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Research Article

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# The Relation of Serum Zonulin with Clinical and Biochemical Parameters in Obese Egyptian Adolescents

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## Abstract

***Background:*** *Changes in intestinal permeability have been related to obesity and its metabolic complications. Zonulin is recognized as a biomarker of impaired intestinal permeability.*

*The study aimed to find out if there is a difference in serum levels of zonulin as a marker of intestinal permeability between obese subjects and those of normal weight in a group of young adolescents and to evaluate its associations with anthropometric measures and obesity-associated biomarkers, such as lipid profile and insulin resistance.*

***Methods:*** *A case-control study enrolled obese Egyptian adolescents with a body mass index ≥ 95th percentile, and normal weight who were of similar age and gender distribution. Fasting insulin, glucose, lipid levels, and zonulin were measured.*

***Results:*** *Serum zonulin in the obese group had significantly higher levels compared to the control group (P= 0.009). There were significant and positive correlations in the obese group between zonulin and insulin (r= 0.393; P= 0.002), homeostasis model assessment of insulin resistance (r= 0.43; P= 0.001), cholesterol (r= 0.299; P= 0.018), and low-density lipoprotein cholesterol (r= 0.447; P=0.000).*

***Conclusion:*** *These findings suggested a probable participation of zonulin in the etiopathogenesis of obesity-associated metabolic disturbances.*

**Keywords:** Public Diplomacy, Soft Power, European Union, Tolerance and Diversity, LGBTQ+ Rights, Identity

## 1. Introduction

Childhood and adolescent obesity continue to be prevailing across the developed world, and its incidence is increasing to pandemic proportions in most developing countries [1]. Discrepancy between energy intake and expenditure along with a sedentary lifestyle are the principal reasons for obesity [2].

Adolescent obesity is related to important cardio-metabolic comorbidities and biochemical changes, such as dysglycemia, hyperinsulinemia, dyslipidemia, and hypertension that track into adulthood with a significant rise in morbidity and mortality later in life [3].

Quantitative and qualitative alterations of obese children and adolescents' diet, specifically the usage of fat-rich food and easily digested carbohydrates and reduced consumption of fiber, considerably influence the intestinal biocenosis, causing alterations in the component of the intestinal microbiota (dysbiosis), which could cause impairment in the function of the intestinal barrier [4].

The intestine has an important role as a barrier to defend the body from unwanted foreign substances, infectious agents, and food particles [5].

In experimental models of animals with obesity, substantial changes in the function of the intestinal barrier arise, causing an increase in the intestinal permeability resulting in an increased translocation of bacteria and their products, particularly lipopolysaccharides (LPS), into the systemic circulation and therefore, via activation of Toll- and Nod-like receptors, results in stimulation of signalling cascades and the liberation of an increased amount of chemokines and cytokines. This initiates the development of chronic low-grade inflammation, linked with obesity, giving impulse to the progression of obesity and its complications [6]. The structure, regulation, and function of interepithelial tight junctions (TJs) are significant for the role of the intestinal barrier. Microbiota dysbiosis is a stimulus for increased synthesis of zonulin, which is a recently discovered 47-kDa protein and is considered one of the regulators of intestinal permeability as it causes a reversible modulation of intercellular tight junctions, and it also acts as an agent of innate immunity of the intestine. Zonulin promotes the opening of tight junctions between epithelial cells, resulting in an increase in the permeability of the intestinal barriers (the so-called "leaky gut syndrome") [7].

Circulating zonulin levels are recognized as a beneficial marker of intestinal permeability. Cross-sectional studies revealed that serum zonulin has been correlated with obesity and its comorbidities [8].

Few studies have examined the effect of disturbed intestinal permeability and the association of zonulin with the pathogenesis of obesity and its complications in obese adolescents.

The study aimed to find out if there is a difference in serum levels of zonulin as a marker of intestinal permeability between obese subjects and those of normal weight in a group of young adolescents and to evaluate its associations with anthropometric measures and obesity-associated biomarkers, such as lipid profile and insulin resistance.

## 2. Subjects and Methods

A case-control study enrolled 153 Egyptian adolescents aged 10-18 years of both sexes. The cutoff body mass index (BMI) was calculated consistent with the World Health Organization (WHO) growth charts.

### 2.1. Inclusion Criteria

Adolescents of both sexes aged 10 to 18 years old (obese and normal weight).

### 2.2. Exclusion Criteria

Adolescents who had a genetic or endocrine cause of obesity (e.g., Prader-Willi Syndrome, Cushing's disease, hypothyroidism), associated chronic diseases, celiac disease, or other diseases characterized by malabsorption.

The obese adolescents were recruited from the Nutrition-Immunotherapy Clinic at the Medical Research Centre of Excellence (National Research Centre), Giza, Egypt, and the control group was recruited from the pediatric outpatient clinic.

### 2.3. Study Design

The study participants were divided into two groups as follows:

- Obese group (the study group): Included obese adolescents with BMI more than or equal to the 95th centile.
- Normal weight group (the control group): Involved healthy age- and gender-matched adolescents who had a BMI between the 5th and less than 85th centiles.

### 2.4. The Study Participants were Subjected to the Following

- History taking: with emphasis on age, sex, parent education, and the inquiry about associated morbidities such as diabetes mellitus, hypertension, and family history of obesity, diabetes mellitus, and hypertension.
- Physical examination: with special emphasis on weight, height, waist circumference, hip circumference measurements, and blood pressure measurement.
- Anthropometric measures: Body weight was assessed wearing light clothes and barefoot on Seca Scale Balance to the nearest 0.1Kg. Height was determined to the nearest 0.1 cm on a Holtain portable anthropometer. The calculation of BMI was done using the equation [weight (kg)/height ($m^2$)]. Data were plotted on WHO curves via the software Anthro Cale v1.66 Home [9]. Waist circumference was assessed at the midpoint between the rib cage and the iliac crest on the midaxillary line using a flexible and inelastic tape. Hip circumference was measured around the largest part of the hips (the widest part

of the buttocks).

- Blood pressure: was estimated 5 minutes after rest in the semi-sitting position using an appropriately sized cuff using a Riester sphygmomanometer from Germany. Data were plotted through the software Anthro Cale v1.66 Home, and the participants were diagnosed to have hypertension consistent with the guidelines of the American Academy of Pediatrics (AAP), 2017 [10].
- Biochemical investigations: The venous blood samples were withdrawn in the morning following 10-12 hours of overnight fasting. Fasting blood glucose, insulin, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), total cholesterol, and triglycerides were analyzed immediately. Fasting blood glucose was analyzed using the colorimetric method [11]. Serum cholesterol and triglycerides were estimated respectively [12,13]. HDL-C was measured by the enzyme method while LDL-C was calculated [14,15]. Serum insulin was measured using an ELISA kit (Sun long kit). Part of the collected blood was centrifuged and stored at -20°C to analyze zonulin levels by the use of an ELISA kit (Sun long kit). Insulin resistance was estimated from fasting plasma measurements using the homeostasis model assessment of insulin resistance (HOMA-IR) using the formula [fasting insulin (µU/L) × fasting glucose (mg/dl) / 405] [16]. Criteria of insulin resistance were HOMA-IR > 4.0 for adolescents [17].

### 2.5. Statistical Analysis

Data entry and analysis were executed by Statistical Package for the Social Sciences (SPSS) software, version 16. Data were shown as mean and standard deviation (SD). To compare between groups for parametric data, an independent sample t-test was utilized, and a Mann-Whitney test was utilized for non-parametric data. Pearson's correlation [correlation coefficient (r)] was utilized for assessment of correlations. A p-value < 0.05 was considered statistically significant.

## 3. Results

According to the WHO cutoff of BMI, the participants were classified into two groups: the obese group (the study group), which included 69 adolescents, and the normal-weight group (control group), which included 84 adolescents. The two groups were matched in terms of age and gender (P=0.048 and P=0.853, respectively). As expected, weight, BMI, waist circumference, hip circumference, waist circumference/hip ratio, and waist circumference/height ratio; moreover, systolic and diastolic blood pressures were significantly higher in the obese group in comparison to the normal weight group (P=0.001). The demographic and clinical data of the study participants were shown in Table (1).

| | Obese group (N=69) | Control group (N=84) | P-values |
|---|---|---|---|
| Gender (Male/Female) | 31/38 | 39/45 | **0.853** |
| Age (years) | 13.49 ± 1.87 | 14.15 ± 2.25 | **0.048** |
| Weight (Kg) | 77.00 ± 16.12 | 48.24 ± 11.43 | **0.001*** |
| Weight centile | 94.61 ± 9.34 | 35.36 ± 23.77 | **0.001*** |
| Height (m) | 1.59 ± 0.10 | 1.57 ± 0.13 | **0.335** |
| Height centile | 51.94 ± 32.32 | 34.74 ± 27.06 | **0.001*** |
| BMI | 30.24 ± 4.75 | 19.22 ± 2.31 | **0.001*** |
| BMI centile | 98.80 ± 1.38 | 42.73 ± 23.92 | **0.001*** |
| Waist circumference (cm) | 90.09 ± 12.65 | 67.90 ± 7.66 | **0.001*** |
| Hip circumference (cm) | 107.14 ± 12.68 | 85.51 ± 8.99 | **0.001*** |
| Waist/ hip ratio | 0.84 ± 0.07 | 0.79 ± 0.06 | **0.001*** |
| Waist/Height ratio | 0.57 ± 0.07 | 0.43 ± 0.05 | **0.001*** |
| Waist/Height centile | 86.99 ± 13.11 | 34.57 ± 22.08 | **0.001*** |
| Systolic blood pressure | 106.16 ± 16.52 | 95.66 ± 8.94 | **0.001*** |
| Diastolic blood pressure | 70.65 ± 9.15 | 63.60 ± 6.35 | **0.001*** |
| *: Significant difference at p< 0.05. | | | |

**Table 1: The Demographic and Clinical Data of the Study Participants**

The control group was normotensive, while 16 participants of the obese group had stage 1 hypertension according to AAP 2017 criteria. No significant difference in height could be identified between the two groups. Control group participants had normal fasting blood glucose levels, while 2 participants in the obese group had fasting blood glucose levels > 126 mg/dl. The serum levels of insulin and HOMA-IR readings were non-significantly higher in the obese group in comparison to the control group. Total cholesterol, triglycerides, and LDL-C were significantly higher in the obese group in comparison to the control group (P<0.001), while HDL-C was significantly lower in the obese group compared to the control group (P=0.001).

Serum zonulin in the obese group had significantly higher levels compared to the control group (P = 0.009). The biochemical data of the study participants were shown in Table (2).

| | Obese group (N=69) | Control group (N=84) | P-values |
|---|---|---|---|
| Zonulin (pg/ml) | 325.92 ± 275.58 | 236.46 ± 89.87 | **0.009*** |
| Insulin (µU/L) | 5.31 ± 5.45 | 3.85 ± 3.80 | 0.189 |
| Fasting blood glucose (mg/dl) | 97.13 ± 38.95 | 92.44 ± 9.18 | 0.799 |
| Total cholesterol(mg/dl) | 160.03 ± 24.20 | 145.00 ± 24.96 | **0.001*** |
| Triglyceride (mg/dl) | 106.15 ± 54.26 | 64.14 ± 22.64 | **0.001*** |
| HDL-C (mg/dl) | 45.88 ± 7.97 | 52.81 ± 11.14 | **0.001*** |
| LDL-C (mg/dl) | 91.92 ± 21.81 | 77.29 ± 19.68 | **0.001*** |
| HOMA-IR | 1.25 ± 1.26 | 0.88 ± 0.86 | **0.141** |
| *: Significant difference at p<0.05. | | | |

**Table 2: The Biochemical Data of the Study Participants**

Correlation analysis revealed significant and positive correlations in the obese group between zonulin and insulin (r= 0.393; P= 0.002), HOMA-IR (r= 0.43; P= 0.001), cholesterol (r= 0.299; P= 0.018), and LDL-C (r= 0.447; P= 0.000).

There were non-significant positive correlations between zonulin and BMI (r = 0.082; P = 0.514), waist circumference (r = 0.070; P = 0.582), fasting blood glucose (r = 0.045; P = 0.724), and triglyceride (r = 0.024; P = 0.854) in the obese group.

A significant negative correlation was identified between zonulin and HDL-C in the obese group (r = 0.369; P = 0.003). The correlations were shown in Table (3).

| | Variables | Correlation Coefficient (r) | P-values |
|---|---|---|---|
| Zonulin (pg/ml) | Body Mass Index | 0.082 | 0.514 |
| | Waist circumference (cm) | 0.070 | 0.582 |
| | Systolic blood pressure | -0.079 | 0.529 |
| | Diastolic blood pressure | -0.032 | 0.799 |
| | Insulin (µU/L) | 0.393 | 0.002* |
| | Fasting blood glucose (mg/dl) | 0.045 | 0.724 |
| | HOMA-IR | 0.413 | 0.001* |
| | Total cholesterol(mg/dl) | 0.299 | 0.018* |
| | Triglyceride (mg/dl) | 0.024 | 0.854 |
| | HDL-C (mg/dl) | -0.369 | 0.003* |
| | LDL-C (mg/dl) | 0.447 | 0.000* |
| *: Significant difference at p<0.05. | | | |

**Table 3: Correlations between Zonulin (pg/ml) and other Variables in Obese Group**

## 4. Discussion

Experimental studies in animals have revealed a direct relationship between intestinal permeability and the pathogenesis of obesity [18]. Though, it is still unknown if this also occurs in individuals with obesity, as studies evaluating intestinal barrier permeability (IBP) in humans, particularly in children and adolescents, are rare and their results inconsistent [19].

Change of intestinal permeability is not easy to assess. Assay of serum zonulin, a protein made by the intestinal epithelium, has been recognized as an easy-to-execute surrogate marker of dysfunction of the intestinal barrier, appropriate also in pediatric age [20].

The present study found that obese adolescents had sig¬nificantly higher values than normal-weight adolescents in all the studied anthropometric measurements (BMI, waist circumference, hip circumference, waist/hip ratio, and waist/height ratio). This is in agreement with what was reported in different studies [21].

The study revealed that obese adolescents had significantly higher zonulin levels compared to normal-weight adolescents. This finding was similar who also reported that serum zonulin levels were significantly higher in obese adolescents in comparison to peers with normal weight [21,22]. On the other hand, revealed that the obese children had significantly a lower value of zonulin than the control one [23]. This difference may be explained by the age differences of studies.

The study showed non-significant correlations between serum zonulin and BMI and waist circumference in obese individuals. Similarly, stated that serum zonulin was not associated with BMI in obese individuals [24]. However, reported a significant and positive relationship between zonulin levels and BMI and waist circumference/height ratio in obese adolescents [22]. Moreover, revealed significant negative correlations between zonulin and BMI, waist circumference, and waist/height ratio [23]. These differences could additionally support the probability of multiple functions of zonulin in children [25].

The microbiota of the intestine is identified to defend the body against different pathogens through the formation of a mechanical barrier on the mucosal surface of the intestine [26]. Several studies have established that the microbiota is impaired and intestinal dysbiosis occurs in subjects who eat a rich-fat and low-fiber diet [27]. Microbiota dysbiosis may initiate the release of zonulin by the lamina propria of the epithelium of the intestine, a modulator of intercellular TJs, which increases the paracellular permeability of the small intestine [28]. The increased permeability of the intestine causes the transfer of substances of bacteria, such as lipopolysaccharide (LPS), and products of their metabolism, such as short-chain fatty acids, from the gut into the systemic circulation, ultimately defining a low-grade inflammatory status that promotes the progress of obesity and its associated pathology [6]. This endorses the damaging impact of obesity, particularly the abdominal type of it, on the status of the barrier of the intestine and the progression of associated pathology.

Studied the relation between the metabolic imbalance with obesity and intestinal permeability, and advocated that gut per¬meability could be the cause, the result, or both of obesity and its metabolic disorders [25].

Reported that serum zonulin was decreased after weight-loss interventions [24]. A reduction in the permeability of the intestinal barrier after treatment for weight reduction in patients with obesity has also been detected in other clinical studies [29].

Among the obese group, the study revealed insignificant correlation between zonulin and blood pressure. In agreement with our results, reported insignificant correlation between zonulin and blood pressure in obese children [23].

The study showed that obese adolescents had non-significantly higher glucose, insulin, and HOMA-IR than normal-weight adolescents ($P>0.05$). Additionally, the study showed that zonulin had a significant and positive correlation with fasting insulin and HOMA-IR in obese adolescents ($P<0.05$). Similarly, reported significant positive relationships between serum zonulin and insulin and HOMA-IR in obese adolescents [22].

It was well established that insulin resistance is related to obesity, particularly with visceral obesity [30]. Intestinal permeability has been suggested as one of the key elements participating in the development of insulin resistance [25]. Increased serum levels of zonulin in obese individuals weaken the cytoskeletal structure between intestinal cells, increasing the intestinal permeability that facilitates the transfer of substances and products, as pro-inflammatory molecules, for instance, interleukin-6 (IL-6), through the intestinal wall to the systemic circulation, causing a change of the insulin receptor signaling through several pathways [31]. Therefore, zonulin is proposed to have a probable contribution to the pathogenesis of metabolic disturbances linked with obesity.

In this study, obese adolescents had significantly higher total cholesterol, triglycerides, and LDL and significantly lower HDL than normal-weight adolescents ($P = 0.001$). A significant and positive correlation between zonulin levels and total cholesterol and LDL ($P<0.05$) and a significant and negative correlation between zonulin levels and HDL ($P<0.05$) were reported in our study. There was a non-significantly positive link between zonulin levels and triglycerides in obese adolescents ($P>0.05$). Similarly, found that obese children had sig¬nificantly higher total cholesterol and sig-nificantly lower values of HDL than normal-weight healthy chil¬dren. Also, they reported insig-nificant correlations between zonulin and triglycerides in obese children [23].

On the other hand, reported significant and positive correlations between zonulin levels and both triglycerides and VLDL-C in obese adolescents [22]. Zonulin was proposed to augment adipose tissue via the endocannabinoid pathway through increasing the permeability of intestines, thus causing the occurrence of dyslipidemia [31].

These findings proposed that a change in paracellular permeability of intestines could be a mediator among the intestinal environment and metabolic disorders related to obesity. Consequently, zonulin inhibitors can decrease the paracellular permeability and can have an impact on some of the metabolic disturbances linked to obesity and may become beneficial as drugs in the future [24].

## 5. Conclusion

The study determined that serum zonulin levels (a plausible marker of altered intestinal permeability) were significantly higher in obese adolescents in comparison to normal-weight ones. Moreover, there were significant positive correlations between serum zonulin levels and insulin, HOMA-IR, cholesterol, and LDL, and a significant negative correlation between zonulin and HDL in obese adolescents.

Consequently, zonulin could be suggested not just as a marker of changed intestinal permeability in obese children and adolescents but also as a probable indicator of metabolism dysregulation, aiding in the early recognition of obese children and adolescents who are most at threat of developing metabolic complications. This is essential in clinical practice to execute targeted early preventive approaches.

## Limitations and Strengths

The limited data about zonulin in the pediatric age group pose a challenge in fully understanding the precise role of zonulin in the

pathogenesis of obesity. This study could help in understanding the underlying etiopathogenesis of metabolic complications associated with obesity in adolescents.

## Declarations

### Ethical Approval and Consent to Participate

The present study was accomplished in agreement with the Code of Ethics of the World Medical Association, consistent with the ethics indicated in the Declaration of Helsinki. The study had an approval by the "Ethics Committee of National Research Centre, Cairo, Egypt" with approval number 19224. An informed written consent was attained from the legal guardian of every participant former to participation in the study.

### Consent for publication

Not applicable.

### Conflict of interest

The authors declare that they have no competing interest.


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### Availability of Data and Materials

The datasets generated and analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

### Authors Contributions

Azza Abd El-Shaheed was responsible for conception, design, project administration, supervision, in addition to the manuscript revision. Reham F. Fahmy contributed in anthropometric assessments, clinical history taking, data entry, writing the manuscript, in addition to the submission of the manuscript to the journal. Nermine N. Mahfouz contributed in conception, design, project administration, anthropometric assessments, clinical history taking, and data entry. Mona A. El-Bana was responsible for laboratory investigations and interpretations. Safa N. Abd El-Fattah was responsible for laboratory investigations and interpretations. Salwa Refat El-Zayat was responsible for laboratory investigations and interpretations. Hiba Sibaii was responsible for laboratory investigations and interpretations. All authors read and approved the final manuscript.


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