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Zonulin level, a marker of intestinal permeability, is increased in association with liver enzymes in young adolescents

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ABSTRACT

Background: Zonulin is acknowledged as the only physiological mediator established to reversibly regulate intestinal permeability through modulation of intercellular tight junctions. We aimed to determine whether there are differences in zonulin levels between 74 subjects with overweight or obesity and 76 with normal-weight and to assess correlations of circulating zonulin levels with anthropometric measures and obesity-related biomarkers.
Methods: We assessed anthropometric and laboratory measures, including body mass index (BMI) z-score, blood pressure, liver enzymes, lipid profiles, and insulin resistance. Serum zonulin levels were measured using an enzyme-linked immunosorbent assay.
Results: The mean age of the participants was 12.8 ± 1.5 years. Circulating serum zonulin levels were significantly increased in subjects with overweight/obesity compared with those of normal-weight ($P = 0.03$). Zonulin levels were significantly and positively associated with BMI z-score, alanine aminotransferase levels, triglyceride, fasting insulin, and insulin resistance as indicated by the homeostatic model assessment of insulin resistance (HOMA-IR) (all $P < 0.05$). In multivariate linear regression analysis, alanine aminotransferase was significantly and positively associated with circulating zonulin levels in adolescents with overweight or obesity ($P < 0.01$) after controlling for the effect of potential confounding factors. BMI z-score tended to be positively associated with serum zonulin levels in this subgroup analysis ($P = 0.06$).
Conclusions: Serum zonulin is a biomarker associated with hepatic metabolic disturbances in young adolescents with overweight or obesity. The positive relationship suggests a potentially relevant pathophysiological mechanism linking zonulin to hepatic metabolism in this age group of young adolescents with overweight or obesity.

1. Introduction

Child and adolescent obesity remains prevalent across the developed world, and its prevalence is increasing in most developing countries [1,2]. There is mounting evidence reporting that childhood overweight and obesity is associated with significantly increased risk of later cardiometabolic morbidity, such as diabetes, hypertension, ischemic heart disease, and stroke, and premature mortality in adulthood [3].

Research work in the pathogenesis of obesity suggests that it may be the end result of increased intestinal permeability and absorption [4] and is closely correlated with sudden increases in intestinal absorptive capacity by increasing in amounts of absorptive mucosa [4]. Recently, it has become apparent that intestinal permeability controls molecular trafficking between the intestinal lumen and the submucosa, inducing either tolerance or immune responses to foreign antigens such as food antigens [5,6]. It is the intercellular tight junctions (TJs) that regulate this paracellular antigen trafficking in a well-orchestrated manner. TJs are thought to be exceptionally effective structures operative in several main functions of the intestinal epithelium under both physiological and pathological circumstances [7]. Chronic high circulating levels of inflammatory cytokines, which are often observed in subjects with obesity, may be an important contributor to intestinal barrier dysfunction through alterations of structure and localization of TJs [8].

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Furthermore, accumulating evidence links the intestinal microbiome and inflammation, the intestinal barrier integrity, and liver diseases [9].

Zonulin is the only physiological protein known to control intestinal permeability reversibly via modulation of intercellular TJs [10,11]. It is a 47-kDa protein that increases intestinal permeability in the small intestine, is actively engaged in intestinal innate immunity [12], and is overexpressed in autoimmune diseases where TJ dysfunction plays a pivotal role, such as celiac disease [13,14] and type 1 diabetes [15]. In this regard, circulating zonulin is considered a useful biomarker of intestinal permeability [11,16]. In humans, increased zonulin levels have been reported to be closely correlated with increased intestinal permeability caused by genetic overexpression of intestinal TJ proteins [15]. Recent evidence have demonstrated higher circulating zonulin concentrations in adults with obesity compared with controls [17] and in adults with glucose intolerance compared with those with normal glucose tolerance [18].

Despite increasing data that zonulin plays a key role in the pathophysiology of obesity [17] and insulin resistance [18], few studies have investigated its relationship with obesity-related clinical factors and/or laboratory biomarkers in adolescent populations [19,20]. Understanding these relationships in this age group may help advance our understanding of the pathophysiology of adolescent obesity and metabolic dysregulation.

Therefore, we aimed to determine whether there is a difference in serum zonulin levels between subjects with overweight/obesity and those of normal-weight and to assess the relationship between serum zonulin levels and anthropometric measures and obesity-related biomarkers, such as liver enzymes, lipid profiles, and insulin resistance in a population of young adolescents.

2. Materials and methods

2.1. Study subjects

One hundred and seventy one adolescents aged 12 to 13 years underwent Student Health Examinations at a designated local clinic in Seoul, Korea from May through October 2015. Subjects meeting any of the following criteria were excluded ($n = 23$): a history of acute infection and/or recent use of antibiotics, cigarette smoking, food allergies [21], celiac disease [22], psychological and/or physical stressful conditions [23], alcoholic beverages, any missing covariate information, a medication history of steroids, aspirin [24], insulin, glucose regulators, or antihypertensive drugs, refusal to take the test, or failure to fast for at least 12 h before blood sampling. After these exclusions, 150 subjects (83 boys and 67 girls) were included in the final analysis.

Health examinations were performed by a single physician according to a standardized procedure. Body weight and height were measured to the nearest 0.1 kg and 0.1 cm, respectively, using an automatic height–weight scale and with the subjects wearing light indoor clothing and no shoes. BMI was calculated as weight (in kilograms) divided by height (in meters) squared. Subjects' waists were measured with a soft tape midway between the lowest rib and the iliac crest; hip circumference was measured at the widest part of the gluteal region; and waist-to-hip ratio was accordingly calculated. The BMI percentiles for sex and age were determined according to the 2007 Korea Growth Charts [25]. The participants were classified into three groups according to their BMI value: a subgroup of normal-weight (BMI: < 85th percentile), a subgroup with overweight (BMI: 85th–95th percentile), and a subgroup with obesity (BMI: ≥ 95th percentile). A registered nurse measured blood pressure (BP) a maximum of three times on the right arm in seated subjects after a 5-min rest using a sphygmomanometer. The sexual maturity rating was determined according to Tanner stages by the physician. The work described in the current study was carried out in accordance with *The Code of Ethics of the World Medical Association (Declaration of Helsinki)*. The institutional review board approved the study, and written informed consent was obtained from parents or guardians and consent/assent from the children after full explanation of the purpose and nature of all procedures used. We assessed that based on a *two-tailed test* at 85% power, a standard deviation of 10, and $\alpha = 0.05$, a difference in means of at least 5 could be detected with a sample size of 72 per group ($n = 2{\cdot}W / [\Delta/\sigma]^2 = [2{\cdot}9.0] / [5/10]^2 = 72$).

2.2. Laboratory measurements for obesity-related biomarkers

Following a 12-h overnight fast, blood samples were obtained from the antecubital vein of each subject by venipuncture and were immediately centrifuged, aliquoted, and frozen at −20 °C. The frozen serum samples were stored at −80 °C until analysis. Fasting serum glucose, total cholesterol, triglyceride, and high-density lipoprotein cholesterol (HDL cholesterol) levels were measured by enzymatic methods using a Hitachi Modular D2400 automated chemistry analyzer (Hitachi, Tokyo, Japan). Levels of low-density lipoprotein cholesterol (LDL cholesterol) were calculated using the following formula: LDL cholesterol = total cholesterol – HDL cholesterol − (triglyceride/5). Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were determined by the catalytic concentration from the rate of decrease of nicotinamide adenine dinucleotide measured at 340 nm by means of lactate dehydrogenase coupled reaction. Fasting insulin levels were measured using a chemiluminescent microparticle immunoassay (Abbott Architect System, Irving, TX, USA). Insulin resistance was estimated using the homeostatic model assessment of insulin resistance (HOMA-IR) and calculated using the following formula: HOMA-IR = [fasting insulin (μIU/mL) × fasting glucose (mg/dL)/18]/22.5 [26]. We also subjected the fasting data to various transformations and ultimately defined quantitative insulin sensitivity check index (QUICKI = $1/[\log(I_0) + \log(G_0)]$), where I_0 is the fasting insulin, and G_0 is the fasting glucose [27].

2.3. Measurement of serum zonulin

Serum samples were collected and stored at −80 °C for zonulin assays. Concentrations of zonulin were measured by using competitive enzyme-linked immunosorbent assay (ELISA) kits (K5600, Immundiagnostik AG, Bensheim, Germany) according to the manufacturer's instructions. The absorbance values for the ELISA assays were determined with an Infinite 2000 Pro multimode plate reader (Tecan, Vienna, VA, USA) at 450 nm.

2.4. Statistical analysis

All continuous variables are presented as mean ± standard deviation or median with interquartile range (IQR), or are shown as scatter plots. Categorical variables are presented as number (%). Between-group differences were compared using the Independent Student *t*-tests, the Mann-Whitney *U* tests, the Kruskal-Wallis tests, or the chi-square tests, as appropriate. Spearman's correlation analyses were used to calculate the correlation between serum zonulin levels and anthropometric measures and obesity-related biomarkers. Anthropometric and biochemical parameters according to zonulin quartiles were summarized using the Kruskal-Wallis test for continuous variables and the chi-square test for categorical variables. Zonulin quartiles were categorized separately as follows: Q1, < 38.6; Q2, 38.6–49.8; Q3, 49.9–58.4; and Q4, > 58.4. To examine independent correlates of serum zonulin levels, a multivariate linear regression analysis was conducted with zonulin level as the dependent variable and clinical and laboratory factors as the independent variables. All statistical analyses were performed by using IBM SPSS Statistics ver. 21.0 (IBM Co., Armonk, NY, USA). All statistical tests were two-sided, with a P value of < 0.05 indicating statistical significance.