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Associations of Adipocyte-derived Versican and Macrophage-derived Biglycan with Body Adipose Tissue and Hepatosteatosis in Obese Children

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What is already known on this topic?

In animal models of obesity, adipocyte-derived versican and macrophage-derived biglycan play a crucial role in mediating adipose tissue inflammation. Inhibition of versican in mouse models reduces macrophage accumulation, inflammatory gene expression, and liver inflammation, leading to improved glucose tolerance and insulin sensitivity.

What this study adds?

This is the first study to report elevated levels of versican in obese children and a positive correlation between versican and inflammatory markers, such as interleukin-6 and high sensitivity C-reactive protein. This suggests that attenuating versican release in obese individuals may have the potential to decelerate the inflammatory process, thereby reducing associated complications.

Abstract

Objective: In animal models of obesity, adipocyte-derived versican, and macrophage-derived biglycan play a crucial role in mediating adipose tissue inflammation. The aim was to investigate levels of versican and biglycan in obese children and any potential association with body adipose tissue and hepatosteatosis.

Methods: Serum levels of versican, biglycan, interleukin-6 (IL-6), and high sensitivity C-reactive protein (hsCRP) were measured by ELISA. Fat deposition in the liver, spleen, and subcutaneous adipose tissue was calculated using the IDEAL-IQ sequences in magnetic resonance images. Bioimpedance analysis was performed using the Tanita BC 418 MA device.

Results: The study included 36 obese and 30 healthy children. The age of obese children was 13.6 (7.5-17.9) years, while the age of normal weight children was 13.0 (7.2-17.9) years ($p = 0.693$). Serum levels of versican, hsCRP, and IL-6 were higher in the obese group ($p = 0.044$, $p = 0.039$, $p = 0.024$, respectively), while no significant difference was found in biglycan levels between the groups. There was a positive correlation between versican, biglycan, hsCRP, and IL-6 ($r = 0.381$ $p = 0.002$, $r = 0.281$ $p = 0.036$, rho = 0.426 $p = 0.001$, $r = 0.424$ $p = 0.001$, rho = 0.305 $p = 0.017$, rho = 0.748 $p < 0.001$, respectively). Magnetic resonance imaging revealed higher segmental and global hepatic steatosis in obese children. There was no relationship between hepatic fat content and versican, biglycan, IL-6, and hsCRP. Versican, biglycan, hsCRP, and IL-6 were not predictive of hepatosteatosis. Body fat percentage > 32% provided a predictive sensitivity of 81.8% and a specificity of 70.5% for hepatosteatosis [area under the curve (AUC): 0.819, $p < 0.001$]. Similarly, a body mass index standard deviation score > 1.75 yielded a predictive sensitivity of 81.8% and a specificity of 69.8% for predicting hepatosteatosis (AUC: 0.789, $p < 0.001$).

Conclusion: Obese children have higher levels of versican, hsCRP, and IL-6, and more fatty liver than their healthy peers.

Keywords: Chronic inflammation, biglycan, hepatosteatosis, obesity, versican

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Introduction

Obesity is an epidemic condition affecting all age groups worldwide, and the prevalence is increasing rapidly (1). Adipose tissue serves not only as a primary site of storage for excess energy but may also trigger a chronic inflammatory process through the secretion of autocrine/paracrine molecules and cytokines (2,3). Lymphocytes and macrophages accumulated in adipose tissue release various proinflammatory/anti-inflammatory molecules such as tissue necrosis factor-alpha (TNF-α), interleukin-1 (IL-1), IL-4, IL-6, IL-10. Moreover, the adipocytes also release molecules, such as leptin, adiponectin, visfatin, resistin, and adipsin, thereby initiating a chronic inflammatory process (2,4,5). This process, originating in adipose tissue, culminates in systemic inflammation and may give rise to complications, such as insulin resistance, metabolic syndrome, and type 2 diabetes mellitus (6,7). In addition, increased extracellular matrix (ECM) molecules and their degradation products function as immunomodulators (6,8,9). Identifying ECM components associated with adipose tissue inflammation and metabolic disturbances is important for a better understanding of this process (10).

Versican, released from hypertrophic adipocytes under inflammatory conditions, functions as a proteoglycan, rich in chondroitin sulfate. Versican functions by binding to serum amyloid A in high density lipoproteins (HDL). Versican is known to regulate events associated with adipose tissue inflammation, including lipoprotein retention, lipid uptake, and foam cell formation. Furthermore, versican interacts with molecules, such as chemokines, growth factors, proteases, and immune cell receptors, including CD44, p-selectin glycoprotein-1 and toll-like receptor-2 (TLR2), facilitating the formation of intracellular signals (6,10,11,12).

Another proinflammatory molecule, biglycan, is a small proteoglycan rich in leucine and serves as a structural scaffold by interacting with collagen and elastin molecules in the ECM under physiological conditions. In addition, biglycan production increases in adipose tissue during inflammatory states due to the accumulation of macrophages in the tissues. Elevated biglycan molecules bind to TLR2 and TLR4, inducing the secretion of proinflammatory cytokines such as TNF-α and IL-1β, thus playing a role in adipose tissue inflammation (6,10,13).

Han et al. (6) investigated the effects of adipose tissue proteoglycans on inflammation and insulin resistance. They examined the molecules versican, released from adipocytes, and biglycan released from macrophages. In their experiments with mice, they observed an increased presence of versican and biglycan molecules in the adipose tissue of obese mice. Through targeted deletion of adipocyte-specific versican, the researchers noted a mitigation of macrophage chemotaxis. This intervention was associated with a reduction in the expression of inflammatory genes, attenuation of hepatic inflammation, augmentation of insulin sensitivity, and improvement in glucose tolerance. These findings suggest that versican exerts a regulatory influence on these processes. Furthermore, deletion of macrophage-specific biglycan led to reduced macrophage accumulation and cytokine/chemokine release. However, while a decrease in liver inflammation and an increase in insulin sensitivity were observed with versican deletion, these effects were not evident in mice with biglycan deletion. This study demonstrated the association of elevated biglycan levels with inflammation, obesity, insulin resistance, and type 2 diabetes in mice (6).

An inflammatory process in adipose tissue contributes to the early development of insulin resistance, dyslipidemia and hepatosteatosis in obesity (14). Although ultrasonography (US) is commonly used for detecting non-alcoholic fatty liver disease (NAFLD), which is the most prevalent chronic liver condition, liver biopsy remains the gold standard diagnostic tool (15). However, biopsy, being an invasive procedure, can yield false negatives in patients without diffuse hepatosteatosis. In recent years, a noninvasive method known as "iterative decomposition of water and fat with an echo asymmetry at least-square estimation-iron quantification (IDEAL-IQ) sequence," utilized through magnetic resonance imaging (MRI), has emerged as a reliable means for the detection of NAFLD (16).

A clinical study investigating the relationship between versican, biglycan, and metabolic parameters related to obesity has not been published to date. In the present study, the levels of versican and biglycan, which are believed to play a significant role in the etiopathogenesis and complications of obesity, were investigated in obese children. A further aim was to explore the association of these molecules with adipose tissue, hepatosteatosis, and inflammation in the context of pediatric obesity.

Methods

The study included obese children aged 7-18 years presenting to a single pediatric endocrinology clinic with complaints of weight gain. These children had a body mass index (BMI) ≥95th percentile, based on national data from Turkish children. Gender and age-matched healthy children with BMI <85th percentile were selected as the control group.

Patients underwent detailed physical examinations and laboratory tests were conducted to assess the possibility of underlying endocrine pathologies. Cases with any chronic diseases, a history of medication use, identified endocrine pathologies, and cases suspected of syndromic or monogenic origins of obesity were excluded. Anthropometric measurements were carried out using a Harpenden stadiometer (Crosswell, Crymych, Pembs., SA41 3UF, UK) with a precision of 0.1 cm for height and a SECA scale (Hammer Steindamm 3-25 22089, Hamburg, Germany) with a precision of 0.1 kg for weight. Patients were evaluated after removing all clothing except thin underwear. BMI was calculated by dividing body weight (kg) by the square of measured height (m) and then transformed into standard deviation score (SDS) using national BMI reference data (17).

Blood pressure measurements were conducted by one of the investigators following a validated protocol. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were taken twice from the right arm after a 10-minute rest in the supine position, using a calibrated sphygmomanometer with appropriate cuff size (18). Waist circumference was measured using a flexible but not stretchable tape, positioned midway between the lowest rib and the superior border of the iliac crest (19). The measurement of triceps skinfold thickness was performed using a Holtain skinfold caliper (Crosswell, Crymych, Pembs., SA41 3UF, UK). One investigator performed triceps skinfold thickness measurements by grasping a fold of skin and subcutaneous adipose tissue approximately 2.0 cm above the mid-arm circumference mark. The procedure involved placing the tips of the caliper jaws over the entire skinfold, followed by releasing the caliper handle to apply full tension on the skinfold. The thickness was then read to the closest 0.1 mm (20). Bioelectrical impedance analysis was performed according to standards using the Tanita BC 418 MA device (Maenocho Itabashi-Ku, Tokyo, 174-0063 Japan). The basal metabolic rate was determined through bioimpedance analysis.

Fasting blood samples were collected from a peripheral vein between 08:00 and 09:00 in the morning after a minimum of 12 hours of fasting. Serum fasting glucose, insulin, glycated hemoglobin (HbA1c), liver and thyroid function tests, total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL), and HDL levels were measured using enzymatic colorimetric methods. Biochemical analyses were performed using original reagents on an auto analyzer with standardized methods at Aydın Adnan Menderes University Faculty of Medicine Hospital. To assess insulin resistance, the Homeostasis Model Assessment-Insulin Resistance (HOMA-IR) index was used. Different cut-off values were employed for prepubertal and pubertal subjects to evaluate insulin resistance. The cut-off values for the HOMA-IR index were 2.22 for prepubertal girls, 2.67 for prepubertal boys, 3.82 for pubertal girls, and 5.22 for pubertal boys (21).

Serum levels of versican (cat no: SL1818Hu, detection range: 16-1000 pg/mL, sensitivity: 4.5 pg/mL; Sunlong, Hangzhou, China), biglycan (cat no: SL2244Hu, detection range: 0.08-4.0 ng/mL, sensitivity: 0.01 ng/mL; Sunlong, Hangzhou, China), IL-6 (cat no: 201-12-0091, detection range: 3-600 ng/L, sensitivity: 2.112 ng/L; Sunred, Shanghai, China), and high sensitivity C-reactive protein (hsCRP) (cat no: 201-12-1816, detection range: 0.15-40 ng/L, sensitivity: 0.112 ng/L; Sunred, Shanghai, China) were measured using commercial kits following the manufacturer's directions. These commercial kits used antibody-coated plates and a sandwich ELISA method. Serum samples were applied onto these plates, followed by incubation following the kit procedure to allow the specific binding of the relevant molecules to the specific antibodies. Subsequent washing steps were conducted to remove unbound molecules, a second antibody with a chromogen was added and measurements were taken at 450 nm using an ELISA reader. The results were then calculated based on the standard curve included.

Hepatosteatosis was assessed by both US and MRI. US was performed in the supine position by an experienced radiologist using a Sonostar C5PL portable handheld ultrasound device (Sonostar Technologies Co. Ltd., Guangzhou, China). The definition of hepatosteatosis was based on the increased difference in echogenicity between the liver and kidney. The evaluation was categorized into no steatosis (grade 0), mild (grade 1), moderate (grade 2), and severe (grade 3) steatosis using a previously published ultrasound steatosis score (22,23). MRI was conducted using a General Electric 3T Sigma Pioneer SW 29.0_R01_2034.a device (General Electric Company Neumann Way Cincinnati, OH 45215). The IDEAL-IQ sequence, a brief imaging protocol without contrast, was employed to obtain cross-sectional images encompassing the liver, spleen, and subcutaneous adipose tissue within the abdominal region. The acquired images were used to calculate the percentages of fat in the liver, spleen, and subcutaneous adipose tissue using the GE AW 4.7 version work station. The liver parenchyma was divided into nine segments and measurements were taken. In the segmental measurement technique, each segment of the liver was measured separately, and the average of the measurements was taken. In the global measurement technique, the entire liver parenchyma was measured in a single session (16).

Statement of Ethics

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the Non-interventional Ethics Committee of Aydın Adnan Menderes University (ethics no: 2021/83, date: 24.06.2021). Informed consent in this study was taken from all participants.

Statistical Analysis

The statistical analysis of the data was conducted using the Statistical Package for Social Sciences, version 21 (IBM Corp., Armonk, NY, USA). The normality of continuous variables was assessed through descriptive statistics, skewness, kurtosis coefficients, histograms, and the Shapiro-Wilk test. Descriptive statistics are presented using counts, percentages, means, and standard deviations for normally distributed data, and medians, minimum, and maximum values for non-normally distributed data. For categorical variables, the chi-squared test was used in the statistical analysis. For independent group comparisons, if the data followed a normal distribution, the t-test was applied, and if not, the Mann-Whitney U test was used. Spearman correlation and receiver operator curve (ROC) analysis were used. The type 1 error level was set at 5%, and p values less than 0.05 were considered statistically significant.

Results

In total, 36 obese and 30 healthy children were included in the study. There were no significant differences between the obese and healthy groups in terms of age, gender, height, and DBP. However, obese individuals exhibited higher weight, BMI, SBP, waist circumference, triceps skinfold thickness, basal metabolic rate, and body fat percentage.

In the obese group, serum insulin levels were higher, and the incidence of insulin resistance was greater (Table 1). In addition, TG, LDL cholesterol, HbA1c, alanine aminotransferase (ALT), and white blood cell count were higher among the obese individuals, while HDL cholesterol levels were lower. TC, aspartate aminotransferase, and thyroid function tests showed similar results between the two groups (Table 1).

When compared to the control group, serum levels of versican, IL-6, and hsCRP were higher in obese children, whereas biglycan levels were similar between the two groups (Table 1).

Table 2 shows the associations between versican, biglycan, hsCRP, and IL-6. There was no relationship between the

Table 1. Clinical, demographic, and laboratory characteristics of enrolled cases

	Obese n = 36	Controls n = 30	p*
	Median (min-max)	Median (min-max)	
Age (year)	13.6 (7.5–17.9)	13.0 (7.2-17.9)	0.693
Height (SDS)	0.8 (-2.1–-3.2)	0.3 (-2.2–2.8)	0.114
Weight (SDS)	2.9 (2.0- – -6)	0.1 (-1.9–1.7)	**< 0.001**
BMI (SDS)	2.6 (1.9–3.3)	0.4 (-0.6–0.7)	**< 0.001**
Systolic BP (mmHg)	120 (100-140)	110 (105-134)	**< 0.001**
Diastolic BP (mmHg)	70 (60-91)	70 (55-99)	0.435
Waist circumference (cm)	101 (42-141)	70.6 (55-96.5)	**< 0.001**
Triceps skinfold thickness (mm)	19.5 (8-37)	10.3 (4-18)	**< 0.001**
Glucose (mg/dL)	89.5 (77-146)	88 (76-115)	0.622
Insulin (µU/mL)	17.2 (7.9-42.8)	10.4 (3-19)	**< 0.001**
HOMA-IR index	3.8 (1.7-9.1)	2.2 (0.6-4.3)	**< 0.001**
Triglyceride (mg/dL)	94.5 (26-559)	65 (25-169)	**0.001**
Total cholesterol (mg/dL)	156.5 (117-214)	151.5 (90-207)	0.123
LDL-C (mg/dL)	86.5 (38-149)	78.5 (37-132)	**0.038**
HDL-C (mg/dL)	48.4 (25.2-73.1)	55.3 (38.1-119.9)	**0.005**
HbA1c (%)	5.5 (4.5-6.2)	4.8 (3.9-5.8)	**0.006**
AST (U/L)	19 (9-173)	20 (13-72)	0.111
ALT (U/L)	18.5 (8-311)	15 (10-25)	**0.022**
Free T_4 (ng/dL)	1 (0.8-1.2)	0.9 (0.8-1.2)	0.086
TSH (uIU/mL)	1.8 (0.7-5.1)	1.8 (0.5-9)	0.359

Table 1. Continued

	Obese n = 36	Controls n = 30	p*
	Median (min-max)	Median (min-max)	
WBC (10^3/μL)	9080 (6070-13410)	6520 (3990-12510)	**< 0.001**
Versican (pg/mL)	63.6 (48.3-78.3)	59.1 (44.3-80.2)	**0.044**
Biglycan (ng/mL)	1.2 (0.4-1.7)	1.0 (0.4-1.5)	**0.176**
IL-6 (ng/L)	48.3 (26.1-119.6)	34.4 (8.3-120.4)	**0.024**
hsCRP (ng/L)	5.1 (2.1-9.7)	3.7 (1.3-8.7)	**0.039**
Basal metabolic rate (kcal)	1632.0 (929-3022)	1230.5 (777-2032)	**< 0.001**
Fat mass (kg)	30.0 (9.9-56.1)	10.3 (3.9-26.9)	**< 0.001**
Fat percentage	36.2 (26.9-61.9)	21.9 (10.8-31.6)	**< 0.001**
Liver FQ global method	6 (1.9-25.9)	2.6 (1.3-6.4)	**< 0.001**
Spleen FQ	2 (0.5-7.1)	2.3 (1.3-9.6)	0.143
Subcutaneous fat FQ	93 (88.9-96.3)	92.7 (81.6-98.3)	0.949

*Mann-Whitney U test was used.
min-max: minimum-maximum, BMI: body mass index, BP: blood pressure, HOMA-IR: Homeostasis Model Assessment-Insulin Resistance, LDL-C: low-density lipoprotein-cholesterol, HDL-C: high-density lipoprotein-cholesterol, hsCRP: high sensitive C-reactive protein, IL-6: interleukin-6, FQ: fat quantity, SDS: standard deviation score

Table 2. Correlation table for versican, biglycan, IL-6, and hsCRP

	Biglycan		hsCRP		IL-6	
	r	p	r	p	rho	p*
Versican	0.381	0.002	0.281	0.036	0.426	**0.001**
Biglycan			0.424	0.001	0.305	**0.017**
hsCRP					0.748	**< 0.001**

*Spearman correlation was used.
hsCRP: high sensitive C-reactive protein, IL-6: interleukin-6

degree of hepatosteatosis and serum levels of versican, biglycan, IL-6, and hsCRP. Furthermore, no correlation was found between metabolic parameters (glucose, HbA1c, insulin, HOMA-IR, lipid profile, thyroid-stimulating hormone, sT4, and leukocyte) and serum levels of versican and biglycan.

Similar results were obtained from both US and MRI for the assessment of hepatic fat content. When comparing groups based on MRI findings, obese children had significantly higher liver fat content than the control group, using both the segmental and global measurement techniques ($p < 0.001$). Spleen fat levels were similar in both groups. Liver fat content was positively correlated with TG, LDL, HbA1c, ALT, white blood cell count, basal metabolic rate, and body fat percentage, while it was negatively correlated with HDL ($r = 0.333$ $p = 0,013$, $r = 0.268$ $p = 0,048$, $r = 0.339$ $p = 0.006$, $r = 0.365$ $p = 0.006$, $r = 0.529$ $p < 0.001$, $r = 0.310$ $p = 0.019$, $r = 0.634$ $p < 0.001$, $r = -0.330$ $p = 0.014$, respectively). Patients with hepatosteatosis had higher levels of HbA1c, white blood cells, insulin, HOMA-IR, TG, LDL, ALT, free thyroxine (fT4), body fat percentage, and body fat weight (all $p < 0.05$). Participants with higher body fat percentages exhibited significantly higher serum versican levels ($rho = 0.318$ $p = 0.012$), while those with more subcutaneous adipose tissue had higher IL-6 levels ($rho = 0.255$ $p = 0.047$).

Serum levels of versican, biglycan, hsCRP, and IL-6 were not significantly predictive of hepatosteatosis ($p > 0.05$). A body fat percentage of over 32% had a predictive sensitivity of 81.8% and specificity of 70.5% [area under the curve (AUC): 0.819, $p < 0.001$] for hepatosteatosis. Similarly, a BMI SDS above 1.75 yielded a predictive sensitivity of 81.8% and specificity of 69.8% (AUC: 0.789, $p < 0.001$) for predicting hepatosteatosis (Figure 1, Table 3).

Discussion

This study, which investigated the relationship between serum versican and biglycan levels with metabolic parameters and hepatosteatosis in obese children, revealed that versican levels were higher in obese children and there was a positive correlation between versican and inflammatory markers, such as IL-6 and hsCRP (2,24).

Due to the chronic inflammatory process, inflammatory markers are known to be elevated in obese individuals (2,5,7,25). Furthermore, several studies have demonstrated

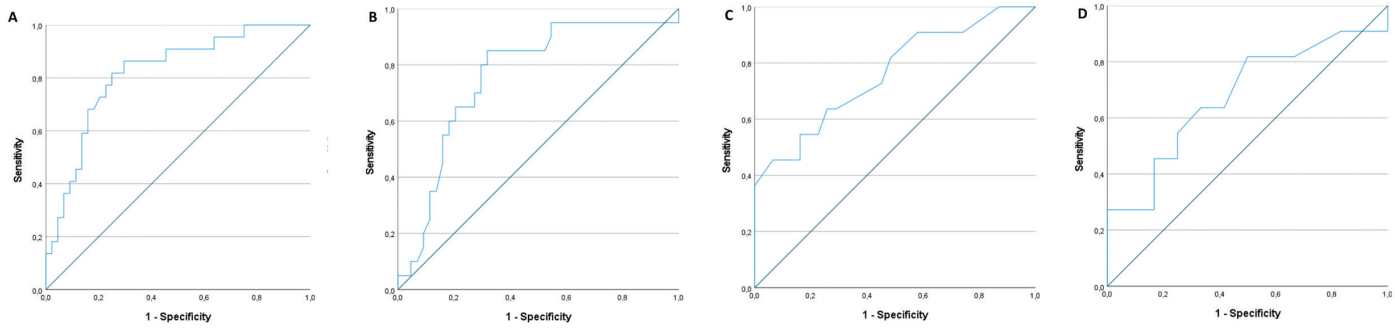

Figure 1. ROC curve of hepatosteatosis for the four parameters: (a) body fat percentage, (b) waist circumference, (c) ALT (girls), (d) ALT (boys)

ROC: receiver operator curve, ALT: alanine aminotransferase

Table 3. Clinical and laboratory predictors of hepatosteatosis

Criteria	Sensitivity	Specificity	AUC	95% CI	p*
Body fat percentage > 32 %	81.8 %	70.5 %	0.819	0.711-0.933	**< 0.001**
BMI SDS > 1.75	81.8 %	69.8 %	0.789	0.659-0.918	**< 0.001**
Waist circumference > 90 cm	70.0 %	70.5 %	0.760	0.631-0.888	**0.001**
ALT > 22 U/L (girls)	45.5 %	83.9 %	0.762	0.590-0.935	**0.010**
ALT > 25 U/L (boys)	45.5 %	75.0 %	0.678	0.451-0.905	0.148

*ROC analysis was used.
BMI: body mass index, SDS: standard deviation score, AUC: area under the curve, CI: confidence interval, ALT: alanine aminotransferase

that complications such as hepatosteatosis, metabolic syndrome, and type 2 diabetes mellitus arise from the chronic inflammation seen in obesity (24,25,26). In our study, inflammatory markers IL-6 and hsCRP were found to be higher in obese children compared to the control group. This finding implies that the origins of complications are established during the early stages of life.

The number of studies focusing on the role of versican in the regulation of inflammation and immunity is steadily increasing. Versican, with five known isoforms, binds to various receptors and components involved in the inflammatory response, playing a pivotal role in both pro- and anti-inflammatory processes (9). In experiments conducted with obese mice, it has been demonstrated that obese mice exhibit increased production of versican from adipocyte cells. Inhibition of versican production from adipocytes has been shown to reduce macrophage accumulation, inflammatory gene expression, and liver inflammation, resulting in improved insulin sensitivity and glucose tolerance (6). In various human studies, the association between versican and inflammation, such as cardiovascular diseases, respiratory diseases, and certain cancer types has been investigated and increased serum versican levels have been reported in these diseases when inflammation is present (9,12,27). However and to the best of our knowledge, there is no published study investigating versican levels in obese humans. In the present study and consistent with published animal studies, serum versican levels in obese children were found to be higher compared to the control group (6). This suggests that interventions aimed at preventing the increased release or accumulation of versican might slow the inflammatory process, thereby reducing the complications caused by chronic inflammation in obese individuals.

High levels of biglycan have been associated with inflammation, obesity, insulin resistance, and type 2 diabetes mellitus (10,28). However, unlike versican, the relationship between biglycan and hepatosteatosis has not been established (6,10). Previous animal studies have shown a link between obesity, insulin resistance, and biglycan levels. Nevertheless, in our study, no significant differences were observed in serum biglycan levels between obese children and the control group. This suggests that the *in vivo* relationship might be different in different species, or this relationship might manifest later in life and may not be evident in the childhood age group. In line with the literature, the present study also found no correlation between serum biglycan levels and liver fat content (6,28).

In obese individuals, the chronic inflammatory process associated with increased adipose tissue, which is both a cause and a consequence of obesity, is known to lead to elevated inflammatory markers, including hsCRP (29,30,31). Furthermore, as previously mentioned, the increased

production and secretion of versican and biglycan due to the expansion of adipose tissue and their relationship with inflammatory cells have been shown to play a role in chronic inflammation (6,8,9). In the present study, a strong positive correlation was observed between hsCRP, versican, and biglycan levels, all of which have functional roles in the chronic inflammatory process. Partial correlation analysis was performed, revealing that the associations between versican, biglycan, IL-6, and hsCRP persisted in a similar manner. The correlation of versican and biglycan levels with hsCRP and IL-6 in obese children suggests a potential role for versican and biglycan in the inflammation process of obesity. Based on insights from animal studies, when evaluating the relationship between serum versican and biglycan levels with metabolic parameters yielded no evident correlation. The lack of correlation between versican and biglycan with metabolic parameters may have been due to the small sample size in this study.

Steatosis involving more than 5% of the weight of hepatocytes or liver tissue is considered abnormal (32). Studies on the accurate detection and grading of NAFLD have been continuing for many years. The gold standard method for the quantitative diagnosis of hepatosteatosis remains biopsy. However, the routine use of biopsy is quite limited due to its invasiveness and sampling error risk (33). Ultrasound is an economical and useful method, but it is highly subjective, and its quantitative and objective criteria are not clear (34). Even though US is relatively easy to perform and interpret, some limitations may be encountered: a quantitative assessment is not performed, when lower than 20% steatosis may not be detected (35). MRI techniques are currently in clinical use for the detection and quantification of hepatic steatosis (36,37). The IDEAL-IQ method of MRI is based on the water and oil separation technique based on chemical change to obtain the proton-dense oil fraction. Many studies have shown that using IDEAL-IQ to test the stability and reproducibility of liver fat is acceptable and has high accuracy (16,38). MRI accurately classifies grades and changes in hepatosteatosis, with 80.0-95.8% sensitivity and 83.6-100% specificity (39,40). However, due to the high cost, time-consuming nature, and limited accessibility of MRI, there is a need for more practical and cost-effective methods to identify hepatosteatosis. Considering this objective, we systematically assessed the relationship between hepatosteatosis identified via MRI, and various biochemical and auxological parameters. In concordance with the existing literature, most of the participants manifesting hepatosteatosis exhibited obesity, with this condition correlating with elevated levels of liver fat accumulation and an augmented ratio of subcutaneous adipose tissue. Similar to previous studies, hepatosteatosis demonstrated positive correlations with TG, LDL, HbA1c, ALT, white blood cell count, basal metabolic rate, and body fat ratio, while exhibiting a negative correlation with HDL (32,40,41). Based on ROC analysis, similar to NASPGHAN, ALT displayed predictive efficacy for hepatosteatosis in females, yielding an AUC of 0.762, 45.5% sensitivity, and 83.9% specificity, utilizing a cutoff of 22 U/L (32). The absence of a significant cut off value in males was most likely due to the smaller numbers involved in our study.

The presence and severity of hepatosteatosis increase with higher waist circumference, BMI SDS, and body fat ratio (42,43). Consistent with these findings, our study identified a relationship between hepatosteatosis and these parameters. Specifically, our results revealed that body fat percentage $>32\%$, BMI SDS >1.75, and waist circumference >90 cm indicating the presence of hepatosteatosis and were in line with previous studies (42,44). However, our study could not establish a significant relationship between hepatosteatosis and IL-6, hsCRP, versican and biglycan, primarily attributed once again to the limited number of participants. Nevertheless, our findings underscored that the most reliable predictors for hepatosteatosis were body fat ratio and BMI SDS.

Study Limitations

The inability to perform a power analysis due to the absence of a similar study in the literature represents a significant limitation of the study. Consequently, the sample size obtained may have been relatively limited as a result of this constraint. Moreover, the patients were not anesthetized during imaging, so movement artifacts occurred in some patients. In the technique we used, the resolution of the liver fat measurement sequence is low,and the presence of fat was not confirmed by biopsy, which is the gold standard method. Additionally, adiposity was measured once by a single radiologist.

Conclusion

This is the first study to report elevated levels of versican in obese children, concomitant with other accepted inflammatory markers. These findings indicated that slowing down the release of versican in obese individuals may mitigate the inflammatory process, as suggested by animal studies, potentially reducing complications. Furthermore, the study showed that waist circumference, BMI SDS and body fat ratio can be used to predict hepatosteatosis identified through the IDEAL-IQ MR sequence. However, further studies with a larger population are needed to identify novel predictive markers for hepatosteatosis.

Ethics

Ethics Committee Approval: The study was approved by the Non-interventional Ethics Committee of Aydın Adnan Menderes University (ethics no: 2021/83, date: 24.06.2021).

Informed Consent: Informed consent in this study was taken from all participants.

Authorship Contributions

Concept: Ahmet Anık, Design: Ahmet Anık, Data Collection or Processing: Reyhan Deveci Sevim, Sebla Güneş, Analysis or Interpretation: Reyhan Deveci Sevim, Mustafa Gök, Özge Çevik, Ömer Erdoğan, Literature Search: Reyhan Deveci Sevim, Tolga Ünüvar, Writing: Reyhan Deveci Sevim, Ahmet Anık.

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