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Association of FTO GENE Polymorphisms, Salivary Oxidative Stress Biomarkers, and Salivary Protein Profiles with Dental Caries in Overweight and Obese Children

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Highlights

What are the main findings?

- In the permanent dentition, children who are overweight or obese exhibited a greater incidence of caries (DMFT 4.41 vs. 3.25; nominal $p = 0.032$) than healthy-weight controls; the difference was attenuated but persisted after adjustment for age, sex, and oral hygiene/dietary variables (rate ratio 1.23–1.29) whereas primary-tooth caries (dft) and total caries (dft + DMFT) did not differ between groups.
- Children with overweight or obesity exhibited notably elevated levels of salivary total oxidant status (TOS) and oxidative stress index (OSI) ($p < 0.001$), whereas FTO rs9939609 and rs11642841 genotype distributions and overall SDS-PAGE protein profiles showed no consistent differences between weight groups. Furthermore, FTO variants and salivary oxidative stress parameters were not significantly associated with caries presence; the estimates were imprecise, so an association cannot be excluded.

What are the implications of the main findings?

- The findings indicate greater caries experience in the permanent dentition and increased salivary oxidative stress among children with overweight/obesity, despite comparable age distributions between groups.
- Saliva may provide a non-invasive biological matrix for investigating obesity-associated oxidative alterations. Larger longitudinal studies incorporating high-resolution proteomic analyses are needed to clarify the relationships among genetic susceptibility, salivary biomarkers, and dental caries.

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Abstract

Background/Objectives: The potential associations of obesity-related genetic variants and salivary biological alterations with dental caries remain insufficiently understood. This study investigated the associations of *FTO* gene polymorphisms, salivary oxidative stress biomarkers, and salivary protein profiles with dental caries in children with overweight/obesity and healthy-weight controls. **Methods:** This cross-sectional study included 88 children aged 6–17 years. Dental caries experience was assessed using the dft/DMFT indices. *FTO* rs9939609 and rs11642841 polymorphisms were analyzed using

TaqMan genotyping assays. Salivary total antioxidant/oxidant status (TAS/TOS) and oxidative stress index (OSI) were evaluated, and salivary protein profiles were examined using sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). DMFT was compared between groups with and without adjustment (count regression), and associations with caries presence were investigated using Firth-penalized logistic regression analyses. **Results:** Children with overweight/obesity had higher DMFT scores than healthy-weight controls (4.41 ± 2.99 vs. 3.25 ± 1.86; $p = 0.032$; adjusted rate ratio 1.29, 95% CI 1.03–1.62). Salivary TOS (11.76 ± 2.58 vs. 8.79 ± 1.82 μmol H_2O_2 Eq/L) and OSI (70.77 ± 24.53 vs. 50.51 ± 17.72) were significantly higher in children with overweight/obesity (both $p < 0.001$). *FTO* genotype distributions and overall SDS-PAGE protein profiles showed no consistent differences between weight groups. In age- and sex-adjusted logistic regression analyses, age and sex remained significantly associated with caries presence, whereas *FTO* variants and salivary oxidative stress parameters were not significantly associated, with wide confidence intervals. **Conclusions:** Children with overweight/obesity exhibited greater caries experience in the permanent dentition and increased salivary oxidative stress. No evidence of an association was found between the investigated *FTO* polymorphisms and salivary oxidative stress parameters, which were not associated with caries presence in this small sample; the study was not powered for these analyses, and caries was almost universal (DMFT > 0 in 89%), which limits the binary outcome.

Keywords: childhood obesity; dental caries; FTO gene polymorphism; saliva; oxidative stress; salivary proteins; DMFT

1. Introduction

The prevalence of childhood overweight and obesity has increased markedly over recent decades, representing a major public health challenge [1–3]. Among children and adolescents, dental caries continues to be one of the most widespread chronic conditions, negatively impacting their quality of life and creating a significant health burden [4,5]. Although these conditions are traditionally considered separate disorders, they share several behavioral, dietary, and socioeconomic determinants, particularly the frequent consumption of sugar-rich, energy-dense foods [6,7]. Consequently, growing attention has been focused on the potential biological and behavioral links between obesity and oral health. Recent evidence, including a cumulative dose–response meta-analysis, indicates that an increased body mass index (BMI) is linked to an increased likelihood of tooth decay in children [8]. Nevertheless, the strength and direction of this association remain inconsistent across studies, likely reflecting differences in study populations, obesity definitions, caries assessment methods, and other confounding factors [6,8].

While an elevated BMI has been linked to a higher incidence of caries in certain populations, this correlation does not inherently imply a direct causal relationship between obesity and dental caries [6–8]. Both conditions are influenced by several shared behavioral and environmental factors [7–28]. Frequent consumption of dietary sugars may lead to excessive energy intake and weight gain, while also promoting acid production by cariogenic biofilms, resulting in enamel demineralization [16,29]. Socioeconomic factors, such as parental education and household income, may affect dietary habits, access to dental care, and preventive measures, potentially confounding the observed associations. Additionally, oral hygiene practices and fluoride exposure may independently influence caries susceptibility, regardless of weight status [4,7]. Conversely, obesity-related systemic inflammation, oxidative stress, and changes in salivary composition have been suggested as potential biological pathways linking obesity to oral health [7,17–19]; however, their direct role in caries devel-

opment remains inadequately established [7,18]. Consequently, the relationship between weight status and dental caries should be understood within a multifactorial framework that considers both shared risk factors and potential biological mechanisms [7,8].

Genetic predispositions and environmental conditions intricately shape eating patterns and food cravings [9]. Among obesity-related genetic variants, *FTO* rs9939609 has been extensively investigated, and meta-analytic evidence supports its association with an increased susceptibility to overweight and obesity during childhood and adolescence [10]. Wardle et al. observed lower food intake after satiety among children with the TT genotype than among A-allele carriers, supporting greater responsiveness to internal satiety signals [11]. More directly relevant to dental caries, Yeum et al. associated the rs9939609 risk allele with greater gummy candy consumption in the absence of hunger, particularly following exposure to food cues [12]. However, Rahimlou et al. found no significant association between rs9939609 and preferences for sweet or fatty foods after adjustment for potential confounders, suggesting that dietary preferences cannot be explained by this genetic variant alone [13]. In a UK Biobank analysis, rs11642841 emerged as a genome-wide significant signal for total sugar intake and was highly correlated with the established obesity-associated rs9939609 variant [14]. Findings from a Swedish cohort further suggested that the relationship between rs11642841 and sugar intake may differ according to BMI status [15]. Collectively, these findings suggest that *FTO* variants may contribute to obesity susceptibility through differences in satiety and sugar-related eating behaviours. Frequent exposure to dietary sugars is a well-established risk factor for dental caries in children [16]; rs9939609 and rs11642841 were selected to examine whether *FTO*-related susceptibility to obesogenic eating behaviors is also associated with dental caries.

Previous studies have associated FTO variants with appetite regulation, satiety responsiveness, and sugar-related eating behaviors [9–13]. However, these associations offer only an indirect basis for examining susceptibility to dental caries. Specifically, FTO variants may affect caries risk through dietary behaviors, particularly the frequency of sugar exposure, rather than through a direct biological impact on cariogenesis. Consequently, the genetic variants rs9939609 and rs11642841 were chosen to investigate whether variants linked to obesogenic eating behaviors are also related to dental caries experience in children.

Saliva provides a non-invasive medium for investigating biological changes associated with both obesity and dental caries. Adolescents who are overweight or obese have been found to experience disruptions in their salivary antioxidant defense, along with heightened oxidative damage [17]. Salivary oxidative balance may also be relevant to caries, although a systematic review found that the available evidence linking alterations in antioxidant and pro-oxidant parameters to dental caries in children and adolescents was of very low certainty [18]. Salivary protein composition may represent an additional link between nutritional status and the oral environment. Differences in the salivary proteome, including altered cystatin levels, have been observed across BMI categories and taste-sensitivity profiles in children [19]. Moreover, a recent systematic review suggested that total protein content and several specific salivary proteins differ between caries-active and caries-free children, although the reported patterns were not consistent for all proteins [20].

Despite these parallel findings, the clinical, genetic, oxidative, and proteomic dimensions of childhood obesity and dental caries have not been well integrated within the same study population. Therefore, this study aimed to investigate the associations of the obesity-related *FTO* polymorphisms rs9939609 and rs11642841, salivary oxidative stress biomarkers, and salivary protein profiles with dental caries experience in children. Children with overweight or obesity were compared with healthy-weight controls, while plaque and gingival indices were assessed to characterize oral hygiene and gingival status.

2. Materials and Methods

2.1. Study Design and Participants

This cross-sectional study took place at the Department of Pediatric Dentistry, Faculty of Dentistry, Marmara University in Istanbul, Türkiye. The sample size was calculated in advance using G*Power software (version 3.1.9.7; Heinrich Heine University Düsseldorf, Düsseldorf, Germany), with the basis being an independent-samples *t*-test. The parameters for this calculation included an effect size (Cohen's d) of 0.65 and a significance level of 0.05; 44 children per group (88 in total) provided a statistical power of approximately 85% (two-sided); a power of 95% would have required 62 children per group. The sample size was determined to facilitate the comparison between two groups and was not powered for the genetic, regression, or protein-profile analyses; these analyses, together with all multivariable models, were considered exploratory. A total of 88 children aged 6–17 years were consecutively recruited and allocated into two groups according to age- and sex-specific BMI percentiles.

The inclusion criteria required the acquisition of written informed consent from parents or legal guardians, assent from the participating children, and a positive or definitely positive cooperation rating as per the Frankl Behavior Rating Scale. Children with BMI values between the 5th and 85th percentiles were categorized as having a healthy weight, those between the 85th and 95th percentiles as overweight, and those at or above the 95th percentile as obese. For statistical analyses, overweight and obese participants were consolidated into a single group.

The study protocol was approved by the Marmara University Faculty of Medicine Clinical Research Ethics Committee (Approval Date: 30 April 2025; Approval No. 2025-05-04/2025-11). Written informed consent was obtained from the parents or legal guardians of all participants prior to enrollment. All study procedures were performed in accordance with the ethical principles of the Declaration of Helsinki.

2.2. Anthropometric Assessment

Body weight and height were measured by trained healthcare personnel using standardized procedures, with the participants wearing light clothing and no shoes. Body weight was recorded to the nearest 0.1 kg using a calibrated digital scale, and height was measured to the nearest 0.1 cm using a wall-mounted stadiometer. The Body Mass Index (BMI) was determined by dividing weight in kilograms by the square of height in meters (m^2). The ÇEDD Çözüm (Child Metrics) online calculator, created by the Turkish Society for Pediatric Endocrinology and Diabetes, was used to calculate age- and sex-specific BMI percentiles and BMI standard deviation scores (BMI-SDS), utilizing the reference values provided by Neyzi et al. for Turkish children [21]. Participants were categorized as having a healthy weight (5th to <85th percentile), overweight (85th to <95th percentile), or obese (≥95th percentile). For the statistical analyses, the categories of overweight and obesity in children were merged into one group labeled as overweight/obesity (22 children with overweight and 22 with obesity). Differences between overweight and obese subgroups were examined descriptively (Section 3.2).

2.3. Oral Examination and General Questionnaire

All oral examinations were carried out by a single experienced examiner (a PhD student in Pediatric Dentistry) at the Department of Pediatric Dentistry, Faculty of Dentistry, Marmara University, to ensure consistency in clinical assessments. Dental caries experience was recorded using the DMFT index for permanent teeth and the dft index for primary teeth. The dft index included decayed and filled primary teeth, excluding the missing component. Therefore, physiologically exfoliated primary teeth were not counted as missing due to

caries. Permanent molars were considered erupted when at least three cusps were clinically visible in the oral cavity. Dental caries was diagnosed based on the presence of clinically detectable cavitated lesions. Non-cavitated enamel lesions, including white spot lesions, were not included in the decayed component of the DMFT and dft indices.

Dental caries experience in both primary and permanent dentitions was assessed using the dft and DMFT indices. Oral hygiene and gingival health were assessed using the Silness and Löe Plaque Index (PI) and the Löe and Silness Gingival Index (GI), respectively. All clinical findings were recorded on standardized examination forms immediately after the examination. Demographic profiles covering dietary habits and brushing routines were created in accordance with feedback from the parents' questionnaire.

2.4. Saliva Collection

Unstimulated whole saliva samples were collected from all participants between 09:00 and 11:00 a.m. under standardized conditions, with an intended target volume of approximately 5 mL, rather than over a fixed collection period. Individual collection times were not recorded. Participants were instructed to refrain from eating and drinking for at least 1 h before sample collection. During saliva collection, the participants were seated comfortably with their heads tilted slightly forward (approximately 45°) and instructed to allow saliva to accumulate naturally in the floor of the mouth before expectorating into sterile polypropylene collection tubes through disposable plastic funnels. Approximately 5 mL of unstimulated whole saliva was collected from each participant.

Saliva samples were collected over a six-month period and immediately stored at −80 °C following collection. Prior to biochemical analyses, the samples were transferred to −20 °C overnight, followed by incubation at 4 °C overnight for gradual thawing. After thawing, the samples were centrifuged, and subsequent sample preparation procedures were performed on ice. All thawed samples were analyzed on the same day, without refreezing or repeated freeze–thaw cycles.

2.5. Determination of Salivary Oxidative Stress Parameters

Salivary total oxidant status (TOS) (Cat. No. E-BC-K802-M, Elabscience, Wuhan, China) and total antioxidant status (TAS) (Cat. No. E-BC-K801-M, Elabscience, Wuhan, China) were determined using commercially available colorimetric assay kits according to the manufacturer's instructions. The oxidative stress index (OSI) was calculated individually for each participant from the TOS/TAS ratio. Because TOS (μmol H_2O_2 Eq/L) and TAS (mmol Trolox Eq/L) are reported in different molar units, TAS values were first converted to μmol Trolox Eq/L by multiplying by 1000 (in the denominator), and a scaling factor of 10,000 was applied in the numerator to express the index in arbitrary units. These two steps simplify to

$$\begin{aligned} \text{OSI (arbitrary units)} &= [\text{TOS (}\mu\text{mol } H_2O_2 \text{ Eq/L)}/(\text{TAS (mmol Trolox Eq/L)} \times 1000)] \times 10,000 \\ &= [\text{TOS (}\mu\text{mol } H_2O_2 \text{ Eq/L)}/\text{TAS (mmol Trolox Eq/L)}] \times 10 \end{aligned} \quad (1)$$

Group values reported in Table 8 are presented as the mean ± SD of individual OSI values rather than the ratio of group means. This approach yields OSI values consistent with those reported in comparable studies [17,22].

2.6. Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis (Sds-Page) Analysis of Salivary Proteins

Salivary protein profiles were analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) as described by Laemmli (1970) [23]. Prior to electrophoresis, the total protein concentration of each saliva sample was determined using the method of

Lowry (1951) [24]. Based on the measured protein concentrations, each sample was mixed with Laemmli sample buffer containing β-mercaptoethanol, and the loading volume was adjusted to ensure that 20 µg of total protein was loaded into each lane. The samples were heated at 95 °C for 5 min to denature the proteins before electrophoresis.

Proteins were separated on a 10% resolving gel with a 5% stacking gel under reducing conditions. Electrophoresis was performed at 30 mA until the bromophenol blue tracking dye reached the bottom of the gel. Following electrophoresis, the gels were stained with Coomassie Brilliant Blue R-250 (Sigma-Aldrich, St. Louis, MO, USA) and destained until the protein bands were clearly visualized.

For electrophoretic analysis, 8 saliva samples were randomly selected from each group (overweight/obesity and healthy-weight controls), yielding a total of 16 samples. The samples were analyzed on two separate gels, each containing 4 control samples, 4 overweight/obesity samples, and a molecular weight marker lane. The gels were photographed, and the resulting images were analyzed using ImageJ software version 1.54 (National Institutes of Health, Bethesda, MD, USA). Lane intensity profiles were generated using the Plot Lanes function, and the areas under the peaks corresponding to individual protein bands were determined by densitometric analysis. These peak areas were used to assess the relative intensities of protein bands across samples. Because equal amounts of total protein were loaded in each lane, peak areas were interpreted as relative quantities; no internal standard lane was used for inter-gel normalization, and band intensities were compared qualitatively between groups. Approximate molecular weights of protein bands were estimated by comparison with the molecular weight markers. Both gels were evaluated to compare protein banding patterns between the groups, and representative gel images and corresponding densitometric profiles were selected for presentation. Each sample was analyzed once, without independent technical replicates. The SDS-PAGE analysis was descriptive; no quantitative between-group comparison of band intensities was performed, and protein assignments based on apparent molecular weight are tentative.

2.7. DNA Extraction and Quality Assessment

Buccal epithelial cell samples were collected using sterile swabs and suspended in 1000 µL phosphate-buffered saline (PBS) until DNA extraction. Genomic DNA was extracted using the PureLink™ Genomic DNA Mini Kit (Cat. No. K182002, Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Briefly, samples were subjected to proteinase K digestion, followed by DNA binding to silica-based spin columns, washing, and elution steps as specified by the kit protocol. The purified genomic DNA was eluted in 50 µL elution buffer and stored at −20 °C until further analysis.

The concentration of the extracted DNA was determined using a Qubit™ 4 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. The Qubit™ working solution was freshly prepared by diluting the Qubit™ reagent in Qubit™ buffer (1:200). Following incubation at room temperature, DNA concentrations were measured fluorometrically, and only samples meeting the required quality criteria were included in the subsequent genotyping analyses. A minimum of 20 ng of DNA per reaction was required for genotyping. DNA purity (e.g., A260/A280 ratio) was not assessed. A minimum of 20 ng of DNA per reaction was required for genotyping; all 88 samples met this criterion, and no sample was excluded.

2.8. Genotyping

FTO rs9939609 and rs11642841 polymorphisms were genotyped using TaqMan SNP Genotyping Assays (Cat. No. 4351379; Assay IDs: C___2031251_10 and C__30090620_10, respectively; Thermo Fisher Scientific, Inc.) on a StepOnePlus Real-Time PCR System

(Applied Biosystems, Thermo Fisher Scientific, Foster City, CA, USA), according to the manufacturer's instructions. Each reaction was performed in a total volume of 10 µL containing 5 µL TaqMan Genotyping Master Mix, 3.5 µL nuclease-free water, 0.5 µL TaqMan SNP Genotyping Assay, and 1 µL template DNA (20 ng). Allelic discrimination was performed based on the fluorescence intensities of allele-specific VIC- and FAM-labelled probes, and genotypes were assigned using the allelic discrimination plots generated by the instrument software. Each plate included positive controls and no-template controls. The genotyping call rate was 100% for both variants. Reproducibility was verified by re-genotyping 10% of the samples, and all repeated samples gave identical genotypes (100% concordance).

2.9. Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics for Windows, version 26.0 (IBM Corp., Armonk, NY, USA). Continuous variables were summarized as mean ± standard deviation (SD) or median (interquartile range [IQR]), as appropriate, whereas categorical variables were presented as frequencies and percentages. The distribution of continuous variables was assessed based on skewness and kurtosis values. Comparisons between two independent groups were performed using the independent-samples *t*-test or Mann–Whitney U test, as appropriate, while comparisons among three or more groups were performed using one-way analysis of variance (ANOVA) or the Kruskal–Wallis test. Associations between categorical variables were assessed using the Pearson chi-square test. Fisher's exact test was used when expected cell counts were <5. FTO genotype and allele frequencies were determined by direct counting. Hardy–Weinberg equilibrium (HWE) was tested for each variant with a chi-square goodness-of-fit test separately in the healthy-weight and overweight/obesity groups. Genotypes were compared under a dominant model (A-allele carriers vs. non-carriers), which maximizes the number of individuals per comparison in a small sample; three-genotype and allele-wise comparisons were also performed. No adjustment for multiple testing was applied, and the genetic findings were considered exploratory. Univariate and multivariate binary logistic regression analyses were performed to identify factors associated with overweight/obesity and with caries presence in the permanent dentition (DMFT > 0), and the Enter method was used for multivariate analysis. Variables that remained statistically significant in the multivariate model were further evaluated using receiver operating characteristic (ROC) curve analysis. The area under the curve (AUC), 95% confidence interval (CI), cut-off value, sensitivity, and specificity were calculated. All statistical tests were two-sided, and a p value of <0.05 was considered statistically significant.

Additional analyses were performed in Python (version 3.14; Python Software Foundation, Wilmington, DE, USA) with the statsmodels (version 0.15.0) and SciPy (version 1.18.1) libraries. (i) Between-group differences are reported with 95% CIs. (ii) Because DMFT is a count with mild overdispersion (variance/mean = 1.69) and 78 of 88 children (89%) had DMFT > 0, DMFT was analyzed as a count: Poisson regression with robust (HC1) standard errors (rate ratios) and, as a sensitivity analysis, linear regression, adjusted for age and sex and then additionally for plaque index, snacking frequency, and toothbrushing frequency (covariates chosen on clinical grounds, independent of univariable p values). (iii) Because few children had DMFT = 0 (n = 10), logistic models for DMFT > 0 used Firth's penalized likelihood, and each exposure (FTO genotype group, TAS, TOS, OSI, BMI-SDS, plaque index, weight group, and the other variables of Table 7) was added separately to a model containing age and sex. (iv) Genotype distributions were tested against Hardy–Weinberg equilibrium (χ^2, 1 df), and linkage between the two SNPs was quantified as the squared correlation of allele counts (r^2). (v) As sensitivity analyses, DMFT was also modeled with a

negative binomial regression, restricted to children aged ≤12 years (n = 67), and compared across three weight categories (Kruskal–Wallis). (vi) Across the ten primary two-group comparisons (DMFT, dft, dft + DMFT, plaque, gingival index, TOS, TAS, OSI, and the two dominant-model genotype comparisons), Holm-adjusted p values were calculated; all other analyses are considered exploratory and nominal p values are presented without correction.

3. Results

A total of 88 children were included in the study, comprising 44 children with overweight/obesity and 44 healthy-weight controls. Participant characteristics are presented first, followed by the findings on dental caries, FTO polymorphisms, and salivary biomarkers.

3.1. Participant Characteristics

Data are presented as mean ± standard deviation (SD) and median (Q1–Q3). Between-group comparisons were performed using the independent-samples t-test. * $p < 0.05$; ** $p < 0.01$.

As shown in Table 1, the two groups were comparable in age (10.57 ± 2.60 vs. 10.32 ± 2.42 years; $p = 0.642$), height ($p = 0.106$), gestational age ($p = 0.679$), maternal age at delivery ($p = 0.795$), number of siblings ($p = 0.530$), and duration of breastfeeding ($p = 0.591$) (all $p > 0.05$). By design, weight, BMI, BMI-SDS, and BMI percentile were markedly higher in children with overweight/obesity (all $p < 0.001$), confirming the effectiveness of the group allocation. Similar mean ages do not establish equivalence in dentition stage, which was not assessed.

Table 1. Demographic, anthropometric, and early-life characteristics of children with overweight/obesity and healthy-weight controls.

Variables	Group	n	Mean ± SD/Median (Q1–Q3)	Test p
Age (years)	Obese/overweight	44	10.57 ± 2.60/10.50 (9.00–13.00)	t = 0.467 0.642
	Healthy weight	44	10.32 ± 2.42/10.50 (9.00–12.00)	
Height (cm)	Obese/overweight	44	147.25 ± 14.07/150.00 (140.25–158.00)	t = 1.631 0.106
	Healthy weight	44	142.43 ± 13.63/140.00 (132.75–154.25)	
Weight (kg)	Obese/overweight	44	56.77 ± 17.77/56.50 (41.90–67.25)	t = 6.665 <0.001 **
	Healthy weight	44	36.32 ± 9.93/34.50 (28.75–45.00)	
BMI	Obese/overweight	44	25.64 ± 4.36/25.24 (22.90–28.89)	t = 10.983 <0.001 **
	Healthy weight	44	17.54 ± 2.21/17.05 (16.02–18.91)	
BMI-SDS	Obese/overweight	44	2.02 ± 0.67/2.00 (1.45–2.58)	t = 16.146 <0.001 **
	Healthy weight	44	−0.23 ± 0.64/−0.17 (−0.69–0.18)	
BMI percentile	Obese/overweight	44	95.06 ± 4.46/94.95 (91.77–99.29)	t = 15.366 <0.001 **
	Healthy weight	44	42.15 ± 22.14/39.74 (22.96–62.07)	
Gestational age (weeks)	Obese/overweight	44	38.07 ± 3.05/39.00 (37.00–40.00)	t = −0.415 0.679
	Healthy weight	44	38.32 ± 2.59/40.00 (37.00–40.00)	
Maternal age at delivery (years)	Obese/overweight	44	29.32 ± 5.56/29.50 (25.00–33.00)	t = −0.261 0.795
	Healthy weight	44	29.61 ± 5.06/29.00 (25.75–33.00)	

Table 1. *Cont.*

Variables	Group	n	Mean ± SD/Median (Q1–Q3)	Test *p*
Number of siblings	Obese/overweight	44	2.50 ± 0.82/2.00 (2.00–3.00)	t = −0.631 0.530
	Healthy weight	44	2.61 ± 0.87/3.00 (2.00-3.00)	
Duration of breastfeeding (months)	Obese/overweight	44	18.16 ± 14.19/18.00 (7.50–24.00)	t = 0.539 0.591
	Healthy weight	44	16.80 ± 8.98/18.00 (11.00–24.00)	

** $p < 0.01$.

Data are presented as n (%). Between-group comparisons were performed using Pearson's chi-square test or Fisher's exact test, as appropriate. χ^2, chi-square statistic.

As presented in Table 2, the two groups did not differ significantly in sex distribution or in any of the sociodemographic characteristics examined, including mother's and father's education levels, parental employment status, and monthly household income (all $p > 0.05$). Accordingly, the groups shared a comparable sociodemographic background, which limits confounding by these variables in the subsequent analyses.

Table 2. Sociodemographic characteristics of children with overweight/obesity and healthy-weight controls.

Variables	Category	Overweight/Obesity n (%)	Healthy Weight n (%)	Test *p*
Gender	Girl	19 (43.2)	23 (52.3)	$\chi^2 = 0.729$ 0.393
	Boy	25 (56.8)	21 (47.7)	
Mother's education level	Primary or lower secondary school	18 (40.9)	21 (47.7)	$\chi^2 = 1.261$ 0.532
	High school or associate degree	16 (36.4)	17 (38.6)	
	Bachelor's degree or higher	10 (22.7)	6 (13.6)	
Mother's employment status	Yes	12 (27.3)	13 (29.5)	$\chi^2 = 0.056$ 0.813
	No	32 (72.7)	31 (70.5)	
Father's education level	Primary or lower secondary school	13 (29.5)	13 (29.5)	$\chi^2 = 0.067$ 0.967
	High school or associate degree	18 (40.9)	19 (43.2)	
	Bachelor's degree or higher	13 (29.5)	12 (27.3)	
Father's employment status	Yes	42 (95.5)	39 (88.6)	Fisher exact 0.434
	No	2 (4.5)	5 (11.4)	
Monthly household income	Below the minimum wage	5 (11.4)	3 (6.8)	$\chi^2 = 0.575$ 0.750
	The minimum wage	8 (18.2)	9 (20.5)	
	Above the minimum wage	31 (70.5)	32 (72.7)	

Regarding the FTO variants (Table 3), A-allele carriage of rs9939609 (AA + AT: 59.1% in the overweight/obesity group vs. 75.0% in controls) and rs11642841 (AA + AC: 63.6% vs. 72.7%) did not differ significantly between the groups ($p = 0.112$ and $p = 0.360$, respectively); however, with 44 children per group, these comparisons had limited precision to detect modest genetic effects. Complete genotype and allele frequencies are given in Table 3; the three-genotype comparisons also showed no significant difference (rs9939609: $p = 0.273$; rs11642841: $p = 0.640$), nor did the allele-wise comparisons (A-allele frequency 34.1% vs.

44.3%, $p = 0.165$; and 37.5% vs. 42.0%, $p = 0.538$, respectively). Genotype distributions were in Hardy–Weinberg equilibrium in both groups (healthy-weight group: rs9939609 $p = 0.107$, rs11642841 $p = 0.086$; overweight/obesity group: $p = 0.455$ and $p = 0.445$, respectively). The genotyping call rate was 100% for both variants.

Table 3. (**A**) Distribution of *FTO* genotypes grouped by A-allele carriage according to weight status. (**B**) Genotype and allele distributions of *FTO* variants according to weight status.

(**A**)				
FTO Variant	**Genotype Group**	**Overweight/Obesity (n = 44), n (%)**	**Healthy Weight (n = 44), n (%)**	***p* Value**
rs9939609	AA + AT	26 (59.1)	33 (75.0)	0.112
	TT	18 (40.9)	11 (25.0)	
rs11642841	AA + AC	28 (63.6)	32 (72.7)	0.360
	CC	16 (36.4)	12 (27.3)	
(**B**)				
FTO variant	**Genotype Group**	**Overweight/Obesity (n = 44), n (%)**	**Healthy Weight (n = 44), n (%)**	***p***
rs9939609	AA	4 (9.1)	6 (13.6)	0.273
	AT	22 (50.0)	27 (61.4)	
	TT	18 (40.9)	11 (25.0)	
	HWE *p*	0.455	0.107	
	A allele, %	34.1	44.3	0.165
	T allele, %	65.9	55.7	
rs11642841	AA	5 (11.4)	5 (11.4)	0.640
	AC	23 (52.3)	27 (61.4)	
	CC	16 (36.4)	12 (27.3)	
	HWE *p*	0.445	0.086	
	A allele, %	37.5	42.0	0.538
	C allele, %	62.5	58.0	

Pearson's chi-square test was employed to conduct comparisons between groups. Dominant model: individuals with the A-allele compared to those without it. Genotypes are given as n (%); allele frequencies are given as % of 2n alleles. Between-group comparisons were performed using Pearson's chi-square test.

3.2. Oral Findings

Dental caries, plaque, and gingival indices were compared by gender and weight status, and factors associated with dental caries were evaluated using univariable and multivariable logistic regression analyses.

When dental caries, plaque, and gingival indices were compared by gender (Table 4), boys showed a significantly higher dft score than girls (3.11 ± 3.71 vs. 1.76 ± 2.42; $p = 0.049$), whereas DMFT ($p = 0.793$), dft + DMFT ($p = 0.071$), plaque index ($p = 0.238$), and gingival index ($p = 0.863$) did not differ significantly between the genders. This borderline dft difference should be interpreted cautiously: it was one of several unadjusted comparisons, and after adjustment for age (dft decreased by 0.79 per year of age, reflecting exfoliation) the sex difference was no longer significant (+0.83, $p = 0.126$).

Table 4. Comparison of dental caries, plaque, and gingival indices by gender.

Variables	Sex	n	Mean ± SD/Median (Q1–Q3)	Test *p*
dft	Girl	42	1.76 ± 2.42/0.00 (0.00–4.00)	t = −1.995 0.049 *
	Boy	46	3.11 ± 3.71/1.00 (0.00–6.75)	
DMFT	Girl	42	3.90 ± 2.15/4.00 (2.25–4.00)	t = 0.264 0.793
	Boy	46	3.76 ± 2.88/4.00 (2.00–5.00)	
dft + DMFT	Girl	42	5.67 ± 2.77/6.00 (4.00–8.00)	t = −1.829 0.071
	Boy	46	6.87 ± 3.34/7.00 (4.00–9.75)	
Plaque Index	Girl	42	0.45 ± 0.25/0.33 (0.25–0.62)	t = −1.188 0.238
	Boy	46	0.53 ± 0.42/0.40 (0.25–0.69)	
Gingival Index	Girl	42	0.40 ± 0.25/0.29 (0.25–0.50)	t = 0.173 0.863
	Boy	46	0.39 ± 0.32/0.30 (0.25–0.50)	

Data are presented as mean ± SD/median (Q1–Q3). Comparisons were performed using the independent-samples *t*-test or Mann–Whitney U test based on the distribution of the variables. * $p < 0.05$.

Comparison of the two groups (Table 5) revealed a significantly higher DMFT score in children with overweight/obesity (4.41 ± 2.99 vs. 3.25 ± 1.86; $p = 0.032$), whereas dft ($p = 0.921$), dft + DMFT ($p = 0.065$), plaque index ($p = 0.147$), and gingival index ($p = 0.126$) were similar between groups. The unadjusted DMFT difference was 1.16 (95% CI 0.10–2.21); the Mann–Whitney p value was 0.051. DMFT was strongly related to age (Spearman $\varrho = 0.56$, $p < 0.001$); although mean age was similar between groups, dentition stage and the number of erupted permanent teeth were not assessed, so the difference could not be adjusted for them. Table 6 presents the adjusted analyses.

Poisson models used robust (HC1) standard errors. Age was a strong predictor (linear model: +0.57 DMFT per year). Erupted permanent teeth/dentition stage and dietary sugar intake were not available.

When weight status was examined in three categories, mean DMFT was 3.25 ± 1.86 in healthy-weight children (n = 44), 4.23 ± 3.41 in children with overweight (n = 22), and 4.59 ± 2.58 in children with obesity (n = 22) (one-way ANOVA $p = 0.090$); mean ages were 10.32, 10.36, and 10.77 years, respectively. The ordering suggests a graded pattern, but the differences between the three groups were not statistically significant, and pooling overweight and obesity may obscure differences by severity. When BMI-SDS was used as a continuous variable, adjusted for age and sex, each 1-SD increase was associated with +0.26 DMFT (95% CI −0.09 to 0.60; $p = 0.145$). A negative binomial model gave a similar estimate (rate ratio 1.29, 95% CI 1.02–1.62; $p = 0.032$), and overdispersion was small after covariate adjustment (Pearson $\chi^2/df = 1.18$). The Kruskal–Wallis test across the three weight categories gave $p = 0.080$. Two analyses weakened the finding: after Holm adjustment across the ten primary two-group comparisons, the DMFT difference was no

longer significant (adjusted $p = 0.25$), whereas the TOS and OSI differences remained highly significant; and when the analysis was restricted to children aged ≤12 years (n = 67), the adjusted rate ratio was 1.20 (95% CI 0.91–1.58; $p = 0.19$).

Table 5. Comparison of dental caries, plaque, and gingival indices between children with overweight/obesity and healthy-weight controls.

Variables	Overweight/Obesity (n = 44): Mean ± SD/Median (Q1–Q3)	Healthy Weight (n = 44): Mean ± SD/Median (Q1–Q3)	Test	*p* Value	Mean Difference (95% CI)
dft	2.50 ± 3.56/ 0.00 (0.00–4.00)	2.43 ± 2.87/ 0.50 (0.00–5.00)	t = 0.099	0.921	0.07 (−1.30 to 1.44)
DMFT	4.41 ± 2.99/ 4.00 (2.00–6.00)	3.25 ± 1.86/ 4.00 (2.00–4.00)	t = 2.184	0.032 *	1.16 (0.10 to 2.21)
dft + DMFT	6.91 ± 2.98/ 7.00 (4.00–9.00)	5.68 ± 3.18/ 4.00 (3.75–8.00)	t = 1.870	0.065	1.23 (−0.08 to 2.53)
Plaque Index	0.55 ± 0.42/ 0.40 (0.25–0.75)	0.44 ± 0.27/ 0.37 (0.25–0.62)	t = 1.464	0.147	0.11 (−0.04 to 0.26)
Gingival Index	0.44 ± 0.31/ 0.33 (0.25–0.54)	0.35 ± 0.26/ 0.25 (0.14–0.50)	t = 1.546	0.126	0.09 (−0.03 to 0.22)

Data are presented as mean ± SD/median (Q1–Q3). Comparisons between groups were performed using the independent-samples *t*-test or Mann–Whitney U test based on the distribution of the variables. * $p < 0.05$. Mean differences are overweight/obesity minus healthy weight.

Table 6. Adjusted comparison of DMFT between children with overweight/obesity and healthy-weight controls.

Model	Estimate (Overweight/ Obesity vs. Healthy Weight)	95% CI	*p*
Poisson, unadjusted	Rate ratio 1.36	1.05–1.76	0.021
Poisson + age, sex	Rate ratio 1.29	1.03–1.62	0.025
Poisson + age, sex, plaque index, snacking, toothbrushing frequency	Rate ratio 1.23	1.00–1.51	0.048
Linear + age, sex	Mean difference 1.00	0.12–1.88	0.026
Linear + age, sex, plaque index, snacking, toothbrushing frequency	Mean difference 0.94	0.11–1.76	0.027

OR, odds ratio; CI, confidence interval. Caries presence was defined as DMFT > 0 (permanent dentition). Of 88 children, 78 had DMFT > 0 (healthy weight 38/44; overweight/obesity 40/44) and 10 had DMFT = 0; among girls 41/42 and among boys 37/46 had DMFT > 0. Dental visit frequency was coded as never (category 1) vs. as needed or regularly (categories 2–3); toothbrushing frequency as once daily vs. more than once daily (including irregular); and snacking frequency as <2 vs. ≥2 times/day. Variables with $p < 0.05$ in the univariable analysis were entered into the multivariable model (Enter method); the gingival index showed quasi-complete separation in univariable analysis (extremely wide CI) and was therefore not entered. Last three columns: Firth penalized logistic regression; each exposure was added separately to a model containing age and sex (the age and sex rows show the age + sex base model). Predictor coding was as stated; reference categories were TT (rs9939609), CC (rs11642841), female, and healthy weight. TAS, TOS and OSI were modeled as continuous variables per unit (OSI on the corrected scale). A dash (–) means the variable was not entered in that model. †The previously reported univariable OSI odds ratio (5.427; 95% CI 0.192–153.121) referred to the old, incorrectly scaled OSI (÷100) and corresponds to 1.017 per unit of the corrected OSI. For the gingival

index, the Firth estimate is expressed per 0.1 unit (Wald confidence interval; imprecise). All 10 caries-free children were aged 6–9 years and 9 of them were boys; BMI percentile was missing for one child (n = 87 in that model).

Univariable logistic regression analysis (Table 7) identified age (OR = 2.72, 95% CI 1.56–4.74; $p < 0.001$), male sex (OR = 0.10, 95% CI 0.01–0.83; $p = 0.033$), and gingival index ($p = 0.012$) as factors associated with the presence of caries experience (DMFT > 0), whereas the association with plaque index was borderline ($p = 0.060$). In the multivariable model, age (OR = 4.20, 95% CI 1.67–10.54; $p = 0.002$) and male sex (OR = 0.03, 95% CI 0.00–0.49; $p = 0.015$) remained independently associated with caries presence. The gingival index displayed quasi-complete separation with an extremely wide confidence interval and was therefore not entered into the multivariable model. FTO genotypes, salivary oxidative stress parameters, BMI-related variables, and behavioral factors showed no significant association with caries presence. These estimates are unstable: only 10 children were caries-free and 41 of 42 girls had DMFT > 0, so the sex estimate largely reflects near-complete separation rather than a reliable effect size, and the univariable gingival index estimate (OR 881) cannot be interpreted as an effect size. All 10 caries-free children were aged 6–9 years (9 were boys), so the age and sex estimates largely describe the youngest children. After adjustment for age and sex, the gingival index remained associated with caries presence in the Firth model (OR 3.89 per 0.1 unit, 95% CI 1.15–13.22; $p = 0.029$), although the interval was wide; the plaque index ($p = 0.074$), toothbrushing frequency, snacking frequency, dental visit frequency, maternal education, BMI, and BMI percentile were not significantly associated (all $p > 0.1$). In Firth-penalized models, the FTO variants, TAS, TOS, and OSI remained unassociated with caries presence after adjustment for age and sex, but the confidence intervals were very wide (e.g., rs9939609 OR 0.33, 95% CI 0.04–2.74). These results indicate a lack of evidence of an association in this sample, not evidence of absence. In continuous analyses, TOS correlated weakly with DMFT (Spearman $\varrho = 0.22$, $p = 0.038$), but this was not retained after adjustment for age, sex, and weight group ($p = 0.60$), indicating that TOS is related to DMFT mainly through weight group.

Table 7. Factors associated with dental caries presence in univariable and multivariable analyses.

Variables	Univariable OR	95% CI	*p*	Multivariable OR (Original Model)	95% CI	*p*	Age- and Sex-Adjusted OR (Firth)	95% CI	*p*
FTO rs9939609 (AA + AT vs. TT)	0.472	0.094–2.382	0.363	–	–	–	0.33	0.04–2.74	0.303
FTO rs11642841 (AA + AC vs. CC)	0.909	0.217–3.811	0.896	–	–	–	0.82	0.12–5.59	0.839
TAS	0.808	0.179–3.639	0.781	–	–	–	0.71	0.08–6.27	0.756
TOS	1.134	0.859–1.498	0.373	–	–	–	1.03	0.72–1.48	0.879
OSI (per unit)	1.017	0.984–1.052	0.321	–	–	–	1.005 (per OSI unit)	0.97–1.04	0.776
Age	2.716	1.555–4.744	<0.001 **	4.196	1.670–10.541	0.002 **	3.31 (age + sex model)	1.58–6.95	0.002
Sex (male vs. female)	0.100	0.012–0.830	0.033 *	0.027	0.002–0.492	0.015 *	0.057 (age + sex model)	0.005–0.64	0.020
Weight group (overweight/obesity vs. healthy weight)	–	–	–	–	–	–	3.87	0.52–28.98	0.188
BMI	1.181	0.990–1.409	0.065	–	–	–	1.09	0.88–1.36	0.431
BMI-SDS	1.241	0.738–2.085	0.415	–	–	–	1.81	0.83–3.93	0.137
BMI percentile	1.006	0.986–1.027	0.553	–	–	–	1.02 (n = 87)	0.99–1.05	0.152
Maternal education level	1.082	0.443–2.643	0.862	–	–	–	1.69	0.45–6.35	0.436

Table 7. *Cont.*

Variables	Univariable OR	95% CI	*p*	Multivariable OR (Original Model)	95% CI	*p*	Age- and Sex-Adjusted OR (Firth)	95% CI	*p*
Plaque index	25.921	0.872–770.236	0.060	–	–	–	26.9	0.72–>999	0.074
Gingival index	881.365	4.376–177,510.642	0.012 *	–	–	–	3.89 (per 0.1 unit)	1.15–13.22	0.029
Toothbrushing frequency (> once vs. once daily)	0.636	0.125–3.235	0.586	–	–	–	3.53	0.43–29.34	0.243
Snacking frequency (≥ 2 vs. <2 times/day)	0.627	0.162–2.433	0.500	–	–	–	1.95	0.25–15.05	0.520
Dental visit frequency (never vs. as needed/regularly)	0.237	0.019–2.879	0.258	–	–	–	0.37	0.01–18.5	0.621

* $p < 0.05$; ** $p < 0.01$.

3.3. Salivary Oxidative Stress Parameters

The salivary oxidative stress parameters are presented in Table 8.

Table 8. Comparison of salivary oxidative stress parameters between children with overweight/obesity and healthy-weight controls.

	Healthy Weight, Mean ± SD (n = 44)	Overweight/Obesity, Mean ± SD (n = 44)	Mean Difference (95% CI)	*p* Value
Total oxidant status (μmol H_2O_2 equiv./L)	8.79 ± 1.82	11.76 ± 2.58	2.96 (2.02 to 3.91)	<0.001 **
Total antioxidant status (mmol Trolox equiv./L)	1.87 ± 0.48	1.75 ± 0.38	−0.12 (−0.30 to 0.06)	0.195
Oxidative stress index (arbitrary units)	50.51 ± 17.72	70.77 ± 24.53	20.26 (11.20 to 29.33)	<0.001 **

** $p < 0.01$.

The values are shown as mean ± standard deviation (SD). The independent-samples *t*-test was used to compare the groups. *p* values that are statistically significant are indicated with **.OSI = TOS/TAS × 10, calculated for each sample and then averaged (the previously reported values of 0.51 and 0.71 were a scaling error). Mann–Whitney tests gave the same conclusions (TOS and OSI $p < 0.001$; TAS $p = 0.248$).

In summary, children with overweight/obesity showed significantly higher salivary TOS (11.76 ± 2.58 vs. 8.79 ± 1.82 μmol H_2O_2 Eq/L) and OSI (70.77 ± 24.53 vs. 50.51 ± 17.72) than healthy-weight controls (both $p < 0.001$), whereas TAS did not differ significantly between the groups (1.75 ± 0.38 vs. 1.87 ± 0.48 mmol Trolox Eq/L; $p = 0.195$). These findings indicate a shift in the salivary redox balance toward oxidation in childhood overweight/obesity. Within the overweight/obesity group, children with overweight and with obesity had similar values (TOS 11.76 ± 2.84 vs. 11.75 ± 2.36 μmol H_2O_2 Eq/L, $p = 0.98$; OSI 69.15 ± 21.14 vs. 72.40 ± 27.92, $p = 0.67$). These between-group differences by weight status are distinct from the association of the same markers with caries, which was not significant (Section 3.2).

3.4. Sds-Page Analysis of Salivary Proteins

Representative SDS-PAGE profiles of randomly selected saliva samples from the control and obese groups are shown in Figure 1, and the corresponding densitometric profiles are presented in Figure 2. Prominent protein bands with approximate molecular weights of 69, 56, 46, and 13–16 kDa were consistently detected in both groups. Additional bands at approximately 39, 37, and 28 kDa were observed in some samples, whereas bands at approximately 75 and 78–80 kDa were detected sporadically. The densitometric analysis

confirmed the molecular weight distribution and relative intensity of the major protein bands observed in the SDS-PAGE profiles. No quantitative between-group comparison of band intensities was performed; the interpretation is therefore restricted to the qualitative patterns of the representative profiles and does not allow the conclusion that the groups do not differ.

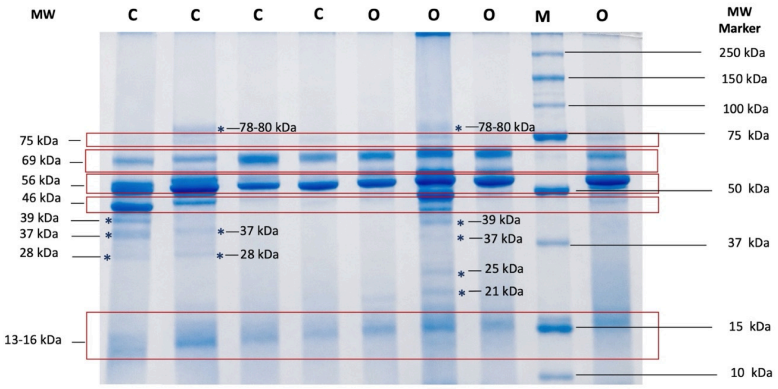


Figure 1. Representative SDS-PAGE profiles of saliva proteins from the control (C) and overweight/obese (O) group; rectangular boxes indicate common protein bands observed in both groups. Additional protein bands (39, 37, 28, 25, and 21 kDa), indicated by asterisks (*), were observed sporadically in individual samples from both groups. M: Marker; MW: Molecular weight.

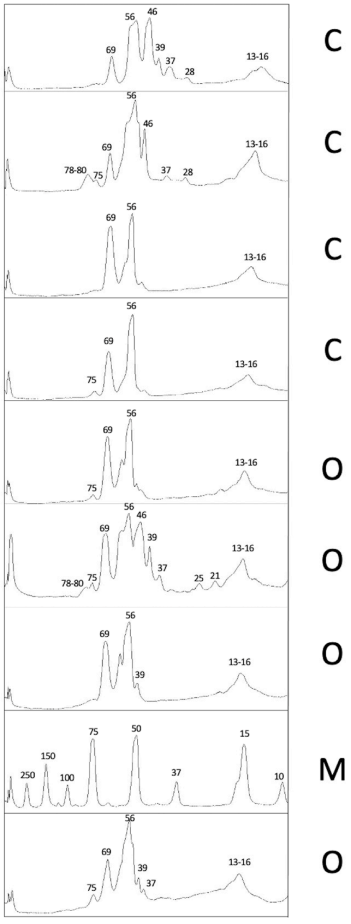


Figure 2. Representative densitometric profiles of SDS-PAGE analysis of saliva samples from control (C) and overweight/obese (O) groups. Lane M represents the molecular weight marker. Numbers indicate the approximate molecular weights (kDa) of the detected protein bands.

4. Discussion

The present study investigated the associations of *FTO* polymorphisms (rs9939609 and rs11642841), salivary oxidative stress biomarkers, and salivary protein profiles with dental caries in children with overweight/obesity and healthy-weight controls. Dental caries is acknowledged as a disease with multiple contributing factors, including genetic, environmental and socioeconomic factors [25]. Therefore, the sociodemographic characteristics of the study population are important when interpreting differences in caries experience between groups. In this study, children with overweight/obesity and those with a healthy weight were comparable regarding age (mean 10.6 vs. 10.3 years), parental education, parental employment, and monthly household income (Tables 1 and 2). The similarity in the measured sociodemographic characteristics between the two groups may have minimized variability in environmental and socioeconomic factors, although residual confounding cannot be excluded.

One of the main findings of our study was a higher DMFT score in children with overweight/obesity (4.41 ± 2.99 vs. 3.25 ± 1.86; $p = 0.032$), indicating greater caries experience in the permanent dentition. This difference persisted after adjustment for age, sex, plaque index, snacking, and toothbrushing frequency (rate ratio 1.23, 95% CI 1.00–1.51), but the lower confidence limit was at the null value and the unadjusted Mann–Whitney test gave $p = 0.051$, so the evidence should be regarded as modest. The difference was also not significant after Holm adjustment across the primary comparisons ($p = 0.25$) and was smaller in children aged ≤12 years (rate ratio 1.20, 95% CI 0.91–1.58). The three-category comparison (healthy weight, overweight, obesity) showed a graded but non-significant pattern. Due to the absence of records on the number of erupted permanent teeth and the stage of dentition, part of the difference may reflect dentition stage, and dietary sugar intake and other behaviors were not measured in detail; residual confounding therefore remains possible. This finding is consistent with a recent cross-sectional study involving 164 children aged 12–15 years, which reported higher DMFT scores among children with overweight/obesity and a positive correlation between BMI and DMFT [26]. Similarly, a German study involving 694 children aged 9–12 years reported higher DMFT scores among children with overweight/obesity and a significant association between BMI and caries experience in permanent teeth [27]. Consistent findings were also reported in another study involving 87 children aged 1–18 years, in which DMFT scores were significantly higher in children with overweight/obesity than in healthy-weight controls [28]. In a different study, in which children aged 7 to 15 years participated, overweight and obese children had significantly higher DMFT, aligning with the findings of our study [30].

In our study, there was no significant difference in dft and dft + DMFT scores between the groups. The absence of a significant difference in primary dentition caries is consistent with an Australian birth cohort study involving 1174 children, which found no association between overweight/obesity and dental caries in early childhood [31], although another study in Brazilian preschool children reported higher odds of early childhood caries in overweight/obese and underweight children [32]. These inconsistent findings may reflect differences in age, dentition stage, dietary habits, socioeconomic characteristics, and caries assessment methods across study populations. Overall, our findings suggest that the association between overweight/obesity and dental caries may differ according to dentition stage, highlighting the importance of considering the multifactorial etiology of dental caries. Because dft and DMFT were combined across ages 6–17 years, and dft necessarily falls with age as primary teeth exfoliate (−0.79 per year in our data), the combined score has limited clinical meaning in this age range.

When dental caries experience was evaluated by gender, boys in our study had significantly higher dft scores than girls (3.11 ± 3.71 vs. 1.76 ± 2.42; $p = 0.049$), whereas DMFT

did not differ significantly between genders. This difference was borderline, arose among several unadjusted comparisons, and was not significant after adjustment for age ($p = 0.126$); it should not be over-interpreted. This finding is consistent with a recent large-scale study of 4630 children aged 6–12 years, which reported significantly higher primary dentition caries experience in boys, with sex-related differences in dft ($p < 0.001$) [33]. Similarly, another study involving 3354 children aged 5–12 years reported significantly higher dft scores among boys, with no significant sex-related difference in DMFT scores [34]. These findings suggest that sex-related differences in caries experience may vary according to dentition stage. A study involving 1491 Turkish children aged 5–15 years reported that permanent teeth generally erupted earlier in girls than in boys [35]. Differences in eruption timing may influence the number of primary teeth remaining and, consequently, the interpretation of dft scores during the mixed dentition period.

A recent cross-sectional study involving 120 children aged 7–12 years reported significantly lower combined dmft + DMFT scores among children with overweight/obesity compared with underweight/normal-weight children [36]. In our study, although DMFT scores were statistically significantly higher among children with overweight/obesity, no statistically significant difference was observed in combined dft + DMFT scores between the groups. The differences in findings between the two studies, even with the larger sample size of the previous study, might underscore the multifactorial etiology of dental caries.

Previous studies have investigated the association between *FTO* polymorphisms and eating behaviors, particularly food intake in the absence of hunger and sugar consumption. Among preschool children, carriers of the rs9939609 A allele consumed more palatable food after a meal than TT homozygotes, even after adjustment for BMI [11]. Further support for this possible behavioral link comes from a study of children aged 9–12 years: gummy candy intake after a meal increased with the number of *FTO* rs9939609 risk alleles, particularly following exposure to food advertisements [12]. The previously reported association between rs11642841 and total sugar intake [14] provides an additional reason for examining this variant in relation to dental caries. In light of the well-documented correlation between frequent dietary sugar exposure and dental caries [16,29,37,38], this study aimed to investigate whether FTO gene variants, which are linked to eating behaviors and sugar consumption, also correlate with dental caries within our study population.

The choice of FTO rs9939609 and rs11642841 was based on research that connects these genetic variants to behaviors related to satiety and the consumption of total sugar, respectively. A comparable mechanism has also been described for sweet taste receptor genes: polymorphisms in TAS1R2 and TAS1R3 were associated with caries experience in schoolchildren [39], and the TAS1R2 rs35874116 TT genotype was linked to both more frequent sweet intake and severe early childhood caries in five-year-old children [40]. However, findings across populations are not entirely consistent, and these genes act through taste perception rather than appetite regulation. Together, these observations provided a reason, though not direct evidence, for examining whether FTO variants are associated with caries experience in our cohort.

In our study, the genotype distributions of *FTO* rs9939609 and rs11642841 did not differ significantly between children with overweight/obesity and healthy-weight controls. Previous studies have reported variability in the associations between *FTO* polymorphisms and obesity-related phenotypes, potentially reflecting differences in genetic background, eating behaviors, and environmental or epigenetic influences [9,41,42]. The multifactorial nature of childhood obesity is further supported by research examining the interplay between genetic susceptibility and environmental factors [43], as well as the contribution of environmental and behavioral factors to BMI and overweight in adolescents [44]. Given the multifactorial etiology of childhood obesity, individual *FTO* variants may contribute to

obesity susceptibility alongside other genetic, dietary, and lifestyle factors. The statistical power to identify subtle genetic associations might have been constrained by the relatively limited sample size of this study. The confidence intervals for the FTO–caries odds ratios were very wide (e.g., OR 0.33, 95% CI 0.04–2.74 for rs9939609), so these results represent a lack of evidence of an association rather than evidence that none exists. These findings should therefore be regarded as inconclusive. Residual confounding by unmeasured or imprecisely measured factors, such as habitual sugar and energy intake, physical activity, pubertal stage, and population substructure, also cannot be excluded. In addition, the two variants were in strong linkage ($r^2 = 0.71$) and are not independent tests of the same hypothesis. It is noteworthy that the T/T genotype of rs9939609 was numerically more frequent in the overweight/obesity group (40.9% vs. 25.0%), which was not statistically significant ($p = 0.112$) with the present sample size.

In metabolic disorders such as obesity, changes in the salivary proteome have been associated with inflammation, oxidative stress, and metabolic dysfunction [17]. Increased lipid peroxidation products and altered antioxidant enzyme activities in saliva suggest that salivary redox balance may serve as another biomarker of obesity and reflect both oral and systemic health status [45,46]. In this study, salivary TOS and OSI values were significantly higher in the overweight/obese group compared to the control group, indicating increased oxidative stress. Excess adipose tissue promotes mitochondrial dysfunction, activation of Nicotinamide Adenine Dinucleotide Phosphate (NADPH) oxidase, and chronic low-grade inflammation, all of which enhance Reactive Oxygen Species (ROS) production and may contribute to the altered salivary redox status [47,48]; this remains a hypothesis, since circulating biomarkers and longitudinal data were not available in this study.

Consistent with the present findings, Zalewska et al. also reported significantly increased salivary TOS and OSI levels in overweight and obese adolescents, supporting the presence of obesity-associated impairment of salivary redox homeostasis [17].

Although the mean salivary TAS value was lower in the overweight/obese group, the difference was not statistically significant. The data do not establish the mechanism underlying the higher TOS and OSI; an adaptive antioxidant response, enhanced oxidant production, or changes in antioxidant defense mechanisms cannot be distinguished or excluded. Our interpretation is therefore limited to the observed differences in salivary oxidative stress parameters. This finding may reflect an adaptive antioxidant response that partially compensates for the increased oxidative stress. Accordingly, the increase in oxidative stress is primarily triggered by increased oxidant production rather than a significant decrease in total antioxidant capacity. Similar observations have been reported previously, indicating that antioxidant responses may vary depending on the severity of obesity, age, metabolic status, and the specific antioxidant parameters assessed [17]. Overall, the present findings suggest that childhood obesity is associated with increased salivary oxidative stress, as evidenced by elevated TOS and OSI levels.

To further investigate whether obesity-associated oxidative stress is accompanied by alterations in salivary proteins, salivary protein profiles were evaluated using SDS-PAGE. This method was chosen to assess the overall salivary protein pattern and the distribution of major protein bands. Considerable inter-individual variability in salivary protein profiles was observed in both the control and overweight/obese groups. This variability may be attributed to several physiological and environmental factors, such as age, sex, circadian rhythm, oral hygiene, dietary habits, and genetic background. Consequently, these factors may have contributed to the differences in band intensities observed among individual samples.

Based on the approximate molecular weights determined by SDS-PAGE, the 69, 56, and 46 kDa bands may correspond to albumin, α-amylase, and carbonic anhydrase VI,

respectively. The 13–16 kDa region may represent members of the cystatin family and/or lysozyme. The sporadically detected 78–80 kDa band may correspond to lactoferrin, whereas the 75 kDa band may represent transferrin or a lower-migrating form of lactoferrin. The 46 kDa band appeared less consistently among children with obesity than in controls. The bands at approximately 39, 37, and 28 kDa may correspond to other salivary proteins, including kallikrein-1, annexins, or cathepsins. These putative protein assignments are consistent with previous studies describing the human salivary proteome [49–51]. These assignments are tentative and have not been independently confirmed.

Nevertheless, these protein assignments represent tentative interpretations based solely on apparent molecular weights estimated by SDS-PAGE. Further confirmation using protein-specific methods, such as Western blotting or mass spectrometry (LC–MS/MS), would be required to establish the definitive identity of the corresponding proteins.

Rodrigues et al. demonstrated that BMI is associated with alterations in the salivary proteome, particularly in cystatin proteins [19]. Similarly, the present study identified minor differences in salivary protein band patterns between groups, although no consistent changes in the overall protein profile were observed. Because the electrophoretic analysis was qualitative and based on representative profiles, no conclusion about the absence of group differences can be drawn. Overall, the combined assessment of salivary oxidative stress parameters and protein profiles provides a comprehensive biochemical perspective on obesity-associated changes in saliva. These findings establish a basis for future high-resolution proteomic studies to further characterize obesity-associated alterations in the salivary proteome.

Salivary proteins and oxidative stress markers may also offer insight into the oral environment associated with dental caries. In a comprehensive analysis of children with mixed dentition, it was found that caries-active children exhibited elevated total salivary protein levels, while caries-free children had higher concentrations of IgA, antimicrobial peptides, and proteinase-3 [20]. Reviews of salivary oxidative stress markers have also identified differences by caries status, although the findings vary across markers and studies [18,52]. The evidence for α-amylase is similarly mixed: systematic reviews in mixed-dentition populations report conflicting results [20]. In the present study, salivary TOS and OSI were not associated with caries presence in the regression models. Higher TOS and OSI by weight status coexisted with no significant association with the binary caries outcome. This is not necessarily contradictory: caries was nearly universal (DMFT > 0 in 78 of 88 children), only 10 children were caries-free, and dichotomizing DMFT discards information, so the binary outcome had little ability to detect an association; in the continuous analysis, TOS correlated weakly with DMFT ($\varrho = 0.22$) but not after adjustment, suggesting that TOS is related to caries experience mainly through weight group. SDS-PAGE analysis showed that the salivary protein profiles of children with overweight or obesity did not differ consistently from those of children with a healthy weight in the representative profiles. These findings indicate that changes in salivary oxidative stress associated with obesity do not necessarily correlate with variations in caries status. This underscores the importance of conducting further research to assess the clinical significance of individual salivary biomarkers.

In our study, the gingival index was significantly associated with caries presence in the univariable analysis ($p = 0.012$), whereas the plaque index showed a borderline association ($p = 0.060$) (Table 7). The univariable gingival index estimate was unstable because of quasi-complete separation (OR 881, 95% CI 4.4–177,511) and is not interpretable as an effect size; after adjustment for age and sex the association persisted but was imprecise (OR 3.89 per 0.1 unit, 95% CI 1.15–13.22). Plaque scores represent plaque accumulation at the time of examination and may have been influenced by toothbrushing immediately before the dental

appointment. In contrast, gingival inflammation may reflect a more sustained response to plaque accumulation and may therefore be less sensitive to recent toothbrushing. This possibility should be considered when interpreting the observed associations between oral hygiene indicators and caries presence.

The current study has several limitations that need to be recognized. One such limitation is the relatively small sample size, which might have reduced the ability to statistically identify modest associations, particularly those involving FTO polymorphisms and salivary biomarkers. The sample size was calculated for a two-group comparison (approximately 85% power), not for these analyses. Caries was almost universal in this sample (DMFT > 0 in 89%), which made logistic models for caries presence unstable and limited the binary outcome; DMFT could not be adjusted for the number of erupted permanent teeth or dentition stage, and dietary sugar intake was not quantified. Weight categories (overweight and obesity) were pooled for the main comparisons, and the age range (6–17 years) spans primary, mixed, and permanent dentition. Multiple comparisons were corrected only for the primary two-group comparisons (the DMFT difference did not remain significant, whereas the TOS and OSI differences did), and the genetic, biochemical, and multivariable analyses were exploratory. All caries-free children were aged 6–9 years. The two FTO variants were in strong linkage and are not independent; haplotype analysis was not performed, and residual confounding (e.g., by physical activity, pubertal stage, or population stratification) cannot be excluded. The SDS-PAGE analysis was qualitative, and information on saliva flow rate was not available, so dilution effects on TOS/TAS concentrations cannot be excluded. Only two FTO variants were examined, mainly under a dominant model, and small genotype cell counts limited genotype-wise analyses; no correction for multiple comparisons was applied, so the genetic findings should be considered exploratory, and residual confounding and population stratification cannot be excluded. In addition, the cross-sectional design does not allow causal relationships to be established. Although SDS-PAGE enabled the comparison of salivary protein profiles, its limited resolution and inability to identify individual proteins restrict the interpretation of these findings. Additionally, salivary flow rate was not measured, which represents a limitation of the present study, as variations in salivary flow may influence the measured concentrations of oxidative stress biomarkers and potentially affect comparisons between groups.

Furthermore, dietary and oral hygiene information was parent-reported and may have been affected by recall and social desirability bias. Future longitudinal studies involving larger cohorts and more advanced proteomic techniques are needed to further investigate the potential relationships among genetic susceptibility, salivary biomarkers, and dental caries in children.

5. Conclusions

In conclusion, children with overweight/obesity in this cohort showed higher caries experience in the permanent dentition (DMFT) than age-comparable healthy-weight controls, a difference that was modest, persisted after adjustment for age, sex, and oral hygiene/dietary variables, but did not remain significant after multiple-comparison correction and could not be adjusted for dentition stage, whereas primary-tooth caries did not differ between the groups. Salivary TOS and OSI were significantly elevated in the overweight/obesity group, supporting saliva as a non-invasive source of obesity-related oxidative stress biomarkers, while *FTO* rs9939609 and rs11642841 were not associated with either weight status or caries presence in this small sample, without excluding modest associations. Because oral health is integral to overall health, dietary, genetic, and salivary factors should be considered together when investigating caries susceptibility in children. Larger, preferably

longitudinal and age-stratified studies with high-resolution proteomic methods are needed to clarify the clinical relevance of these salivary alterations for caries susceptibility.

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Abbreviations

BMI	body mass index
BMI-SDS	body mass index standard deviation score
CI	confidence interval
dft	decayed and filled teeth (primary dentition)
DMFT	decayed, missing, and filled teeth (permanent dentition)
FTO	fat mass and obesity-associated gene
GI	gingival index
IQR	interquartile range
OR	odds ratio
OSI	oxidative stress index
PI	plaque index
ROC	receiver operating characteristic
SDS-PAGE	sodium dodecyl sulfate–polyacrylamide gel electrophoresis
TAS	total antioxidant status
TOS	total oxidant status
WHO	World Health Organization

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