

The Relationship between Serum Zonulin Level and Clinical and Laboratory Parameters of Childhood Obesity

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What is already known on this topic?

Zonulin is a protein that increases the intestinal permeability in small intestinal system by modulating the intracellular tight junctions.

What this study adds?

The present study is the first study to compare the serum zonulin levels between obese and non-obese children. We demonstrated that serum zonulin levels were higher in obese children when compared to healthy children, which may play a role in the pathogenesis of obesity and related metabolic disturbances.

Abstract

Objective: To investigate the relationship between zonulin levels and clinical and laboratory parameters of childhood obesity.

Methods: The study included obese children with a body mass index (BMI) > 95th percentile and healthy children who were of similar age and gender distribution. Clinical (BMI, waist circumferences, mid-arm circumference, triceps skinfold, percentage of body fat, systolic blood pressure, diastolic blood pressure) and biochemical (glucose, insulin, lipid levels, thyroid function tests, cortisol, zonulin and leptin levels) parameters were measured.

Results: A total of 43 obese subjects (23 males, mean age: 11.1 ± 3.1 years) and 37 healthy subjects (18 males, mean age: 11.5 ± 3.5 years) were included in this study. Obese children had significantly higher insulin, homeostasis model assessment of insulin resistance, triglyceride, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol (HDL-C), zonulin and leptin levels than healthy children ($p < 0.05$), while glucose levels were not different ($p > 0.05$). Comparison of the obese children with and without insulin resistance showed no statistically significant differences for zonulin levels ($p > 0.05$). Zonulin levels were found to negatively correlate with HDL-C and positively correlate with leptin levels, after adjusting for age and BMI.

Conclusion: To the best of our knowledge, this is the first study investigating the relationship between circulating zonulin level (as a marker of intestinal permeability) and insulin resistance and leptin (as markers of metabolic disturbances associated with obesity) in childhood obesity. The results showed that zonulin was significantly higher in obese children when compared to healthy children, a finding indicating a potential role of zonulin in the etiopathogenesis of obesity and related disturbances.

Keywords: Tight junctions, zonulin, insulin resistance, obesity, childhood

Introduction

The prevalence of an overweight state, which is an important cause of morbidity and mortality in the world, has increased to pandemic proportions among children and adolescents. Imbalance between energy intake and expenditure as well as sedentary lifestyle are the leading causes of obesity (1). Obesity is associated with systemic microinflammation due to the

release of proinflammatory peptides in visceral adipose tissue. Systemic microinflammation in obesity is the major cause in the pathogenesis of metabolic disorders such as insulin resistance (IR), dyslipidemia, type 2 diabetes (2). Recent evidence suggests a possible role of intestinal barrier dysfunction from releasing proinflammatory peptides in obesity (3).

The intestines have a function as a barrier to protect the body from infectious, toxic, allergic agents as well as from



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antigenic loads and also from caloric loads. Recent studies which report an increase in intestinal permeability and absorption together with a decrease in intestinal motility in patients with metabolic disorders indicate that there is a link between intestinal barrier dysfunction and metabolic disorders (3).

The structure, function, and regulation of interepithelial zonula occludens [tight junction (TJ)] are important for intestinal barrier function. Firstly, the zonula occludens toxin (zot), an enterotoxin secreted by *Vibrio Cholera*, was reported to affect the TJs. Subsequently, human zonulin, a physiological mediator in 47 kDa protein structure which regulates the permeability of intestinal TJ and acts as an agent of innate immunity, was isolated as a homolog of zot. Intestinal TJ dysfunction and upregulation of zonulin were found to be the primary defects in these diseases (4). Circulating zonulin levels are considered to be a useful marker of intestinal permeability. Recently, higher circulating zonulin levels were reported in obese adult subjects as compared to the non-obese and also in adults with glucose intolerance as compared to those with normal glucose tolerance (3,5). Significantly higher serum zonulin levels were also reported in obese subjects with biopsy-confirmed nonalcoholic fatty liver disease (NAFLD) than in obese subjects without NAFLD (4).

Leptin, the protein product of the *ob* gene, was shown to be involved in the regulation of food intake, energy consumption, body weight, and glucose metabolism (6). It was shown that leptin concentrations change in nutritional states such as fasting followed by a subsequent increase in food intake (7). However, there are no studies which evaluate the association between serum zonulin and leptin levels.

In the present study, we aimed to investigate the relationship of circulating zonulin level as an intestinal permeability marker and that of leptin as a metabolic disorders marker in childhood obesity.

Methods

Forty-three obese children with a body mass index (BMI) greater than the 95th percentile according to the standards of the Centers for Disease Control and Prevention (CDC-2000) and 37 healthy children of similar age, gender, and pubertal stage distribution with a BMI between the 5th and 85th percentile were consecutively enrolled in the study (8). Patients and control subjects with chronic diseases (cardiovascular, gastrointestinal, and respiratory), a history of drug use (steroids and antipsychotics), endocrine pathology (Cushing syndrome and hypothyroidism), or suspected syndromes associated with obesity (Prader-Willi and Laurence-Moon-Biedl syndromes) were excluded from

the study. Subjects with a recent history of upper airway infection, gastroenteritis, and use of antibiotics were also excluded.

All subjects underwent a thorough physical examination. A biochemical evaluation including thyroid function tests and serum cortisol measurement for probable endocrine pathology was performed in all obese subjects.

The study was initiated after the approval of the local ethics committee of Dokuz Eylül University Faculty of Medicine. A written informed consent of the parent(s) of each subject was also obtained before the study.

Height was measured using a Harpenden stadiometer with a sensitivity of 0.1 cm. Weight was measured using a SECA scale with a sensitivity of 0.1 kg, with all clothing removed except undergarments. BMI was calculated by dividing weight (kg) by height squared (m²). Waist circumference (WC) and mid-upper arm circumferences (MAC) were measured using standard techniques. Triceps skinfold thickness (TSF) (in millimeters) was measured with a Harpenden skinfold caliper. The percentage of body fat (PBF) was measured using bioelectric impedance analysis (Tanita BC-418, Tokyo, Japan).

Findings for pubertal development were evaluated according to Tanner staging (9). A testicular volume of ≥ 4 mL in males and breast development of stage 2 and over in females were considered to be findings of puberty.

Blood pressure was measured by one of the investigators using a validated protocol. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured twice at the right arm after a 10-min rest in the supine position using a calibrated sphygmomanometer. Hypertension was defined as blood pressure values above the 95th percentile for height, age, and gender (10).

The venous blood samples were collected in plane tubes after 10-12 h of night fasting. The plane tubes were centrifuged at 1200xg for 10 minutes and serum samples were transferred into the Eppendorf tubes using plastic Pasteur pipettes. Routine parameters (glucose, insulin, lipids, thyroid function tests, cortisol) were analyzed on the same day. Samples to be analyzed for special parameters (zonulin, leptin) were stored at -80 °C until analysis.

Fasting serum glucose, triglyceride (TG), total cholesterol (TC), and high-density lipoprotein cholesterol (HDL-C) concentrations were measured enzymatically using DP Modular Systems (Roche Diagnostic Corp., Indianapolis, IN). Low-density lipoprotein cholesterol (LDL-C) levels were calculated using the Friedewald formula when plasma TGs were < 400 mg/dL. Serum insulin was measured according to the electrochemiluminescence immunoassay method, using an automated immunoassay analyzer (Immulite 2500 Insulin, Diagnostic Products Corporation, Los Angeles,

CA, USA). IR was evaluated according to the homeostasis model assessment of IR (HOMA-IR) index. Different cut-off values for prepubertal and pubertal subjects were determined based on 85th percentile values of control cases (prepubertal > 2.5, pubertal > 4) (11).

Serum leptin levels (catalog no: EK0595 and EK0437, Boster Biological Technology Co Ltd, Wuhan, China) and zonulin levels (catalog no: CSB-EL028107HU and CSBEQ27649HU, CUSABIO Biotech Co Ltd, Wuhan, China) were measured by enzyme linked immunosorbent assay kit (ELISA) based on the principle of competitive enzyme immunoassay. In this assay, the microplate in the kit is pre-coated with antibody specific to the analyte. The standard is reconstituted and prepared by serial dilution with sample diluents. The serum samples are diluted 1:10 with sample diluents for leptin and adiponectin assays and 1:2000 for zonulin. Standards and samples are loaded into the appropriate microtiter plate wells and any analyte present is bound by immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific to the analyte is added to the wells. After washing, avidin-conjugated peroxidase is added to the wells. Following a wash to remove any unbound avidin-enzyme reagent, a substrate solution is added and color develops in proportion to the amount of analyte. The color development is stopped and the intensity of the color is measured spectrophotometrically at a wavelength of 450 nm. A standard curve of known concentration of analyte is established and the concentration of analyte in the samples is calculated accordingly. The ELISA assays for leptin and zonulin had a sensitivity of < 10 pg/mL and 0.156 ng/mL, a detection range of 62.5-4000 pg/mL and 0.625-40 ng/mL, an intraassay coefficient of variation (CV) of < 10% and < 8%, as well as interassay CV of < 10% and < 10%, respectively.

Statistical Analysis

The analyses of the data were conducted with Statistical Package for the Social Sciences 16.0.1 (SPSS Inc., Chicago, IL, USA). The distribution of the data was evaluated with the Kolmogorov-Smirnov test. For numerical comparisons, the student's t-test or Mann-Whitney U-test were used according to the distribution of the measured parameters. Categorical variables were compared using the chi-square test. A correlation analysis was performed using Spearman's correlation analysis. Since zonulin levels were not normally distributed, they were transformed to logarithmic values for multivariate linear regression analysis. Variables with a p-value < 0.05 in the bivariate correlation analysis were included in a multivariate linear regression analysis model to assess the independent determinants of serum zonulin level. A partial correlation was also performed, with serum

zonulin as a dependent variable, controlling for potential confounders such as age and BMI. The data were presented as mean ± standard deviation or median and interquartile range (IQR). In all statistical tests, p-values < 0.05 were considered significant.

Results

A total of 43 obese subjects (23 males, 20 pubertal, mean age: 11.1 ± 3.1 years) and 37 healthy subjects (14 males, 23 pubertal, mean age: 11.5 ± 3.6 years) were included in the study. The groups were similar for age, gender, and pubertal status. There were significant differences between obese and healthy children in terms of BMI, BMI- standard deviation score (SDS), WC, MAC, TSF, fat mass, PBF, SBP, and DBP (p < 0.05), as shown in Table 1. Obese children had significantly higher insulin, HOMA-IR, TG, TC, LDL-C, HDL-C, zonulin, and leptin levels than the healthy children (p < 0.05), while glucose levels were similar in the two groups (p > 0.05) (Figure 1, Table 2).

Comparison of the obese children regarding their findings on IR showed statistically significant differences for BMI, MAC, WC, fat mass, PBF, insulin, and HOMA-IR (p < 0.05). Age, sex, BMI-SDS, TSF, SBP, DBP, serum glucose, TG, TC, LDL-C, HDL-C, zonulin, and leptin levels were similar in the insulin resistant and non-resistant obese children (p > 0.05) (Table 3).

Spearman's correlation analysis revealed that serum zonulin levels negatively correlated with age and HDL-C, while positive correlated with BMI-SDS, PBF, LDL-C, HDL-C, and leptin levels in the entire cohort. Zonulin level were significantly associated with only HDL-C and leptin levels, after adjusting for age and BMI (r = -0.348, p = 0.026; r = 0.417, p = 0.007, respectively) (Table 4). In the multivariate backward regression analysis (r² = 0.503, p < 0.001), log-transformed zonulin was significantly

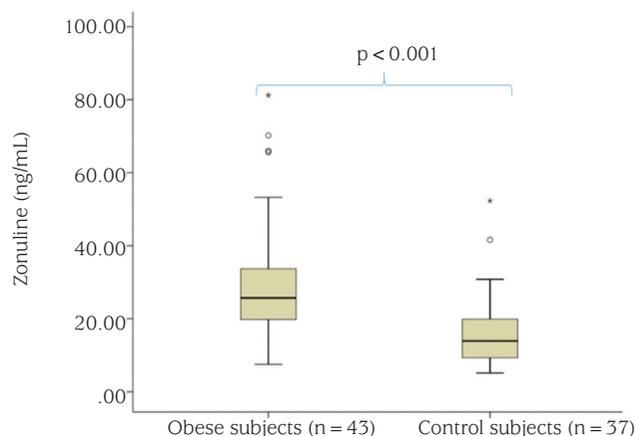


Figure 1. Zonulin levels in obese and healthy subjects

Table 1. Clinical characteristics in the obese and control groups

	Obese subjects (n = 43)		Control subjects (n = 37)		p-value
	Mean ± SD	Median (IQR)	Mean ± SD	Median (IQR)	
Age (years)	11.1 ± 3.1	10.8 (4.5)	11.5 ± 3.6	12 (6.3)	0.635 ^a
Sex (Male/Female)	23/20		14/23		0.823 ^c
Pubertal/prepubertal	20/23		23/14		0.184 ^c
BMI (kg/m ²)	28.9 ± 4.6	27.4 (6.2)	18.3 ± 2.8	18 (3.75)	< 0.001 ^a
BMI SDS	2.2 ± 0.3	2.2 (0.3)	0.02 ± 0.8	0.12 (1.4)	< 0.001 ^b
WC (cm)	96.1 ± 14.2	96.5 (20)	66.6 ± 9.7	67 (12.6)	< 0.001 ^a
MAC (cm)	30.0 ± 4.2	29 (6.0)	20.9 ± 3.8	21 (6.2)	< 0.001 ^a
TSF (mm)	29.7 ± 5.9	30 (11.5)	13.1 ± 4.9	11.6 (8.0)	< 0.001 ^a
Fat mass (kg)	26.4 ± 11.8	24.2 (15.0)	8.1 ± 3.2	7.7 (3.1)	< 0.001 ^b
PBF (%)	36.0 ± 6.4	35.2 (9.4)	18.3 ± 5.4	18.6 (6.5)	< 0.001 ^a
SBP (mmHg)	122.2 ± 15.2	120 (20)	103.9 ± 13.0	100 (18.7)	< 0.001 ^b
DBP (mmHg)	76.7 ± 11.7	75 (10)	66.9 ± 10.2	65 (15)	0.004 ^b

^aStudent's t-test, ^bMann-Whitney U-test, ^cChi-square test.

IQR: interquartile range, BMI: body mass index, BMI-SDS: standard deviation score of body mass index, WC: waist circumferences, MAC: mid-arm circumference, TSF: triceps skinfold, PBF: percent body fat, SBP: systolic blood pressure, DBP: diastolic blood pressure, SD: standard deviation

Table 2. Laboratory findings in the obese and control groups

	Obese subjects (n = 43)		Control subjects (n = 37)		p-value
	Mean ± SD	Median (IQR)	Mean ± SD	Median (IQR)	
Glucose (mg/dL)	82.8 ± 12.6	81 (17)	85.1 ± 8.2	86 (13.5)	0.339 ^a
Insulin (uIU/mL)	19.9 ± 12.7	16.8 (18.3)	5.7 ± 3.2	5.3 (5.5)	< 0.001 ^b
HOMA-IR	4.1 ± 2.7	3.3 (3.9)	1.2 ± 0.7	1.1 (1.2)	< 0.001
Triglyceride (mg/dL)	133.2 ± 56.8	123 (71)	74.1 ± 24.9	73 (40.5)	< 0.001 ^b
TC (mg/dL)	180.3 ± 33.2	168 (43)	155.1 ± 21.9	150 (29)	0.001 ^b
LDL-C (mg/dL)	108.0 ± 31.0	104 (39)	87.2 ± 19.5	90 (27)	0.001 ^b
HDL-C (mg/dL)	45.7 ± 12.1	45 (9)	53.7 ± 13.3	53 (12)	< 0.001 ^b
Zonulin (ng/mL)	30.1 ± 16.7	25.7 (15.8)	16.4 ± 9.8	13.9(10.8)	< 0.001 ^b
Leptin (ng/mL)	10.7 ± 4.9	10.7 (7.1)	3.3 ± 1.8	2.9 (2.2)	< 0.001 ^b

^aStudent's t-test, ^bMann-Whitney U-test, ^cChi-square test

IQR: interquartile range, HOMA-IR: homeostasis model assessment of insulin resistance, LDL-C: low-density lipoprotein cholesterol, TC: total cholesterol, HDL-C: high-density lipoprotein cholesterol, SD: standard deviation

associated with BMI-SDS (β -coefficient 0.398, $p < 0.001$), and HDL-C (β -coefficient -0.379, $p < 0.001$), which explained 47.5% of the variance.

Discussion

Experimental studies have demonstrated a close association between intestinal permeability and the pathogenesis of obesity (12,13,14,15,16). The molecular transport between the intestinal lumen and the submucosa is regulated by dynamic TJ structures found between the intestinal cells (17). The recently described peptide, zonulin, is known to increase the intestinal permeability by altering the

paracellular TJs. The weakening of the intestinal barrier leads to an increased exposure to pathogens and allergens (18,19). On the other hand, various organisms are known to have an effect on intestinal permeability and to live symbiotically in the intestinal system (20). This structure, referred to as the intestinal microbiota, is known to protect the body against various pathogens by creating a mechanic barrier on the surface mucosa of the intestinal system (20). Many studies have demonstrated that the microbiota is damaged and intestinal permeability increased in individuals exposed to a high-fat diet, leading to obesity and metabolic abnormalities (12,21). The mechanical barrier formed by the microbiota of the intestinal system is altered by changes in dietary habits

Table 3. Clinical and laboratory findings in obese patients with and without insulin resistance

	IR (-) Group (n = 22)	IR (+) Group (n = 21)	p ^a
Age (years)	10 (5.0)	12.2 (5.6)	0.063
Sex (Male/Female)	14/8	9/12	0.227 ^b
BMI (kg/m ²)	26.8 (5.2)	31 (7.2)	0.003
BMI SDS	2.2 (0.4)	2.3 (0.4)	0.166
MAC (cm)	27.5 (4.2)	32 (6.0)	0.008
TSF (mm)	27.2 (9.4)	31.0 (10.5)	0.192
WC (cm)	88.5 (20.5)	105 (22.2)	0.006
Fat mass (kg)	18.6 (9.8)	30.1 (18.0)	0.001
PBF (%)	33.4 (6.6)	39.7 (7.5)	0.006
Glucose (mg/dL)	76.5 (18.5)	85 (16.5)	0.125
Insulin (uIU/mL)	10.6 (6.7)	28.7 (14.4)	<0.001
Triglyceride (mg/dL)	113.5 (64.5)	128 (86)	0.211
TC (mg/dL)	165.5 (35.7)	177 (54)	0.258
LDL-C (mg/dL)	97.5 (40.5)	110 (40.2)	0.206
HDL-C (mg/dL)	47 (9.2)	42 (9.5)	0.058
HOMA-IR	2.1 (1.1)	5.9 (3.2)	<0.001
SBP (mmHg)	120 (12.5)	122.5 (27.5)	0.564
DBP (mmHg)	75 (10)	77.5 (17.5)	0.815
Zonulin (ng/mL)	27.5 (17.6)	22.9 (13.2)	0.194
Leptin (ng/mL)	10.2 (8.0)	12.5 (5.7)	0.120

*Mann-Whitney U-test, ^bChi-square test, Data are as given median (interquartile range)

BMI: body mass index, BMI-SDS: standard deviation score of body mass index, MAC: mid-arm circumference, TSF: triceps skin fold, WC: waist circumference, PBF: percentage of body fat, TC: total cholesterol, LDL-C: low-density lipoprotein cholesterol, HDL-C: high-density lipoprotein cholesterol, HOMA-IR: homeostasis model assessment of insulin resistance, SBP: systolic blood pressure, DBP: diastolic blood pressure,

and by increased exposure of intestinal cells to antigens and pathogens. Consequently, the development of inflammation in the intestinal microbiota is suggested to increase zonulin expression (22,23,24). Furthermore, adipokines and cytokines [tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6), and transforming growth factor- β], induced by fat tissues in obese individuals, trigger a metabolic imbalance (IR, type 2 diabetes mellitus, etc.) by causing subclinical systemic inflammation (25). Markers of subclinical inflammation, particularly IL-6, have been suggested to regulate the gene expression of haptoglobin-2 (HP-2) known to encode zonulin protein (3,26). In conclusion, zonulin expression is thought to be regulated by subclinical systemic inflammation as well as by local intestinal inflammation.

Increased serum zonulin levels, by weakening the cytoskeletal structure between intestinal cells, are suggested to lead to IR and to also affect the other aspects of the metabolic syndrome in obese individuals through

an increase in mucosal absorption surface (5). Studies investigating the relationship between zonulin, which has an effect on increased intestinal permeability, and IR, have yielded varying results (3,5,27). Moreno-Navarrete et al (3) were the first to investigate the association between zonulin and IR and demonstrated that serum zonulin levels were significantly higher in obese individuals when compared to non-obese individuals. In the same study, serum zonulin levels were found to correlate with anthropometric (BMI and waist-hip ratio), metabolic (fasting TG levels, uric acid, HDL-C levels, and insulin sensitivity index) and inflammatory parameters (IL-6). On the other hand, multi-regression analyses have shown that the fundamental relationship of zonulin levels is with IR index and have also demonstrated that this relationship is made possible by the subclinical inflammatory marker IL-6. In a study conducted by Zhang et al (5) on three adult patient groups with normal glucose tolerance, impaired glucose tolerance, and type 2 diabetes mellitus, zonulin was found to have a positive correlation with IR. However, Zak-Golab et al (27) could not demonstrate any relationship between zonulin levels and IR in obese adult patients and interpreted their results to be due to the small sample size and also to their inability to use oral glucose tolerance test (OGTT) instead of the HOMA-IR index in the evaluation of IR. Zonulin, which is considered a marker of intestinal permeability, is suggested to play a role in the development of the metabolic syndrome (IR, type 2 diabetes mellitus, and dyslipidemia) found in obese individuals. Similar to the findings reported by Moreno-Navarrete et al (3), our study demonstrated that serum zonulin levels were higher in obese children when compared to healthy children. This result suggests that zonulin, which is known to increase intestinal permeability, may play a role in the pathogenesis of obesity and related metabolic disturbances. Our findings, similar to those reported by Zak-Golab et al (27), also failed to show any relationship between serum zonulin levels and the IR index, HOMA-IR. However, this finding, namely, the absence of a relationship with IR index, may also be attributed to the small sample size in our study and also to the fact that metabolic syndrome was not as prominent in children as in adults.

Patients with IR have been reported to have a statistically significantly high level of TC and a low HDL-C level (28). Zhang et al (5) demonstrated that serum zonulin levels and IR have a positive correlation with TG and TC, but a negative correlation with HDL-C. In this study, zonulin was suggested to cause an increase in adipose tissue through the endocannabinoid pathway by increasing intestinal permeability, thereby leading to the development of dyslipidemia (5). However, in our study, serum zonulin levels

Table 4. Correlation coefficients and partial correlation coefficients between zonulin levels and anthropometrics and laboratory parameters

Parameters	Spearman's rho	*p-value	Partial correlation	**p-value
Age (year)	-0.287	0.01		
BMI (kg/m ²)	0.210	0.06		
BMI SDS	0.373	0.001	0.306	0.05
WC (cm)	0.192	0.094	0.202	0.206
MAC (cm)	0.164	0.154	-0.051	0.750
TSF (mm)	0.217	0.058	-0.073	0.651
Fat mass (kg)	0.217	0.105	-0.001	0.994
PBF (%)	0.423	0.001	0.120	0.456
Systolic BP (mmHg)	0.084	0.542	0.105	0.514
Diastolic BP (mmHg)	0.137	0.317	0.109	0.497
Glucose (mg/dL)	-0.147	0.193	-0.249	0.116
Insulin (uIU/mL)	0.174	0.122	0.007	0.965
HOMA-IR	0.176	0.117	-0.035	0.830
TG (mg/dL)	0.188	0.104	0.074	0.647
TC (mg/dL)	0.128	0.272	0.082	0.609
LDL-C (mg/dL)	0.246	0.032	0.197	0.216
HDL-C (mg/dL)	-0.422	< 0.001	-0.348	0.026
Leptin (pg/mL)	0.408	< 0.001	0.417	0.007

*Spearman's correlation analysis; Serum zonulin levels as dependent variable

**Partial correlation coefficient; controlling for age and body mass index

BMI: body mass index, BMI-SDS: standard deviation score of body mass index, WC: waist circumference, PBF: percentage of body fat, TC: total cholesterol, LDL-C: low-density lipoprotein cholesterol, HDL-C: high-density lipoprotein cholesterol, HOMA-IR: homeostasis model assessment of insulin resistance, SBP: systolic blood pressure, DBP: diastolic blood pressure, MAC: mid-arm circumference

and IR were not shown to have a relationship with LDL-C, TG, and TC, but were shown to have a negative correlation with HDL-C, which is in line with previous reports (5,28). Although the mechanism of the relationship of serum zonulin with HDL-C is not well understood, it is thought to be a precursor in the development of dyslipidemia and cardiometabolic imbalance.

Leptin is secreted by adipose tissue and its levels increase with the amount of adipose tissue (29). Zonulin is a peptide that increases intestinal permeability by altering the structure of TJs and is suggested to have a potential role in the etiopathogenesis of obesity (3). Hence, a close relationship between zonulin and leptin levels might be expected. However, to the best of our knowledge, there are no studies investigating the correlation between serum zonulin concentrations and serum leptin levels in children. In the present study, we found a significant positive correlation between serum zonulin concentrations and leptin levels in obese individuals.

Some limitations need to be acknowledged regarding the present study. Firstly, inflammatory markers (IL-6, TNF- α , C-reactive protein, etc.) were not measured and hence their relationship with serum zonulin could not be evaluated due

to financial constraints. Moreover, the evaluation of IR was made using the HOMA-IR index, instead of the more sensitive OGGT. Although OGTT is known to be a more sensitive index than HOMA-IR in the evaluation of IR, a strong correlation was demonstrated between IR indices detected by HOMA-IR and OGTT (30). Another point is the relationship between serum zonulin levels and celiac disease. It has been claimed that celiac disease may lead to obesity by increasing serum zonulin levels (31). Our study did not include evaluation of the obese children in terms of celiac disease.

In conclusion, the results of the present study showed that zonulin and leptin were significantly higher in obese children when compared to healthy children. Furthermore, it was reported for the first time in obese children that zonulin did not correlate with any of the anthropometric and metabolic parameters, except with serum HDL-C levels. However, further studies identifying the zonulin receptor and/or other possible cofactors will be required to elucidate the exact role of zonulin in obesity and/or IR.

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Ethics

Ethics Committee Approval: Dokuz Eylül University; Date: 24.07.2014, Number: 2014/25-04. Informed Consent: A written informed consent of the parent(s) of each subject was also obtained before the study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: Ayhan Abacı, Tuncay Küme, Ece Böber, Design: Ece Böber, Ayhan Abacı, Tuncay Küme, Gönül Çatlı, Hale Tuhan, Data Collection or Processing: Sezer Acar, Hale Tuhan, Ahmet Anık, Özlem Gürsoy Çalan, Analysis or Interpretation: Tuncay Küme, Ayhan Abacı, Özlem Gürsoy Çalan, Sezer Acar, Hale Tuhan, Literature Search: Ahmet Anık, Gönül Çatlı, Hale Tuhan, Sezer Acar, Writing: Ayhan Abacı, Sezer Acar, Hale Tuhan, Özlem Gürsoy Çalan.

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