (BMI).<sup>22,23</sup> Chemerin is an adipokine that is linked to inflammation and adipogenesis. It also has a vital role in glucose and lipid metabolism<sup>24,25</sup> by modulating the expression of adipocyte genes involved in glucose and lipid homeostases such as glucose transporter-4, adiponectin, fatty acid synthase, and leptin.<sup>24</sup> Consequently, chemerin is linked to obesity and inflammation in humans.<sup>25</sup>

Obesity is associated with 25-hydroxy vitamin D (25-OHD) deficiency.<sup>26</sup> Vitamin D (25-OHD) deficiency is also associated with insulin resistance in obese children<sup>27,28</sup>, and adults.<sup>26</sup> PWS patients show a higher percent of body fat, more subcutaneous adipose tissue, and frequently develop insulin resistance, glycemic alterations, and metabolic syndrome as a consequence of obesity, which results in a 25-OHD deficiency.<sup>29</sup>

The aim of the work is to assess the role of oxidative stress (ox-LDL) and chronic low-grade inflammation (chemerin) as comorbidity factors affecting obesity in PWS patients in comparison to nongenetic obesity. In addition, the study aims to

compare 25-OHD levels in obese PWS patients and obese healthy children. To the best of our knowledge, this is the first study to test for ox-LDL and chemerin markers in PWS children.

## Patient and Method

### Patient

This case–control study was conducted on patients referred to the outpatient clinic of the Clinical Genetics Department, National Research Centre, Cairo, Egypt. Their parents gave written informed consent. The study was approved by the Ethical Committee of the National Research Centre in accordance with the Declaration of Helsinki protocols. The study included three groups: group I (26 children clinically diagnosed as PWS), group II (20 nonobese healthy controls), and group III (26 obese healthy children) with matching age and sex. All children enrolled in the study were subjected to full history taking, three-generation pedigree construction, a thorough clinical examination of all body systems to detect any system abnormality, and essential anthropometric measures. Exclusion criteria for all the participants were type 1 diabetes, presence of metabolic disease (confirmed by the use of drugs), pulmonary diseases, and those on medications. Anthropometric measurements were performed as follows: height was measured to the nearest 0.5 cm, body weight was measured to the nearest 0.1 kg, BMI was calculated as weight/height<sup>2</sup> (kg/m<sup>2</sup>), and Z-score BMI was calculated according to the WHO Anthro Plus program<sup>10</sup>.<sup>30</sup>

### Method

All 26 clinically diagnosed children were of the deletion subtype confirmed by routine cytogenetic and fluorescence in-situ hybridization (FISH) analysis. Diagnosis of PWS depends on the neonatal history, characteristic clinical features followed by chromosomal, and FISH analysis. FISH was performed on metaphase spread from peripheral blood according to modification of Pinkel et al<sup>31</sup> and manufacturer's instructions by using a locus-specific probe (LSI) Prader–Willi SNRPN (15q11) supplied by Cytocell FISH probes.

The cytogenetic study was performed by the conventional G-banding technique.<sup>32</sup> At least 25 metaphases were karyotyped and nomenclature, according to ISCN, 2016.

FISH studies: FISH was applied using 170 kb SNRPN Prader–Willi/Angelman chromosome region probe labeled in red and covers the whole SNRPN gene as well as the entire imprinting center. The 15qter subtelomere specific probe (clone 154P1), labeled in green, allows identification of chromosome 15 and acts as a control probe.<sup>33</sup>

Serum chemerin was determined by ELISA assay supplied by SinoGeneClonBiotech, Co., Ltd. Ox-LDL was determined by ELISA assay catalog No. SG-11266. Serum 25 hydroxy-vitamin D (25-OHD) was assessed by vitamin D direct ELISA kit (EIA-4696) DRG International, Inc.; NJ, USA, according to Wielders and Wijnberg.<sup>34</sup>

### Statistical Analysis

Analysis of data was performed using SPSS 23 (Statistical Package for Scientific Studies) for Windows. The description

of the quantitative variables was presented in the form of mean and standard deviation (SD). The description of qualitative variables was in the form of numbers (No.) and percent (%). Comparing means of the quantitative variable between the three groups were performed after data were explored for normality using Shapiro–Wilk tests of normality. Whenever the results of the test indicated that the data were normally distributed, the one-way analysis of variance (ANOVA) test (parametric tests) was used to carry out the comparisons, followed by the Tukey test for multiple comparisons whenever a statistical significance was detected by the ANOVA. When the data were not normally distributed, comparisons were performed using the Kruskal–Wallis H test (nonparametric tests) followed by a series of Mann–Whitney U tests for every two groups to detect the group(s) responsible for the significance of the Kruskal–Wallis H test. Chi-squared test ( $\chi^2$ ) was used to detect the independence between groups and the qualitative variable (Gender). Binary correlations were performed by Pearson correlation. Results were expressed in the form of correlation coefficient (R) and *p*-values. The following points are the accepted guidelines for interpreting the correlation coefficient:

- 0 indicates no linear relationship.
- +1 indicates a perfect positive linear relationship: as one variable increases in its values, the other variable also increases in its values via an exact linear rule.
- –1 indicates a perfect negative linear relationship: as one variable increases in its values, the other variable decreases in its values via an exact linear rule.
- Values between 0 and 0.3 (0 and -0.3) indicate no or a weak positive (negative) linear relationship, respectively.
- Values between 0.3 and 0.7 (0.3 and -0.7) indicate a moderate positive (negative) linear relationship, respectively.
- Values between 0.7 and 1.0 (-0.7 and -1.0) indicate a strong positive (negative) linear relationship

The level of significance for all tests was set at  $p \leq 0.05$ .

## Results

This case–control study included three groups: group I (26 children clinically diagnosed as PWS), group II (20 nonobese healthy controls), and group III (26 obese healthy children). Group I comprised 26 patients clinically diagnosed as PWS

**Table 2** Investigating the independence between the three groups and gender

		Group I No. (%)	Group II No. (%)	Group III No. (%)	<i>p</i> -Value
Gender	Male	12 (46.2)	10 (50)	13 (50)	0.952
	Female	14 (53.8)	10 (50)	13 (50)	

and cytogenetically confirmed as PWS-deletion subtype using FISH analysis. Their ages ranged from 3 to 15 years of age, with a mean of 8.54 years  $\pm$  3.42 SD (**Table 1**). Group II comprised 20 nonobese healthy children. Their ages ranged from 3 to 15 years of age, with a mean of 7.8 years  $\pm$  3.47 SD. Group III comprised 26 healthy obese children. Their ages ranged from 4 to 15 years of age, with a mean of 9.58 years  $\pm$  3.30 SD. There were 12 males and 14 females among the PWS cohort. Gender as a variable was found to be independently and almost equally distributed among the patients and control groups, which was confirmed by the insignificant result with *p*-value = 1.000 (**Table 2**). Anthropometric measurements, including weight, height, and Z-score BMI, were done for all children enrolled in the study. As shown in **Table 1**, analysis of the measurements revealed obesity with elevated BMI in PWS patients as compared with the normal controls with no significant difference with the obese healthy controls.

Demographic and clinical data of patients are summarized in **Table 3**. Seventeen patients (65.3%) were the offspring of consanguineous marriage. Dysmorphic features were present in 20/26 (76.9%) patients, including a round face, full cheeks, broad bossing forehead, and almond eyes. Epicanthic fold was present in 12/26 (46.1%) patients, and nystagmus was present in 6/26 (23%) of cases. Hand anomalies in the form of short fingers (42.3%), syndactyly (53.8%), and Simian crease (34.6%) were also detected in PWS patients. Neurological examination revealed hypotonia (100%) in all patients and microcephaly in 23% of cases. Dermatological changes were observed in some patients in the form of skin hyperpigmentation (38.4%) and café au lait patches (19.2%). Abdominal and pelvic ultrasonography showed hepatosplenomegaly in four patients. External genitalia showed absent labia minora and/or hypoplastic clitoris in eight females. Micropenis and hypospadias were observed in 9 and 5 males, respectively, and undescended

**Table 1** Comparing the mean and SD of age, weight, height, and Z-BMI between PWS patients, obese controls, and nonobese control

	Group I Mean $\pm$ SD	Group II Mean $\pm$ SD	Group III Mean $\pm$ SD	<i>p</i> -Value
Age (y)	8.54 $\pm$ 3.42	7.8 $\pm$ 3.47	9.58 $\pm$ 3.30	0.209
Weight (kg)	58.43 $\pm$ 17.53 <sup>a</sup>	33.5 $\pm$ 12.68 <sup>b</sup>	70.52 $\pm$ 15.61 <sup>c</sup>	< 0.0001*
Height (cm)	126.58 $\pm$ 18.68	125.3 $\pm$ 22.3	129.85 $\pm$ 22.59	0.454
Z-BMI (kg/m <sup>2</sup> )	10.25 $\pm$ 4.02 <sup>a</sup>	20.78 $\pm$ 3.39 <sup>b</sup>	11.03 $\pm$ 4.46 <sup>a</sup>	< 0.0001*

Abbreviations: PWS, Prader–Willi syndrome; SD, standard deviation; Z-BMI, Z-score body mass index.

\*Statistically significant *p*-value  $\leq 0.05$ .

<sup>a</sup>, <sup>b</sup>, <sup>c</sup>Different small letters indicate statistical significance between the groups.

**Table 3** Clinical data of the PWS patients

Parameter	Number (n = 26)	Frequency (%)
Male/female	12/14	1/1.16
Positive parental consanguinity	17/26	65.3
Similarly affected family members	6/26	23
Dysmorphic facies (round face, broad bossing forehead, almond eyes, full cheek)	20/26	76.9
Epicanthic fold	12/26	46.1
Nystagmus	6/26	23
Short fingers	11/26	42.3
Syndactyly	14/26	53.8
Simian crease	9/26	34.6
Skin hyperpigmentation	10/26	38.4
Café au lait patches	5/26	19.2
Microcephaly	6/26	23
Hypotonia	26/26	100
Intellectual disability	14/26	53.8
Hepatosplenomegaly	4/26	15.3
Undescended testis	6/14	42.8
Micropenis	9/14	64.2
Hypospadias	5/14	35.7
Absent labia minora and/or hypoplastic clitoris	8/12	66.6

Abbreviation: PWS, Prader-Willi syndrome.

testis in 6 males. ID was present in 14 patients ranging from mild to moderate ID.

Chemerin levels, which denoted ongoing chronic inflammatory processes, were significantly elevated in PWS patients as compared with the healthy nonobese controls ( $85.04 \pm 15.13$  versus  $58.25 \pm 1.79$ ;  $p < 0.0001$ ). There was also a significant difference in chemerin levels between PWS patients and the obese healthy controls ( $85.04 \pm 15.13$  vs.  $71.42 \pm 8.02$ ;  $p \leq 0.0001$ ) (–Table 4), (–Fig. 1).

Concerning oxidative damage, children with PWS showed higher Ox-LDL levels compared with the healthy nonobese

controls ( $444.69 \pm 33.43$  vs.  $263.07 \pm 9.25$ ;  $p < 0.0001$ ). Higher Ox-LDL levels were also noticed in PWS patients when compared with the obese healthy controls ( $444.69 \pm 33.43$  vs.  $350.69 \pm 79.83$ ;  $p < 0.0001$ ) (–Table 4; –Fig. 1). In addition, vitamin D levels were significantly affected in PWS patients in comparison with the normal healthy nonobese controls ( $16.39 \pm 2.90$  vs.  $23.10 \pm 2.22$ ;  $p \leq 0.0001$ ). A significant difference was also observed between PWS patients and the obese healthy controls ( $16.39 \pm 2.90$  vs.  $18.46 \pm 1.45$ ;  $p \leq 0.0001$ ) (–Table 4; –Fig. 1). The results in –Table 5 indicate that BMI positively correlated with the levels of chemerin and ox-LDL, while it was negatively correlated with vitamin D levels (–Figs. 2–4).

## Discussion

PWS is a genetic disorder that affects many systems in the body and is the most common obesity genetic syndrome. However, clinical diagnosis of patients is still challenging, as many features of PWS are nonspecific, and patients may present with various features depending on patients' age. In this study, 68% of the clinically diagnosed patients were confirmed by cytogenetic analysis. Routine cytogenetic analysis is essential to identify chromosomal abnormalities that contribute to Prader-Willi like phenotypes, known as Prader-Willi like syndromes (PWLS).<sup>35</sup> Many PWLS were reported with imbalances of chromosomes 1, 2, 3, 6, 10, 12, 14, and X.<sup>36,37</sup> However, the polymerase chain reaction-based methylation test and Multiplex ligation probe dependent amplification of 15q11-q13 region are the most specific tests for confirming the diagnosis of PWS.<sup>9</sup>

In our study, positive consanguinity was present in 65.3% of cases denoting a high incidence of consanguinity in our population, which is estimated to be around 60%.<sup>38</sup> The patients presented with dysmorphic facies, hypotonia, hypogonadism, and ID, ranging from mild to moderate. These findings were consistent with other studies.<sup>8,39</sup> Abdominal ultrasound showed hepatosplenomegaly in four patients. This could be due to extreme obesity and liver function impairment.

Excessive weight gain and obesity, especially in early childhood, are considered major complications of this disease. Obesity was the main problem in our patients as the measures of weight and BMI, when compared with the healthy controls, were significantly above the normal values. This is in agreement

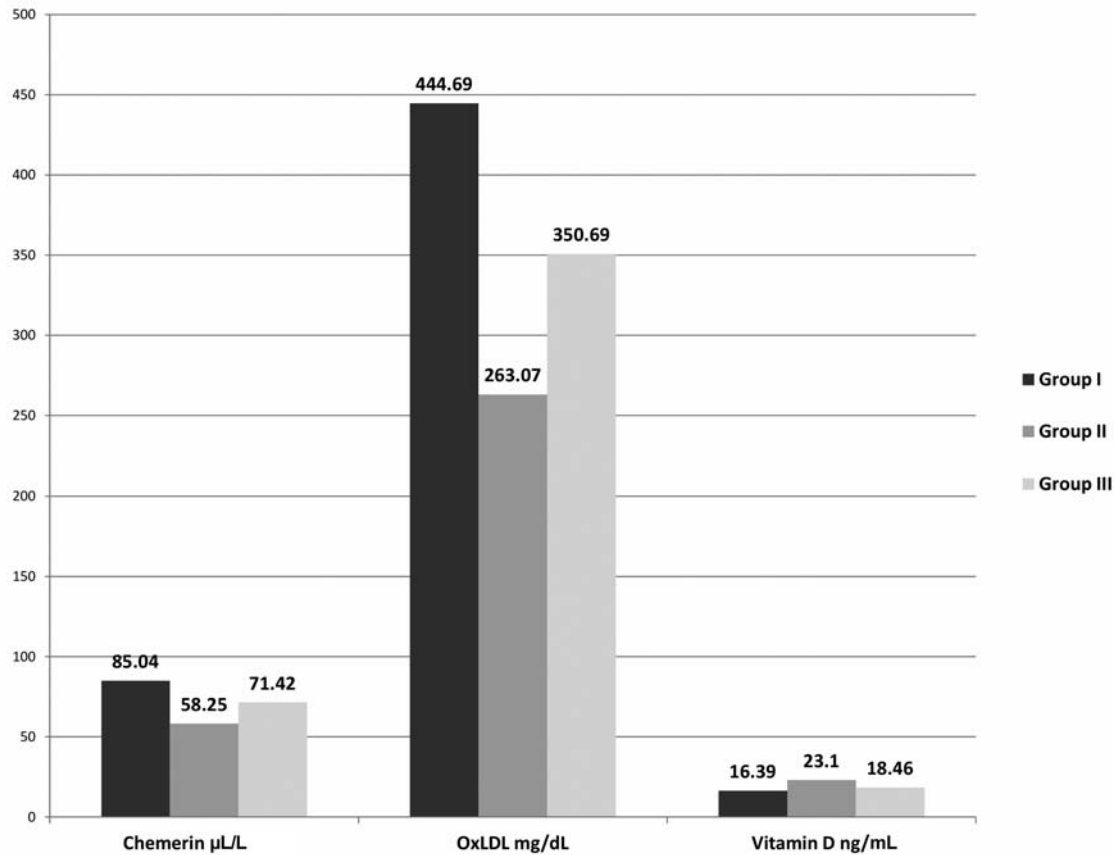
**Table 4** Levels of oxidized LDL, chemerin, and vitamin D level in the studied patients as compared with the healthy nonobese controls and healthy obese controls

	Group I Mean $\pm$ SD	Group II Mean $\pm$ SD	Group III Mean $\pm$ SD	p-Value
Chemerin ( $\mu$ g/L)	$85.04 \pm 15.13^a$	$58.25 \pm 1.79^b$	$71.42 \pm 8.02^c$	$< 0.0001^*$
Oxidized LDL (mg/dL)	$444.69 \pm 33.43^a$	$263.07 \pm 9.25^b$	$350.69 \pm 79.83^c$	$< 0.0001^*$
Vitamin D level (ng/mL)	$16.39 \pm 2.90^a$	$23.10 \pm 2.22^b$	$18.46 \pm 1.45^c$	$< 0.0001^*$

Abbreviations: LDL, low density lipoprotein; SD, standard deviation.

\*Statistically significant p-value  $\leq 0.05$ .

<sup>a, b, c</sup>Different small letters indicate statistical significance between the groups.



**Fig. 1** Mean levels of chemerin, oxidized low-density lipoprotein (ox-LDL), and vitamin D in Prader–Willi syndrome patients (group I), non-obese controls (group II), and obese controls (group III).

**Table 5** Pearson correlation of Z-BMI with chemerin, oxidized LDL, and vitamin D in PWS patients

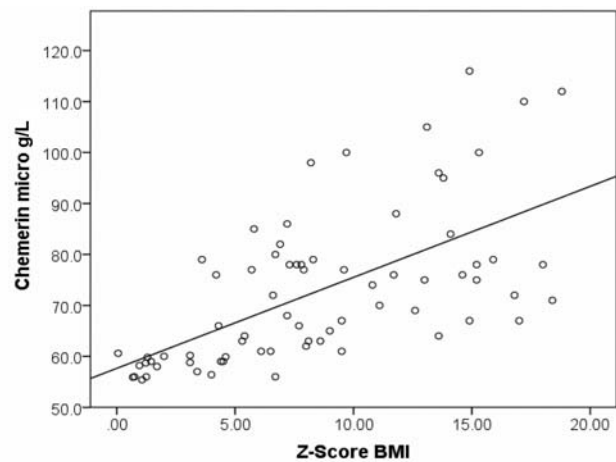
	Correlation coefficient “R”	p-Value
Chemerin	0.625	< 0.0001*
Oxidized LDL	0.561	< 0.0001*
Vitamin D	-0.653	< 0.001*

Abbreviations: BMI, body mass index; LDL, low density lipoprotein; PWS, Prader–Willi syndrome.

\*Statistically significant  $p$ -value  $\leq 0.05$ .

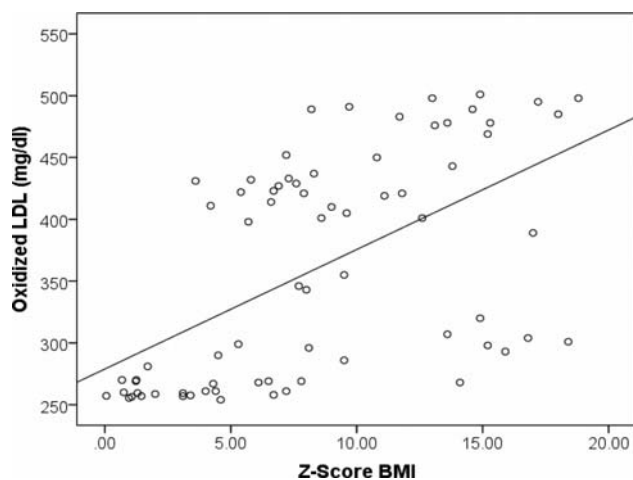
with previous studies denoting that obesity is a prominent feature of PWS and could be severe and life-threatening.<sup>6,40</sup>

Oxidative stress is involved in the pathogenesis of PWS<sup>41</sup> and is linked to obesity<sup>42</sup> with the consequent lipid peroxidation and production of ox-LDL. In our study, ox-LDL levels were significantly elevated in comparison to the healthy nonobese controls and were significantly (positively) correlated with higher BMI and obesity. These results confirm the association of an oxidative stress state in obese PWS patients. Several observations reported the association between oxidative stress and PWS.<sup>16,17</sup> Kelly et al<sup>43</sup> highlighted that overweight and obese children and adolescents had higher levels of ox-LDL as compared with controls. Many studies demonstrated that adipocytes could have a role in the metabolism of circulating

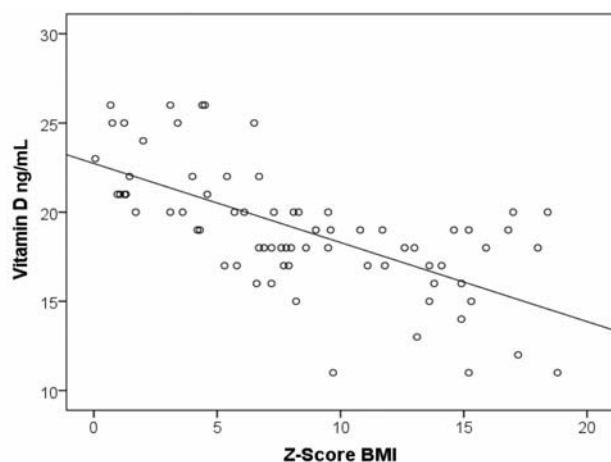


**Fig. 2** Pearson correlation of Z-score body mass index (Z-BMI) with chemerin in Prader–Willi syndrome patients.

lipoproteins, including ox-LDL.<sup>44,45</sup> The sensitivity of biomarkers of oxidative damage is higher in obese individuals and correlate directly with BMI. Oxidative stress in obesity among children requires serious consideration. Infants may exhibit peculiar susceptibilities to the effects of oxidative stress as they are undergoing rapid tissue growth and development.<sup>46</sup> In fact, individuals with PWS harbor a higher fat mass than non-PWS



**Fig. 3** Pearson correlation of Z-score body mass index (Z-BMI) with oxidized low-density lipoprotein (ox-LDL) in Prader-Willi syndrome patients.



**Fig. 4** Pearson correlation of Z-score body mass index (Z-BMI) with vitamin D in Prader-Willi syndrome patients.

obese subjects. Therefore, oxidative stress is higher in PWS than non-PWS obese children. This finding might be related to the different amounts of fat tissue in the two obese groups. Furthermore, subjects with PWS are known to be GH deficient; GH is reported to be associated with increased systemic inflammation and oxidative stress<sup>47</sup> therefore, our results demonstrate metabolic differences between PWS and non-PWS obese subjects.

Obesity is considered a pro-inflammatory process, and chronic low-grade inflammation is proposed as one of the mechanisms involved in the pathogenesis of obesity.<sup>48</sup> Chemerin is a pro-inflammatory adipokine.<sup>49</sup> In our study, chemerin levels were significantly elevated in PWS patients as compared with the controls ( $p < 0.0001$ ). These results indicate a state of ongoing chronic inflammation in those patients. Also, chemerin levels positively correlated with higher BMI and obesity with statistical significance in PWS patients ( $p < 0.0001$ ). This is in accordance with other studies that highlighted the role of chemerin as an adipokine marker of chronic inflammation and has an effect on adipogenesis and lipid metabolism.<sup>24,25</sup> A study by Sell et al<sup>50</sup> reported that overweight and obese

patients show higher chemerin levels than normal weight, whose plasma levels decrease after diet. A study by Perumalsamy et al<sup>51</sup> reported increased levels of chemerin in patients with obesity, diabetes, and cardiovascular diseases. Interestingly, many studies showed that chemerin gene expression and its levels positively correlated with higher BMI and obesity-related biomarkers.<sup>50,52–54</sup> In addition, Helfer and Wu<sup>54</sup> added that chemerin affects cell expansion and increases inflammation and angiogenesis in the adipose tissue, leading to adiposity. The association of obesity and metabolic syndrome has been reported in many studies.<sup>55,56</sup> Stejskal et al<sup>57</sup> confirmed that chemerin is a marker of obesity and metabolic syndrome. Additionally, Ross et al<sup>58</sup> identified an association between chemerin and appetite with body weight regulation in the hypothalamus in genome-wide expression analysis. Another study showed that the release of chemerin from the adipose tissue in obese individuals was higher than that in the normal controls and that it was positively correlated with BMI, waist-hip ratio, and fat cell mass.<sup>52</sup> Consistent with these studies, chemerin levels were lowered in obese patients who lost weight by diet regimen or bariatric surgery.<sup>50,53</sup> Interestingly, weight loss by exercise leads to more decrease in chemerin levels, implicating that chemerin could be used as a predictor for insulin resistance in obese individuals.<sup>53,59,60</sup> On the other hand, a study conducted by Buechler et al<sup>61</sup> detected higher chemerin levels in obese mice and humans, while its bioactivity was not concomitantly changed. Contradictory results were reported on the association of chemerin with obesity and insulin resistance with no or little effect on body composition and glucose homeostasis.<sup>62–64</sup>

As previous studies demonstrated the occurrence of chronic low-grade systemic inflammation in obese individuals,<sup>47,65,66</sup> it is assumed that chemerin being a pro-inflammatory adipokine marker has a superadded role in the association of chronic low-grade inflammation in obese individuals. Previous studies reported the association between increased levels of pro-inflammatory adipokines and low-grade chronic inflammation.<sup>21,22,40,48</sup> Huang et al<sup>67</sup> revealed that inflammation plays a vital role in the pathogenesis of obesity and its related complications. In accordance, our results showed significantly higher chemerin levels in obese PWS patients as compared with the controls ( $p < 0.0001$ ). In contrast, some studies that investigated the correlation between obesity with low-grade inflammation and insulin resistance did not detect elevated chemerin levels in all patients.<sup>57,68–70</sup> An additional study by Catalán et al<sup>71</sup> linked elevated levels of Chemerin and its receptor, Chemokine-Like receptor-1, in obese patients to inflammation and reported that TNF- $\alpha$  stimulates the production of chemerin through mRNA in visceral adipocytes in these patients. Other studies reported a positive correlation of chemerin with other inflammatory cytokines and C-reactive protein in chronic inflammatory diseases.<sup>72,73</sup>

PWS patients exhibit excess body fat and more subcutaneous adipose tissue leading to increased sequestration of 25-hydroxy vitamin D (25-OHD) levels.<sup>74</sup> Moreover, PWS patients suffer from GH deficiency<sup>11,15</sup>, which contributes to 25-OHD deficiency as GH and insulin growth factor-I (IGF-I) play a role in stimulating the hydroxylation of vitamin D to its active form.

In addition, vitamin D regulates gene expression of the GH/IGF-I axis and promotes the effect of IGF-I by increasing IGF-I receptors.<sup>75,76</sup> In the present study, the estimated 25-OHD levels were significantly lower in comparison to the healthy controls ( $p < 0.0001$ ). The study also demonstrated a statistically significant negative correlation between BMI and 25-OHD levels ( $p < 0.001$ ). These results indicate an inverse relationship between obesity and vitamin D levels in PWS patients. This is in agreement with previous studies showing an inverse correlation between 25-OHD levels with BMI and fat mass in different ethnic and geographical groups of obese children and adults.<sup>77-79</sup> A growing body of evidence suggests that adipocytes from obese people with insulin-resistant might have impaired release function of 25-OHD.<sup>80,81</sup> Only a few studies on 25-OHD levels in PWS were available. A study done by Brunetti et al<sup>82</sup> showed a significantly lower 25-OHD serum level in PWS patients than the controls. Purtell et al<sup>83</sup> demonstrated in their study deficiency of vitamin D in adult PWS patients correlated with obesity and GH deficiency. Interestingly, Rubin et al<sup>84</sup> observed a deficiency of 25-OHD alongside calcium in adolescents with PWS, although 33% were taking vitamin D and calcium supplements. On the other hand, Fintini et al<sup>74</sup> showed no difference in 25-OHD levels between PWS pediatric patients and BMI matched control subjects. They owed it to the smaller sample size and other factors determining 25-OHD serum levels such as dietary intake and sunlight exposure.

Interestingly, when comparing genetic obesity (PWS patients) and nongenetic obesity (obese healthy children), we found a significant difference in the studied parameters (chemerin, ox-LDL, and vitamin D), denoting that PWS patients were more affected than obese healthy children.

In conclusion, obesity in PWS is associated with oxidative stress and chronic low-grade inflammation and ox-LDL is considered as a sensitive marker for oxidative stress. Since chemerin acts as a pro-inflammatory adipokine, it could be used as a biomarker reflecting the chronic pro-inflammatory status. Vitamin D supplementation is recommended in PWS patients.

#### Conflict of Interest

None declared.

#### References

- 1 OMIM. (Online Mendelian Inheritance in Man). 2018McKusick-Nathans Institute for Genetic Medicine, John Hopkins University (Baltimore, MD) and National Center for Biotechnology Information National Library of Medicine (Bethesda, MD). WorldWide-WebURL: <http://www.ncbi.nlm.nih.gov/omim>. Accessed July 20, 2019
- 2 Lioni T, Reid SM, White SM, Rowell MM. A population-based profile of 160 Australians with Prader-Willi syndrome: trends in diagnosis, birth prevalence and birth characteristics. *Am J Med Genet A* 2015;167A(02):371-378
- 3 Butler MG, Weaver DD, Meaney FJ. Prader-Willi syndrome: are there population differences? *Clin Genet* 1982;22(05):292-294
- 4 Angulo MA, Butler MG, Cataletto ME. Prader-Willi syndrome: a review of clinical, genetic, and endocrine findings. *J Endocrinol Invest* 2015;38(12):1249-1263
- 5 El-Bassyouni HT, Hassan N, Mahfouz I, Abd-Elnaby AE, Mostafa MI, Tosson AMS. Early detection and management of Prader-Willi syndrome in Egyptian Patients. *J Pediatr Genet* 2019;8(04):179-186
- 6 Butler MG, Miller JL, Forster JL. Prader-Willi syndrome - clinical genetics, diagnosis and treatment approaches: an update. *Curr Pediatr Rev* 2019;15(04):207-244
- 7 Butler MG, Lee PDK, Whitman BY. Management of Prader-Willi Syndrome. 3rd edition. New York, NY: Springer Verlag Inc; 2006
- 8 Cassidy SB, Driscoll DJ. Prader-Willi syndrome. *Eur J Hum Genet* 2009;17(01):3-13
- 9 Cassidy SB, Schwartz S, Miller JL, Driscoll DJ. Prader-Willi syndrome. *Genet Med* 2012;14(01):10-26
- 10 Salah EM, El-Bassyouni HT, Kholoussi S, Marwa S, Kandeel WA. Behavioral problems, biochemical and anthropometric characteristics of patients with Prader-Willi syndrome. *Middle East J Med Genet* 2015;4:63-69
- 11 Butler MG. Single gene and syndromic causes of obesity: Illustrative examples. *Prog Mol Biol Transl Sci* 2016;140:1-45
- 12 Alves C, Franco RR. Prader-Willi syndrome: endocrine manifestations and management. *Arch Endocrinol Metab* 2020;64(03):223-234
- 13 Butler MG, Manzardo AM, Forster JL. Prader-Willi syndrome: clinical genetics and diagnostic aspects with treatment approaches. *Curr Pediatr Rev* 2016;12(02):136-166
- 14 Irizarry KA, Bain J, Butler MG, et al. Metabolic profiling in Prader-Willi syndrome and nonsyndromic obesity: sex differences and the role of growth hormone. *Clin Endocrinol (Oxf)* 2015;83(06):797-805
- 15 Heksch R, Kamboj M, Anglin K, Obrynba K. Review of Prader-Willi syndrome: the endocrine approach. *Transl Pediatr* 2017;6(04):274-285
- 16 Dalle-Donne I, Rossi R, Colombo R, Giustarini D, Milzani A. Biomarkers of oxidative damage in human disease. *Clin Chem* 2006;52(04):601-623
- 17 Weinbrenner T, Schröder H, Escurriol V, et al. Circulating oxidized LDL is associated with increased waist circumference independent of body mass index in men and women. *Am J Clin Nutr* 2006;83(01):30-35, quiz 181-182
- 18 Linna MS, Ahotupa M, Kukkonen-Harjula K, Fogelholm M, Vasankari TJ. Co-existence of insulin resistance and high concentrations of circulating oxidized LDL lipids. *Ann Med* 2015;47(05):394-398
- 19 Holvoet P, Lee DH, Steffes M, Gross M, Jacobs DR Jr. Association between circulating oxidized low-density lipoprotein and incidence of the metabolic syndrome. *JAMA* 2008;299(19):2287-2293
- 20 Holvoet P, Jenny NS, Schreiner PJ, Tracy RP, Jacobs DR. Multi-Ethnic Study of Atherosclerosis. The relationship between oxidized LDL and other cardiovascular risk factors and subclinical CVD in different ethnic groups: the Multi-Ethnic Study of Atherosclerosis (MESA). *Atherosclerosis* 2007;194(01):245-252
- 21 Rondinone CM. Adipocyte-derived hormones, cytokines, and mediators. *Endocrine* 2006;29(01):81-90
- 22 Panagiotakos DB, Pitsavos C, Yannakoulia M, Chrysohou C, Stefanadis C. The implication of obesity and central fat on markers of chronic inflammation: the ATTICA study. *Atherosclerosis* 2005;183(02):308-315
- 23 Zaki ME, Youness E, Gadelhak M, et al. DNA damage and neutrophil elastase in children with Prader-Willi syndrome. *Biomed Pharmacol J* 2019;12:1967-1974
- 24 Goralski KB, McCarthy TC, Hanniman EA, et al. Chemerin, a novel adipokine that regulates adipogenesis and adipocyte metabolism. *J Biol Chem* 2007;282(38):28175-28188
- 25 Zabel BA, Kwitniewski M, Banas M, Zabieglo K, Murzyn K, Cichy J. Chemerin regulation and role in host defense. *Am J Clin Exp Immunol* 2014;3(01):1-19
- 26 Saneei P, Salehi-Abargouei A, Esmailzadeh A. Serum 25-hydroxy vitamin D levels in relation to body mass index: a systematic review and meta-analysis. *Obes Rev* 2013;14(05):393-404

- 27 Walker GE, Ricotti R, Roccio M, et al. Pediatric obesity and vitamin D deficiency: a proteomic approach identifies multimeric adiponectin as a key link between these conditions. *PLoS One* 2014;9(01):e83685
- 28 Cediél G, Corvalán C, Aguirre C, de Romaña DL, Uauy R. Serum 25-Hydroxyvitamin D associated with indicators of body fat and insulin resistance in prepubertal Chilean children. *Int J Obes* 2016;40(01):147–152
- 29 Fintini D, Grugni G, Bocchini S, et al; Genetic Obesity Study Group of the Italian Society of Pediatric Endocrinology and Diabetology (ISPED). Disorders of glucose metabolism in Prader-Willi syndrome: results of a multicenter Italian cohort study. *Nutr Metab Cardiovasc Dis* 2016;26(09):842–847
- 30 WHO AnthroPlus for personal computers Manual: Software for assessing growth of the world's children and adolescents. Geneva: WHO, 2009. Available at: <http://www.who.int/growthref/tools/en/>. Accessed July 3, 2020
- 31 Pinkel D, Gray JW, Trask B, van den Engh G, Fuscoe J, van Dekken H. Cytogenetic analysis by in situ hybridization with fluorescently labeled nucleic acid probes. *Cold Spring Harb Symp Quant Biol* 1986;51(Pt 1):151–157
- 32 Verma RS, Babu A. Tissue culture techniques and chromosome preparation. In: Verma RS, Babu A, eds. *Human Chromosomes Principles and Technique*. 2nd edition. New York: McGraw-Hill; 1995:6–71
- 33 Haaf T. Fluorescence in situ hybridization. In: Meyers RA, ed. *Encyclopedia of Analytical Chemistry*. Genetic Diagnosis of Prader-Willi Syndrome Chichester, UK: John Wiley & sons Ltd; 2000: 4984–5006
- 34 Wienders JP, Wijnberg FA. Preanalytical stability of 25(OH)-vitamin D3 in human blood or serum at room temperature: solid as a rock. *Clin Chem* 2009;55(08):1584–1585
- 35 Rocha CF, Paiva CLA. Prader-Willi-like phenotypes: a systematic review of their chromosomal abnormalities. *Genet Mol Res* 2014; 13(01):2290–2298
- 36 Tsuyasaki Y, Yoshihashi H, Furuya N, et al. 1p36 deletion syndrome associated with Prader-Willi-like phenotype. *Pediatr Int* 2010;52(04):547–550
- 37 D'Angelo CS, Kohl I, Varela MC, et al. Obesity with associated developmental delay and/or learning disability in patients exhibiting additional features: report of novel pathogenic copy number variants. *Am J Med Genet A* 2013;161A(03):479–486
- 38 Afifi HH, El-Ruby MO, El-Bassyouni HT, et al. The most encountered groups of genetic disorders in Giza Governorate, Egypt. *Bratisl Lek Listy* 2010;111(02):62–69
- 39 Khedr AA, Meguid NA, Mohamed AM, et al. Genetic diagnosis of Prader-Willi syndrome. *Middle East J Med Genet* 2016;5:45–53
- 40 Khan MJ, Gerasimidis K, Edwards CA, Shaikh MG. Mechanisms of obesity in Prader-Willi syndrome. *Pediatr Obes* 2018;13(01):3–13
- 41 Anichini C, Lotti F, Longini M, et al. Antioxidant effects of potassium ascorbate with ribose therapy in a case with Prader-Willi syndrome. *Dis Markers* 2012;33(04):179–183
- 42 Bondia-Pons I, Ryan L, Martinez JA. Oxidative stress and inflammation interactions in human obesity. *J Physiol Biochem* 2012;68(04):701–711
- 43 Kelly AS, Jacobs DR Jr, Sinaiko AR, Moran A, Steffen LM, Steinberger J. Relation of circulating oxidized LDL to obesity and insulin resistance in children. *Pediatr Diabetes* 2010;11(08):552–555
- 44 Zhao SP, Wu J, Zhang DQ, Ye HJ, Liu L, Li JQ. Fenofibrate enhances CD36 mediated endocytic uptake and degradation of oxidized low density lipoprotein in adipocytes from hypercholesterolemia rabbit. *Atherosclerosis* 2004;177(02):255–262
- 45 Chui PC, Guan HP, Lehrke M, Lazar MA. PPARgamma regulates adipocyte cholesterol metabolism via oxidized LDL receptor 1. *J Clin Invest* 2005;115(08):2244–2256
- 46 Marseglia L, Manti S, D'Angelo G, et al. Oxidative stress in obesity: a critical component in human diseases. *Int J Mol Sci* 2014;16(01): 378–400
- 47 Ferretti G, Bacchetti T, Masciangelo S, Grugni G, Bicchiega V. Altered inflammation, paraoxonase-1 activity and HDL physicochemical properties in obese humans with and without Prader-Willi syndrome. *Dis Model Mech* 2012;5(05):698–705
- 48 Hassan NE, El-Masry SA, Abu Shady MM, et al. Relation of circulating oxidized LDL with fat distribution and insulin resistance among obese school students. *RJMMS* 2013;8:16–22
- 49 Moreno-Indias I, Oliva-Olivera W, Omiste A, et al. Adipose tissue infiltration in normal-weight subjects and its impact on metabolic function. *Transl Res* 2016;172:6–17.e3
- 50 Sell H, Divoux A, Poitou C, et al. Chemerin correlates with markers for fatty liver in morbidly obese patients and strongly decreases after weight loss induced by bariatric surgery. *J Clin Endocrinol Metab* 2010;95(06):2892–2896
- 51 Perumalsamy S, Aqilah Mohd Zin NA, Widodo RT, Wan Ahmad WA, Vethakkan SRDB, Huri HZ. Chemokine like receptor-1 (CMKLR-1) receptor: a potential therapeutic target in management of chemerin induced type 2 diabetes mellitus and cancer. *Curr Pharm Des* 2017;23(25):3689–3698
- 52 Sell H, Laurencikiene J, Taube A, et al. Chemerin is a novel adipocyte-derived factor inducing insulin resistance in primary human skeletal muscle cells. *Diabetes* 2009;58(12):2731–2740
- 53 Chakaroun R, Raschpichler M, Klöting N, et al. Effects of weight loss and exercise on chemerin serum concentrations and adipose tissue expression in human obesity. *Metabolism* 2012;61(05): 706–714
- 54 Helfer G, Wu QF. Chemerin: a multifaceted adipokine involved in metabolic disorders. *J Endocrinol* 2018;238(02):R79–R94
- 55 Lehrke M, Becker A, Greif M, et al. Chemerin is associated with markers of inflammation and components of the metabolic syndrome but does not predict coronary atherosclerosis. *Eur J Endocrinol* 2009;161(02):339–344
- 56 Rourke JL, Dranse HJ, Sinal CJ. Towards an integrative approach to understanding the role of chemerin in human health and disease. *Obes Rev* 2013;14(03):245–262
- 57 Stejskal D, Karpisek M, Hanulova Z, Svestak M. Chemerin is an independent marker of the metabolic syndrome in a Caucasian population—a pilot study. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub* 2008;152(02):217–221
- 58 Ross AW, Helfer G, Russell L, Darras VM, Morgan PJ. Thyroid hormone signalling genes are regulated by photoperiod in the hypothalamus of F344 rats. *PLoS One* 2011;6(06):e21351
- 59 Khoo J, Dhamodaran S, Chen D-D, Yap S-Y, Chen RY-T, Tian RH-H. Exercise-induced weight loss is more effective than dieting for improving adipokine profile, insulin resistance, and inflammation in obese men. *Int J Sport Nutr Exerc Metab* 2015;25(06): 566–575
- 60 Faramarzi M, Banitalebi E, Nori S, Farzin S, Taghavian Z. Effects of rhythmic aerobic exercise plus core stability training on serum omentin, chemerin and vaspin levels and insulin resistance of overweight women. *J Sports Med Phys Fitness* 2016;56(04):476–482
- 61 Buechler C, Feder S, Haberl EM, Aslanidis C. Chemerin isoforms and activity in obesity. *Int J Mol Sci* 2019;20(05):1128
- 62 Becker M, Rabe K, Leberher C, et al. Expression of human chemerin induces insulin resistance in the skeletal muscle but does not affect weight, lipid levels, and atherosclerosis in LDL receptor knockout mice on high-fat diet. *Diabetes* 2010;59(11): 2898–2903
- 63 Rouger L, Denis GR, Luangsay S, Parmentier M. ChemR23 knockout mice display mild obesity but no deficit in adipocyte differentiation. *J Endocrinol* 2013;219(03):279–289
- 64 Gruben N, Aparicio Vergara M, Kloosterhuis NJ, et al. Chemokine-like receptor 1 deficiency does not affect the development of insulin resistance and nonalcoholic fatty liver disease in mice. *PLoS One* 2014;9(04):e96345
- 65 Tam CS, Clément K, Baur LA, Tordjman J. Obesity and low-grade inflammation: a paediatric perspective. *Obes Rev* 2010;11(02): 118–126



- 66 Catrysse L, van Loo G. Inflammation and the metabolic syndrome: the tissue-specific functions of NF-kappaB. *Trends Cell Biol* 2017; 27(06):417–429
- 67 Huang J-Y, Zhou QL, Huang C-H, et al. Neutrophil elastase regulates emergency myelopoiesis preceding systemic inflammation in diet-induced obesity. *J Biol Chem* 2017;292(12):4770–4776
- 68 Weigert J, Neumeier M, Wanninger J, et al. Systemic chemerin is related to inflammation rather than obesity in type 2 diabetes. *Clin Endocrinol (Oxf)* 2010;72(03):342–348
- 69 Coimbra S, Brandão Proença J, Santos-Silva A, Neuparth MJ. Adiponectin, leptin, and chemerin in elderly patients with type 2 diabetes mellitus: a close linkage with obesity and length of the disease. *BioMed Res Int* 2014;2014:701915
- 70 Zhuang XH, Sun FD, Chen SH, et al. Circulating chemerin levels are increased in first-degree relatives of type 2 diabetic patients. *Clin Lab* 2014;60(06):983–988
- 71 Catalán V, Gómez-Ambrosi J, Rodríguez A, et al. Increased levels of chemerin and its receptor, chemokine-like receptor-1, in obesity are related to inflammation: tumor necrosis factor- $\alpha$  stimulates mRNA levels of chemerin in visceral adipocytes from obese patients. *Surg Obes Relat Dis* 2013;9(02):306–314
- 72 Fatima SS, Rehman R, Baig M, Khan TA. New roles of the multidimensional adipokine: chemerin. *Peptides* 2014;62:15–20
- 73 Mariani F, Roncucci L. Chemerin/chemR23 axis in inflammation onset and resolution. *Inflamm Res* 2015;64(02):85–95
- 74 Fintini D, Pedicelli S, Bocchini S, et al. 25OH vitamin D levels in pediatric patients affected by Prader-Willi syndrome. *J Endocrinol Invest* 2018;41(06):739–742
- 75 Ameri P, Giusti A, Boschetti M, Murialdo G, Minuto F, Ferone D. Interactions between vitamin D and IGF-I: from physiology to clinical practice. *Clin Endocrinol (Oxf)* 2013;79(04):457–463
- 76 Savanelli MC, Scarano E, Muscogiuri G, et al. Cardiovascular risk in adult hypopituitary patients with growth hormone deficiency: is there a role for vitamin D? *Endocrine* 2016;52(01):111–119
- 77 Samuel L, Borrell LN. The effect of body mass index on optimal vitamin D status in U.S. adults: the National Health and Nutrition Examination Survey 2001–2006. *Ann Epidemiol* 2013;23(07):409–414
- 78 Walsh JS, Evans AL, Bowles S, et al. Free 25-hydroxyvitamin D is low in obesity, but there are no adverse associations with bone health. *Am J Clin Nutr* 2016;103(06):1465–1471
- 79 Walsh JS, Bowles S, Evans AL. Vitamin D in obesity. *Curr Opin Endocrinol Diabetes Obes* 2017;24(06):389–394
- 80 Wamberg L, Christiansen T, Paulsen SK, et al. Expression of vitamin D-metabolizing enzymes in human adipose tissue – the effect of obesity and diet-induced weight loss. *Int J Obes* 2013;37(05):651–657
- 81 Di Nisio A, De Toni L, Sabovic I, et al. Impaired release of vitamin D in dysfunctional adipose tissue: new cues on vitamin D supplementation in obesity. *J Clin Endocrinol Metab* 2017;102(07):2564–2574
- 82 Brunetti G, Grugni G, Piacente L, et al. Analysis of circulating mediators of bone remodeling in Prader-Willi syndrome. *Calcif Tissue Int* 2018;102(06):635–643
- 83 Purtell L, Viardot A, Campbell LV. Vitamin D levels in primary growth hormone deficiency disorder Prader-Willi syndrome. *Endocrine* 2016;53(02):619–620
- 84 Rubin DA, Nowak J, McLaren E, Patiño M, Castner DM, Dumont-Driscoll MC. Nutritional intakes in children with Prader-Willi syndrome and non-congenital obesity. *Food Nutr Res* 2015; 59:29427