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Morphological study of the mucosal epithelium of the palatal gingiva of the maxillary first molars in obese type 2 diabetes mellitus model rats

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In Japanese, obesity is a lifestyle-related disease and major risk factor for type 2 diabetes mellitus. Asians, including the Japanese, are reported to have a higher incidence of diabetes mellitus because they have a lower body mass index and higher body fat percentage. In this study, we performed a morphological analysis of the mucosal epithelium of the palatal gingiva of the maxillary first molar in normal rats and in Spontaneously Diabetic Torii fatty rats, a model of obese type 2 diabetes mellitus (ODM). The superficial morphology of the mucosal epithelium showed epithelial cells of various sizes and nearly exfoliated epithelial cells in the ODM group. The mucosal epithelium was significantly thicker in the ODM group than in the normal group ($p < 0.01$). The number of cells in the granular and prickle layers was significantly greater in the ODM group than in the normal group ($p < 0.01$), and the number of epithelial papilla was significantly lower in the ODM group than in the normal group ($p < 0.01$). In conclusion, hyperglycemia with ODM thickens the mucosal epithelium of the palatal gingiva of the maxillary first molar causing the epithelial papillae to be lower and epithelial cells of the keratinized layer to detach more easily. (J Osaka Dent Univ 2023; 57: 301-312)

Key words: Obese type 2 diabetes mellitus; SDT fatty rat; Gingiva; Maxillary first molar; Mucosal epithelium

INTRODUCTION

In Japanese, obesity is a lifestyle-related disease and major risk factor for type 2 diabetes mellitus (DM). Approximately 26% of Japanese adults have a body mass index (BMI) of 25 kg/m^2 or greater, and this proportion is increasing.[1] Asians, including Japanese, are reported to have a higher incidence of diabetes mellitus because they have a lower BMI and higher body fat percentage than Caucasians and Europeans.[2] In the Goto-Kakizaki (GK) rat[3], an animal model of spontaneous non-obese type 2 DM, hyperglycemia has been reported to cause atrophic changes in various oral organs (gingiva,[4-6] tongue,[7] palate,[8] mandibular glands[9] and connective tissue of the posterior part of the articular disc[10]). We have also reported on the subepithelial connective tissue and capillaries of the palatal gingiva of the maxillary first molar in the Spontaneously Diabetic Torii (SDT) fatty rat[11] as a model rat of obese type 2 DM (ODM);[12] however, to our knowledge, investigation on the mucosal epithelium has not been reported. Therefore, we performed a morphological study of the mucosal epithelium of the palatal gingiva of the maxillary first molars in ODM model and normal (N) rats.

MATERIALS AND METHODS

Experimental Animals

We used 12 rats in this study: six 8-week-old male SDT fatty rats with a body weight of 298.8 ± 13.0 g in the ODM group, and six 8-week-old male Sprague-Dawley rats with a body weight of 242.0 ± 28.8 g in the N group (Fig. 1 and Table 1). The animals were purchased from CLEA (Tokyo, Japan). The study was approved by the Osaka Dental Uni-

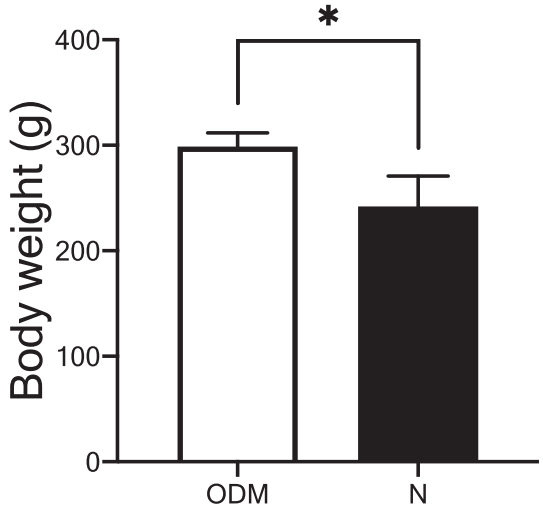

Fig. 1 Comparison of the body weight in obese type 2 diabetes mellitus (ODM) and normal (N) rats (*$p < 0.01$).

Table 1 Comparison of body weight and serum levels of fasting blood glucose and hemoglobin $A1_c$ in ODM and N rats (*$p < 0.01$)

Parameter	ODM	N
Body weight (g)	298.8±13.0*	242.0±28.8
Fasting blood glucose levels (mg/dL)	128.8±14.8*	71.0±8.7
Hemoglobin $A1_c$ levels (%)	5.7±0.6*	3.7±0.1

*$p < 0.01$

versity Institutional Animal Care and Use Committee (Approval Nos. 21-03008 and 22-01003).

Measurements of fasting blood glucose and hemoglobin $A1_c$ levels

All rats were fasted for 24 h, intraperitoneally injected with Novo Heparin Injection 5000® heparin sodium (Mochida Pharmaceutical, Tokyo, Japan), and weighed. After 30 min, the rats were euthanized by an intraperitoneal overdose of Somnopentyl® barbiturates (Kyoritsu Pharmaceutical, Tokyo, Japan). Four milliliters of blood was collected from the left ventricle using a 5-mL Terumo® syringe (Terumo, Tokyo, Japan) after the thorax had been opened. The serum sample was collected, and fasting blood glucose (FBG) levels were measured via the hexokinase / glucose-6-phosphate dehydrogenase method using a Quick Auto Neo GLU-HK kit (Shino Test, Tokyo, Japan) after centrifugation of 2 mL of the collected blood at 3,000 rpm for 5 min in a H-201FR high-speed cooling centrifuge (Kokusan, Saitama, Japan).[13] The $HbA1_c$ levels of the remaining 2 mL of blood were measured using a RAPIDIA® Auto $HbA1_c$-L kit latex agglutination-based assay (Fujirebio, Tokyo, Japan).[14] The $HbA1_c$ levels were determined using National Glycohemoglobin Standardization Program values. A cannula was then inserted from the left ventricle into the ascending aorta to rinse blood from the right atrium using a 0.1-M phosphate buffer solution (PBS) to prepare the specimens as described below.

Collection and preparation of superficial morphological specimens

Three rats from each group were used to observe the superficial morphology of the mucosal epithelium of the palatal gingiva of the maxillary first molars. The solutions were adjusted to 2.5% (w/v) glutaraldehyde (Kishida Chemical, Osaka, Japan) and 5% (w/v) neutral-buffered formalin (Sigma-Aldrich, Osaka, Japan) with 0.1-M PBS, respectively, and the mixed solutions were infused into the ascending aorta. A BS-3000 diamond bandsaw (EXAKT, Norderstedt, Germany) was used for en bloc removal of the maxillary first molars together with the surrounding mucosa and bone. The samples were fixed via soaking in a glutaraldehyde/formalin solution at 4°C for 24 h. The samples were then soaked in a 1% (w/v) tannic acid solution (Fujifilm Wako Pure Chemical, Osaka, Japan) for 2 h for pretreatment after washing with 0.1-M PBS for 1 h. They were then washed in 0.1-M PBS for 2 h and further washed in 0.1-M PBS using an UT-105HS® ultrasonic washer (Sharp, Osaka, Japan) at 35°C for 5 min. The samples were subsequently dehydrated using an increasing series of ethanol concentrations. The ethanol was replaced with t-butyl alcohol (Kishida Chemical) for 12 h, and the samples were freeze-dried using a JFD-310® freeze-dryer (Jeol, Tokyo, Japan). The samples were fixed onto a metal stage using conductive NEM TAPE® (Nisshin Em, Tokyo, Japan) and DOTITE® silver paste (Fujikura Kasei, Tochigi, Japan). Finally, an

osmium coating was applied to the superficial morphological specimens to a thickness of 2 nm using an HPC-20® osmium coater (Vacuum Device, Ibaraki, Japan). For the superficial morphological specimens, the mucosal epithelium of the palatal gingiva of the maxillary first molar was photographed from the palatal side using a JSM-5500® scanning electron microscope (SEM) (Jeol), and the obtained digital images were used to evaluate the superficial morphology of the mucosal epithelium.

Collection and preparation of histological specimens

Three rats from each group were used to observe the histological morphology of the mucosal epithelium of the palatal gingiva of the maxillary first molars. A 10% (w/v) neutral-buffered formalin solution (Sigma-Aldrich) was infused into the ascending aorta. A diamond bandsaw was used for en bloc tissue removal as described for superficial morphological specimens. The samples were soaked in the aforementioned formalin solution at 4°C for 24 h followed by decalcification with a 10% (w/v) ethylenediaminetetraacetic acid disodium solution (Kishida Chemical) for 14 days using an ML-77® microwave rapid sample processor (Azumaya, Tokyo, Japan). They were dehydrated with an increasing series of ethanol concentrations after washing with 0.1-M PBS followed by soaking for 4.5 h in a G-Nox low-toxicity solvent (Genostaff, Tokyo, Japan) as a xylene substitute. The specimens were subsequently embedded in paraffin pellets (Genostaff) using a CT-Pro 20® paraffin-embedding device (Genostaff). Serial frontal sections (5 μm were sliced distally from the medial surface of the maxillary first molar using an HM 430® sliding microtome (Thermo Fisher Scientific, Shanghai, China). Sections for histological analysis were prepared using conventional hematoxylin and eosin staining.

In the histological specimens, the palatal gingiva of the maxillary first molars of the ODM and N group rats was photographed using a BZ-9000® digital light microscope (Keyence, Osaka, Japan), and 10 digital images per specimen were selected for histological evaluation of the mucosal epithelium. A standard line (line S) was drawn through the cementoenamel junction (CEJ) of the palatal gingiva of the right and left maxillary first molars in the digital images (Fig. 2 A). Next, a perpendicular line (P) was drawn from the top of the gingiva to line S and divided into three equal regions, referred to as the upper gingiva (U), middle gingiva (M), and lower gingiva (L). The lowest point of the mucosal epithelium in the selected area was named point z (Fig. 2 B). From point z, a perpendicular line (line u) was drawn perpendicular to the tangent line of the most superficial portion of the mucosal epithelium of the palatal gingiva of the maxillary first molar. The intersection point of line u and deepest part of the keratinized layer is denoted by point w, and the distance from point v to w is the thickness of the keratinized layer (k). The intersection point of line u and deepest part of the granular layer was named point x, and the distance from point w to point x was named the thickness of the granular layer (g). The intersection point of line u and deepest part of the prickle layer is denoted by point y, and the distance from point x to y is the thickness of the prickle layer (p). The distance from point y to z is defined as the thickness of the basal layer (b). The distance from point v to z is defined as the thickness of the mucosal epithelium (e). The number of cells in the granular and prickle layers of line u were counted. A cotangent line q was then drawn to the uppermost points of the adjacent connective tissue papilla with reference to the epithelial papilla at the chosen location, a vertical line was drawn from the cotangent line to the lowest point r of the epithelial papilla, and the intersection of line q and vertical line was named point t (Fig. 2 C). The distance from point r to t was defined as the height of the epithelial papilla (H). The thickness and cell count of the tissue were measured using Image-Pro Plus® 5.1 J image analysis software (Nippon Roper, Tokyo, Japan). The mean and standard deviation of the measured levels were calculated, and Student's *t*-test was performed for analysis of differences among the groups at a significance level of 1%.

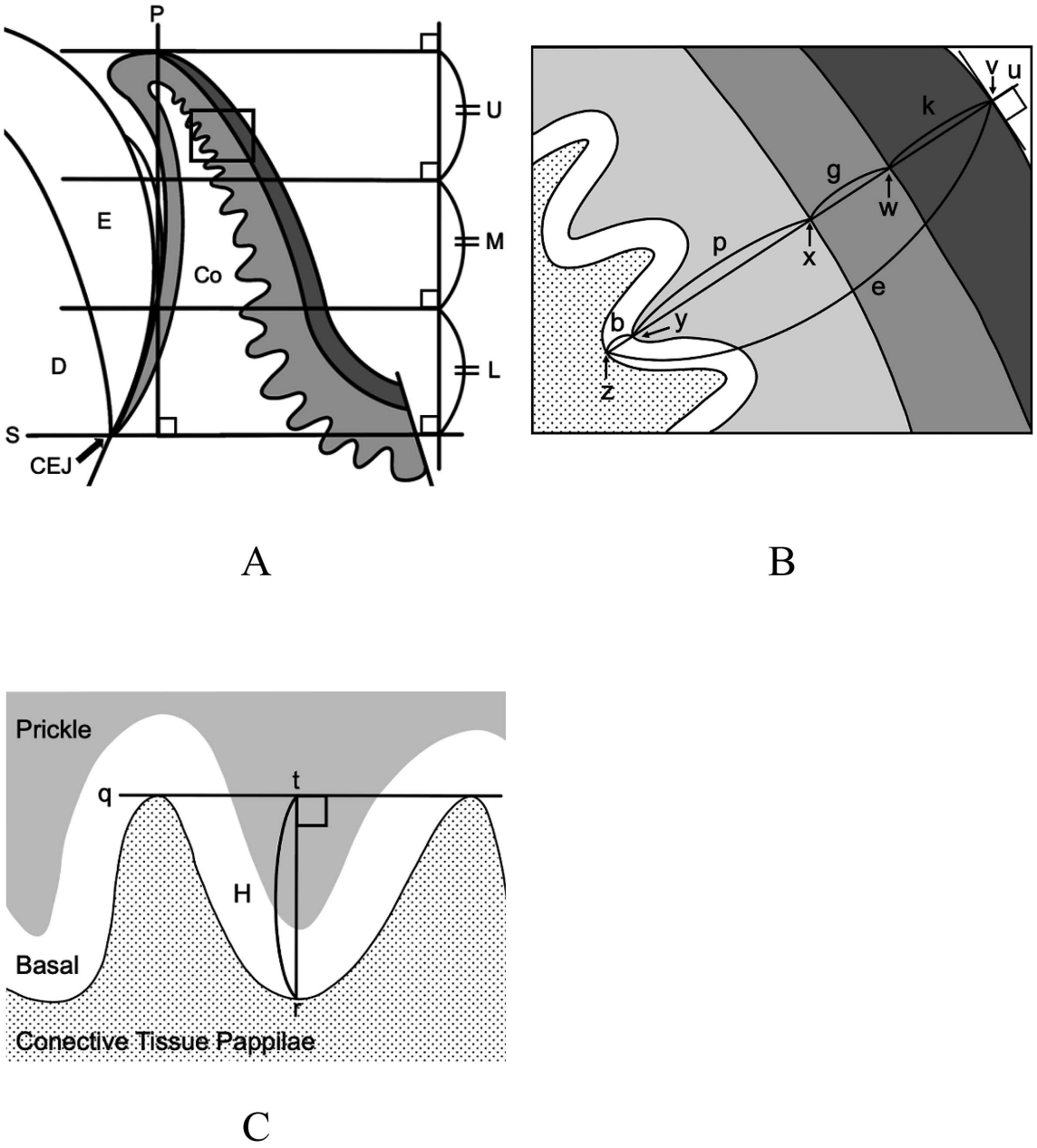

Fig. 2 Palatal gingiva of the maxillary first molar. (**A**) Measurement of the thickness of the mucosal epithelium in the palatal gingiva of the maxillary first molar (CEJ: Cementoenamel junction, Co: Connective tissue beneath the mucosal epithelium, D: Dentin, E: Decalcified enamel, P: Perpendicular line, S: Standard line, U: Upper gingiva, M: Middle gingiva, L: Lower gingiva). (**B**) Higher magnification of the black frame in **A** showing measurement of the thickness of the keratinized (k), granular (g), prickle (p), and basal (b) layers, and the mucosal epithelium (e). The most inferior point of the epithelial papilla is named point z. A perpendicular line (line u) has been drawn from point z to the tangent line on the surface of the maxillary gingival mucosa (v: Intersection of line u and surface of keratinized layer, w: Intersection of line u and deepest point of keratinized layer, x: Intersection of line u and deepest point of the granular layer, y: Intersection of line u and deepest point of prickle layer). (**C**) Measurement of the height of the epithelial papilla (H) (q: Line cotangent to the uppermost point of the adjacent connective tissue process, r: Lowest point of the epithelial papilla, t: Intersection of the cotangent line q and the perpendicular line).

RESULTS

Measurements of fasting blood glucose and hemoglobin A1c levels

The FBG levels were significantly greater in the ODM than in the N group (128.8 ± 14.8 vs. 71.0 ± 8.7 mg/dL, $p < 0.01$, Fig. 3 and Table 1). Similarly, the HbA1c levels were significantly greater in the ODM than in the N group (5.7 ± 0.6 % vs. 3.7 ± 0.1 %, $p < 0.01$, Fig. 4 and Table 1).

Specimen Findings

Gross findings

No characteristic morphological changes were observed in the ODM and N groups in the mucosal epithelium of the palatal gingiva of the maxillary first molars, and the two groups could not be distinguished in a blinded manner.

Superficial morphological specimens

Epithelial cells of various sizes were observed on the surface of the mucosal epithelium of the palatal

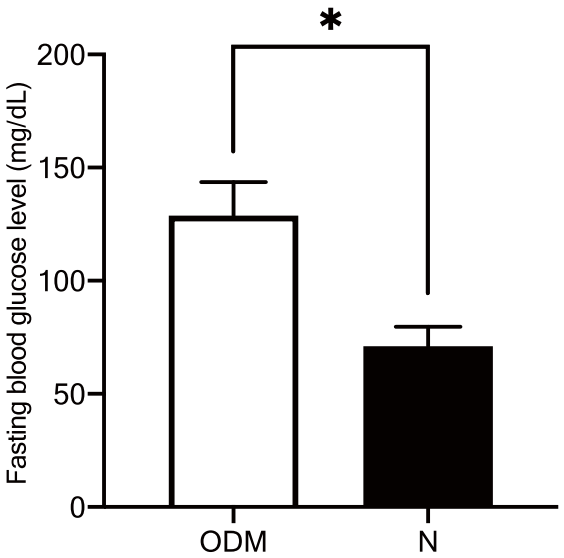

Fig. 3 Comparison of fasting blood glucose levels in the ODM and N rats (*$p < 0.01$).

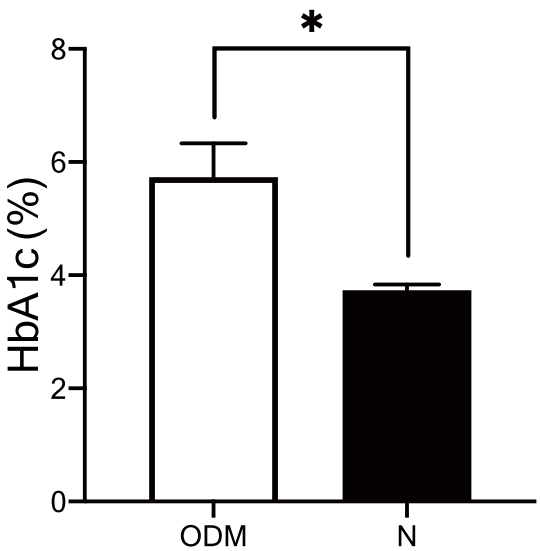

Fig. 4 Comparison of hemoglobin A1c (HbA1c) levels in the ODM and N rats (*$p < 0.01$).

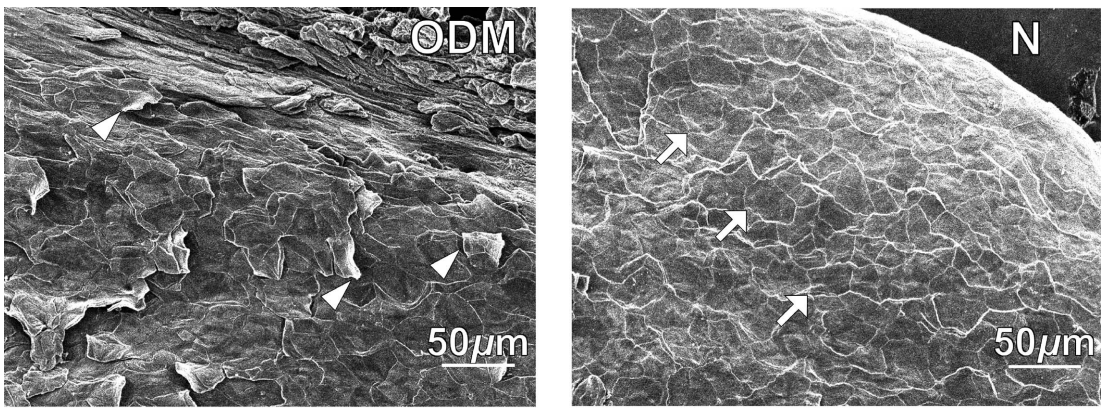

Fig. 5 Scanning electron micrographs of superficial morphological specimens in the ODM rats (**A**) and in N rats (**B**).

gingival margin of the maxillary first molars in the ODM group (arrowheads in Fig. 5 A), and many epithelial cells were on the verge of detachment. Conversely, epithelial cells of uniform size with a tortoise-shell pattern were observed with few epithelial cells on the verge of exfoliation in the N group (arrows in Fig. 5 B).

Histological specimens

The mucosal epithelium of the palatal gingiva of the maxillary first molars was thicker in the ODM group than in the N group (Figs. 6 A and C). In addition, the structures of the keratinized, granular, prickle, and basal layers were observed in both groups; however, no inflammatory cell infiltration was seen

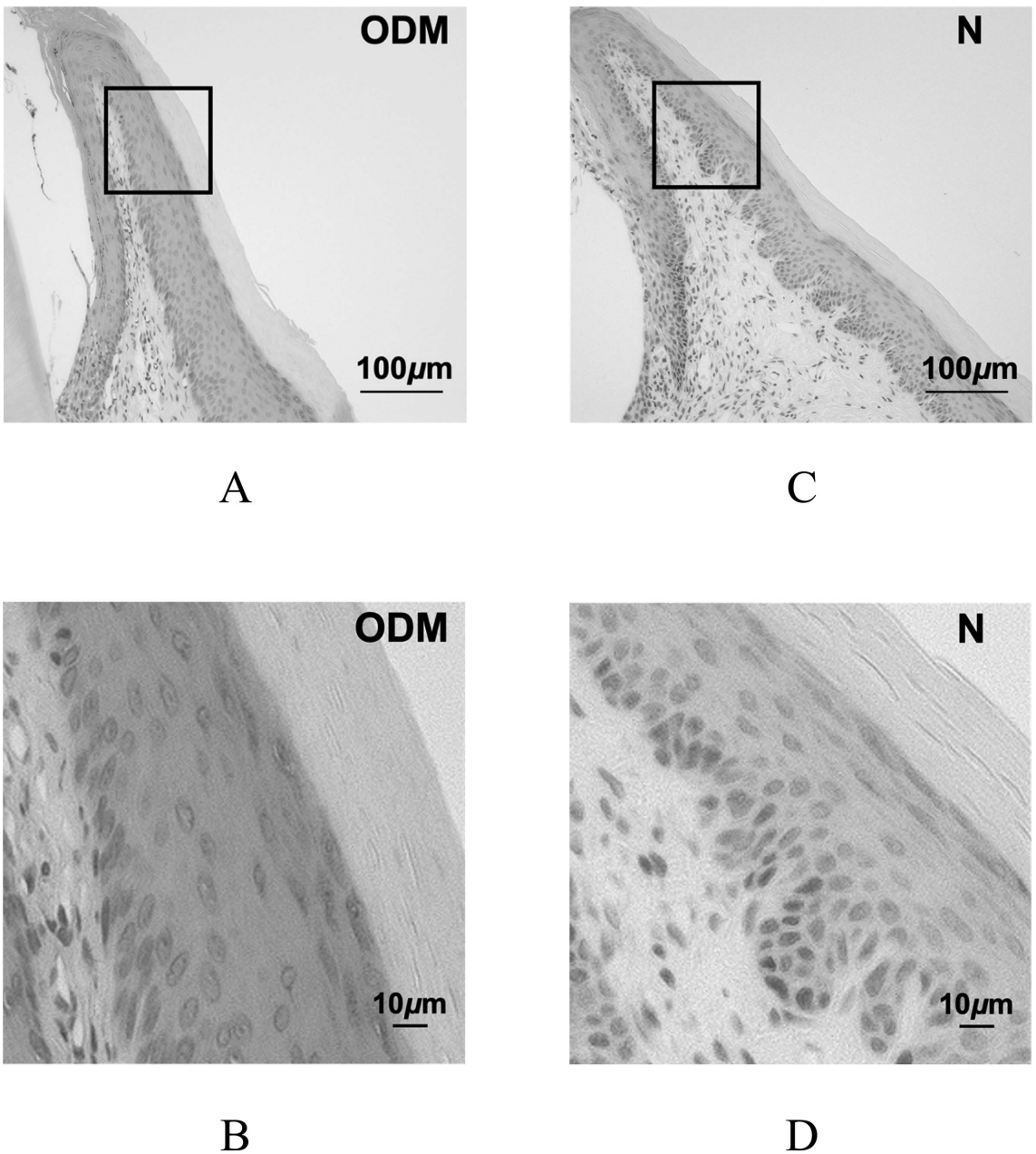

Fig. 6 Light micrographs of histological specimens from an ODM rat (**A**) and a higher magnification (**B**) of the black frame area in **A**. Similar low- and high-magnification images of histological specimens from an N rat are shown in (**C**) and (**D**).

(Figs. 6 B and D). Anucleated keratinocytes stained with eosin were observed in the keratinized layer, whereas epithelial cells containing keratohyalin granules stained dark with hematoxylin were observed in the granular layer. The basal layer was present as a single layer in the lowest layer of the mucosal epithelium, and a prickle layer was observed between the granular and basal layers. The epithelial papillae had an acute apical shape in the ODM group (Figs. 6 A and B), whereas they had a rounded apical shape in the N group (Figs. 6 C and D).

Imaging analysis and statistical processing

Histological specimens

The keratinized layer (k) was significantly thicker in the ODM group than in the N group (37.1 ± 7.2 μm vs. 29.6 ± 9.2 μm, $p < 0.01$, Fig. 7 A and Table 2). The granular layer (g) was significantly thicker in the ODM group than in the N group (20.8 ± 5.1 μm vs. 15.2 ± 3.7 μm, $p < 0.01$, Fig. 7 A and Table 2). The prickle layer (p) was significantly thicker in the ODM group than in the N group (62.2 ± 17.0 μm vs. 40.4 ± 16.6 μm, $p < 0.01$, Fig. 7 A and Table 2). The basal layer (b) was not significantly different between the two groups (7.6 ± 1.6 μm vs. 7.5 ± 1.7 μm, Fig. 7 A and Table 2). The thickness of the mucosal epithelium (e), and sum of

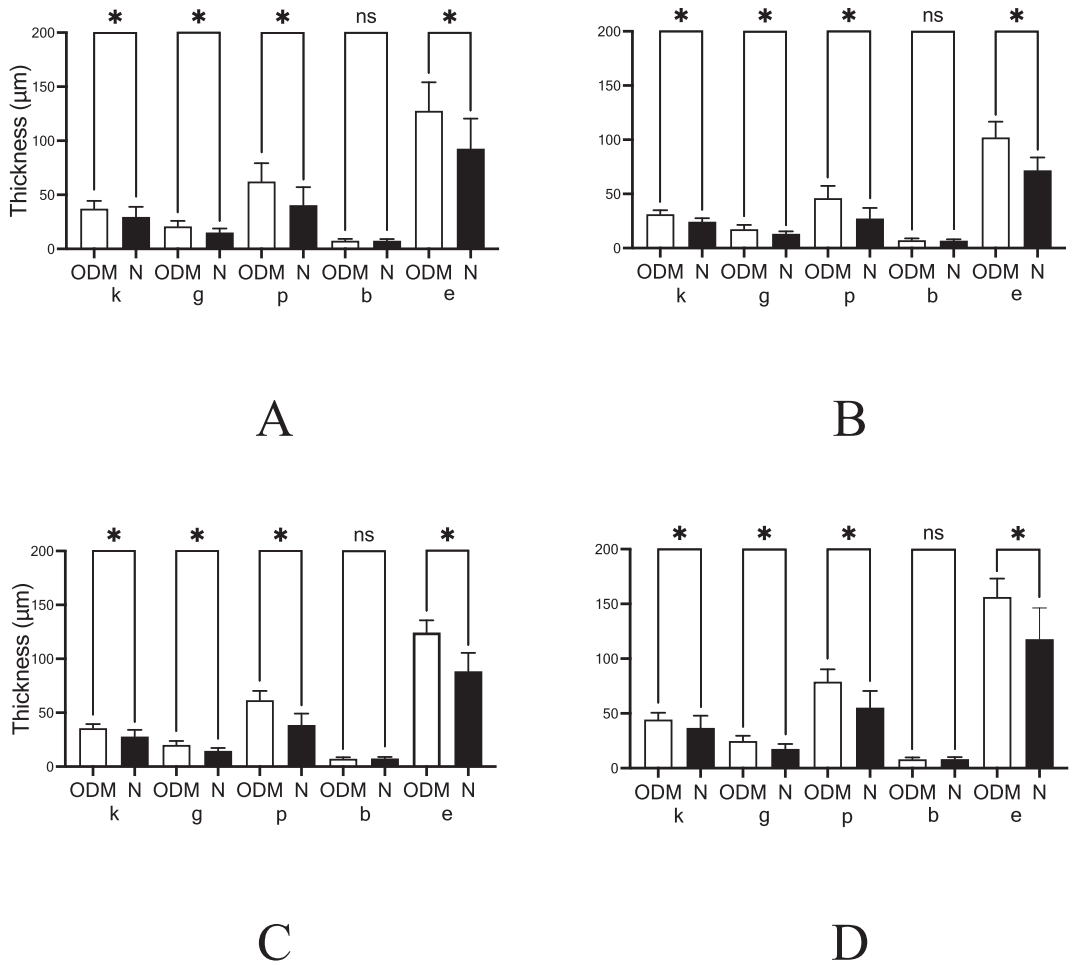

Fig. 7 Comparison of the thickness of the mucosal epithelium and the keratinized (k), granular (g), prickle (p), and basal (b) layers, and the mucosal epithelium (e) in various regions of the ODM and N rats in the (**A**) average region, (**B**) upper gingiva, (**C**) middle gingiva, and (**D**) lower gingiva (*$p < 0.01$).

Table 2 Comparison of the thickness of the keratinized, granular, prickle, basal layers, and mucosal epithelium in various regions of the ODM and N rats in the average region, upper gingiva, middle gingiva, and lower gingiva (*$p<0.01$)

Parameter		ODM	N
Keratinized layer	Average	37.1±7.2*	29.6±9.2
	Upper	31.3±3.7*	24.4±3.2
	Middle	35.6±3.8*	27.7±6.3
	Lower	44.4±6.2*	36.7±11.2
Granular layer	Average	20.8±5.1*	15.2±3.7
	Upper	17.5±3.9*	13.3±2.2
	Middle	20.1±3.7*	14.6±2.7
	Lower	24.7±4.8*	17.6±4.5
Prickle layer	Average	62.2±17.0*	40.4±16.6
	Upper	46.1±11.3*	27.4±9.6
	Middle	61.6±8.6*	38.6±10.6
	Lower	79.0±11.2*	55.2±15.3
Basal layer	Average	7.6±1.6	7.5±1.7
	Upper	7.3±1.7	6.8±1.3
	Middle	7.2±1.4	7.5±1.4
	Lower	8.2±1.6	8.2±1.9
Mucosal epithelium	Average	127.6±26.5*	92.7±27.8
	Upper	102.1±14.5*	71.8±11.8
	Middle	124.5±11.2*	88.4±17.1
	Lower	156.3±16.8*	117.8±28.6

*$p<0.01$ (μm)

Table 3 Comparison of the cell population of the granular and prickle layers in various regions of the ODM and N rats in the average region, upper gingiva, middle gingiva, and lower gingiva (*$p<0.01$)

Parameter		ODM	N
Granular laye	Average	3.9±0.9*	2.9±0.7
	Upper	3.5±0.9*	2.6±0.5
	Middle	3.7±0.6*	2.9±0.6
	Lower	4.4±0.7*	3.2±0.8
Prickle layer	Average	7.7±1.9*	5.6±2.2
	Upper	6.2±1.5*	4±1.1
	Middle	7.8±1.2*	5.3±1.6
	Lower	9.3±1.7*	7.4±2.1

*$p<0.01$ (cells)

Table 4 Comparison of the height of the epithelial papilla in the ODM and N rats (*$p<0.01$)

Parameter	ODM	N
Height of the epithelial papillae	13.1±7.7*	17.6±9.0

*$p<0.01$ (μm)

the thicknesses of the keratinized (k), granular (g), prickle (p), and basal (b) layers were significantly greater in the ODM group than in the N group (127.6 ± 26.5 μm vs. 92.7 ± 27.8 μm, $p<0.01$, Fig. 7 A and Table 2). The number of cells in the granular layer was significantly greater in the ODM group than in the N group (3.9 ± 0.9 cells vs. 2.9 ± 0.7 cells, $p<0.01$, Fig. 8 A and Table 3). The number of cells in the prickle layer was significantly greater in the ODM group than in the N group (7.7 ± 1.9 cells vs. 5.6 ± 2.2 cells, $p<0.01$, Fig. 8 A and Table 3). The basal layer was defined as a single layer. The number of cells in the keratinized layer could not be counted because the nuclei could not be observed. All measurements in the upper (U), middle (M), and lower (L) gingival sections showed similar trends ($p<0.01$, Figs. 7 B-D, Figs. 8 B-D, Tables 2 and 3). The height of the epithelial papilla (H) was significantly lower in the ODM group than in the N group (13.1 ± 7.7 μm vs. 17.6 ± 9.0 μm, $p<0.01$, Fig. 9 and Table 4).

DISCUSSION

Experimental animals

DM is of two types: type 1 and type 2. Type 1 DM has a rapid and irreversible decrease in insulin secretion resulting in hyperglycemia due to destruction of the pancreatic islet β cells, while type 2 DM has a chronic hyperglycemic state resulting from varying degrees of impaired insulin secretion and increased insulin resistance. A streptozotocin (STZ) -induced type 1 DM model rat has been studied as a model of type 1 DM. STZ induces type 1 DM in animals through destruction of the β cells of the pancreatic islets, and has also been reported to have adverse effects on the kidneys and liver.[15, 16] This STZ-induced type 1 DM model rat has been reported to have extremely high FBG levels of approximately 400 mg/dL or higher.[17, 18] The GK rat has also been used as a non-obese type 2 DM model that tends to have the same FBG levels as adult Japanese type 2 DM or borderline DM. Relatively lower FBG levels of 240.9 ± 58.0 mg/dL have been reported in the GK rats[9] than in the STZ -induced type 1 DM model rats, as well as atrophic

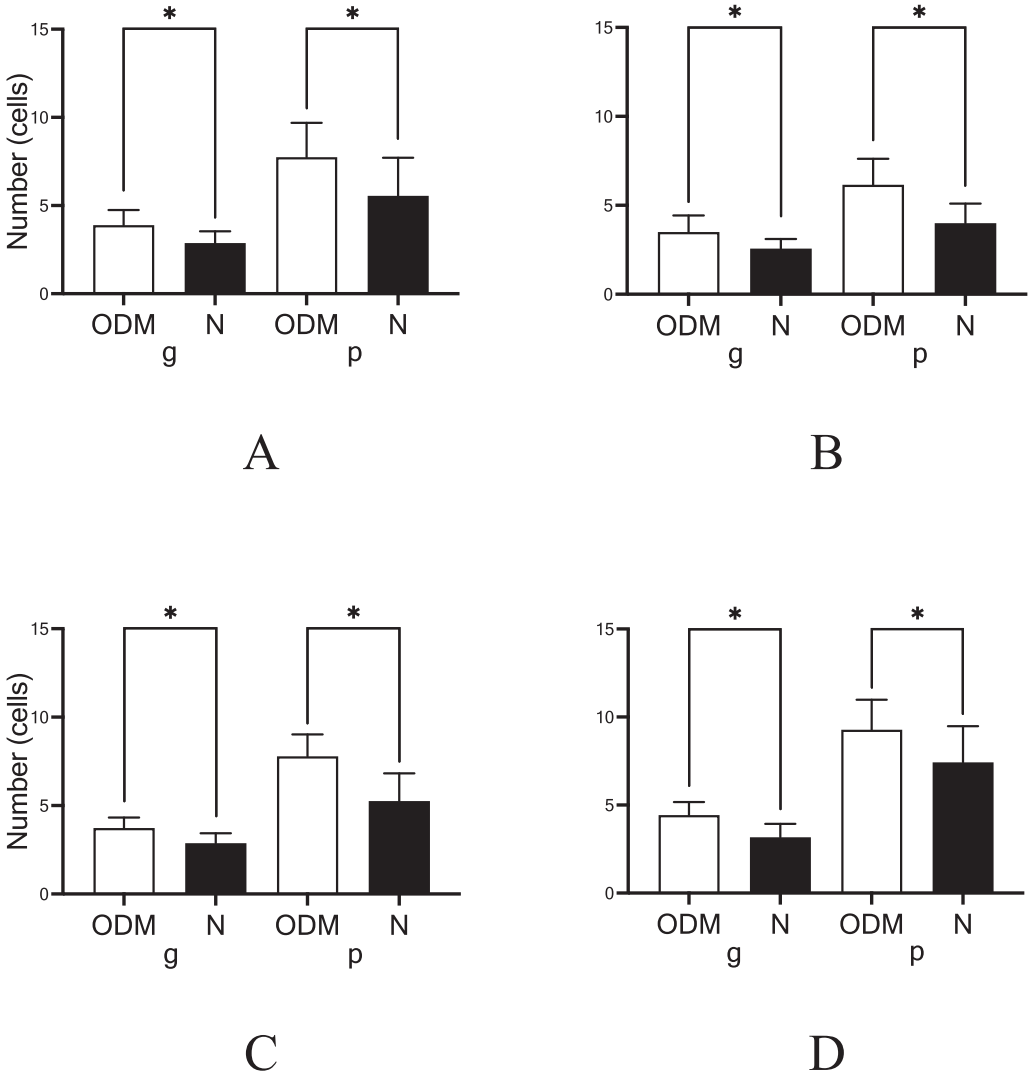

Fig. 8 Comparison of the number of cells in the granular (g) and prickle (p) layers in various regions of the ODM and N rats in the (**A**) average region, (**B**) upper gingiva, (**C**) middle gingiva, and (**D**) lower gingiva (*$p < 0.01$).

changes in the oral mucosa.[4-6, 8]

We previously reported that hyperglycemia induced atrophic changes in the subepithelial connective tissue and capillaries of the palatal gingiva of the maxillary first molars in the SDT fatty rats,[12] the ODM model rat. However, most other studies have focused on the STZ-induced type 1 DM model rats and the non-obese type 2 DM model rats. The SDT fatty rat (FBG level: 122.4 ± 15.5 mg/dL, HbA 1_c level: 5.4 ± 0.7 %) was also used as an ODM model in this report, as the SDT fatty rats have been reported to develop diabetes mellitus and diabetic complications earlier than SDT rats, which are considered a non-obese type 2 DM model.[19] Therefore, the SDT fatty rats were selected for this study because they are a suitable model rat to examine the morphological differences in the mucosal epithelium of the palatal gingiva of the maxillary first molars caused by ODM.

Body weight

Total fat weight, which represents the combined weight of visceral and subcutaneous fat, was reported to be approximately 5 fold greater in 6-week -old SDT fatty rats than in the same age SDT rats, which are considered a non-obese type 2 DM

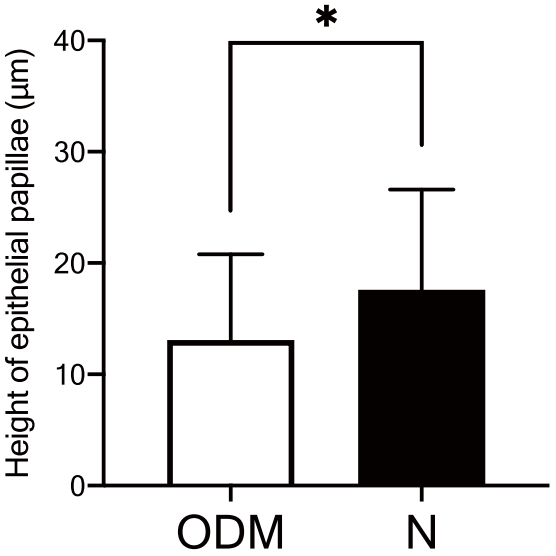

Fig. 9 Comparison of the height of epithelial papillae in the palatal gingiva of the maxillary first molar in the ODM and N rats (*$p < 0.01$).

model.[20] The body weight of the ODM group in this study was significantly greater than that of the N group by approximately 1.2 fold. Therefore, the heavier body weight of the ODM group in this study than in the N group was thought to be due to the greater total fat weight associated with obesity. Thus, the SDT fatty rat is a useful model of DM with obesity and high body fat percentage, which is increasing in the Japanese population.

Fasting blood glucose and hemoglobin A1c levels

In an experiment using GK rats, FBG levels have been reported to be significantly higher in the DM group than in the N group by approximately 1.5 fold. However, the HbA1c levels were not significantly different.[21] Serum levels of tumor necrosis factor alpha (TNF-α), which inhibits the action of insulin, has also been reported to be secreted by adipocytes and is positively correlated with insulin resistance.[22] The FBG levels in the ODM group in this study were significantly higher than those in the N group by approximately 1.6 fold. The HbA1c levels in the ODM group were also significantly higher than those in the N group by approximately 1.5 fold. Therefore, the higher FBG and HbA1c levels in the ODM group than in the N group in this study were thought caused by an increase in adipocytes caused by obesity, which in turn increased TNF-α in the serum and increased insulin resistance. Therefore, the STD fatty rat is a suitable model for FBG and HbA1c levels in ODM, which are increasing in the Japanese population.

Keratinized layer

A decrease in desmosomal molecules, the intercellular adhesion molecules that connect adjacent cells, has been reported in experiments using obese mice with hyperglycemia.[23] Many epithelial cells in the keratinized layer of the palatal gingiva of the maxillary first molars were observed to be on the verge of exfoliation in the ODM group in the superficial morphological specimens of this study. Therefore, the keratinized layer of the ODM group in this study was more likely to be exfoliated by epithelial cells, owing to a decrease in desmosomal molecules caused by the hyperglycemia associated with ODM. In addition, we observed that the exfoliation of epithelial cells exposed the epithelial cells in the underlying layer, and epithelial cells of various sizes.

The keratinocytes cultured under low glucose conditions of 2 mmol/L were small and organized *in vitro,* while those cultured under high glucose conditions of 20 mmol/L were large and flattened, and also swelled with increased keratin expression.[24] The keratinized layer was approximately 1.9 fold thicker in the palatal gingiva of maxillary first molars than in the N group in an experiment using GK rats.[6] In this study, the keratinized layer was significantly 1.3 fold thicker in the ODM group than in the N group. Therefore, the thicker keratinized layer in the ODM group in this study was thought to be caused by the significant flattening of keratinocyte morphology due to hyperglycemia associated with ODM, as well as by swelling associated with increased keratin expression.

Granular layer

Oral epithelial cells show enhanced cell proliferation under high glucose conditions *in vitro.*[25] Keratin also

accumulates in the cytoplasmic granules of the granular layer in a structure called keratohyaline granules.[26] As previously mentioned, keratin increases in keratinocytes under high glucose conditions *in vitro* with a greatly flattened and swollen cell morphology.[24] The DM group had a significantly thicker granular layer than the N group by approximately 2.3 fold in an experiment using GK rats, and the number of cells was significantly greater in the DM group than in the N group by approximately 1.6 fold.[6] In this study, the granular layer was significantly 1.4 fold thicker in the ODM group than in the N group, and the number of cells was 1.4 fold greater in the ODM group than in the N group, showing a significant difference. Therefore, the thicker granular layer and the greater number of cells in the ODM group in this study were thought to be the result of an increase in the number of cells in the granular layer caused by hyperglycemia associated with ODM, as well as by an increase in keratin and greater flattening and swelling cell morphology.

Prickle layer

Oral epithelial cells have been reported to show enhanced cell proliferation under high glucose conditions *in vitro* as mentioned previously.[25] The prickle layer was significantly thicker in the ODM group than in the N group in the experiment using GK rats, and the number of cells in the ODM group was significantly greater than in the N group by approximately 1.6 fold.[6] In this study, the prickle layer was significantly 1.5 fold thicker in the ODM group than in the N group, and the number of cells was significantly 1.4 fold greater in the ODM group than in the N group. Therefore, the thicker prickle layer and higher cell counts in the ODM group in this study were thought to be due to the hyperglycemia associated with ODM, which promoted cell proliferation of epithelial cells in the prickle layer.

Basal layer

One layer of the basal layer was observed in both groups with no significant difference in thickness in the experiment using GK rats.[6] In our study, one basal layer was observed in both groups, and no significant difference in thickness was noted between the two groups. Therefore, the basal layer of the ODM group in our study was not morphologically affected by hyperglycemia associated with ODM.

Mucosal epithelium

The keratinized, granular, and prickle layers were thickened in the ODM group as mentioned previously, whereas the basal layer was not morphologically affected. In our study, the mucosal epithelium was approximately 1.4 fold thicker in the ODM group than in the N group. Therefore, the mucosal epithelium of the ODM group in the present study was considered to be thickened by the hyperglycemia associated with ODM, which thickened the keratinized, granular, and prickle layers.

Epithelial papillae

In our previous study, we reported that hyperglycemia significantly decreased the height of the connective tissue papillae in the ODM group compared to that of the N group.[12] In this study, the height of the epithelial papilla was significantly lower in the ODM group than in the N group by approximately 0.7 fold. These results suggested that the height of the epithelial papilla was reduced owing to a decrease in the height of the connective tissue papilla caused by hyperglycemia associated with ODM.

These findings suggest that in clinical practice, the oral health of these patients should be carefully monitored because there may actually be a thickening of the mucosal epithelium and a greater number of nearly exfoliated epithelial cells, although no significant changes were seen grossly on the surface of the mucosal epithelium in the palatal gingiva of patients with ODM.

Our results suggest that in obese type 2 diabetes, the ODM group gained more weight and had higher FBG and $HbA1_C$ levels than the N group. In the palatal gingiva of the maxillary first molar, hyperglycemia associated with obesity-related type 2 diabetes mellitus can cause thickening of the mucosal epithelium (especially the keratinized, granu-

lar, and prickle layers), reduction of the epithelial papilla, and easy detachment of epithelial cells in the keratinized layer.

Conflict of Interst

The authors declare no conflicts of interest directly relevant to the content of this article.

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REFERENCES

1. OECD. Overweight and obesity among adults. in Health at a Glance 2019: OECD Indicators, OECD Publishing, Paris: doi. 10.1787/055833f6-en.
2. WHO expert consultation. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. *Lancet* 2004; **363**: 157-163.
3. Goto Y, Kakizaki M. The spontaneous-diabetes rat: a model of noninsulin dependent diabetes mellitus. *Proc Japan Acad* 1981; **57**: 381-384.
4. Hata Y, Suwa F, Imai H. Histological study of gingival epithelium in a type II diabetes mellitus rat model. *J Osaka Dent Univ* 2004; **38**: 23-29.
5. Kanemura N, Suwa F, Ueda M. Morphological study of gingival connective tissue papillae in type II diabetes model rats. *J Osaka Dent Univ* 2007; **41**: 7-14.
6. Yasuda K, Uemura M, Suwa F. Morphological study of the palatal gingiva of the maxillary first molar in the type 2 diabetes mellitus model rat. *Okajimas Folia Anat Jpn* 2011; **88**: 65-74.
7. Uemura M, Tamada Y, Suwa F. Morphological study of the connective tissue papillae and the capillary loops on the lingual dorsum in the type 2 diabetes mellitus model rats. *Okajimas Folia Anat Jpn* 2009; **85**: 139-149.
8. Akai K, Uemura M, Suwa F. Morphological study of the palatine mucosa in the type 2 diabetes mellitus model rat. *J Osaka Dent Univ* 2013; **47**: 185-194.
9. Morishita A, Uemura M, Suwa F. Morphological study of the submandibular gland in the type 2 diabetes mellitus model rat. *J Osaka Dent Univ* 2014; **48**: 1-8.
10. Uemura M, Toda I, Kawashima W, Yoshimoto G, Fang YR, Xu YJ, Liu Y, Zhang L, Takemura A. Morphological study of the articular disc and capillary of the retrodiscal tissue in a type 2 spontaneous diabetes mellitus rat model. *Okajimas Folia Anat Jpn* 2016; **92**: 53-59.
11. Masuyama T, Katsuda Y, Shinohara M. A novel model of obesity-related diabetes: introgression of the Lepr (fa) allele of the Zucker fatty rat into nonobese Spontaneously Diabetic Torii (SDT) rats. *Exp Anim* 2005; **54**: 13-20.
12. Sumi Y, Kamimura T, Okusa N, Toda I, Uemura M. Morphological study of subepithelial connective tissue and capillaries in the palatal gingiva of the maxillary first molar in obese type 2 diabetes mellitus model rats. *J Osaka Dent Univ* 2022; **56**: 37-53
13. Slein M. D-glucose: Determination with hexokinase and glucose-6-phosphate dehydrogenase. in Bergmeyer H, ed. Methods of enzymatic analysis. Methods of Enzymatic Analysis, Academic Press, 1965: 117-123.
14. Hirata M, Takanashi N, Oka M, Tsukada Y. Application of unsensitized soap-free latex to a new assay principle for $HbA1_c$ and its evaluation. *Japanese Journal of Medicine and Pharmaceutical Science* 1995; **34**: 125-136. (Japanese)
15. Liao D, Liu YQ, Xiong LY, Zhang L. Renoprotective effect of atorvastatin on STZ-diabetic rats through inhibiting inflammatory factors expression in diabetic rat. *Eur Rev Med Pharmacol Sci* 2016; **20**: 1888-1893.
16. Mahata LE, Ali H, Murni AW. Effect of streptozotocin on liver histology damage in rats model of gestational diabetes mellitus. *International Journal of Research and Review.* 2021; **15**: 562-571.
17. Satyaprakash RJ, Rajesh MS, Bhanumathy M, Harish MS, Shivananda TN, Shivaprasad HN, Sushma G. Hypoglycemic and antihyperglycemic effect of Ceiba pentandra L. Gaertn in normal and streptozotocin-induced diabetic rats. *Ghana Med J* 2013; **47**: 121-127.
18. Liu K, Sun T, Luan Y, Chen Y, Song J, Ling L, Yuan P, Li R, Cui K, Ruan Y, Lan R, Wang T, Wang S, Liu J, Rao K. Berberine ameliorates erectile dysfunction in rats with streptozotocin-induced diabetes mellitus through the attenuation of apoptosis by inhibiting the SPHK1/S1P/S1PR2 and MAPK pathways. *Andrology* 2022; **10**: 404-418.
19. Matsui K, Ohta T, Oda T, Sasase T, Ueda N, Miyajima K, Masuyama T, Shinohara M, Matsushita M. Diabetes-associated complications in Spontaneously Diabetic Torii fatty rats. *Exp Anim* 2008; **57**: 111-121.
20. Morinaga H, Ohta T, Matsui K, Sasase T, Fukuda S, Ito M, Ueda M, Ishii Y, Miyajima K, Matsushita M. Effect of food restriction on adipose tissue in spontaneously diabetic Torii fatty rats. *Exp Diabetes Res* 2009; Article ID 715057, 9 pages.
21. Orihara H, Uemura M, Takemura A. Palatal mucosal wound healing in type 2 diabetes model rats after administration of an antibacterial drug. *J Osaka Dent Univ* 2019; **53**: 15-23.
22. Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM. Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. *J Clin Invest* 1995; **95**: 2409-2415.
23. Aoki M, Murase T. Obesity-associated insulin resistance adversely affects skin function. *PLoS One* 2019; **14**: doi. 10.1371/journal.pone.0223528.
24. Spravchikov N, Sizyakov G, Gartsbein M, Accili D, Tennenbaum T, Wertheimer E. Glucose effects on skin keratinocytes: implications for diabetes skin complications. *Diabetes* 2001; **50**: 1627-1635.
25. Tazawa K. Effects of high glucose concentration on cell proliferation, in vitro wound healing and IL-1α production by the EGF-stimulated oral epithelial cell line, Ca9-22. *Nihon Univ Dent J* 2002; **76**: 25-30 (in Japanese).
26. Freeman SC, Sonthalia S. Histology, Keratohyalin Granules. StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022. Updated May 8, 2022. Available from: https://www.ncbi.nlm.nih.gov/books/NBK537049/