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# The Level of Inflammatory Markers and Their Relationship with Fat Tissue Distribution in Children with Obesity and Type 2 Diabetes Mellitus

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## What is already known on this topic?

- *Obesity induces systemic, chronic, and low-grade inflammation characterized by high/normal concentrations of inflammatory markers in children and adolescents. Insulin resistance and pathogenesis of type 2 diabetes mellitus have been associated with subclinical chronic inflammation and activation of the immune system.*

## What this study adds on this topic?

- *Proinflammatory markers in the type 2 diabetes group were similar to those in the obese/overweight and control groups, and transforming growth factor-β level, an anti-inflammatory marker, was lower than those in the control group.*

## ABSTRACT

**Objective:** This study aimed to determine the changes in proinflammatory and anti-inflammatory markers in children aged 10-18, who were not diagnosed with type 2 diabetes mellitus, were obese/overweight, and children with type 2 diabetes mellitus. In addition, we aimed to investigate whether these markers were associated with clinical and laboratory parameters, subcutaneous adipose tissue, preperitoneal adipose tissue, visceral adipose tissue, and hepatosteatosis.

**Materials and Methods:** Children between the ages of 10 and 18, obese/overweight, with type 2 diabetes mellitus, and with a normal body mass index were included. Fat tissue thickness was measured. Tumor necrosis factor-α, interleukin-1β, interleukin-6, interleukin-18, and interferon-γ as proinflammatory markers and transforming growth factor-β and interleukin-10 levels as anti-inflammatory markers were studied.

**Results:** Twenty-eight (31.8%) controls, 44 (50%) obese/overweight, and 16 (18.2%) patients with type 2 diabetes mellitus were included in our study. Age, sex, and puberty were similar between the groups. In the type 2 diabetes mellitus group, the subcutaneous fat tissue thickness was higher than that in the obese group, and the preperitoneal and visceral fat tissue thicknesses were similar to those in the obese group. Proinflammatory markers and interleukin-10 levels were similar in the obese/overweight, type 2 diabetes mellitus, and control groups. Transforming growth factor-β levels were significantly lower in the type 2 diabetes mellitus group than in the control group ($P = .039$). Transforming growth factor-β levels and other laboratory variables did not differ significantly in the type 2 diabetes mellitus group.

**Conclusion:** While there was no change in all markers in the obese/overweight group compared with the control group, proinflammatory markers in the type 2 diabetes mellitus group were similar to those in the obese/overweight and control groups, and transforming growth factor-β level, an anti-inflammatory marker, was lower in the type 2 diabetes mellitus group than in the control group.

**Keywords:** Obesity, type 2 diabetes mellitus, proinflammatory, anti-inflammatory, adipose tissue

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## INTRODUCTION

Obesity is characterized by excessive fat accumulation in the body.[1] Today, adipose tissue is considered an endocrine organ with immune function.[2] Excessive fat deposition in obese patients leads to significant changes in the number and function of immune cells in the adipose

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tissue.[3] The number of macrophages, mastocytes, neutrophils, T-lymphocytes, and B lymphocytes increased, whereas the number of eosinophils and some subgroups of T lymphocytes decreased. These changes are associated with the development of local and systemic inflammation.[4] In obesity, the number of macrophages in adipose tissue is high.[5] Although lean adipose tissue contains less than 10% macrophages, the number of macrophages increases to approximately 40% in obese adipose tissue.[3] Macrophages are generally divided into 2 phenotypes: M1 and M2 macrophages.[6] In obese individuals, there is a shift from anti-inflammatory M2 macrophages to proinflammatory M1 macrophages.[5] Macrophages are a source of elevated inflammatory cytokines, and their accumulation in adipose tissue is associated with insulin resistance.[4] It has been shown that weight reduction is accompanied by decreased adipose tissue macrophage count.[7] It has been reported that the infiltration of adipose tissue by macrophages is positively correlated with the expression of proinflammatory markers associated with body mass index (BMI), body fat amount, adipocyte size, and insulin resistance in obese patients.[8] The macrophage content in adipose tissue has been reported to be higher in visceral adipose tissue than in subcutaneous adipose tissue, and visceral adipose tissue plays a more prominent role in the development of insulin resistance.[7]

Chronic inflammation due to abnormal cytokine production and activation of inflammatory signaling pathways is closely associated with metabolic disorders, such as obesity, insulin resistance, and type 2 diabetes mellitus (DM).[9]

This study aimed to determine the changes in proinflammatory [tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, IL-18, and interferon (IFN)-γ] and anti-inflammatory [transforming growth factor (TGF)-β and IL-10] markers in children aged 10-18, who were not diagnosed with type 2 DM, were obese/overweight, and children with type 2 DM. In addition, we aimed to investigate whether these markers were associated with clinical and laboratory parameters, subcutaneous adipose tissue, preperitoneal adipose tissue, visceral adipose tissue, and hepatosteatosis.

## MATERIALS AND METHODS

Ethics committee approval, numbered 2019-050, was obtained from the Clinical Research Ethics Committee of the University of Health Sciences, Ankara Child Health and Diseases, Hematology Oncology Health Application and Research Center (Date: 04.03.2019). We planned to include children between the ages of 10 and 18, overweight/obese, with type 2 DM and normal BMI,[10] who applied to the Pediatric Endocrinology Polyclinic of our hospital between May 2019 and June 2021, after obtaining voluntary consent. This study was supported by the University of Health Sciences as a Scientific Research Project (2019/085).

### Determination of Working Groups

Diabetes mellitus was diagnosed based on one of the following criteria according to the guidelines of the International Pediatric and Adolescent Diabetes Association: (i) ≥126 mg/dL fasting plasma glucose, (ii) ≥200 mg/dL plasma glucose at the 120th minute after the oral glucose tolerance test, (iii) randomly measured plasma glucose ≥200 mg/dL in the presence of diabetes symptoms such as polyuria, polydipsia, nocturia, and unexplained weight loss, and (iv) ≥6.5% hemoglobin A1c.[11,12] Children who met the diagnostic criteria for diabetes, with a BMI Z-score of ≥1 SD for age and sex with negative pancreatic autoantibodies, and those whose pancreatic autoantibodies were not available but who had a fasting C-peptide level >1.2 ng/mL (0.4 nmol/L) at the time of diagnosis were included.[13] The obese/overweight group consisted of patients with BMI above the 85th percentile who were not diagnosed with type 2 diabetes. The control group consisted of subjects with BMI below the 85th percentile. Patients with no history of chronic disease, continuous drug use, normal thyroid function tests, and no history of acute infection were considered for patient selection. Patients with genetic and syndromic diagnoses, obesity, and type 2 DM due to endocrine causes were excluded from the study.

The age, sex, week of birth, and birth weight of all patients were assessed. Obese/overweight and type 2 diabetes patients were asked about the time of breast milk intake, time of starting cow's milk intake, and age at which weight gain started. Information about whether obesity, type 2 diabetes, hyperlipidemia, and atherosclerotic cardiovascular disease in the first- and second-degree relatives of obese/overweight and type 2 diabetes patients was also recorded.

### Anthropometric Assessment

All anthropometric measurements were performed by the same physician at the Pediatric Endocrinology Polyclinic. The height from the top of the head to the sole of the foot was measured while paying attention to parallelism. Weight measurements were performed after the portable, sensitive, electronic scale sensitive to 50 g was adjusted to zero on a flat surface. The values on the scale were recorded in kilograms. The BMI was calculated by dividing the body weight in kilograms by the height in square meters. A BMI between the 85th and 95th percentiles was defined as overweight, and a BMI of the 95th percentile was defined as obese.[1] The standard deviation scores (SDS) of the patients' height, weight, and BMI were calculated automatically using the Child Metrics developed by the Society of Pediatric Endocrinology and Diabetes.[14] Waist circumference was measured at the end of normal expiration while the patient was standing, with the abdomen relaxed, arms at the sides, and feet together. The measurement was made with a non-flexible tape over the umbilicus, where the body is the thinnest, just above the uppermost lateral border of the right iliac crest. The hip circumference was measured using a non-stretchable tape measure, with the patient standing, over the greater trochanter of the gluteal region at the widest level parallel to the ground. In all cases, blood pressure was measured with a mercury sphygmomanometer using a cuff suitable for the patient's age and arm length. Puberty was staged according to the Tanner Marshall criteria.[15] Detailed physical examinations were performed in all cases and the presence of acanthosis nigricans was noted.

## COLLECTION OF SAMPLES AND WORKING METHODS

In our study, blood samples were collected after at least 8 hours of fasting. Fasting glucose, fasting insulin, cholesterol,

triglyceride, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) levels in the obese/overweight and type 2 DM groups in the laboratory of Dr Sami Ulus Obstetrics, Gynecology and Pediatrics Training and Research Hospital. Glucose, total cholesterol, LDL cholesterol, HDL cholesterol, triglyceride, AST, and ALT levels were measured with the automation method Beckman Coulter AU500, insulin levels were measured with the chemiluminescence method (Advia Centaur X-P).

To measure serum TNF-α, IL-1β, IL-6, IL-18, IFN-γ, TGF-β, and IL-10 levels in all groups, blood was centrifuged for 10 minutes at 3000 rpm 30 minutes after it was collected in yellow capped gel tubes. The obtained sera were placed in Eppendorf tubes, immediately frozen at −20°C and then stored at −80°C until further analysis. It was analyzed using a commercial kit (Bioassay Technology Laboratory Shanghai Korain Biotech Co., China) with an enzyme-linked immunoassay method. The intra-assay and inter-assay coefficient of variation (CV) values of the kits were <8% and <10%, respectively. Washing processes during the study were performed with a BIOTEK brand washing device (ELx50 Bioelisa Washer, Bio-Tec. Instruments, Inc., Winooski, Vermont, USA), and the absorbance readings were recorded using a BIOTEK brand reader (ELx800 UV Universal Microplate Reader, Bio-Tec. Instruments, Inc.).

### Ultrasonographic Evaluation

Ultrasonographic measurements were performed by a single experienced pediatric radiologist using a Toshiba Aplio 500 device (Toshiba, Tokyo, Japan) to evaluate the intra-abdominal fat distribution and hepatosteatosis. Subcutaneous and preperitoneal fat tissue thickness measurements were made in the supine position in the midline just below the xiphoid, in the longitudinal plane with a linear 7.5 Mhz probe. The thinnest adipose tissue thickness was measured between the skin and linea alba for subcutaneous adipose tissue, and the thickest adipose tissue thickness was measured between the linea alba and liver for preperitoneal adipose tissue. Visceral fat tissue thickness was evaluated by measuring the fat tissue thickness between the anterior wall of the aorta and the inner surface of the abdominal muscles in the midline axial plane, approximately 5 cm above the umbilicus, using a 3.5 MHz convex probe.[16] Three measurements were performed for each patient and the mean values were determined. Hepatosteatosis was classified based on the echo pattern of the liver parenchyma.

### Liver echopattern was graded as follows[17]:

- Grade 1 (mild): a slightly diffuse increase in fine echoes in the hepatic parenchyma with normal visualization of the diaphragm and intrahepatic vessel borders.
- Grade 2 (moderate): a moderate diffuse increase in fine echoes with slightly impaired visualization of the intrahepatic vessels and diaphragm.
- Grade 3 (marked): a marked increase in fine echoes with poor or no visualization of the intrahepatic vessel borders, diaphragm, and posterior portion of the right lobe of the liver.

### Statistical Analysis

The Statistical Package for the Social Sciences version 24.0 package program (IBM Corp.; Armonk, NY, USA) was used for statistical analyses. For the level of significance, a *P*-value less than .05 was accepted. Chi-square and Fisher's exact tests were used to analyze independent categorical variables. The conformity of continuous variables to normal distribution was evaluated using the Shapiro–Wilk test. Normally distributed variables are presented as means and SD, and non-normally distributed data are presented as medians (minimum-maximum). Student's *t*-test and 1-way analysis of variance (ANOVA) were used for normally distributed variables, and Mann–Whitney *U*-test and Kruskal–Wallis test were used in cases where there was no normal distribution. For posthoc analysis of the ANOVA test regarding the homogeneity of variances, the Bonferroni or Dunnett's T3 test was used, and for Kruskal–Wallis pairwise analysis, the Mann–Whitney *U*-test was used. Spearman correlation test, one of the correlation tests, was used in the comparison of 2 measurement types of data. A correlation >0.7 was considered strong, 0.3-0.7 moderate, and <0.3 weak correlation.

## RESULTS

### Demographic and Clinical Characteristics

Eighty-eight patients, including 28 (31.8%) controls, 44 (50%) obese/overweight, and 16 (18.2%) type 2 DM patients, were included in our study. The age, sex, puberty, week of birth, and birth weight were similar between the groups. Breast milk intake time, cow milk initiation time, and weight gain onset time were similar between the obese/overweight and type 2 DM groups (Table 1).

Family histories of obesity, hyperlipidemia, and atherosclerotic cardiovascular diseases were similar in the obese/weight group and type 2 DM group. Family and maternal history of DM was significantly higher in the type 2 DM group than in the obese group (Table 2).

Weight SDS, BMI SDS, waist circumference, and hip circumference were similar in the obese/overweight and type 2 DM groups, and they were significantly higher in both groups than in the control group. The systolic and diastolic blood pressures in the obese group were similar to those in the control group. Systolic and diastolic blood pressures were significantly higher in the type 2 DM group than in the control group (Table 3). Acanthosis nigricans was present in 11 (68.8%) of 16 patients in the type 2 DM group and in 19 (43.3%) of 44 patients in the obese group.

### Ultrasonographic Findings

Of the 16 patients with type 2 DM, 14 presented with hyperglycemia, 1 with ketosis, and 1 with ketoacidosis. In the type 2 DM group, the subcutaneous fat tissue thickness was higher than that in the obese group, and the preperitoneal and visceral fat tissue thickness was similar to that in the obese group. While hepatosteatosis was present in all 16 patients with type 2 DM, it was detected in 36 (81.8%) of the 44 obese/overweight patients (Table 4).

### Proinflammatory and Anti-Inflammatory Cytokines and Related Factors

Proinflammatory markers (TNF-α, IL-1β, IL-6, IL-18, and IFN-γ) and anti-inflammatory markers (IL-10 and TGF-β) were found to be similar in the obese/overweight, type 2 DM, and control

**Table 1.** Demographic Characteristics of the Obese/Overweight, Type 2 DM, and Control Group

| | Control Group (n = 28) | Obese/Overweight Group (n = 44) | Type 2 DM Group (n = 16) | Total Group (n = 88) | *P* |
|---|---|---|---|---|---|
| Age (years), median (minimum–maximum) | 14.0 (10 .1-17.8) | 13.1 (10.0-17.9) | 14.0 (10.0-17.2) | 13.6 (10.0-17.9) | .953[a] |
| Male, n (%) | 7 (25.0%) | 18 (40.9%) | 4 (25.0%) | 29 (33.0%) | .284[b] |
| Female | 21 (75.0%) | 26 (59.1%) | 12 (75.0%) | 59 (67.0%) | |
| Pubertal, n (%)* | 26 (92.9%) | 39 (88.6%) | 16 (100.0%) | 81 (92.0%) | - |
| *Prepubertal** | 2 (7.1%) | 5 (11.4%) | 0 (0.0%) | 7 (8.0%) | |
| Term, n (%) | 26 (92 .9%) | 40 (90.9%) | 14 (100.0%) | 80 (93.0%) | - |
| Preterm | 2 (7.1%) | 4 (9.1%) | 0 (0.0%) | 6 (7.0%) | |
| Birthweight (kg), median (minimum–maximum) | 3.2 (2.0-4 0) | 3.2 (2.1-5.5) | 3.5 (2.4-4.5) | 3.2 (2.0-5.5) | .256[a] |
| Breast milk intake time (months), median (minimum–maximum) | — | 15.5 (1-36) | 9.0 (2-36) | 14.0 (1-36) | .147[c] |
| Cow's milk initiation time (months), median (minimum–maximum) | — | 12.0 (6-36) | 12.0 (7-36) | 12.0 (6-36) | .190[c] |
| Weight gain onset time (years), median (minimum–maximum) | — | 7.0 (1-14) | 6.5 (1-13) | 7.0 (1-14) | .847[c] |

*P* < .05 was considered significant.
[a]Kruskal–Wallis test was used.
[b]Chi-square test was used.
[c]Mann–Whitney U-test was used.
*Pubertal was defined as thelarche stages 2, 3, 4, and 5 in girls and testicular volume of 4 mL and above in boys.
**Absence of thelarche in girls and testicular volume less than 4 mL in boys were defined as prepubertal.
DM, diabetes mellitus; n, number of cases; Preterm, babies born before 37 weeks of gestation; Term, babies born between 37 and 42 weeks of gestation.

groups. Transforming growth factor-β, an anti-inflammatory marker, was significantly lower in the type 2 DM group than in the control group (*P* = .039) (Table 5).

## DISCUSSION

Obesity induces systemic, chronic, and low-grade inflammation characterized by high/normal concentrations of inflammatory markers in children and adolescents.[18] Insulin resistance and the pathogenesis of type 2 DM have been associated with subclinical chronic inflammation and activation of the immune system; however, what triggers this inflammation remains unclear.[19] Some studies have shown that patients with type 2 DM have higher levels of inflammatory markers, such as IL-6, C-reactive protein, plasminogen activator inhibitor-1, and TNF-α.[20,21] In addition to the direct link between chronic inflammation and insulin resistance, the roles of adipokines, hepatokines, and cytokines in other tissues in the pathogenesis of metabolic syndrome and type 2 DM have also been discussed.[22,23]

Adipose tissue produces various proinflammatory and anti-inflammatory cytokines. Proinflammatory cytokines increase insulin resistance by inducing insulin receptor substrates,

**Table 2.** Family History in the Obese/Overweight Group and Type 2 DM Groups

| | Obese/Overweight Group (n = 44) | Type 2 DM Group (n = 16) | |
|---|---|---|---|
| | n (%) | n (%) | *P* |
| Obesity in the family | 30 (68.2%) | 10 (62.5%) | .680 |
| Presence of maternal obesity | 23 (52.3%) | 8 (50.0%) | .876 |
| Presence of obesity in father | 16 (36.4%) | 5 (31.2%) | .713 |
| Obesity in a sibling | 4 (9.1%) | 2 (12.5%) | .653[a] |
| Presence of obesity in other family members | 8 (18.2%) | 4 (25.0%) | .716[a] |
| Presence of diabetes mellitus in the family | 31 (70.5%) | 16 (100.0%) | **.013**[a,b] |
| Presence of diabetes mellitus in the mother | 7 (15.9%) | 9 (56.2%) | **.006**[a,b] |
| Presence of diabetes mellitus in the father | 7 (15.9%) | 4 (25.0%) | .462[a] |
| Presence of diabetes mellitus in other family members | 27 (61.4%) | 13 (81.2%) | .148 |
| Presence of hyperlipidemia in the family | 15 (34.1%) | 8 (50.0%) | .262 |
| Family history of atherosclerotic cardiovascular disease | 16 (36.4%) | 9 (56.2%) | .167 |

Unmarked *P*-values were obtained by chi-square test. *P* < .05 values in bold considered significant.
[a]Fisher's exact test was used.
[b]Type 2 DM group is statistically different from the obese/overweight group (*P* < .05).
DM, diabetes mellitus.

**Table 3.** Anthropometric Features, Physical Examination Findings of the Obese/Overweight, Type 2 DM, and Control Group

| | Control Group (n = 28) | Obese/Overweight Group (n = 44) | Type 2 DM Group (n = 16) | *P* |
|---|---|---|---|---|
| Height (cm), mean ± SD | 157.9 ± 12.6 | 158.5 ± 9.7 | 160.8 ± 10.4 | .685[a] |
| Height SDS, median (minimum–maximum) | 0.02 (−1.2–2.7) | 0.21 (−3.1–3.2) | 0.51 (−2.7–3.4) | .635[b] |
| Weight (kg), median (minimum–maximum) | 48.5 (25–72) | 70.5 (59–111) | 87.5 (46–139) | **<.001**[b,c] |
| Weight SDS, mean ± SD | −0.3 ± 0.9 | 2.4 ± 0.7 | 3.2 ± 1.6 | **<.001**[a,c] |
| BMI (kg/m²), mean ± SD | 18.6 ± 3.4 | 29.7 ± 2.9 | 33.8 ± 8.0 | **<.001**[a,c] |
| BMI SDS, median (minimum–maximum) | −0.4 (−2.1–1.0) | 2.5 (1.3–3.0) | 2.9 (0.4–4.5) | **<.001**[b,c] |
| Waist circumference (cm), median (minimum–maximum) | 67.5 (50–90) | 94.5 (81–121) | 98.0 (81–150) | **<.001**[b,c] |
| Hip circumference (cm), median (minimum–maximum) | 81.0 (60–102) | 104.5 (93–133) | 111.5 (90–147) | **<.001**[b,c] |
| Systolic blood pressure (mmHg), median (minimum–maximum) | 110 (90–125) | 119 (100–135) | 120 (100–150) | **.009**[b,d] |
| Diastolic blood pressure (mmHg), median (minimum–maximum) | 70 (60–80) | 70 (58–90) | 70 (60–100) | **.039**[b,d] |
| Acanthosis nigricans n (%) | | 19 (43.2%) | 11 (68.8%) | .080[e] |

[a]ANOVA test was used.
[b]Kruskal–Wallis test was used.
[c]The control group is significantly different from the obese/overweight and type 2 DM group ($P < .05$).
[d]Type 2 DM group is significantly different from the control group ($P < .05$).
[e]Chi-square test was used.
ANOVA, analysis of variance; BMI, body mass index; DM, diabetes mellitus; SDS, standard deviation scores.

**Table 4.** Ultrasonography Findings in the Obese/Overweight Group and Type 2 DM Group

| | Obese/Overweight Group (n = 44) | Type 2 DM Group (n = 16) | *P* |
|---|---|---|---|
| Subcutaneous adipose tissue (mm), median (minimum–maximum) | 16.2 (10.2–22.7) | 17.9 (12.1–38.5) | **.021**[a,b] |
| Preperitoneal adipose tissue (mm), mean ± SD | 14.9 ± 3.6 | 15.6 ± 4.9 | .541[c] |
| Visceral adipose tissue (mm), median (minimum–maximum) | 38.5 (14.1–69.3) | 46.5 (18.1–85.4) | .088[a] |
| Hepatosteatosis, n (%) | 36 (81.8%) | 16 (100.0%) | .095[d] |
| Stage 1 | 20 (45.5%) | 4 (25.0%) | |
| Stage 2 | 13 (29.5%) | 8 (50.0%) | |
| Stage 3 | 3 (6.8%) | 4 (25.0%) | |

[a]Mann–Whitney *U*-test was used.
[b]Type 2 DM group is statistically different from the obese/overweight group ($P < .05$).
[c]*T*-test was used.
[d]Fisher's exact test was used.
DM, diabetes mellitus.

**Table 5.** Proinflammatory and Anti-Inflammatory Markers in Obese/Overweight, Type 2 DM, and Control Groups

| | Control Group (n = 28) | Obese/Overweight Group (n = 44) | Type 2 DM Group (n = 16) | *P* |
|---|---|---|---|---|
| TNF-α (ng/L) | 126.9 (29.5–960.0) | 144.5 (29.3–960.0) | 130.2 (29.3–960.0) | .992 |
| IL-1β (ng/L) | 1634.6 (1212.5–2037.3) | 1581.9 (1157.7–2999.8) | 1575.9 (843.3–3095.2) | .414 |
| IL-6 (ng/L) | 33.0 (3.5–914.9) | 46.1 (1.0–640.0) | 23.4 (3.5–640.0) | .951 |
| IL-18 (ng/L) | 9.3 (0.2–30.6) | 7.1 (0.6–85.7) | 4.2 (1.3–128.0) | .542 |
| IFN-γ (ng/mL) | 26.6 (6.9–480.0) | 30.5 (7.7–480.0) | 21.7 (8.4–480.0) | .341 |
| TGF-β (ng/L) | 218.8 (94.0–1683.8) | 223.0 (88.8–3414.8) | 131.4 (101.6–4185.5) | **.039**[*] |
| IL-10 (pg/mL) | 69.4 (34.5–734.4) | 93.4 (18.9–1522.3) | 73.7 (32.9–1600.0) | .788 |

Kruskal–Wallis test was used in all analyses.
*Type 2 DM group is lower than the control group.
DM, diabetes mellitus; IFN, interferon; IL, interleukin; TGF, transforming growth factor; TNF, tumor necrosis factor.The parameters and correlations in the groups are presented in Tables 6, 7, and 8, respectively.

whereas anti-inflammatory cytokines have the opposite effect.[24] Proinflammatory cytokines activate nuclear factor-κB and its efflux pathways.[25] In the liver and adipose tissue, nuclear factor-κB inactivates the insulin receptor by reducing the formation of insulin receptor substrates, thus leading to insulin resistance.[26] Adipocytes are the main cells involved in inflammation and insulin resistance in type 2 DM. Although enlarged adipocytes have been shown to produce proinflammatory cytokines, adipose tissue macrophages are crucial for the production of proinflammatory cytokines from adipose tissue.[27]

**Table 6.** Parameters Correlated with Subcutaneous Adipose Tissue Thickness in the Obese/Overweight and Type 2 DM Groups

| **Subcutaneous Adipose Tissue** | | | | |
|---|---|---|---|---|
| | **Obese/Overweight Group (n = 44)** | | **Type 2 DM Group (n = 16)** | |
| | **Mean ± SD/Correlation Coefficient** | ***P*** | **Mean ± SD/Correlation Coefficient** | ***P*** |
| Weight SDS | 0.340 | **.024*** | 0.587 | **.017*** |
| BMI SDS | 0.409 | **.006*** | 0.564 | **.023*** |
| Waist circumference (cm) | 0.399 | **.007*** | 0.636 | **.008*** |
| Hip circumference (cm) | 0.421 | **.004*** | 0.521 | **.039*** |
| Acanthosis nigricans | | | | |
| Not available | 15.6 ± 3.0 | .635 | 18.5 ± 2.6 | .777 |
| Available | 16.2 ± 3.4 | | 20.7 ± 7.8 | |
| Preperitoneal adipose tissue (mm) | 0.171 | .268 | 0.532 | **.034*** |
| Visceral adipose tissue (mm) | 0.310 | **.041*** | 0.519 | **.039*** |
| LDL cholesterol (mg/dL) | 0.321 | **.033*** | −0.171 | .527 |
| HDL cholesterol (mg/dL) | −0.070 | .654 | −0.684 | **.003*** |
| AST (U/L) | −0.052 | .739 | 0.290 | .276 |
| ALT (U/L) | 0.081 | .600 | 0.230 | .392 |
| IL-6 (ng/L) | 0.380 | **.011*** | 0.104 | .701 |
| IL-18 (ng/L) | 0.365 | **.015*** | 0.461 | .073 |
| IFN-γ (ng/mL) | 0.320 | **.034*** | −0.274 | .304 |
| TGF-β (ng/L) | 0.358 | **.017*** | 0.258 | .335 |

Spearman correlation test was used in all analyses.
The parameters and correlations in the groups are presented in Tables 6, 7, and 8, respectively.
*P-values are considered statistically significant, $P < .05$.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; DM, diabetes mellitus; HDL, high-density lipoprotein; IFN, interferon; IL, interleukin; LDL, low-density lipoprotein; SDS, standard deviation scores; TGF, transforming growth factor; TNF, tumor necrosis factor.

In the adult population, increased concentrations of proinflammatory and reduced anti-inflammatory markers are significantly associated with the incidence of type 2 DM. However, these associations must be confirmed for causality, as Mendelian randomization studies have yielded inconsistent results for some inflammatory markers.[28] Similar to adults, there are inconsistencies in the results of studies conducted on children.

As there were studies reporting that TNF-α is higher in the obese group than in the control and Type 2 DM groups[29]; when the group with type 2 DM was compared with the control group, there were studies that did not find a significant relationship with TNF-α level.[30] In our study, TNF-α levels were similar in the obese/overweight, type 2 DM, and control groups. Subcutaneous, preperitoneal, and visceral adipose

**Table 7.** Parameters Correlated with Preperitoneal Adipose Tissue Thickness in the Obese/Overweight and Type 2 DM Groups

| **Preperitoneal Adipose Tissue** | | | | |
|---|---|---|---|---|
| | **Obese/Overweight Group (n = 44)** | | **Type 2 DM Group (n = 16)** | |
| | **Mean ± SD/Correlation Coefficient** | ***P*** | **Mean ± SD/Correlation Coefficient** | ***P*** |
| Weight SDS | 0.297 | **.050*** | 0.559 | **.024*** |
| BMI SDS | 0.348 | **.021*** | 0.745 | **.001*** |
| Waist circumference (cm) | 0.393 | **.008*** | 0.837 | **<.001*** |
| Hip circumference (cm) | 0.302 | **.046*** | 0.656 | **.006*** |
| Acanthosis nigricans | | | | |
| Not available | 13.9 ± 3.7 | **.019*** | 12.2 ± 3.4 | **.031*** |
| Available | 16.2 ± 3.1 | | 17.2 ± 4.8 | |
| Subcutaneous adipose tissue (mm) | 0.171 | .268 | 0.532 | **.034*** |
| Visceral adipose tissue (mm) | 0.164 | .287 | 0.628 | **.009*** |
| Fasting insulin (mIU/mL) | 0.324 | **.032*** | 0.589 | **.016*** |
| HOMA-IR | 0.326 | **.031*** | 0.271 | .310 |
| LDL cholesterol (mg/dL) | −0.014 | .928 | −0.244 | .362 |
| HDL cholesterol (mg/dL) | −0.214 | .163 | −0.858 | **<.001*** |

Spearman correlation test was used in all analyses.
*P-values marked with are considered statistically significant $P < .05$.
DM, diabetes mellitus; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment for insulin resistance; LDL, low-density lipoprotein; SDS, standard deviation scores.

**Table 8.** Parameters Correlated with Visceral Adipose Tissue Thickness in the Obese/Overweight and Type 2 DM Groups

| **Visceral Adipose Tissue** | | | | |
|---|---|---|---|---|
| | **Obese/Overweight Group (n = 44)** | | **Type 2 DM Group (n = 16)** | |
| | **Mean ± SD/Correlation Coefficient** | ***P*** | **Mean ± SD/Correlation Coefficient** | ***P*** |
| Weight SDS | 0.289 | .057 | 0.747 | **.001*** |
| BMI SDS | 0.368 | **.014*** | 0.762 | **.001*** |
| Waist circumference (cm) | 0.519 | **<.001*** | 0.837 | **<.001*** |
| Hip circumference (cm) | 0.133 | .390 | 0.626 | **.009*** |
| Acanthosis nigricans | | | | |
| Not available | 34.6 ± 8.4 | **.008*** | 33.8 ± 10.5 | **.036*** |
| Available | 45.0 ± 13.1 | | 53.7 ± 17.7 | |
| Subcutaneous adipose tissue (mm) | 0.310 | **.041*** | 0.519 | **.039*** |
| Preperitoneal adipose tissue (mm) | 0.164 | .287 | 0.628 | **.009*** |
| Fasting insulin (mIU/mL) | 0.368 | **.014*** | 0.535 | **.033*** |
| HOMA-IR | 0.378 | **.011*** | 0.632 | **.009*** |
| LDL cholesterol (mg/dL) | −0.014 | .930 | −0.362 | .169 |
| HDL cholesterol (mg/dL) | −0.154 | .320 | −0.608 | **.013*** |
| AST (U/L) | −0.154 | .320 | −0.608 | **.013*** |
| ALT (U/L) | 0.487 | **.001*** | −0.015 | .957 |

Spearman correlation test was used in all analyses.
*$P$-values are considered statistically significant, $P < .05$.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; DM, diabetes mellitus; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment for insulin resistance; LDL, low-density lipoprotein; SDS, standard deviation scores.

tissue thicknesses and TNF-α levels were not significantly associated.

It has been shown that IL-1β is also increased in obese children.[31] It has been reported that higher IL-1β levels are detected in obese children with metabolic syndrome than in the control group.[32] Another study, reported that IL-1β concentrations did not differ between obese adolescents with or without type 2 DM.[30] In our study, proinflammatory IL-1β levels were similar in obese/overweight patients.

One of the proinflammatory markers, IL-6 was found to be higher in the obese and type 2 DM groups than in the control group, and was reported to be similar in the obese and type 2 DM groups.[29] Similarly, it has been reported that the IL-6 concentration is higher in obese children with insulin resistance compared to the control group.[33] However, another study found that IL-6 levels were similar between children with and without type 2 DM.[34] In our study, proinflammatory IL-6 levels were similar in the obese/overweight, type 2 DM, and control groups. A positive correlation was observed between subcutaneous fat tissue thickness and IL-6 levels in the obese/overweight group.

In a study by He Zhuang et al,[35] the causal effect of proinflammatory cytokines on type 2 DM was investigated, and it was confirmed that type 2 DM is caused by high IL-18 levels. In another study, high IL-18 levels were observed in adult patients with type 2 DM.[36] In our study, the proinflammatory cytokine IL-18 levels were similar in the obese/overweight, type 2 DM, and control groups. A positive correlation was observed between subcutaneous fat tissue thickness and IL-18 levels in the obese/overweight group.

In a study of obese and type 2 DM adolescents, it was reported that there was no increase in IFN-γ levels.[30] Another study reported that increased IFN-γ concentrations were associated with insulin resistance and other metabolic syndrome parameters in obese children.[37] These results suggest that IFN-γ may have a role in insulin resistance and metabolic syndrome but may not have an effect on β-cell failure. In our study, IFN-γ levels were found to be similar in the obese/overweight, type 2 DM, and control groups. A positive correlation was observed between subcutaneous fat tissue thickness and IFN-γ levels in the obese/overweight group.

In 2013, Al-Shukaili et al[38] reported higher IL-10 levels in patients with type 2 DM than in the control group. In a study by Rodrigues et al[39] in 2017, IL-10 levels were found to be similar when type 2 DM patients were compared with the control group. In another study conducted in adults, the IL-10 level in the type 2 DM group and the obese group was found to be lower than that in the control group, and it was reported that the IL-10 level was lower in obese type 2 DM individuals compared to non-obese type 2 DM individuals.[40] In our study, IL-10 levels were similar among the 3 groups.

In a study conducted in healthy women, serum TGF-β levels were significantly associated with BMI, waist circumference, fat mass, and ALT levels.[41] In a study by Yoo et al[42] on preschool children, it was reported that TGF-β1 gene polymorphism may affect susceptibility to obesity, and it was found to be higher in obese children than in controls. Contrary to all these data, in a study conducted in adults, TGF-β levels were found to be lower in the type 2 DM group than in the control group, and it was found to be lower in the obese type 2 DM group than in the non-obese type 2 DM group, but the difference did not reach the level of significance.[40] In another study conducted in adult men, TGF-β levels were similar in type 2 DM and control groups.[43] In our study, TGF-β levels were found to be significantly lower in the type 2 DM group than in the control group. These levels were similar among the other groups.

Transforming growth factor-β levels were not associated with adipose tissue distribution.

In our study, all 3 adipose tissue thicknesses in the type 2 DM group showed a negative correlation with HDL cholesterol. In type 2 DM patients with acanthosis nigricans, visceral and preperitoneal adipose tissue thickness was found to be statistically significantly higher. Both conditions were evaluated to be effective in the development of metabolic syndrome. Subcutaneous adipose tissue was not found to be significant in type 2 DM patients with acanthosis nigricans.

While the rate of hepatocytosis was found to be 44.5% in obese children in a previous study,[44] hepatosteatosis was found to be 81.8% in the obese/overweight group and 100% in the type 2 DM group ($P$ = .095).

Although there are studies examining proinflammatory and anti-inflammatory markers in obese children, the number of studies evaluating the levels of these markers in type 2 DM in childhood is limited. In our clinic, 16 patients were diagnosed with type 2 diabetes over a 2-year period. In a recent study from our country, the data of 367 children aged 6-18 years diagnosed with type 2 diabetes from 37 different pediatric endocrinology centers were retrospectively evaluated.[45] When we look at this study from the perspective of the frequency of patients in our country, it is valuable in this understudied group that there are 16 pediatric patients diagnosed with type 2 diabetes in our clinic within 2 years and that we look at the levels of proinflammatory and anti-inflammatory markers in these cases. In our study, the decrease in TGF-β levels in the type 2 DM group is important for investigating the etiological factors and guiding treatment. Targeting inflammation represents a new treatment for obesity-related diseases including insulin resistance and type 2 DM. Numerous animal studies have demonstrated the benefits of reducing or suppressing inflammation in obesity-related insulin resistance and metabolic diseases.[46] Many antidiabetic drugs have anti-inflammatory properties. Promising results have been obtained from preclinical studies testing the efficacy of anti-inflammatory drugs for the treatment of insulin resistance and type 2 DM. Based on our study, it can be said that TGF-B-based treatments will be candidates to prevent the progression to type 2 DM.

Proinflammatory and anti-inflammatory markers have only been studied for diagnosis. If these markers were also studied during follow-up, it would be possible to obtain more precise results. In addition, due to limited resources, the fact that the study was conducted not by selecting a sample, but by the inclusion of patients who applied between certain dates and who were eligible for the study may be the reason for the inability to find a difference between inflammatory markers. Therefore, our results may have been biased due to sample selection. Despite this, our study is valuable because the number of studies evaluating the levels of proinflammatory and anti-inflammatory markers in type 2 diabetes in childhood is very low.

## CONCLUSION

As a result, while there was no change in proinflammatory and anti-inflammatory markers in the obese/overweight group compared to the control group, proinflammatory markers in the type 2 DM group were similar to the obese/overweight group and control group, and TGF-β level, an anti-inflammatory marker, was lower in the type 2 DM group than in the control group. Comparisons between TGF-β levels and other laboratory parameters in the type 2 DM group were not statistically significant. More studies and molecular evidence are needed on proinflammatory and anti-inflammatory cytokine levels and their relationship with body fat distribution in childhood and adolescence.

**Ethics Committee Approval:** This study was approved by the Ethics Committee of SUAM Clinical Research of Health Sciences University Ankara (Approval No: 2019-050).

**Informed Consent:** Written informed consent was obtained from the participants who agreed to take part in the study.

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