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The Effect of Type 1 Diabetes Mellitus on the Dento-Craniofacial Complex

Mona Aly Abbassy, Ippei Watari,
Ahmed Samir Bakry and Takashi Ono

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1. Introduction

Diabetes mellitus (DM) is one of the systemic diseases affecting a considerable number of patients worldwide [1]. Numerous experimental and clinical studies on the complications of DM have demonstrated extensive alterations in bone and mineral metabolism, linear growth, and body composition [2]. Depletion of insulin in Type 1 Diabetes Mellitus (T1DM) causes a reduction of bone mineral density and decreases skeletal mass, thus altering linear growth, body composition and delaying fracture healing. A considerable sector of literature was dedicated to studying the effects of T1DM on long bones; however, fewer studies discussed the effects of T1DM type on the craniofacial complex which is regulated by hormones, nutrients, mechanical forces, and various local growth factors.

Bone metabolism in the craniofacial complex involves a mosaic growth sites that grow at different rates and mature at different times, its growth and by analogy, the response to growth disruption is much more complex than that of the appendicular skeleton. Previous studies showed that T1DM may significantly affect the bone remodeling process which is observed during conducting treatments involving the application of mechanical or functional forces to the craniofacial complex and the teeth as those applied during orthodontic movement. Moreover, it is expected that the T1DM may alter the general growth of patients due to insulin deficiency and consequently leads to delayed skeletal maturation.

Type 1 diabetes mellitus (T1DM) is an endocrine–metabolic syndrome of childhood and adolescence, characterized by hyperglycemia as a cardinal biochemical feature, with important consequences for physical and emotional development. Several mechanisms have been reported to explain the altered bone remodeling in diabetes, one of which is diminished

bone formation as a result of decreased osteoblastic activity or enhanced apoptosis of osteoblastic cells. Another contributing factor may be increased bone resorptive activity. However, it is still controversial whether osteoclastic recruitment and function are altered in diabetes, because no change or decrease in the activity of osteoclasts has been reported [1].

Among researchers, there is lack of consensus about the impact of this disease on dental health. It has been suggested that hyperglycemia is associated with decreased salivary secretion and high salivary glucose levels, particularly in cases of severe insulin deficiency. Consequently, an increased cariogenic challenge in such individuals can be expected. However, no clear evidence has been found for an association between dental caries and diabetes mellitus [3].

The main aim of this chapter is to discuss the complexity of the dento-craniofacial system and how it is affected by T1DM condition. Moreover, the various detrimental effects of T1DM on the dento-craniofacial complex will be explored using the dynamic histomorphometric analysis and a histological study that will demonstrate that T1DM condition induced various detrimental effects on the quality of bone and on the bone turnover process observed in the dento-craniofacial complex.

2. Effect of T1DM on Bone

2.1. Types of Bone formation in craniofacial complex

Bone forms in two ways, resulting in two types of mature bone – intramembranous and cartilage. Cartilage bone forms in a replacement process within the cartilage models of the embryo and infant. Intramembranous bone forms through the activation of the osteoblast cell or specialized bone forming cells in one of the layers of fetal connective tissue. The bones of the cranial vault, the face, and the clavicle are intramembranous in origin. All other bones of the body form from cartilage. Intramembranous bone include the mandible, the maxilla, the premaxilla, the frontal bone, the palatine bone, the squamous part of temporal bone, the zygomatic bone, the medial plate of the pterygoid process, the vomer, the tympanic part of the temporal bone, the nasal bone, the lacrimal bone, and the parietal bone. The original pattern of intramembranous bone changes with progressive maturative growth when these bones begin to adapt to environmental influences. This accounts for deformities due to malfunction, disease and other environmental factors [4].

2.2. Causes of growth problems

Growth disturbances can be associated with specific anatomic or functional defects. They may be of endocrine or non endocrine origin and may result from genetic, nutritional or environmental factors. Disturbances in somatic growth show themselves in retardation or acceleration of the skeletal system, including the facial and cranial bones. Causes for growth problems usually fall into the following categories [5]:

- **Familial short stature**

Familial short stature is a tendency to follow the family's inherited short stature (shortness).

- **Constitutional growth delay with delayed adolescence or delayed maturation**

A child who tends to be shorter than average and who enters puberty later than average, but is growing at a normal rate. Most of these children tend to eventually grow to approximately the same height as their parents.

- **Illnesses that affect the whole body (Also called systemic diseases.)**

Constant malnutrition, digestive tract diseases, kidney disease, heart disease, lung disease, hepatic disease, diabetes, and severe stress can cause growth problems.

- **Endocrine (hormone) diseases**

Adequate production of the thyroid hormone is necessary for normal bone growth. Cushing's syndrome can be caused by a myriad of abnormalities that are the result of hypersecretion of corticosteroids by the adrenal gland. Growth hormone deficiency involves a problem with the pituitary gland (small gland at the base of the brain) that secretes several hormones, including growth hormone.

- **Congenital (present at birth) problems in the tissues where growth occurs**

A condition called intrauterine growth restriction (IUGR), slow growth within the uterus occurs during a pregnancy. The baby is born smaller in weight and length than normal, in proportion to his/her short stature.

2.3. Effect of DM on Bone and Growth

Hand-wrist radiographs have been studied in juvenile diabetics [6]. There is delayed appearance or delayed development of a center of ossification, usually of a carpal bone. These defects occur twice as frequently in boys than in girls, and the total incidence of juvenile diabetics with anomalies and developmental defects is 24.3%. There is also retardation of bone growth in 60% of diabetic males and 51% of diabetic females. The longer the duration of diabetes, the greater the tendency to bone growth retardation. The decreased bone mass in diabetics has been explained by decreased proliferative capacity of the diabetic fibroblasts, and early senescence of all cells has been suggested as basic to the diabetic problem. This degeneration would lead to early osteopenia in bone [6]. The yearly bone loss was reported to be 1.35% in patients with T1DM [7]. In addition, the rate of bone mineral loss is significantly greater among patients with a deterioration of the metabolic state, despite increasing insulin dosage, when compared with patients with unchanged or improved insulin secretion. This may indicate that the exogenous insulin administration does not fully compensate for the decrease in endogenous insulin secretion. These studies also showed increased bone resorption in T1DM patients with no signs of vitamin D deficiency associated with the disease. Vertebral bone density has been studied in T1DM children [7]. It was found that diabetic children exhibited cortical bone density that was slightly, but significantly, lower than the controls. The decrease in cortical bone density in the diabetic group did not correlate with

age, sex, duration of diabetes, or glycosylated hemoglobin levels. These results suggested that in children with uncomplicated T1DM, decreased vertebral bone density is a minor abnormality that affects only cortical bone [6].

2.4. Outline of studying the effect of diabetes mellitus on craniofacial growth

Approximately 60% of adult bone mass including craniofacial bone is gained during the peak of the growth period which coincides with the onset of T1DM condition affecting the bone formation process [8]. It is worth mentioning here that although T1DM condition exact etiological factors are totally unknown however; understanding the course of T1DM condition and its impact on craniofacial development may lead to improving the oral health for a large sector of the population worldwide.

Numerous experimental and clinical studies on the complications of DM have demonstrated extensive alterations in bone and mineral metabolism, linear growth, and body composition. Investigators in the fields of bone biology including orthodontics have long been interested in the general causes that affect the normal growth of the craniofacial region. T1DM has been shown to affect the general growth of patients with earlier onset of the disease, especially onset before or around the circumpubertal growth spurts [9].

In general, growth of the craniofacial complex is controlled by genetic and environmental factors [3, 10]. Regulatory mechanisms responsible for normal morphogenesis of the face and head involve hormones, nutrients, mechanical forces, and various local growth factors. The poor growth and alterations in bone metabolism have been associated with T1DM in both humans and experimental animals [3]. It is of prime importance investigating the changes in craniofacial bone structure and dynamic bone formation in DM condition to explore the impact of the diabetic condition on various mandibular growth elements and bone quality.

The following parts of this chapter are going to focus on these points:

- Investigating the effects of juvenile diabetes on general craniofacial growth and skeletal maturation.
- Analyzing the pattern of association between craniofacial morphology and skeletal maturation.
- Determination of the changes in bone morphology in diabetic rat mandible using micro-C.T.
- Determination of the mineral apposition rate and the bone formation rate in diabetic rat mandible using histomorphometric analysis.

2.5. Animal and Experimental diabetic Model

The animal studies using diabetic model presents various advantages when compared to studies carried out on human diabetic cases. Human studies can be limited by small sample sizes, cross-sectional designs, uncontrolled variables, and often retrospective nature, animal models have been used to yield more rigorous analyses [11].

2.6. Diabetic model

Experimental diabetic models include the streptozotocin-induced diabetic rat and the spontaneously diabetic BioBreeding [12] rat. The occurrence of different abnormalities indicating altered bone formation after inducing DM with streptozotocin (STZ) is well documented [3, 13, 14]. Streptozotocin-induced diabetes mellitus (STZ-DM), caused by the destruction of pancreatic β -cells and is similar to T1DM in human, is characterized by mild to moderate hyperglycemia, glucosuria, polyphagia, hypoinsulinemia, hyperlipidemia, and weight loss. STZ-DM also exhibits many of the complications observed in human DM including enhanced susceptibility to infection and cardiovascular disease, retinopathy, alterations in angiogenesis, delayed wound healing, diminished growth factor expression, and reduced bone formation [15].

2.7. Importance of testing the uncontrolled diabetic condition

In the usual clinical situation, although T1DM patient is treated with insulin, patient may still suffer from an overall poor diabetic metabolic state with an uncontrollable blood glucose level and a high and sometimes changing insulin requirement [16].

2.8. Inducing diabetic condition

All the experimental protocols followed had been approved by the Institutional Animal Care and Use Committee of Tokyo Medical and Dental University, and the experiments were carried out under the control of the University's Guidelines for Animal Experimentation. In our investigation we explored the various effects of DM using the streptozotocin DM model. Twelve 3-week old male Wistar rats were used for this study. They were randomly divided into two groups, the control group and the diabetes group (DM group), each group consists of 6 rats. The rats in the control group were injected intra-peritoneal with a single dose of 0.1M sodium citrate buffer (pH 4.5), while the rats in the DM group were injected intra-peritoneal with a single dose of citrate buffer containing 60mg/kg body weight of streptozotocin (Sigma Chemical Co., St. Louis, MO, USA). [13, 16 - 18] All animals were fed on standard Rodent diet (Rodent Diet CE-2; Japan Clea Inc., Shizuoka, Japan) with free access to water. Body weights, the presence of glucose in urine and blood glucose levels were recorded on day 0,2,7,14,21 and 28 after STZ injection.

Diabetes condition was determined by the presence of glucose in urine and blood. The urine of the rats was tested using reagent strips (Uriace Ga; TERUMO). [20, 21] Blood samples of the rats were obtained via vein puncture of a tail vein, and blood glucose levels were determined using a glucometer (Ascensia Brio. Bayer Medical). Positive urine test and a blood glucose level greater than 200 mg/dl was considered DM. [3]

Fig. 1. shows the weights of the rats (mean $\pm$ SD) in both groups. DM group showed a significant decrease in weight. After STZ injection by 48 hours the urine test showed that the entire DM group had a high glucose level and this was confirmed by the high blood glucose measurements as shown in Fig. 2. A Student's t-test was used to compare the mean of weights and blood glucose levels in both groups.

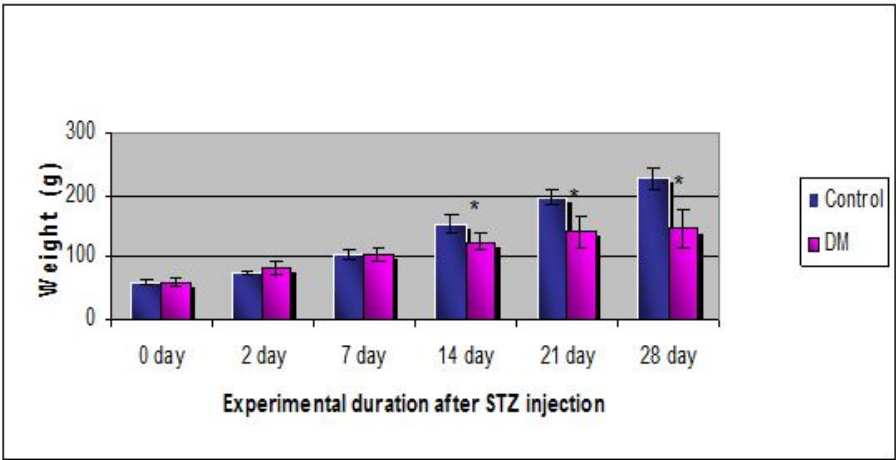


Figure 1. Comparison between the changes of the rat’s weight in the control and DM group. * The weights of the DM group are significantly decreased as compared to the weights of the control group ($p<0.05$).

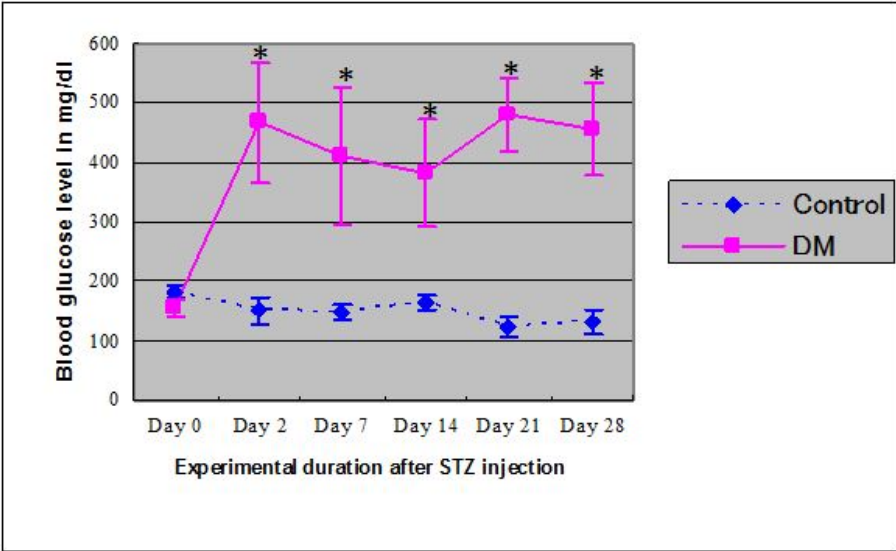


Figure 2. Line graph represents the blood glucose levels for the control and DM group. The blood glucose level in DM group increased significantly 48h post-STZ injection and during the entire experimental period. Values are mean $\pm$ SD. Significant differences between the two groups are marked with asterisks ($p<0.05$).

2.9. Analytic studies conducted to test the effect of diabetic condition on craniofacial growth

Cephalometric analysis

Cephalometric measurements are still one of the most widely spread diagnostic aids crucial for the diagnosis of various abnormalities in the craniofacial complex [22].

The protocol for examining the cephalometric measurements in DM rats involves the following steps:

- Prior to each radiographic session, the rats are anaesthetized with diethyl ether and intraperitoneal injection of 8% chloral hydrate using 0.5ml/100g of body weight.
- Each animal is then placed in this specially-designed apparatus (Fig. 3) to maintain standardized head posture and contact with the film (SGP-3, Mitsutoy, Tokyo, Japan) where the head of each rat is fixed firmly with a pair of ear rods oriented vertically to the sagittal plane and the incisors are fixed into a plastic ring.
- The settings of lateral and dorsoventral cephalometric radiographs are 50/55kVp, 15/10mA, and 20/60-sec impulses respectively.[11, 23]
- A 10 mm steel calibration rod is incorporated into the clear acrylic table on which the animals are positioned for the radiographs.
- All the radiographs are developed and scanned at high resolution by the same operator.[11]



Figure 3. Apparatus for roentgenographic cephalometry

The cephalometric landmarks (Table 1; Fig. 4) were derived from previous studies on rodents.[11, 24-26] Selected linear measurements were then obtained (Table 2). To ensure reliability and replicability of each measurement, each distance was digitized twice and the two values were averaged.

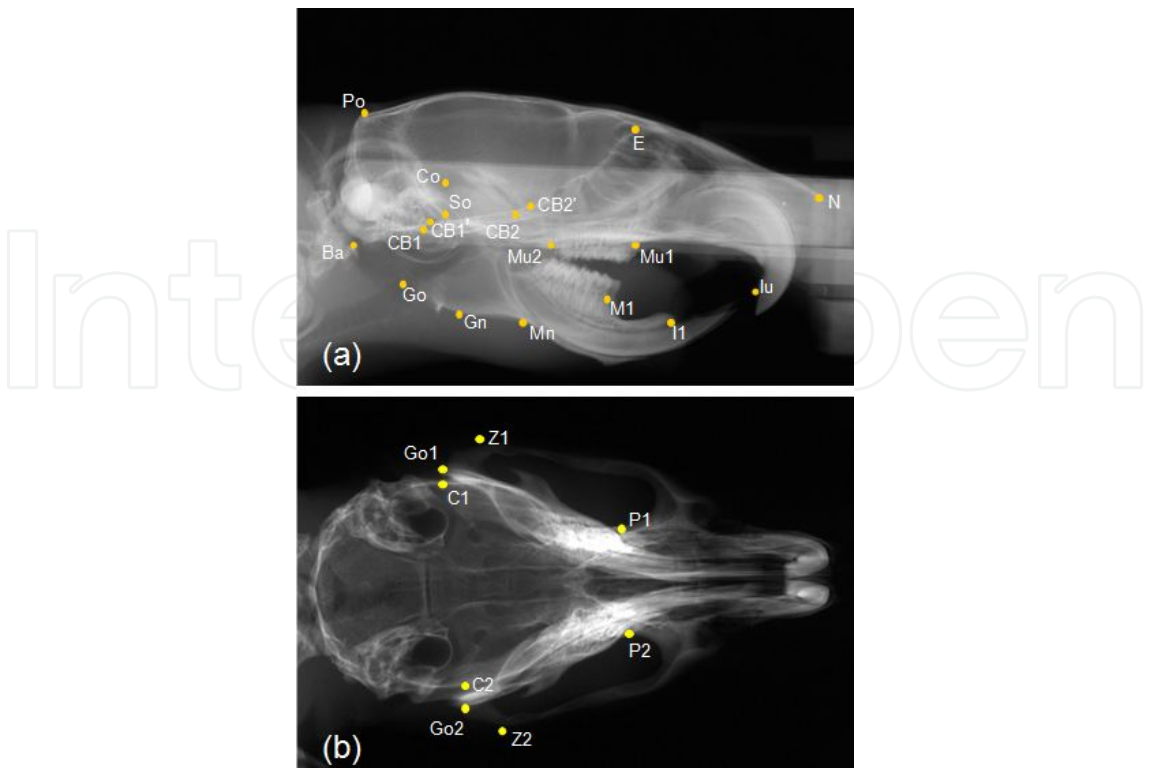


Figure 4. Location of cephalometric points on radiographs: (A) Sagittal and (B) transverse.

On the sagittal radiograph	
N:	The most anterior point on the nasal bone
E:	The intersection of the frontal bone and floor of anterior cranial fossa
Po:	The most posterior and superior point on the skull
Ba:	The most posterior and inferior point on the occipital condyle
Co:	The most posterior and superior point on the mandibular condyle
Go:	The most posterior point on the mandibular ramus
Mn:	The most concave portion of the concavity on the inferior border of the mandibular corpus
Gn:	The most inferior point on the ramus that lies on a perpendicular bisector of the line Go-Mn
I1:	The most anterior and superior point on the alveolar bone of the mandibular incisor
So:	The intersection of the most anterior tympanic bulla and the superior border of the sphenoid bone
CB1:	The most anterior point on the occipital bone at the spheno-occipital synchondrosis
CB1' :	The most posterior point on the sphenoid bone at the spheno-occipital synchondrosis
CB2:	The most anterior point on the sphenoid bone at the spheno-basispheno synchondrosis
CB2' :	The most posterior point on the basisphenoid bone at the spheno-basispheno-synchondrosis
M1:	The junction of the alveolar bone and the mesial surface of the first mandibular molar
Mu1:	The junction of the alveolar bone and the mesial surface of the first maxillary molar
Mu2:	The junction of the alveolar bone and the distal surface of the third maxillary molar
Iu:	The most anterior-inferior point on the maxilla posterior to the maxillary incisors
On the transverse radiograph	
Z1 & Z2:	The points on the lateral portion of the zygomatic arch that produce the widest width
Go1 & Go2:	The points on the angle of the mandible that produce the widest width
P1 & P2:	The most anterior and medial points within the temporal fossae that produce the most narrow palatal width
C1 & C2:	The points on the cranium that produce the widest cranial width

Table 1. Definitions of radiographic points

Neurocranium

Po-N: total skull length
 Po-E: cranial vault length
 Ba-E: total cranial base length
 So-E: anterior cranial base length
 Ba-CB1: occipital bone length
 CB1'-CB2: sphenoid bone length
 Ba-So: posterior cranial base length
 Po-Ba: posterior neurocranium height

Viscerocranium

E-N: nasal length
 Mu2-Iu: palate length
 CB2-Iu: midface length
 E-Mu1: viscerocranial height

Mandible

Go-Mn: posterior corpus length
 Ml-Il: anterior corpus length
 Co-Il: total mandibular length
 Co-Gn: ramus height

Transverse X-ray

Go1-Go2: Bigonial width
 C1-C2: Maximum cranial width
 P1-P2: Palatal width
 Z1-Z2: Bizygomatic width

Table 2. Measurements of craniofacial skeleton

In our studies, evaluation of the craniofacial growth of diabetic rats at the age of 7 weeks was done using lateral and dorsoventral cephalometric radiographs. All of the data in each experiment were confirmed the normal distribution, so a Student's t-test was used to compare the mean of each data recorded in the control group and in the DM group. All statistical analyses were performed at a 5% significance level using statistic software (v. 10; SPSS, Chicago, IL, USA).

2.9.1. Changes in the Total Skull

The size of total skull, denoted by Po-N, was significantly smaller in the DM group than in the control group.

2.9.2. Changes in the Neurocranium

Cranial vault length (Po-E), total cranial base length (Ba-E), anterior cranial base length (So-E), Occipital bone length (Ba-CB1), and posterior cranial base length (Ba-So) showed statistically significant decrease in DM group (Table 3, Fig. 5, 6), while other dimensions exhibited no significant differences.

Table 3: Significant changes in the Total skull and Neurocranium	
Po-N	total skull length
Po-E	cranial vault length
Ba-E	total cranial base length
So-E	anterior cranial base length
Ba-CB1	occipital bone length
Ba-So	posterior cranial base length

Table 3. Significant changes in the Total skull and Neurocranium

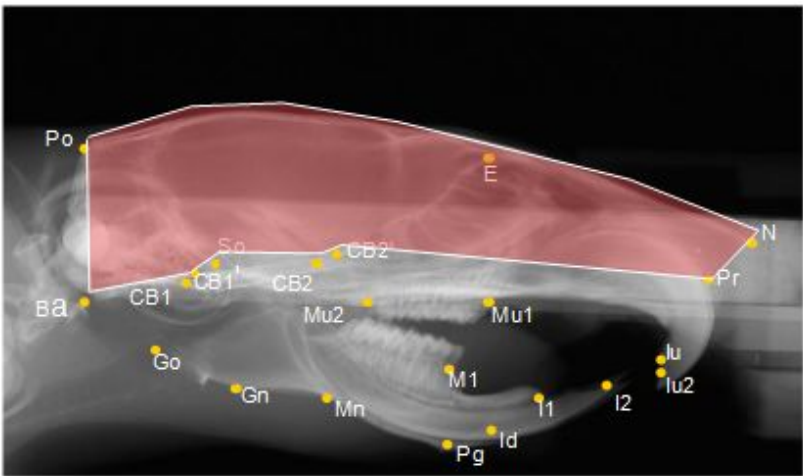


Figure 5. Neurocranium

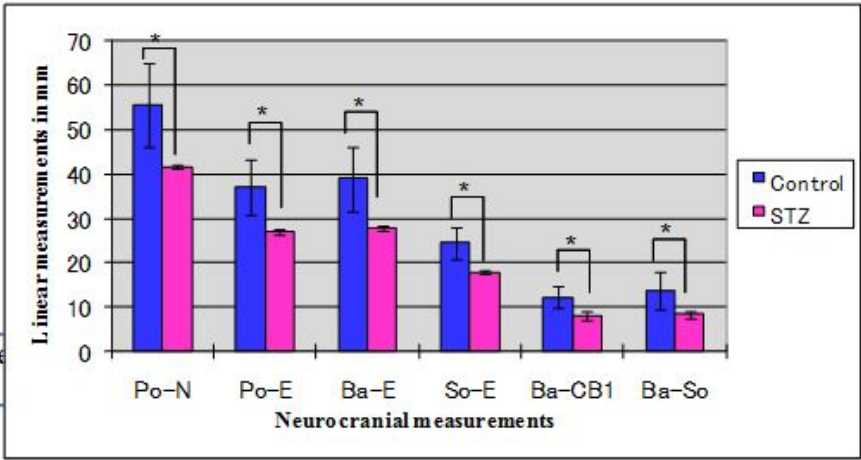


Figure 6. Changes in the neurocranial measurements of the control and DM group. All the significant measurements are shown in this figure. Values are mean±S.D. Significant differences between the two groups are marked with asterisks (p<0.05).

2.9.3. Changes in the Viscerocranium

All measurements of the viscerocranium, including the nasal length (E-N), palatal length (Mu2-Iu), midface length (CB2-Iu), and viscerocranial height (E-Mu1) showed a statistically significant decrease in DM group (Table 4, Fig. 7,8)

Table 4 : Significant changes in the Viscerocranium	
E-N	Nasal length
Mu2-Iu	Palate length
Cb2-Iu	midface length
E-Mu1	Posterior viscerocranial height

Table 4. Significant changes in the Viscerocranium

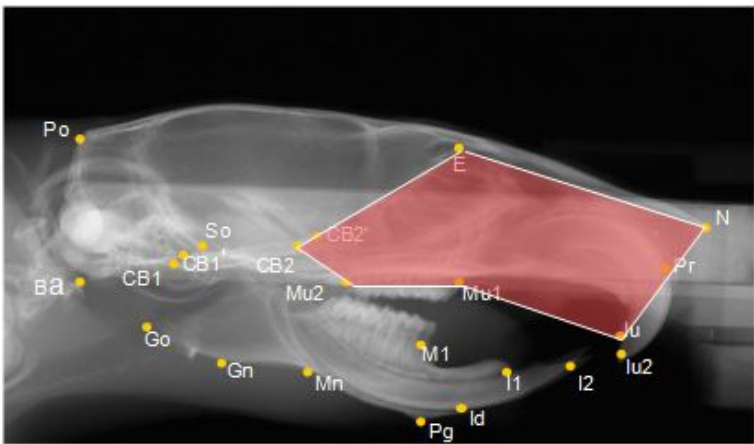


Figure 7. Viscerocranium

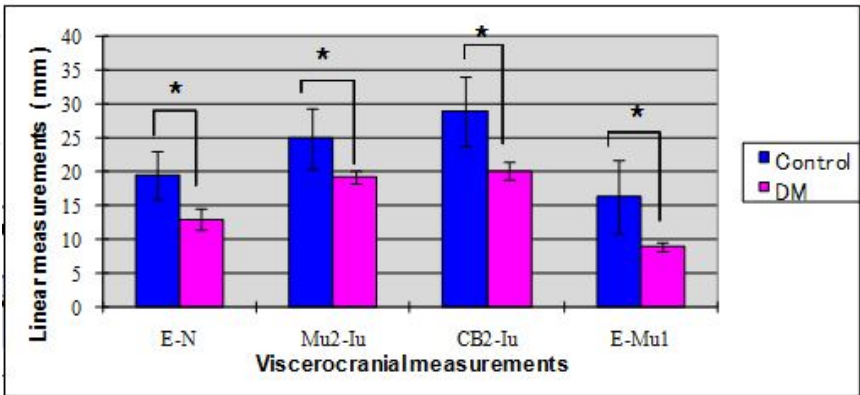


Figure 8. Changes in the viscerocranial measurements of the control and DM group. All the viscerocranial measurements are significant. Values are mean±S.D. Significant differences between the two groups are marked with asterisks ($p<0.05$).

2.9.4. Changes in the Mandible

In the DM group, the posterior corpus length (Go-Mn), total mandibular length (Co-II) and the ramus height (Co-Gn) were significantly shorter than in the control group (Table 5, Fig. 9, 10), whereas no remarkable differences were found in the remaining dimensions.

Table 5: Significant changes in the Mandible	
Go-Mn	Posterior corpus length
Co-II	Total mandibular length
Co-Gn	Ramus height

Table 5. Significant changes in the Mandible



Figure 9. Mandible

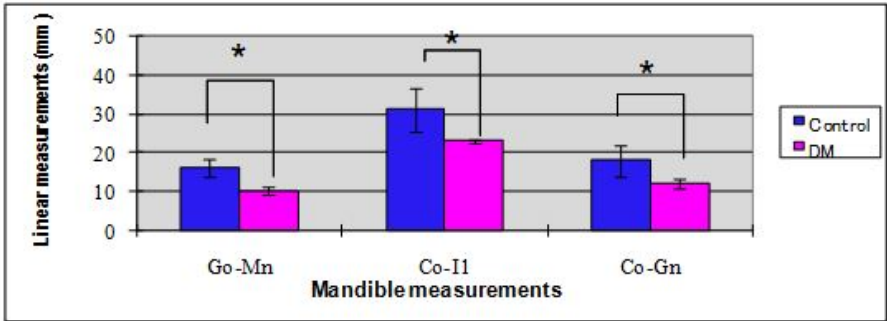


Figure 10. Changes in the mandible measurements of the control and DM group. Values are mean $\pm$ S.D. Significant differences between the two groups are marked with asterisks ($p < 0.05$).

2.9.5. Changes in the Transverse X-ray

In transverse X-ray only the maximum cranial width (C1-C2) and the bizygomatic width (Z1-Z2) were statistically decreased in DM group (Table 6, Fig. 11, 12).

All other linear measurements showed no significant differences between both groups

Table 6: Significant changes in the transverse X-ray	
C1-C2	Maximum cranial width
Z1-Z2	Bizygomatic width

Table 6. Significant changes in the transverse X-ray

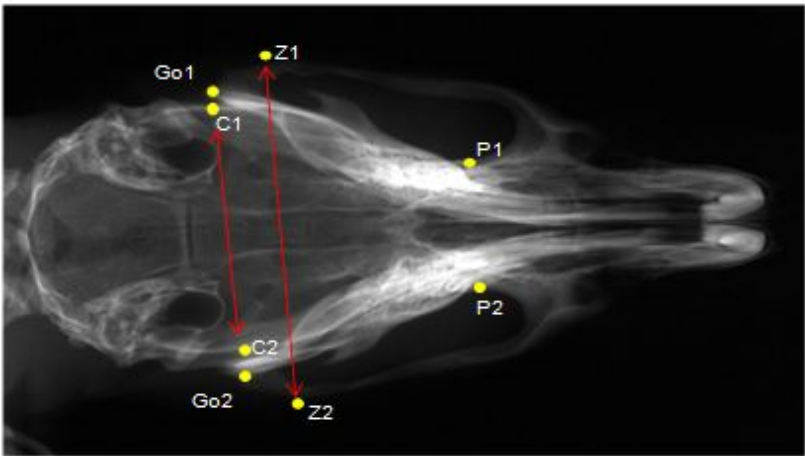


Figure 11. Transverse X-ray measurements

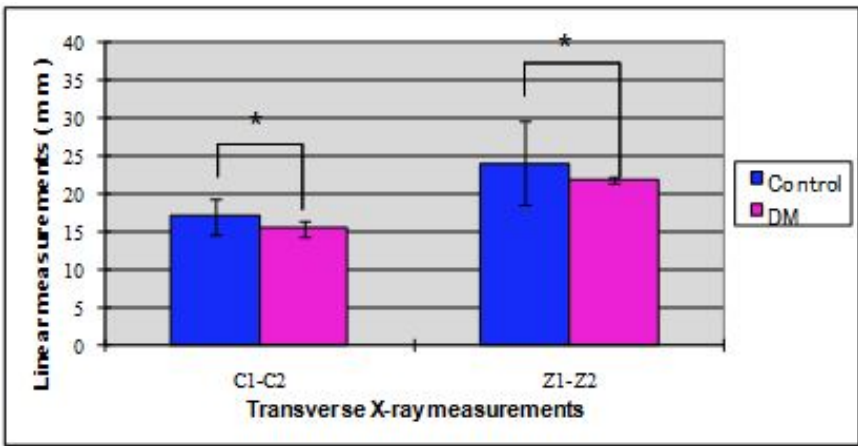


Figure 12. Changes in the transverse X-ray measurements of the control and DM group. Two measurements in the transverse X-ray were significant. Values are mean±S.D. Significant differences between the two groups are marked with asterisks ($p<0.05$)

2.10. Histomorphometric analysis

2.10.1. Fluorescent dyes used for double labeling in histomorphometric analysis

Fluorochromes are calcium binding substances that are preferentially taken up at the site of active mineralization of bone known as the calcification front, thus labeling sites of new bone formation. They are detected using fluorescent microscopy on undecalcified sections. Labeling bones with fluorochrome markers provides a means to study the dynamics of bone formation. The rate and extent of bone deposition and resorption can be determined using double and triple fluorochrome labeling sequences. The sequential use of fluorochromes of clearly contrasting colors permits a more detailed record of events relating to calcification. Fluorochromes commonly used in mammals include tetracycline, calcein green, xylenol orange, alirazin red, and hematoporphyrin. Calcein is a fluorochrome that fluoresces bright green when combined with calcium [27].

2.10.2. Calcein administrations and sections preparation

The steps needed for detecting the double labeling involves the following:

- Rats are subcutaneously injected with 50 mg/kg body weight calcein fluorescent marker on day 21 and day 28 after STZ injection [28]. The time difference between the 2 injections is one week to be able to compare the amount of bone formed during this period (Fig. 13).
- Sacrifice of all animals by transcardiac perfusion under deep anesthesia using 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4).
- Mandibles are dissected and fixed in the same solution for 24 hours.
- All specimens are embedded in polystyrene resin (Rigolac, Nisshin EM Co. Ltd., Tokyo, Japan).
- Undemineralized ground frontal sections are processed to show the crown and both apices of buccal and lingual roots of the lower second molar [28].

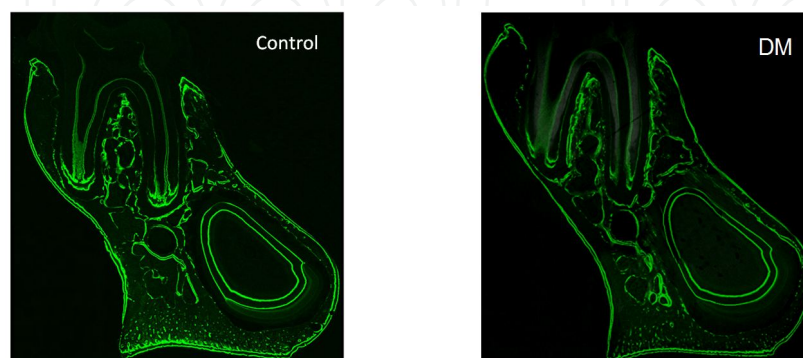


Figure 13. Frontal sections of the mandibular second molar area. (A) Control; (B) DM. Fluorescent labeling on the periosteal surface indicates new bone formation.

2.10.3. Method of analysis

The bone around the lower second molar is centrally located within the mandibular arch, and because of the parallel alignment of the buccal and lingual roots this made a precise reference when frontal sections are produced [29]. To conduct the histomorphometric analysis it is essential to use a digitizing morphometry system to measure bone formation indices. The system consists of a confocal laser scanning microscope (LSM510, Carl Zeiss Co. Ltd., Jena, Germany), and a morphometry program (LSM Image Browser, Carl Zeiss Co. Ltd., Jena, Germany). Bone formation indices of the periosteal surfaces of the alveolar/jaw bone include mineral apposition rate ($\mu\text{m}/\text{day}$) and bone formation rate ($\mu\text{m}^3/\mu\text{m}^2/\text{day}$), according to the standard nomenclature described by [30]. The calcein-labeled surface (CLS, in mm) is calculated as the sum of the length of double labels (Thomas *et al.*) plus one half of the length of single labels (sL) along the entire endosteal or periosteal bone surfaces; that is, $\text{CLS} = \text{dL} + 0.5\text{sL}$ [31]. The mineral apposition rate (MAR, in μ / day) is determined by dividing the mean of the width of the double labels by the interlabel time (7 days). The bone formation rate (BFR) is calculated by multiplying MAR by CLS [32]. Based on the reference line along the long axis of the buccal root, the area superior to the root apex was considered alveolar bone, while the area inferior to the root apex was considered the jaw bone. The lingual side of the bone is excluded, because the existence of the incisor root may influence bone formation.

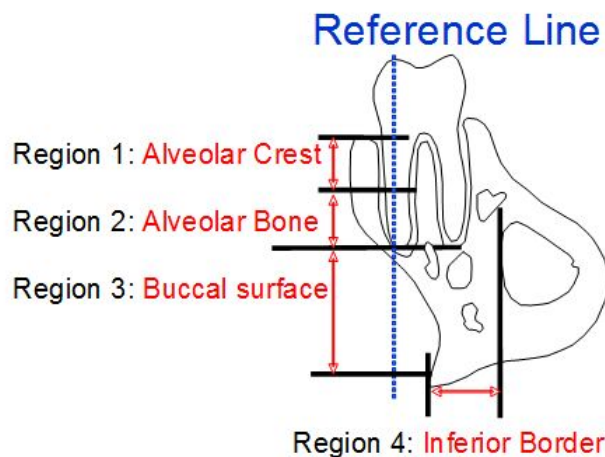


Figure 14. Schematic drawing of observation regions for dynamic bone histomorphometry. The periosteal surfaces were delimited into 4 areas as alveolar crest (region 1), alveolar bone (region 2), buccal surface of the jaw bone (region 3), and inferior border of the jaw bone (region 4).

The periosteal surfaces of the mandible are divided into four regions for analysis (Fig. 14.):

2.10.4. Histomorphometric indices

The obtained results in our study showed that in the alveolar bone (region 2), there was a significant decrease in the MAR (Fig. 15A) BFR (Fig.15B) recorded in the DM group compared to the control group. However, in the alveolar crest (region 1), the MAR and the BFR in the control and the DM groups were not significantly different. ($P < 0.05$). In the buccal surface

(region 3) and inferior borders (region 4) of the jaw bone the MAR (Fig.15A) and BFR (Fig. 15B) were significantly suppressed compared with those in the control group ($P < 0.05$). Most of the periosteal surfaces in the mandibular regions of the control group showed significantly higher values recorded for the mineral apposition rate and the bone formation rate when compared to the DM group. These results agree with previous studies that recorded diminished lamellar bone formation in DM rats' femur and may suggest an association between the DM condition and the decreased number and function of osteoblasts [16, 19]. The alveolar crest region was the only region that did not show a significant difference in the mineral apposition rate and the bone formation rate parameters among the two groups; this may be attributed to the unique nature of this region exhibiting a highly intensive bone remodeling process especially during the teeth eruption that decreases toward the base of the socket [33], however further studies are needed to elaborate the detailed pattern of bone growth at the alveolar crest region.

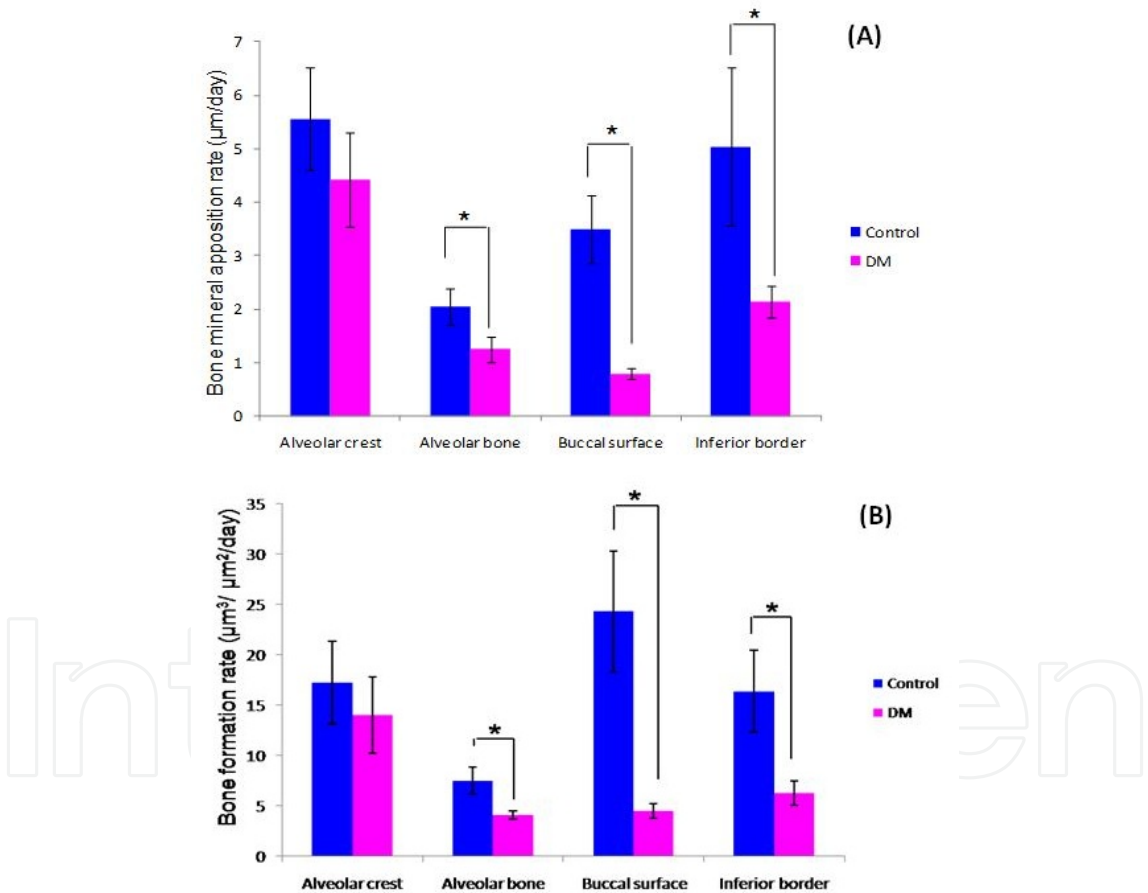


Figure 15. A) The changes in mineral apposition rate (MAR) of the mandible between the control group and the DM group. Alveolar crest (region 1, upper 1/2 of the tooth root, near the tooth crown). Alveolar bone (region 2, lower 1/2 of the tooth root, near the root apex). Buccal surface of the jaw bone (region 3). Inferior border of the jaw bone (region 4). The data are expressed as means $\pm$ SD. N = 5 for each group. *Significantly different from controls, with ($p < 0.05$). (B) The changes in the bone formation rate (BFR/BS) of the mandible between the control group and the DM group. Alveolar crest (region 1, upper 1/2 of the tooth root, near the tooth crown). Alveolar bone (region 2, lower 1/2 of the tooth root, near the root apex). Buccal surface of the jaw bone (region 3). Inferior border of the jaw bone (region 4). The data are expressed as means $\pm$ S.D. N = 5 for each group. *Significantly different from controls, with ($p < 0.05$).

2.10.5. Microtomography of the mandible (Micro-CT)

Micro-computed tomography (micro-CT) has rapidly become a standard technique for the visualization and quantification of the 3D structure of trabecular bone. Bone architecture and mineralization are generally considered to be important components of bone quality, and determine bone strength in conjunction with bone mineral density.

2.10.6. Protocol adopted to examine the mandible using Micro-CT

In our study all specimens were imaged by micro-CT (inspeXio SMX-90CT; Shimadzu Science East Corporation, Tokyo, Japan)

- After removing only the soft tissue, the mandibular plane is set orthogonal to the sample stage.
- Three dimensional images of each hemi mandible are acquired with a resolution voxel size of 15 μm / pixel.
- Raw data are obtained by rotating the sample stage 360 degrees. Then, slice images are prepared using multi-tomographic image reconstruction software (MultiBP; Imagescript, Tokyo, Japan).
- The resulting gray-scale images are segmented using a low-pass filter to remove noise and a fixed threshold to extract the mineralized bone phase.
- The volume of interest (VOI) is drawn on a slice-based method starting from the first slice containing the crown of the first molar and moving dorsally 100 slices [34, 35], in the area of the alveolar crest (Between the buccal and lingual roots of the second molar at the cervical region); and the buccal surface of the jaw bone [29]. Trabecular bone was carefully contoured on the first and the last slice, while the intermediate slices were first interpolated by morphing.
- For observation and analysis of reconstructed 3D images, 3D trabecular structure analysis software (TRI/3D-BON; RATOC System Engineering, Tokyo, Japan) is used [36]. Reconstructed 3D images were prepared from slice images using the volume rendering method, to analyze the microstructure of the bone (Fig. 16).
- The following parameters are measured: tissue volume (TV), bone volume (BV), bone surface (BS), bone surface / bone volume (BS/BV), bone-volume fraction (BV/TV).
- Four properties of the trabeculae are evaluated: trabecular thickness (Tb.Th), trabecular number (Tb.N), trabecular separation (Tb.Sp), and Trabecular space (Tb.S) [36].

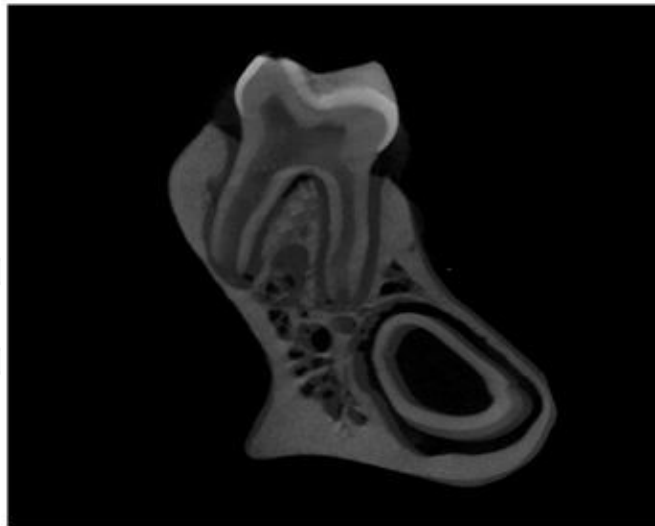


Figure 16. The left mandible was imaged by micro-CT

2.10.7. Microtomography of the DM mandible

The quantification of micro-CT trabecular bone changes (mean $\pm$ SD) is shown for the DM and the control groups in (Table 4). All trabecular parameters in both alveolar bone and buccal surface of jaw bone showed significant changes. Compared with the control group, bone volume fraction (BV/TV) was significantly decreased only in the alveolar bone; however, trabecular thickness (Tb.Th) and trabecular numbers (Tb.N) were significantly decreased both in alveolar and buccal surface of jaw bone, in the DM group. Correspondingly, significantly higher trabecular separation (Tb.Sp) and trabecular space (Tb.S) were revealed both in alveolar and buccal surface of jaw bone for the DM group when compared with that of the control group. Also, the bone surface / bone volume (BS/BV) was significantly increased only in alveolar bone ($P < 0.05$). These findings indicate deterioration of the bone quality in the DM group. These results agree with other research work suggesting that the glycaemic levels play an important role in modulating the trabecular architecture especially in mandibular bone [15].

The DM condition resulted in alteration of the trabecular distance and thickness as compared to the control group indicating profound impact on the histological integrity of the bone. The reduction in trabecular bone volume accompanied by the expansion of the bone marrow space is in agreement with another investigation [37]. In this context, these results may describe a state of osteopenia in experimental diabetic rats, which might be the result of an imbalance between bone formation and resorption

2.10.8. Procedure for preparing mandibles for histological analysis

- Mandibles for all groups were decalcified in 10% EDTA solution pH 7.4 for 5 weeks at 4°C [38].

- Specimens are then dehydrated in an ascending ethanol series and embedded in paraffin (Fig. 17).
- Serial horizontal sections (5 μm thick parallel to the occlusal plane) are prepared using a microtome (Leica RM 2155, Nussloch, Germany) (Fig. 17)

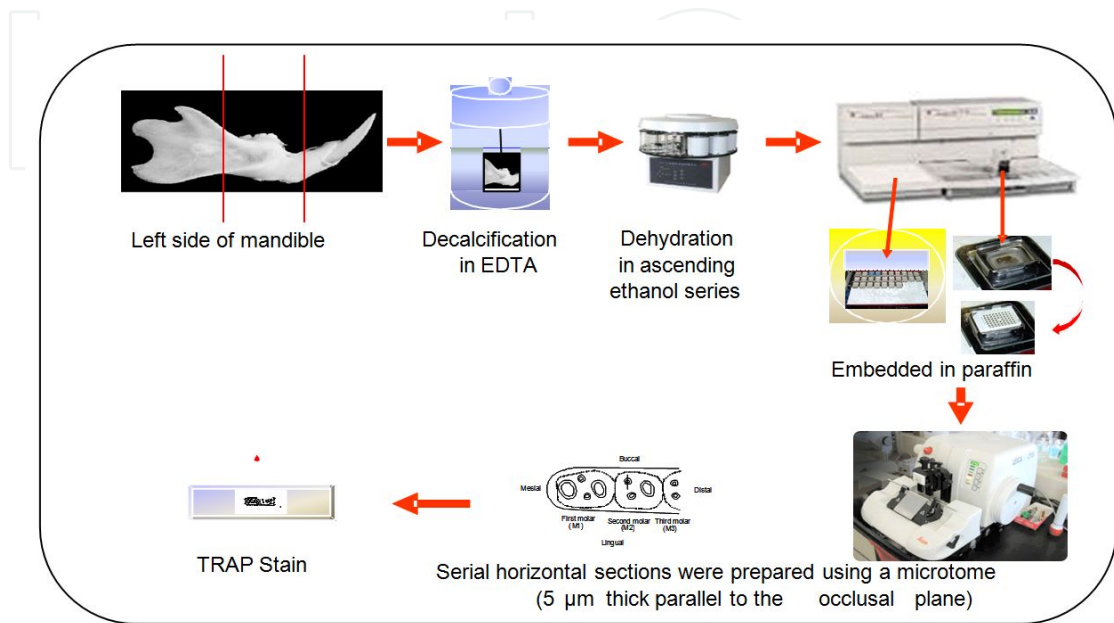


Figure 17. Experimental procedure for histological section preparation

2.10.9. TRAP staining

Histological sections are incubated for 30–60 min at 37°C in a mixture of 0.8% naphthol AS-BI phosphate (Sigma, St Louis, MO, USA), 0.7% fast red violet salt (Sigma, St Louis, MO, USA) and 50mM sodium tartate diluted in 0.2M sodium acetate buffer (pH 5.4). Sections were examined under a light microscope. For the histomorphometric assessment of resorption, the number of tartrate-resistant acid phosphatase-positive multinucleated cells (osteoclasts) on the distal surface of the alveolar bone adjacent to the mesio-buccal root of the second molar were counted in each 540 μm x 120 μm area in five consecutive sections, at the middle third of the root selected at least 25 μm apart from each specimen ($n = 5$) of each group [39, 40].

2.10.10. Histological analysis

Bone-resorption activity was assessed by counting the number of tartrate-resistant acid phosphatase-positive multinucleate cells (osteoclasts) on the distal surface of the alveolar bone adjacent to the mesio-buccal root of the second molar (Fig. 18A-D). Statistical analysis demonstrated a significantly higher number of osteoclast cells in the control group when compared with the DM group ($P < 0.05$) (Fig. 18 E). Results revealed that the number of osteoclasts was significantly lower in the DM rats than in the controls, in line with previous

studies on DM rats’ mandible [40] and long bones [41, 42]. These results confirm that the decreased rate of bone turnover may be associated with the DM condition.

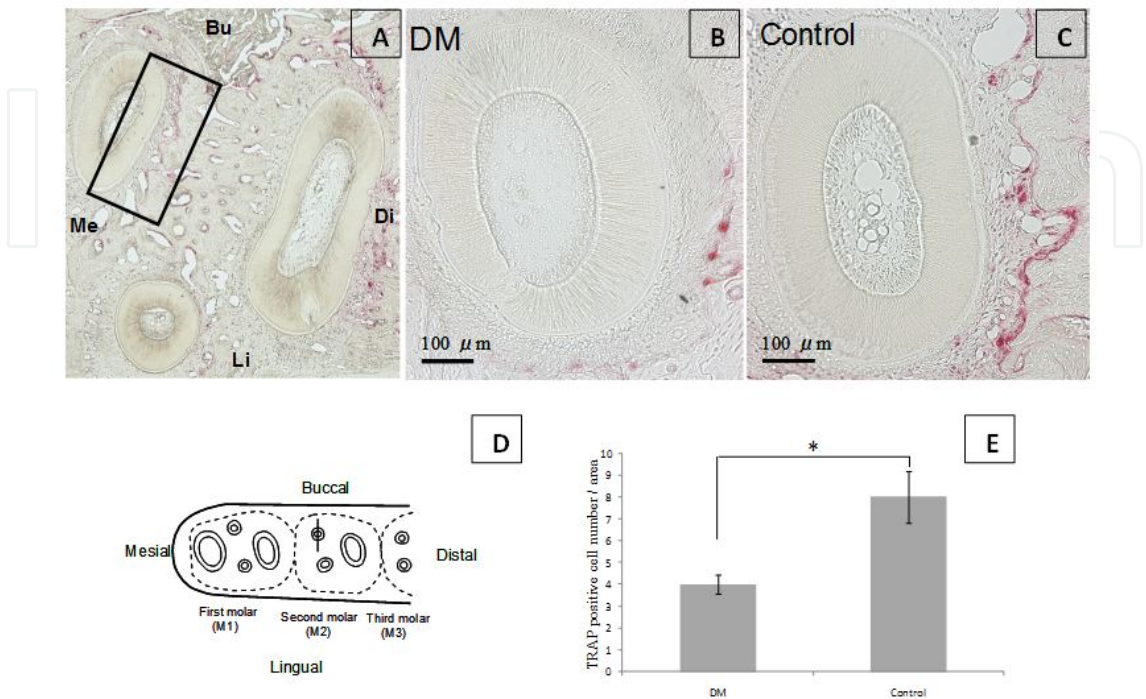


Figure 18. Osteoclast counts in a horizontal section of the mandibular second molar region stained with Tartarate-resistant acid phosphatase (TRAP). (A) Low magnification photograph of the three roots of the second molar stained with TRAP stain. The black rectangle (540 X 120 μm) indicates the area on the distal surface of the alveolar bone adjacent to the middle third of the mesio-buccal root of the second molar in which the osteoclast cells were counted. Bu, buccal; Li, lingual; Me, mesial; Di, distal. (B) The mesio-buccal root of the control rat (original magnification 100X). (C) The mesio-buccal root of the DM rat (original magnification 100X). (D) A schematic drawing showing the observation area on the distal surface of the alveolar bone adjacent to the mesio-buccal root of the second molar in which the osteoclast cells were counted. (E) Statistical analysis demonstrated a significantly higher number of osteoclast cells in the control group when compared with the DM group ($P < 0.05$).

2.11. Suggested mechanisms for the effect of diabetic condition on craniofacial complex

Growth of the craniofacial complex is controlled by genetic and environmental factors [2, 10]. Regulatory mechanisms responsible for normal morphogenesis of the face and head involve hormones, nutrients, mechanical forces, and various local growth factors. The poor growth and alterations in bone metabolism have been associated with T1DM in both humans and experimental animals [2]. Because human studies can be limited by small sample sizes, cross-sectional designs, uncontrolled variables, and often retrospective nature, animal models have been used to yield more rigorous analyses [10]. In our studies we observed the rat growth from the age of 3 weeks old till 7 weeks old. This time period is corresponding to early growth stage in human according to previous craniofacial growth studies [43, 44]. Consequently, in the current study STZ-DM model was used to investigate the effect of T1DM on craniofacial growth.

The studied STZ-DM rats showed significantly reduced growth in most of the craniofacial skeletal units but no significant differences were observed between controls and DM group as regards the remaining craniofacial skeletal units (Sphenoid bone length, posterior neurocranium height, anterior corpus length, bigonial width and palatal width). Craniofacial growth as a whole was also significantly lower in DM group compared to controls in all three dimensions. Previous study investigated the DM effect exclusively on the growth of the mandible and suggested that the diabetic condition had a differential effect on the osseous components and / or its associated non-skeletal tissues. They found that the disharmonious growth of the mandible was due to DM condition and might not be associated with diabetic condition complications such as renal failure, anemia, body weight change or alteration in the food intake qualities [2]. Thus we hypothesize that the deficiency in the craniofacial growth in our experiment might be attributed to the diabetic condition in the DM group as it was reported that specific alterations in bone metabolism are associated with DM. Moreover, several pathogenic possibilities have been proposed, such as insulinopenia, bone microangiopathy, impaired regulation of mineral metabolism, alterations in local factors that regulate bone remodeling, and even an intrinsic disorder associated with T1DM [37, 45]. The aforementioned insulin hormone deficiency that is associated with T1DM cases may have direct effect on bone metabolism. It was mentioned in literature that normal insulin hormone level exerts direct anabolic effects on bone cells [37]. Multiple osteoblast-like cell lines express insulin receptors on the cell surface and have a high capacity for insulin binding [46]. Moreover, osteoclasts exhibit reduced bone resorption in response to insulin stimulation [47]. These findings support the idea that the actions of insulin in bone could be mediated directly via stimulation of osteoblasts in combination with inhibition of osteoclasts, [15, 47] and this mechanism of action may explain the retardation of craniofacial growth in STZ-DM.

Diabetes has a deleterious effect on osseous turnover due to decreased osteoblast and osteoclast activities and numbers and, a lower percentage of osteoid surface and osteocalcin synthesis, as well as increased time for mineralization of osteoid [37]. It was reported that the influence of diabetes on discrete stages of matrix-induced endochondral bone formation could have profound effects on the biomechanical behavior of bone. Also, chondrogenesis and calcification of bone were reduced by 50% in diabetic animals [48]. This was evident in the current study results that showed a significant decrease in the craniofacial linear measurements of the DM group.

In addition to this, insulin may exert synergistic effects with other anabolic agents in bone, such as parathyroid hormone (PTH) [15, 47]. An animal model of T1DM has frequently demonstrated alteration in bone turnover, retarded growth, increased concentration of PTH, and reduced concentration of 1,25-dihydroxvitamin D [37, 49]. The effects of PTH on the bones are complex; it stimulates resorption or bone formation depending on the concentration used, the duration of the exhibition, and the administration method [37, 48, 49, 50]. Also, 1,25-dihydroxvitamin D, like PTH, belongs to the most important group of bone regulatory hormones. It regulates osteoclastic differentiation from hematopoietic mononuclear cells, and osteoblastic functions and activity [37, 51].

Moreover, Insulin may indirectly regulate the enhancement of growth hormone (GH) serum concentration by direct regulation of the hepatic growth hormone receptor, this results in abnormalities in the insulin growth factor-1 (IGF-1) in T1DM [52] which consequently may have lead to the retarded growth in uncontrolled DM in the current study.

In the present study most of the periosteal surfaces in the mandibular regions of the control group showed significantly higher values recorded for the mineral apposition rate and the bone formation rate when compared to the DM group. These results agree with previous studies that recorded diminished lamellar bone formation in DM rats' femur and may suggest an association between the DM condition and the decreased number and function of osteoblasts [16, 19]. The alveolar crest region was the only region that did not show a significant difference in the mineral apposition rate and the bone formation rate parameters among the two groups; this may be attributed to the unique nature of this region exhibiting a highly intensive bone remodeling process especially during the teeth eruption that decreases toward the base of the socket [33].

A significant decrease of bone volume fraction, trabecular thickness, and trabecular number confirmed by Micro-CT analysis in DM rats, there was Also, it showed a significant increase of the trabecular separation and the trabecular space in the DM group when compared with the control group. This finding indicates deterioration of the bone quality in the DM group. These results agree with other research work suggesting that the glycaemic levels play an important role in modulating the trabecular architecture especially in mandibular bone [15]. In this context, these results may describe a state of osteopenia in experimental diabetic rats, which might be the result of an imbalance between bone formation and resorption.

A histometric evaluation of bone resorption was performed by counting the number of osteoclast cells on the distal surface of the alveolar bone adjacent to the mesio-buccal root of the second molar. These evaluations revealed that the number of osteoclasts was significantly lower in the DM rats than in the controls, in line with previous studies on DM rats' mandible [40] and long bones [41, 42]. These studies confirm that the decreased rate of bone turnover may be associated with the DM condition.

This deteriorating effect on mandibular bone structure and dynamic bone formation might be attributed to several pathogenic possibilities, such as insulinopenia, bone microangiopathy, impaired regulation of mineral metabolism, alterations in local factors that regulate bone remodeling, and even an intrinsic disorder associated with DM [12, 37]. However, the detrimental effects observed may not be associated with the significant loss of rats' weights observed in the diabetic group starting from day 14 because previous research [2, 12, 15, 42] showed that the mandibular growth was not affected in normal rats supplied with restricted diet and having same pattern of weight loss resembling weight loss pattern observed in DM rats.

3. Effect of Type 1 Diabetes on teeth susceptibility to caries

3.1. Tooth formation

Tooth growth begins before birth and continues throughout adolescence. The dental development is a continuous process of tooth initiation, matrix secretion, crown mineralization, dental eruption, and root completion. Primate dental development begins before birth with initiation of the deciduous dentition, followed by initiation of the permanent dentition. During the process of dental eruption the tooth must move past the bone margin (alveolar eruption) and the gum (gingival eruption) in order to emerge into the oral cavity and eventually into functional occlusion. Dentine is formed when odontoblasts secrete a collagenous matrix predentine which rapidly undergoes mineralization to form primary dentine. Dentine formation begins at the dentine horn underlying the future cusp tip and progresses inward through secretion and downward through extension until it reaches the apex of the root. Enamel is formed when ameloblasts secrete enamel matrix proteins that mineralize into long, thin bundles of hydroxyapatite crystallites known as enamel prisms. As the secretory cells progress outward toward the future tooth surface, additional adjacent cells are activated through extension until the forming front reaches the cervix of the crown [3].

The resistance of the dental tissues to caries is determined by the quantitative content of the inorganic and organic matrices. Numerous reports have showed that teeth with a correct macro- and microstructure and a proper degree of mineralization are more resistant to the activity of external cariogenic factors [3].

Several biological complications in patients suffering from type 1 diabetes mellitus have been widely investigated over the previous years, however, the scientific data available on the possible effects of T1DM on teeth are scant. These data suggest that T1DM condition may be associated with a change in the chemical composition of the teeth or alteration in the total thickness of enamel or dentin.

Various clinical studies on children reported high caries prevalence in diabetic children when compared with healthy controls [3]. This high caries prevalence may be associated with factors affecting the tooth structure itself or due to some changes in the oral environment causing the increase of the susceptibility of the teeth to dental caries.

3.2. Effects of Type 1 Diabetes on tooth mineral composition

Proper mineralization of teeth during its development is the key factor for the proper resistance of teeth to cariogenic challenge and thus any metabolic disorder affecting the teeth mineralization during its development may render these teeth more prone to be involved by caries. Rat experimental model provides an excellent model for the study of the various metabolic disorders on the mineralization of teeth hard tissues due the fact that the rats incisors are continuously growing and erupting during its life and thus it is possible to study various effects of metabolic disorders which can be induced artificially during the growth period of these rats [53]. The results obtained from the aforementioned experimental rat model can be compared to the results obtained from control rats living under identical situations

and thus can easily eliminate many variables that can affect the outcome of the results of any experiments conducted on humans.

In vitro studies on animal models affected by the streptozotocin induced diabetic condition showed that there is a low concentration of calcium and fluoride in the growing rats' incisors after inducing the diabetic condition by the streptozotocin injection [54]. Moreover, there was a concomitant increase in the concentration of calcium in blood serum and a decrease in fluoride concentration in blood serum. It was suggested that the decrease of calcium concentration in the teeth structure in this study was due to decrease of the cellular activity of ameloblasts during the enamel development [54]. This suggestion may be backed by the results obtained from a similar streptozotocin induced diabetic rat model which showed that the diabetic condition is associated with a decrease in the calcium deposition in bone due to a generalized metabolic activity decrease [12, 28]. The recorded increase in calcium in blood serum was attributed to the absence of insulin which aids in the transfer of calcium from the blood serum to the body tissues. The low concentration of fluoride observed in the tooth structure and in the blood serum may be attributed to the incorporation of the fluoride in bone matrix which is induced by the high concentration of the estrogen observed in the blood serum [54]. Moreover, the increase in fluoride elimination in urine may have caused this decrease in fluoride concentration in the diabetic rats.

3.3. Effects of Type 1 Diabetes on salivary status

The saliva contents and rate of flow are among the critical factors that predict the caries incidence in the oral cavity. Type 1 DM is among the endocrine disease that elevates the glucose level concentration in saliva and decreases the salivary rate of flow.

The mechanism by which the glucose level may increase in saliva may be explained by previous research showed that the increase of blood glucose level in T1DM cases is associated with a concomitant increase of glucose level in saliva. Moreover, it was previously suggested that T1DM causes an increase in the permeability of the basement membrane of the parotid gland that favors the transfer of glucose from the parotid gland to the saliva in the oral cavity [3].

The mechanism by which T1DM decreases the salivary flow may be attributed to the detrimental effects induced by the T1DM condition on the salivary gland tissues itself causing a generalized decrease in the saliva in the oral cavities.

The factors of increased glucose level and decrease in salivary flow cause the improper clearance of glucose from the oral cavity and impairs the buffering capacity of saliva rendering it more acidic, these aforementioned effects consequently increase the metabolic activities of the bacterial biofilm in the oral cavity and render teeth more susceptible to dental caries [3].

Previous research work [3] showed that the dietary habits conducted by the diabetic patients do not have a significant effect on the prevalence of caries in these patients when compared to normal individuals and thus it may be concluded that the increase in caries prevalence in diabetic patients is likely associated with factors affecting the development of teeth or due to some detrimental effects induced by the oral environment that increases the risk of caries occurrence in oral cavities of T1DM patients.

4. Conclusions

It is obvious that the T1DM condition significantly affects craniofacial growth, bone formation mechanism and the quality of the bone formed which may alter many aspects of planning and treatment of orthodontic patients affected by this globally increasing hormonal disturbance. Moreover, T1DM condition impairs the proper tooth development and alters the oral environment rendering teeth more susceptible to dental caries.

There should be a new strategy for treating orthodontic patients suffering from metabolic disorders specially those disorders having direct and indirect effects on bone growth as the diabetic condition. The orthodontic craniofacial linear measurements were significantly decreased in the T1DM cases when compared to normal cases. Moreover, greater risks of developing dental caries and possible tooth loss are associated with patients suffering from T1DM; these risks may complicate the outcome of orthodontic treatment which is associated with less ability of orthodontic patients to implement proper oral hygiene measures due to increased areas of bacterial biofilm formation around orthodontic brackets

These comprehensive studies done on bone and craniofacial growth suggest that planning the treatment in craniofacial region for patients affected with hormonal disorders is a more complex procedure when compared to the treatment of normal patients, moreover it is suggested that it is of prime importance to keep close attention to the general systemic condition of these patients and administer the proper hormonal therapy for these patients when needed to avoid any detrimental effects on bone resulting from any hormonal imbalance. Moreover, it is highly recommended to closely monitor the salivary and the dental conditions of these patients to allow early intervention for treating any developing dental caries.

Within the limitations of this *in vitro* study, it was concluded that:

1. T1DM reduces craniofacial growth, resulting in retardation of skeletal development.
2. DM in the rat affects the bone architecture, as shown by Micro-CT, and impairs the rate of the mandibular bone formation, as examined by the dynamic histomorphometric analysis.
3. All of these results were verified on the cellular level by a histological study that showed the diminished number of osteoclasts on the alveolar wall, suggesting that the early stage of DM resulted in low bone turnover.
4. These findings should be considered when conducting any treatment in the craniofacial region in T1DM since the better understanding of how diabetes affects bone will improve our ability to protect bone health in diabetic patients.
5. The tooth structure and the saliva condition are negatively influenced by the T1DM condition and thus strict oral hygiene measures should be conducted with orthodontic patients suffering from T1DM condition to decrease the risk of developing dental caries in these patients.

6. Good control of diabetes mellitus should be considered before orthodontic treatment by a long time in order to obtain the best outcome.

Author details

Mona Aly Abbassy^{1,2,3,4*}, Ippei Watari¹, Ahmed Samir Bakry^{5,6,7} and Takashi Ono¹

*Address all correspondence to: monaabbassy@gmail.com

1 Orthodontic Science, Department of Orofacial Development and Function, Division of Oral Health Sciences, Graduate School, Tokyo Medical and Dental University, Japan

2 Global Center of Excellence Program; International Research Center For Molecular Science in Tooth and Bone Disease at Tokyo Medical and Dental University, Tokyo Medical and Dental University, Japan

3 Orthodontic Department, Faculty of Dentistry, King Abdulaziz University, Saudi Arabia

4 Alexandria University, Alexandria, Egypt

5 Cariology and Operative Dentistry Department, Graduate School of Medical and Dental Sciences, Tokyo Medical & Dental University, Japan

6 Conservative Dentistry Department, Faculty of Dentistry, Alexandria University, Egypt

7 Conservative Dentistry Department, Faculty of Dentistry, King Abdulaziz University, Saudi Arabia

References

- [1] Bensch, L., Braem, M., Van Acker, K., & Willems, G. (2003). Orthodontic treatment considerations in patients with diabetes mellitus. *American Journal of Orthodontics and Dentofacial Orthopedics*, 123(1), 74-78.
- [2] Giglio, M. J., & Lama, M. A. (2001). Effect of experimental diabetes on mandible growth in rats. *European Journal of Oral Sciences*, 109(3), 193-197.
- [3] Siudikiene, J., Machiulskiene, V., Nyvad, B., Tenovou, J., & Nedzelskiene, I. (2006). Dental caries and salivary status in children with type 1 diabetes mellitus, related to the metabolic control of the disease. *European Journal of Oral Sciences*, 114(1), 8-14.
- [4] Salzmann, J. A. (1979). Practice of orthodontics under public health guidance. *American Journal of Orthodontics*, 76(1), 103-104.

- [5] Kumar, P., & Clark, M. (2009). *Kumar and Clark's Clinical Medicine* (7th edition), Elsevier.
- [6] El -Bialy, T., Aboul-Azm, S. F., & El -Sakhawy, M. (2000). Study of craniofacial morphology and skeletal maturation in juvenile diabetics (Type I). *American Journal of Orthodontics and Dentofacial Orthopedics*, 118(2), 189-195.
- [7] Roe, T. F., Mora, S., Costen, G., Kaufman, F., Carlson, M., & Gilsanz, V. (1991). Vertebral bone density in insulin-dependent diabetic children. *Metabolism*, 40(9), 967-971.
- [8] Eastell, R., & Lambert, H. (2002). Diet and healthy bones. *Calcified Tissue International*, 70(5), 400-404.
- [9] Chew, F. S. (1991). Radiologic manifestations in the musculoskeletal system of miscellaneous endocrine disorders. *Radiologic Clinics of North America*, 29(1), 135-147.
- [10] Yonemitsu, I., Muramoto, T., & Soma, K. (2007). The influence of masseter activity on rat mandibular growth. *Archives of Oral Biology*, 52(5), 487-493.
- [11] Singleton, D. A., Buschang, P. H., Behrents, R. G., & Hinton, R. J. (2006). Craniofacial growth in growth hormone-deficient rats after growth hormone supplementation. *American Journal of Orthodontics and Dentofacial Orthopedics*, 130(1), 69-82.
- [12] Abbassy, M. A., Watari, I., & Soma, K. (2008). Effect of experimental diabetes on craniofacial growth in rats. *Archives of Oral Biology*, 53(9), 819-825.
- [13] Hough, S., Avioli, L. V., Bergfeld, M. A., Fallon, M. D., Slatopolsky, E., & Teitelbaum, S. L. (1981). Correction of abnormal bone and mineral metabolism in chronic streptozotocin-induced diabetes mellitus in the rat by insulin therapy. *Endocrinology*, 108(6), 2228-2234.
- [14] Tein, MS, Breen, S. A., Loveday, B. E., Devlin, H., Balment, R. J., Boyd, R. D., et al. (1998). Bone mineral density and composition in rat pregnancy: effects of streptozotocin-induced diabetes mellitus and insulin replacement. *Experimental Physiology*, 83(2), 165-174.
- [15] Thrailkill, K. M., Liu, L., Wahl, E. C., Bunn, R. C., Perrien, D. S., Cockrell, G. E., et al. (2005). Bone formation is impaired in a model of type 1 diabetes. *Diabetes*, 54(10), 2875-2881.
- [16] Follak, N., Kloting, I., Wolf, E., & Merk, H. (2004). Histomorphometric evaluation of the influence of the diabetic metabolic state on bone defect healing depending on the defect size in spontaneously diabetic BB/OK rats. *Bone*, 35(1), 144-152.
- [17] Alkan, A., Erdem, E., Gunhan, O., & Karasu, C. (2002). Histomorphometric evaluation of the effect of doxycycline on the healing of bone defects in experimental diabetes mellitus: a pilot study. *Journal of Oral and Maxillofacial Surgery*, 60(8), 898-904.
- [18] Mc Cracken-Wesson, MS, Aponte, R., Chavali, R., & Lemons, J. E. (2006). Bone associated with implants in diabetic and insulin-treated rats. *Clinical Oral Implants Research*, 17(5), 495-500.

- [19] Shyng, Y. C., Devlin, H., & Sloan, P. (2001). The effect of streptozotocin-induced experimental diabetes mellitus on calvarial defect healing and bone turnover in the rat. *International Journal of Oral and Maxillofacial Surgery*, 30(1), 70-74.
- [20] Abdus, Salam. M., Matsumoto, N., Matin, K., Tsuha, Y., Nakao, R., Hanada, N., & Senpuku, H. (2004). Establishment of an animal model using recombinant NOD.B10.D2 mice to study initial adhesion of oral streptococci. *Clinical and Diagnostic Laboratory Immunology*, 11(2), 379-386.
- [21] Matin, K., Salam, M. A., Akhter, J., Hanada, N., & Senpuku, H. (2002). Role of stromal-cell derived factor-1 in the development of autoimmune diseases in non-obese diabetic mice. *Immunology*, 107(2), 222-232.
- [22] Chidiac, J. J., Shofer, F. S., Al-Kutoub, A., Laster, L. L., & Ghafari, J. (2002). Comparison of CT scanograms and cephalometric radiographs in craniofacial imaging. *Orthodontics and Craniofacial Research*, 5(2), 104-113.
- [23] Vande Berg, J. R., Buschang, P. H., & Hinton, R. J. (2004b). Absolute and relative growth of the rat craniofacial skeleton. *Archives of Oral Biology*, 49(6), 477-484.
- [24] Engstrom, C., Jennings, J., Lundy, M., & Baylink, D. J. (1988). Effect of bone matrix-derived growth factors on skull and tibia in the growing rat. *Journal of Oral Pathology*, 17(7), 334-340.
- [25] Kiliaridis, S. E. C., & Thilander, B. (1985). The relationship between masticatory function and craniofacial morphology. I. A cephalometric longitudinal analysis in the growing rat fed a soft diet. *European Journal of Orthodontics*, 7, 273-283.
- [26] Vandeberg, J. R., Buschang, P. H., & Hinton, R. J. (2004a). Craniofacial growth in growth hormone-deficient rats. *The Anatomical Record. Part A, Discoveries in Molecular, Cellular and Evolutionary Biology*, 278(2), 561-570.
- [27] Stuart, A., & Smith, D. (1992). Use of the Fluorochromes xylene orange, calcein green, and tetracycline to document bone deposition and remodeling in healing fractures in chickens. *Avian diseases*, 36, July, 447-449.
- [28] Abbassy, M. A., Watari, I., & Soma, K. (2010). The effect of diabetes mellitus on rat mandibular bone formation and microarchitecture. *European Journal of Oral Sciences*, 118(4), 364-369.
- [29] Shimomoto, Y., Chung, C. J., Iwasaki-Hayashi, Y., Muramoto, T., & Soma, K. (2007). Effects of occlusal stimuli on alveolar/jaw bone formation. *Journal of Dental Research*, 86(1), 47-51.
- [30] Parfitt, A. M. (1988). Bone histomorphometry: standardization of nomenclature, symbols and units (summary of proposed system). *Bone*, 9(1), 67-69.
- [31] Keshawarz, N. M., & Recker, R. R. (1986). The label escape error: comparison of measured and theoretical fraction of total bone-trabecular surface covered by single label in normals and patients with osteoporosis. *Bone*, 7(2), 83-87.

- [32] Sheng, M. H., Baylink, D. J., Beamer, W. G., Donahue, L. R., Rosen, C. J., Lau, K. H., et al. (1999). Histomorphometric studies show that bone formation and bone mineral apposition rates are greater in C3H/HeJ (high-density) than C57BL/6J (low-density) mice during growth. *Bone*, 25(4), 421-429.
- [33] Gerlach, R. F., Toledo, D. B., Fonseca, R. B., Novaes, P. D., Line, S. R., & Merzel, J. (2002). Alveolar bone remodelling pattern of the rat incisor under different functional conditions as shown by minocycline administration. *Archives of Oral Biology*, 47(3), 203-209.
- [34] Ding, M., Odgaard, A., & Hvid, I. (1999). Accuracy of cancellous bone volume fraction measured by micro-CT scanning. *Journal of Biomechanics*, 32(3), 323-326.
- [35] Laib, A., & Ruegsegger, P. (1999). Calibration of trabecular bone structure measurements of in vivo three-dimensional peripheral quantitative computed tomography with 28-microm-resolution microcomputed tomography. *Bone*, 24(1), January, 35-39.
- [36] Takada, H., Abe, S., Tamatsu, Y., Mitarashi, S., Saka, H., & Ide, Y. (2006). Three-dimensional bone microstructures of the mandibular angle using micro-CT and finite element analysis: relationship between partially impacted mandibular third molars and angle fractures. *Dent Traumatol*, 22(1), 18-24.
- [37] Duarte, V. M., Ramos, A. M., Rezende, L. A., Macedo, U. B., Brandao-Neto, J., Almeida, M. G., & Rezende, A. A. (2005). Osteopenia: a bone disorder associated with diabetes mellitus. *Journal of Bone and Mineral Metabolism*, 23(1), 58-68.
- [38] Yokoyama, M., Atsumi, T., Tsuchiya, M., Koyama, S., & Sasaki, K. (2009). Dynamic changes in bone metabolism in the rat temporomandibular joint after molar extraction using bone scintigraphy. *European Journal of Oral Sciences*, 117(4), 374-379.
- [39] Misawa, Y., Kageyama, T., Moriyama, K., Kurihara, S., Yagasaki, H., Deguchi, T., Ozawa, H., & Sahara, N. (2007). Effect of age on alveolar bone turnover adjacent to maxillary molar roots in male rats: A histomorphometric study. *Archives of Oral Biology*, 52(1), 44-50.
- [40] Mishima, N., Sahara, N., Shirakawa, M., & Ozawa, H. (2002). Effect of streptozotocin-induced diabetes mellitus on alveolar bone deposition in the rat. *Archives of Oral Biology*, 47(12), 843-849.
- [41] Glajchen, N., Epstein, S., Ismail, F., Thomas, S., Fallon, M., & Chakrabarti, S. (1988). Bone mineral metabolism in experimental diabetes mellitus: osteocalcin as a measure of bone remodeling. *Endocrinology*, 123(1), 290-295.
- [42] Shires, R., Teitelbaum, S. L., Bergfeld, M. A., Fallon, M. D., Slatopolsky, E., & Avioli, L. V. (1981). The effect of streptozotocin-induced chronic diabetes mellitus on bone and mineral homeostasis in the rat. *Journal of Laboratory Clinical Medicine*, 97(2), 231-240.

- [43] Losken, A., Mooney, M. P., & Siegel, M. I. (1994). Comparative cephalometric study of nasal cavity growth patterns in seven animal models. *Cleft Palate Craniofacial Journal*, 31(1), 17-23.
- [44] Siegel, M. I., & Mooney, M. P. (1990). Appropriate animal models for craniofacial biology. *Cleft Palate Journal*, 27(1), 18-25.
- [45] Ward, D. T., Yau, S. K., Mee, A. P., Mawer, E. B., Miller, CA, Garland, H. O., et al. (2001). Functional, molecular, and biochemical characterization of streptozotocin-induced diabetes. *Journal of the American Society of Nephrology*, 12(4), 779-790.
- [46] Pun, K. K., Lau, P., & Ho, P. W. (1989). The characterization, regulation, and function of insulin receptors on osteoblast-like clonal osteosarcoma cell line. *Journal of Bone and Mineral Research*, 4(6), 853-862.
- [47] Thomas, D. M., Udagawa, N., Hards, D. K., Quinn, J. M., Moseley, J. M., Findlay, D. M., et al. (1998). Insulin receptor expression in primary and cultured osteoclast-like cells. *Bone*, 23(3), 181-186.
- [48] Reddy, G. K., Stehno-Bittel, L., Hamade, S., & Enwemeka, C. S. (2001). The biomechanical integrity of bone in experimental diabetes. *Diabetes Research and Clinical Practice*, 54(1), 1-8.
- [49] Tsuchida, T., Sato, K., Miyakoshi, N., Abe, T., Kudo, T., Tamura, Y., et al. (2000). Histomorphometric evaluation of the recovering effect of human parathyroid hormone (1-34) on bone structure and turnover in streptozotocin-induced diabetic rats. *Calcified Tissue International*, 66(3), 229-233.
- [50] Toromanoff, A., Ammann, P., Mosekilde, L., Thomsen, J. S., & Riond, J. L. (1997). Parathyroid hormone increases bone formation and improves mineral balance in vitamin D-deficient female rats. *Endocrinology*, 138(6), 2449-2457.
- [51] Collins, D., Jasani, C., Fogelman, I., & Swaminathan, R. (1998). Vitamin D and bone mineral density. *Osteoporosis International*, 8(2), 110-114.
- [52] Chiarelli, F., Giannini, C., & Mohn, A. (2004). Growth, growth factors and diabetes. *European Journal of Endocrinology*, 151(3), U109-U117.
- [53] Ozbek, M., Dural, S., Kanli, A., Tuncel, M., & Orhan, K. (2009). Morphological evaluation of rat incisor enamel and dentin induced by pregnancy and lactation using a scanning electron microscope. *Journal of Veterinary Medical Science*, 71(10), 1273-1277.
- [54] Balint, E., Marshall, C. F., & Sprague, S. M. (2001). Glucose-induced inhibition of in vitro bone mineralization. *Bone*, 28(1), 21-28.