

BOSTON UNIVERSITY
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THESIS

**ROLE OF $GS\alpha$ -DEPENDENT SIGNALING IN BONE HOMEOSTASIS,
CONDYLAR REMODELING AND ENAMEL MINERALIZATION**

by

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DEDICATIONS

To my mom

whom I love and taught me how to love

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ROLE OF G_sα-DEPENDENT SIGNALING IN BONE HOMEOSTASIS,
CONDYLAR REMODELING AND ENAMEL MINERALIZATION

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ABSTRACT

The Dentin Matrix Protein (DMP1) is a critical regulator of bone and dentin mineralization and this protein is highly expressed in osteocytes and odontoblasts. G_s alpha (G_sα) protein, the main intracellular signal of a broad class of G-protein coupled receptors (GPCRs), is highly expressed in bone cells, including osteocytes. We and others have demonstrated that mice lacking the G_sα expression, predominantly in osteocytes (DMP1-G_sαKO mice), develop severe osteopenia driven by a marked reduction in osteoblast activity associated with a significant increase in SOST/sclerostin expression. In this study, we have examined the role of G_sα in the jaws and teeth of DMP1-G_sαKO mice to investigate if the absence of G_sα expression in osteocytes and odontoblasts altered teeth and jaws morphology. Our previous studies showed that DMP1-G_sαKO leads to a significant decrease in both trabecular and cortical bone content in the skeleton, as assessed by μCT and histomorphometric analysis. Here we characterize the dental and craniofacial phenotype of DMP1-G_sαKO mice. Results showed that DMP1-G_sαKO had decreased total mandibular bone mineral density (BMD), total mandibular mineral content (BMC), condylar BMD and total tooth mineralization as assessed by DEXA using a Lunar PIXImus II densitometer.

Furthermore, μ CT analysis revealed that condylar bone volume and tooth mineralization is reduced in DMP1-Gs α KO mice compared to control littermate. μ CT also showed that the overall skull size and specifically the zygomatic bone is larger in the control group. Next, we examined H&E histological sections of the jaws of DMP1-Gs α KO and control mice, which confirmed the osteopenic phenotype. Tartrate-resistant acid phosphatase (TRAP) staining showed that the number of TRAP-positive osteoclasts was increased in the DMP1-Gs α KO mice compared to controls, suggesting increased bone resorption. In conclusion, our studies identified Gs α signaling in osteocytes and odontoblasts as important in maintaining normal bone and tooth homeostasis.

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INTRODUCTION

Osteocytes were long thought to be quiescent cells, but in the early 2000s they have been identified as the central regulator of bone re-modeling by modulating activities of both osteoblasts and osteocytes (1). Similar to osteocytes, bone lining cells which have a long life span, also appear as quiescent cells. Lineage of the bone lining cells is still unclear. A recent lineage tracing study showed that bone lining cells arise from mature osteoblasts (2) whereas, another study presented that they have the characteristics of both osteoblasts and mesenchymal cells (3), making them a good source of functioning osteoblasts during newly initiating bone remodeling (4).

Role of DMP1

DMP1 is essential in regulating the mineralization process and thus plays an important role in tooth formation, bone, and cartilage development (5, 6). Several studies reported that enamel mineral composition does not vary much between incisors and molars (7). However, tooth mineralization occurs at different timings (8). Moreover, this protein is highly expressed in osteocytes and odontoblasts. Gs alpha ($G_s\alpha$), the main intracellular signaling protein of a wide class of G-protein coupled receptors (GPCRs), is highly expressed in bone cells, including osteocytes (9).

Parathyroid hormone and bone homeostasis

GPCRs couple with many receptors such as parathyroid hormone receptors (PTHr) and the prostaglandin receptor. PTHr are critical regulators of bone formation, as shown by a genetic manipulation study targeting early osteoblast lineage cells (10). Parathyroid hormone (PTH) stimulates new bone formation (11), which

signals through the PTHR, which is also a G-protein coupled receptor (GPCR) highly expressed in bone, condylar cartilage, and kidney (12, 13). PTH has a critical role in calcium and phosphate homeostasis. The receptor signals through several pathways, including the stimulatory subunit of G-proteins, G α . PTH signaling in early osteoblast lineage cells is a critical regulator of bone and cartilage formation (10). A recent study showed that intermittent PTH administration leads to alteration of the subchondral bone microarchitecture and an increased mineralization/calcification of the temporomandibular joint cartilage and condyles (14). Here we focus on the condyles as they play a significant role in mandibular growth, craniofacial anomalies and malocclusions (15).

Overexpression of PTH leads to osteoporosis, which is characterized by low bone mass and micro-architectural defects that lead to decreased bone strength and increased fracture risk. However, in terminally differentiated and most abundant bone cells, osteocytes, loss of PTH signaling results in an increase in bone mineral density (16). On the other hand, G α signaling in early osteoblast lineage cells has more profound effects on skeletal homeostasis (17, 18). Mechanical stimulation is also a well-known anabolic signal for bone formation. Ultrastructural studies showed that mechanical stimulation induces bone lining cells along the trabecular bone surface to differentiate into osteoblastic cells of which characteristics include increased cuboidal shape, rounded nuclei, and abundant rough endoplasmic reticulum (19).

Sclerostin and PTH

Expression of sclerostin, a glycoprotein secreted by osteocytes, is highly suppressed by PTH and mechanical forces (20, 21). It has been shown that higher

sclerostin levels reduce endosteal osteoblasts and that the normalization of high sclerostin levels by anti-sclerostin antibody restores these cells. These findings are consistent with the effects of PTH and mechanical stimulation in these cells (19, 22, 23). In a previous study, the treatment of DMP1-Gs α KO mice with anti-sclerostin antibody showed a significant increase in the numbers of osteocalcin-positive cells on the endosteal bone surface. This was accompanied by an increase in serum levels of PINP, indicating an increase in active bone-forming osteoblasts. Interestingly, serum levels of CTX were also increased, suggesting the osteoblast-osteoclast coupling was also increased (4).

As the central regulator of bone remodeling, osteocytes are thought to maintain the bone lining cells in the quiescence state via sclerostin secretion. They suppress sclerostin secretion, in response to numerous signals, when a new bone remodeling cycle is needed. Numerous studies have shown that Gs α signaling in osteocytes is a potent regulator of sclerostin expression and secretion (24-26). Upstream of the Gs α signaling, PTH signaling through its G-protein coupled receptor, PTHR, is a major ligand influencing bone homeostasis, at least in part, by inhibiting sclerostin (16, 17, 27, 28). Downstream of the Gs α signaling, transcription factors such as Mef2C, HDAC4, and HDAC5 have been shown to regulate sclerostin expression in osteocytes (26).

Effect of Gs α KO in osteocytes and bone homeostasis

To better understand the Gs α signaling pathway in craniofacial bone homeostasis, we examined the role of Gs α signaling in osteocytes. Previous studies have reported that mice lacking Gs α in osteocytes (DMP1-Gs α KO mice) and mature

osteoblasts (Oc-Gs α KO) had increased SOST/sclerostin expression and marked osteopenia (24, 25). It has been previously reported that intermittent PTH administration converts quiescent endosteal bone lining cells into functional osteoblasts (22) and that bone lining cells are a major source of proliferating preosteoblasts and mature osteoblasts (2). These newly formed osteoblasts from bone lining cells actively contribute to bone remodeling in adults and respond to both catabolic and anabolic bone stimuli (2). The bone lining cells express genetic signature markers of both MSCs and osteoblasts such as RANKL (2).

The severe osteopenia present in DMP1-Gs α KO mice is due to dramatic suppression of bone formation with loss of trabecular and endosteal osteoblasts, partially driven by elevated SOST/sclerostin levels. A previous study examined the skeletal phenotype of mice lacking Gs α in osteocytes and some mature osteoblasts (DMP1-Gs α KO mice) and reported that these mice have severe osteopenia, increased myeloid cells and browning of white adipose tissue (24, 25). This is consistent with other studies showing dramatic osteopenia in mice lacking Gs α in pre-osteoblasts (osterix-Gs α KO) osteoblasts (Coll1-Gs α KO) and mature osteoblasts (Oc-Gs α KO) (17, 25, 29). Also, it has been shown clinically that patients whom have reduced Gsa expression suffer from Pseudohypoparathyroidism (30, 31).

Given the role of GS α signaling in regulating both endosteal osteoblasts and cartilage, we hypothesized that this protein is critical for tooth mineralization, craniofacial bone, and condylar remodeling.

MATERIALS AND METHODS

Mice

Twenty-eight, 6-week-old mice were used (Gsa fl/fl or Control: N=16; 7M and 9F, DMP1-Cre+Gsa fl/fl or KO: N=12; 3M and 9F). All experimental animal procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of Boston University Medical Campus (protocol #15522). DMP1-Cre⁽⁺⁾: Gsa^(flox/flox) mice were generated by crossing Gsa^{flox/flox} mice (32) with mice expressing Cre-recombinase driven by a 10 kb DMP1-promoter (33). Littermates lacking Cre-expression were used as controls for all experiments (Gsa fl/fl). The genotype of all experimental mice was determined by PCR analysis of genomic DNA extracted from tail biopsies using following primers: Cre- transgene: forward 5'-CGCGGTCTGGCAGTAAAACTATC-3' and reverse: 5'-CCCACCGTCAGTACGTGAGATATC-3'; Gsa allele: forward: 5'-GAGAGCGAGACGAAGACAGC-3' and reverse: 5'-TCGGGCCTCTGGC GGAGCTT-3'. Mice were euthanized at six weeks of age, and mice heads were harvested and processed for further analyses.

Dual Energy X-ray Absorptiometry (DEXA)

Total bone mineral density (BMD), total mineral content (BMC), condyles BMD, and incisor mineralization were quantitatively assessed by DEXA using a Lunar PIXImus II densitometer Prodigy Advance machine with software package 13.60. DEXA values were based on three regions of interest (ROI). The first ROI (ROI1) was the whole mandible. The second ROI (ROI2) was the condyles, and the third ROI

(ROI3) was the incisors.

mCT

Bone microarchitecture was quantitatively assessed with high-resolution micro-computed tomography (μ CT40; Scanco Medical, Brüttisellen, Switzerland). The mandible and full head of mice were scanned using 70 kVp, 50 mAs, and 12- μ m isotropic voxel size. For the condylar region, we assessed total mineral density (TMD) and bone volume fraction (BV/TV, %) by contouring and measuring the condyle's mushroom-shaped head. Scanning and analyses adhered to recently published guidelines (34). Also, three specific linear measurements of the mandible were performed; mandibular length, condyle head length, and condyle head width using ImageJ, as described by O' Brien (14).

Tissue Processing

The head and the lower jaw were harvested, weighed and processed for μ CT, histology, immunohistochemistry analyses when the mice were six-week old, where we have seen the most effect of osteopenia (4). The mandibles were dissected free by cutting the muscular attachments and were cut in half for μ CT analysis while the other half was decalcified with 20% EDTA. After decalcification, samples were processed, and paraffin-embedded, and 5 μ m serial sections were placed on OptiPlus Positive-Charged Barrier Slides (BioGenex, Fremont, CA), deparaffinized with xylene/ ethanol and processed for histological analyses.

Histology

Mice mandibles were fixed overnight in 4% paraformaldehyde in PBS and dehydrated. Samples were processed, decalcified in 20 % EDTA for four weeks and later embedded in paraffin and sectioned (5- μ m thickness) and stained with hematoxylin and eosin (H&E). Digital images of stained slides were acquired with EVOS® FL Cell Imaging System (Thermo Fisher Scientific, Waltham, MA).

Tartrate-resistant acid phosphatase (TRAP) staining

Paraffin sections were deparaffinized and rehydrated in TBS. The slides were then incubated for 45 min at 37 °C in 0.92% sodium acetate buffer, pH 5.0, containing 0.01% naphthol AS-BI phosphate, and 1.14% L-(+)Tartaric acid. Then, the sections were incubated in the same buffer containing 0.1% pararosaniline chloride for 10 min, followed by washing in distilled water. The sections were dehydrated in ethanol and xylene, and coverslipped with mounting media. TRAP-positive cells were quantified in 6-fields near the growth plate. Digital images of stained slides were acquired with EVOS® FL Cell Imaging System from Thermo Fisher Scientific.

Statistical Analyses

Statistics for comparing relative abundances of BMD, BMC, mineralization content, and osteocyte counts obtained using DEXA, μ CT, H&E and TRAP assays, between DMP1-Cre⁽⁺⁾; Gs α ^(flox/flox) and Gs α ^(flox/flox) (control) groups were obtained using an unpaired two-tailed *t*-test. All data shown represent the mean and standard error of the mean (SEM). Statistically significant differences between groups were determined by using GraphPad Prism. The level of significance is designated as follows: *: $p < 0.05$, **: $p < 0.005$, ***: $p < 0.0005$.

RESULTS

Mice lacking Gsa in osteocytes have decreased craniofacial bone volume

We have previously generated mice lacking Gs α in osteocytes by mating Gs α ^(flox/flox) mice (35) with mice expressing Cre-recombinase driven by dentin matrix protein 1 (DMP1) (36). Therefore, DMP1-Cre⁽⁺⁾; Gs α ^(flox/flox) mice are referred to as DMP1-Gs α KO, and littermates lacking DMP1-Cre expression were used as controls (WT). We have reported that the 10KbDMP1-Cre, albeit highly expressed in osteocytes, is also expressed in some mature osteoblasts and skeletal muscle (25). As previously reported, DMP1-Gs α KO animals have low bone mass, decrease adipose tissue, and develop a myeloproliferative phenotype (24). To examine the effect of Gs α -deletion in osteocytes and some mature osteoblasts on craniofacial homeostasis, we examined the quantitative bone analysis by DEXA using a Lunar PIXImus II densitometer. We also examined the alteration of the craniofacial bones by micro-computed tomography. We analyzed the bones in young (6-week old) male and female animals. DEXA quantitative evaluation of full jaw showed that both the total bone mineral density (BMD) and total bone mineral content (BMC) were decreased in the mandible of 6-week old DMP1-Gs α KO female mice compared to controls (Figure 1).

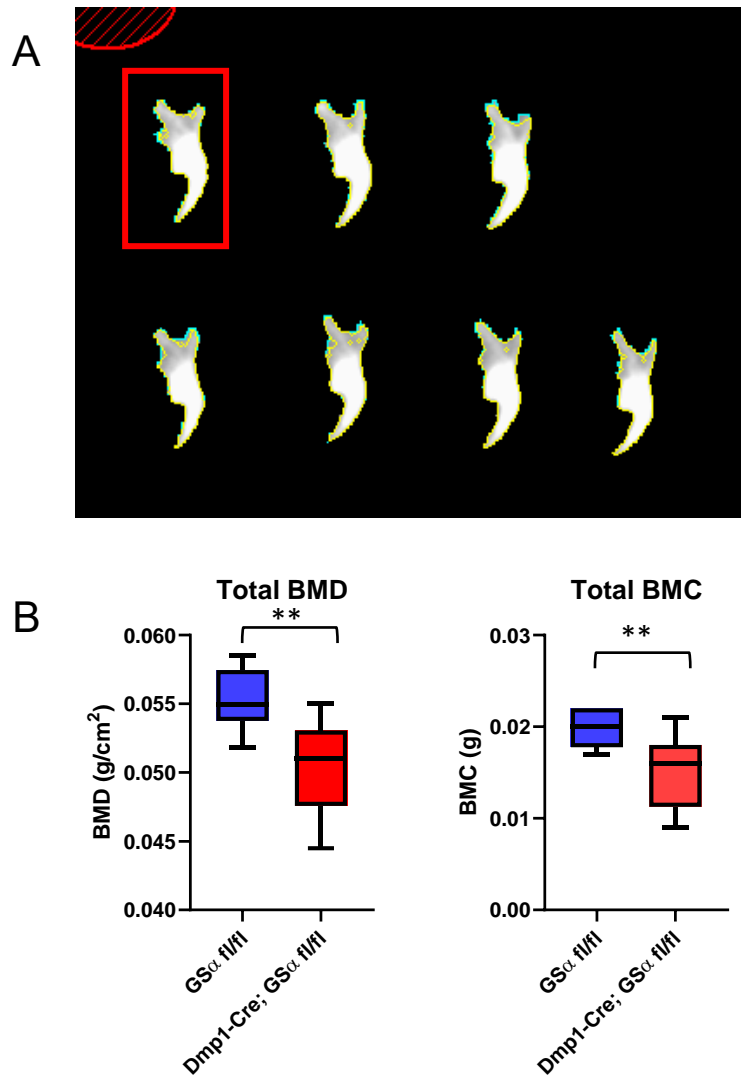


Figure 1. Mice lacking *Gs α* in osteocytes are osteopenic (A). Region of interest (ROI) 1 used to calculate total BMD and BMC for the mandible by DEXA scan. (B). Quantitative DEXA parameters for the mandible showing 6-week old female control and DMP1-*Gs α* KO mice (WT: N=8, KO: N=6). All data: shown as the mean (+SEM) (Unpaired t-test; ** P < 0.005).

Also, qualitative micro-CT reconstructions of the mandible showed that the overall bone structure phenotype appeared to have lower mineral density in DMP1-*Gs α* KO compared to control in both the buccal and lingual view (Figure 2).

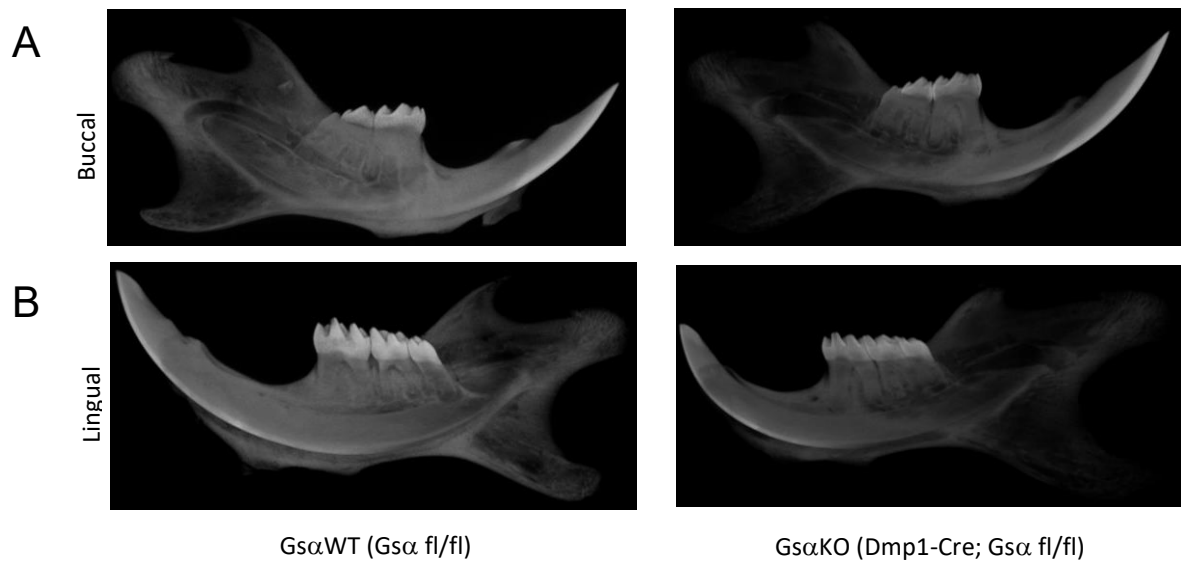


Figure 2. Mice lacking $G\alpha$ in osteocytes are osteopenic. (A, B). Buccal and lingual view of the mandible showing the phenotype with reconstruction by μ CT scan for 6-week old female control and DMP1- $G\alpha$ KO mice.

In addition, the overall skull size of DMP1- $G\alpha$ KO mice was smaller than controls, especially in the zygomatic region (Figure 3). These data show that the loss of $G\alpha$ signaling in osteocytes is critical for both cortical and trabecular bone accumulation in craniofacial bones.

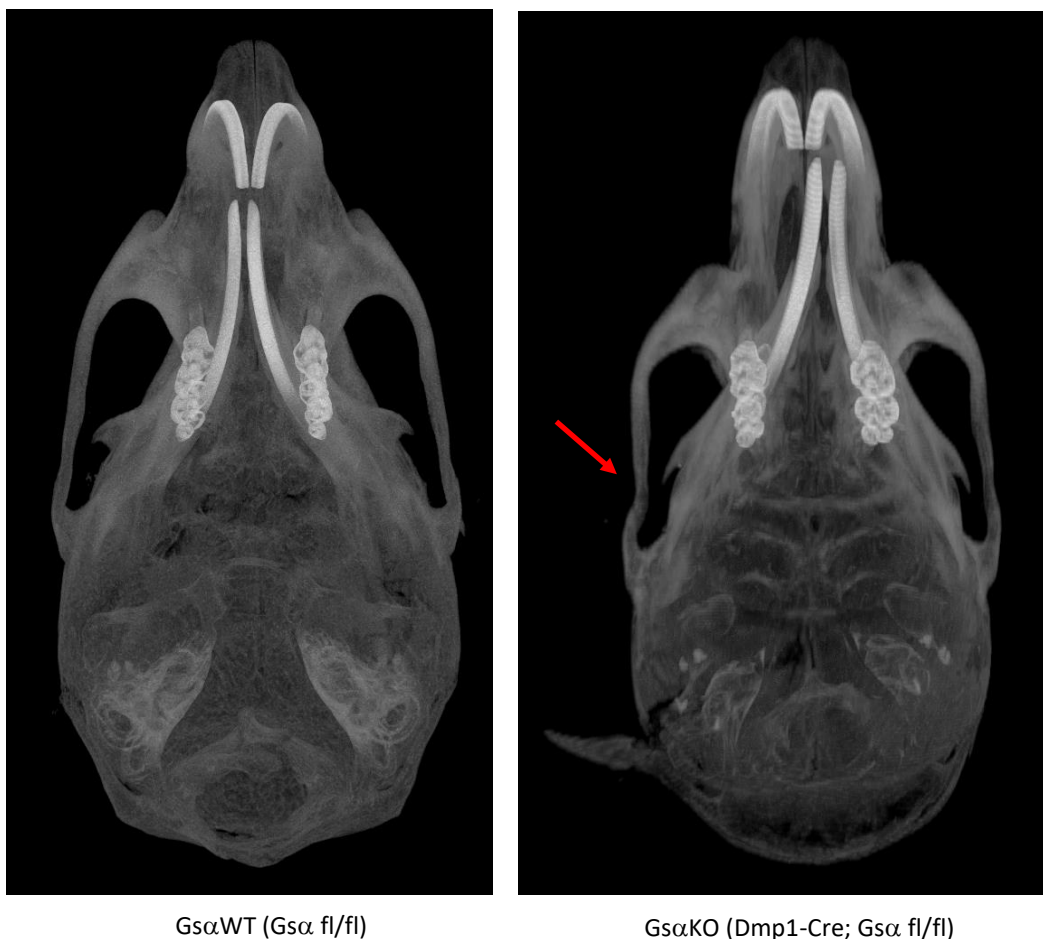
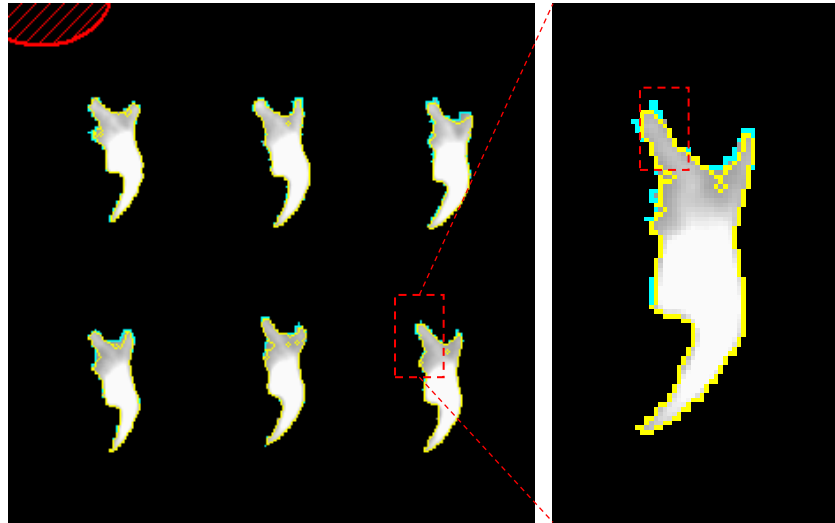


Figure 3. Mice lacking *Gsa* in osteocytes are osteopenic. Top view of the whole head showing the phenotype with reconstruction by μ CT scan. Overall, skull size was smaller, especially in the zygomatic region (Arrow) of DMP1-*Gsa*KO mice compared to *Gsa*WT.

Condylar bone lining cells are decreased in DMP1-Gs α KO mice

Given the critical role condyles play in mandibular growth and craniofacial anomalies, we examined the condyle alone to specifically study the effect of Gs α . In order to examine the effect of Gs α -deletion in osteocytes and some mature osteoblasts on the condyle, we further analyzed the outcome of that region with DEXA and μ CT scans. Also, we stained the joint with H&E to examine them histologically. We examined bones in young (6-week old) female animals for quantitative bone analysis by DEXA using a Lunar PIXImus II densitometer. We also carried on with quantitative and qualitative bone analysis by micro-computed tomography. DEXA quantitative evaluation of the condyles, ROI 2, showed a significant reduction in the bone mineral density (BMD) in the condyles of 6-week old DMP1-Gs α KO female mice compared to wild type (Figure 4).

A



B

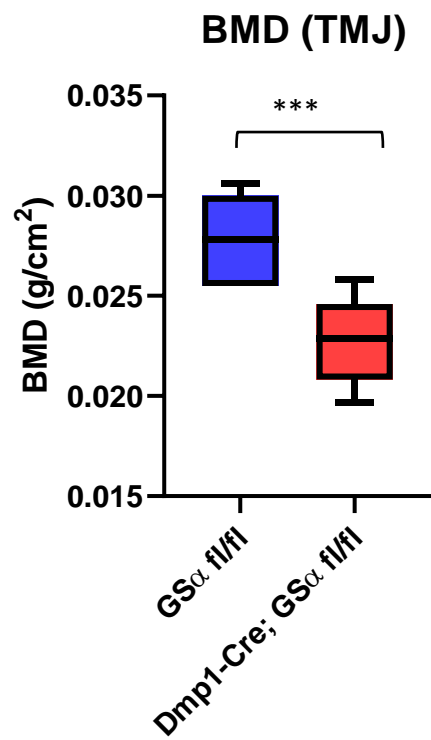
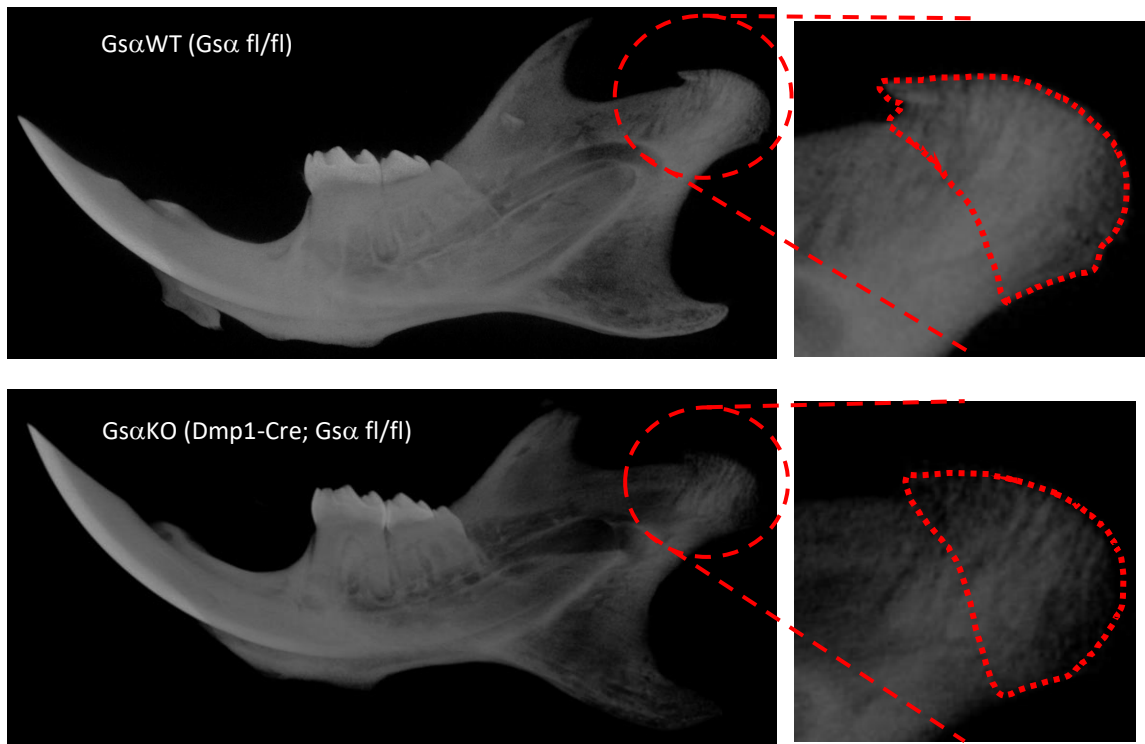


Figure 4. Degenerative changes in DMP1-Gs α KO condyles. (A). Region of interest (ROI) 2 used to calculate the BMD for the condylar head by DEXA scan. (B). Quantitative DEXA parameters for the condyles showing 6-week old female control and DMP1-Gs α KO mice (WT: N=8, KO: N=6). All data: shown as the mean (+SEM) (Unpaired t-test; *** P < 0.0005).

Moreover, μ CT quantitative analysis also showed a reduction in the condyles BMD of 6-week old DMP1-Gs α KO female mice compared to control as we saw in the DEXA scan (Figure 5). Also, we can appreciate the differences in bone quality that we can observe between both groups. Interestingly we see remarkable osteopenia in the condyle of DMP1-Gs α KO compared to the control group (Figure 5A). μ CT quantitative analysis shows a significant reduction in the BMD of the condyles and the BV/TV, which explains the changes in the condylar trabecular bone, as shown in Figure 7.

A



B

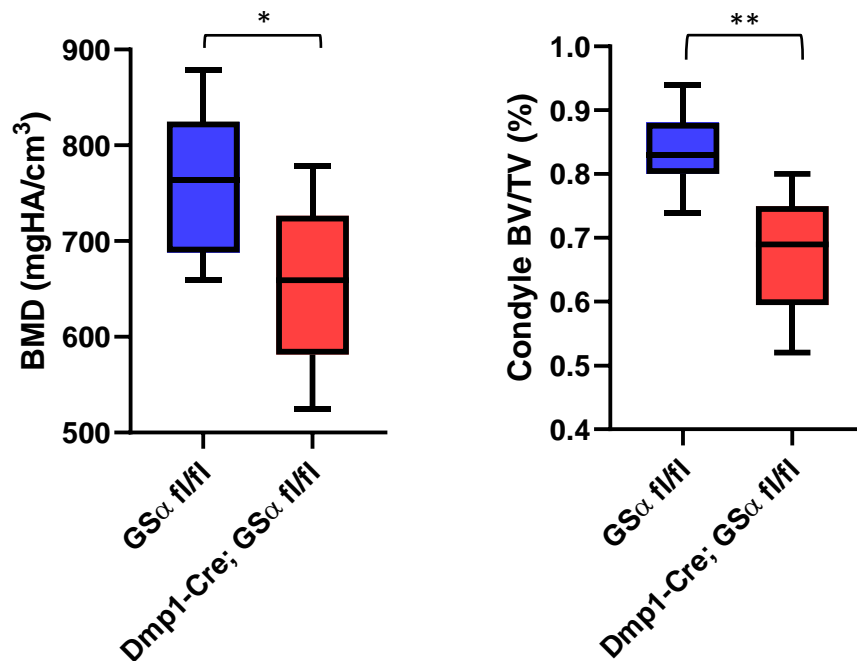


Figure 5. Degenerative changes in DMP1-Gs α KO condyles. (A) Reconstruction of the mandible by μ CT scan showing the difference in bone quality and highlighting the condyles as contoured with the red dots (B) Quantitative measurement for the condyles BMD for the 6-week old female control and DMP1-Gs α KO mice (WT: N=8, KO: N=6). All data: shown as the mean (+SEM) (Unpaired t-test; * P < 0.05).

The mandibular length, condylar head length and condylar head width linear measurements were further assessed. Interestingly, there was a statistically significant reduction in the mandibular length and the condylar head length of 6-week old DMP1-Gs α KO female mice compared to control (Figure 6). However, the condylar width of 6-week old DMP1-Gs α KO female mice compared to wild type did not significantly reduce, as shown in Figure 6.

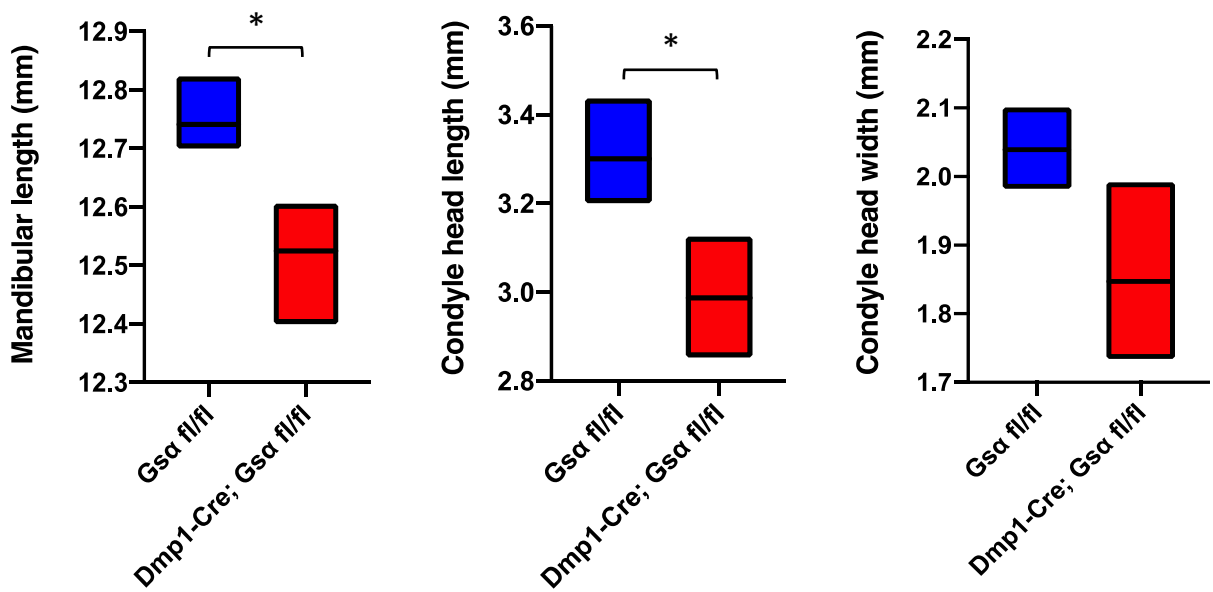
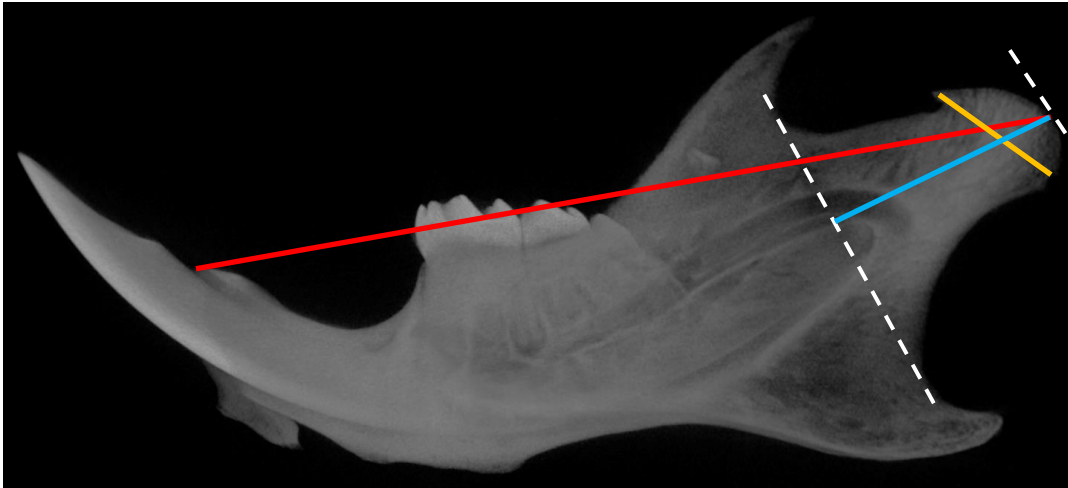
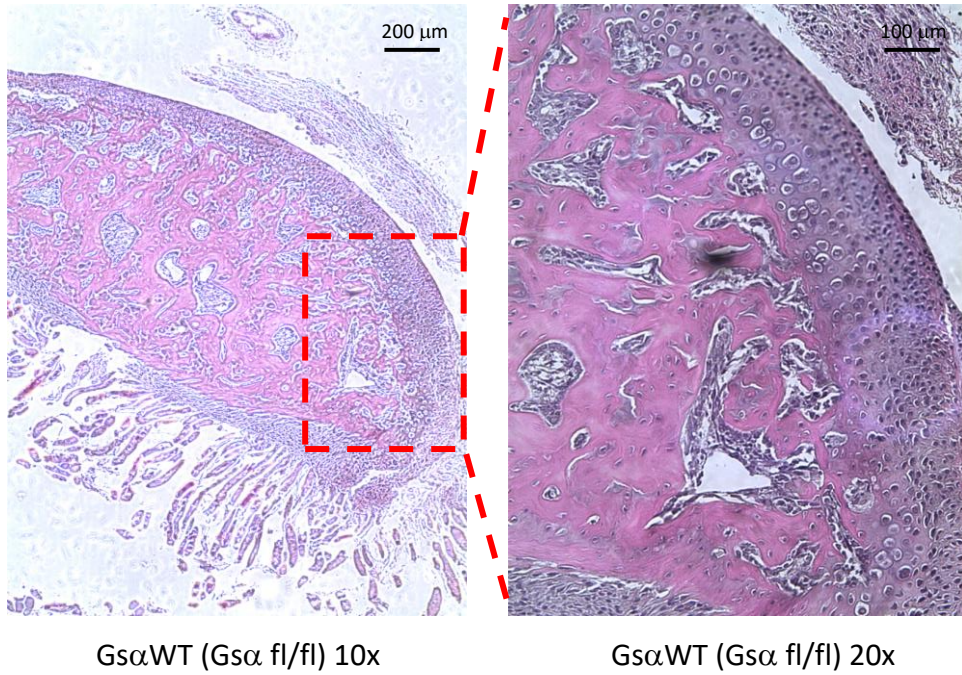


Figure 6. The mandibular length (red), condylar head length (blue) and condylar head width (yellow) linear measurements. Mandibular length and the condylar head length of 6-week old DMP1-GsaKO female mice showing a significant reduction in length compared to control. and DMP1-GsaKO mice (WT: N=3, KO: N=3). Condylar width of 6-week old DMP1-GsaKO female mice is less compared to wild type but it's not significant. *Statistically significant (Unpaired t-test; * P < 0.05).

H&E staining showed that endosteal osteoblasts in cortical bone regions were significantly reduced in the condyles of DMP1-Gs α KO mice compared to Gs α WT (Figure. 7). Tartrate resistant acid phosphatase staining further confirmed that the number of TRAP-positive osteoclasts was significantly increased in the DMP1-Gs α KO mice (Figure 8). Interestingly, this overlaps with the area with the most bone resorption we observed in the H&E staining suggests bone resorption, as shown in Figure 7.

A



B

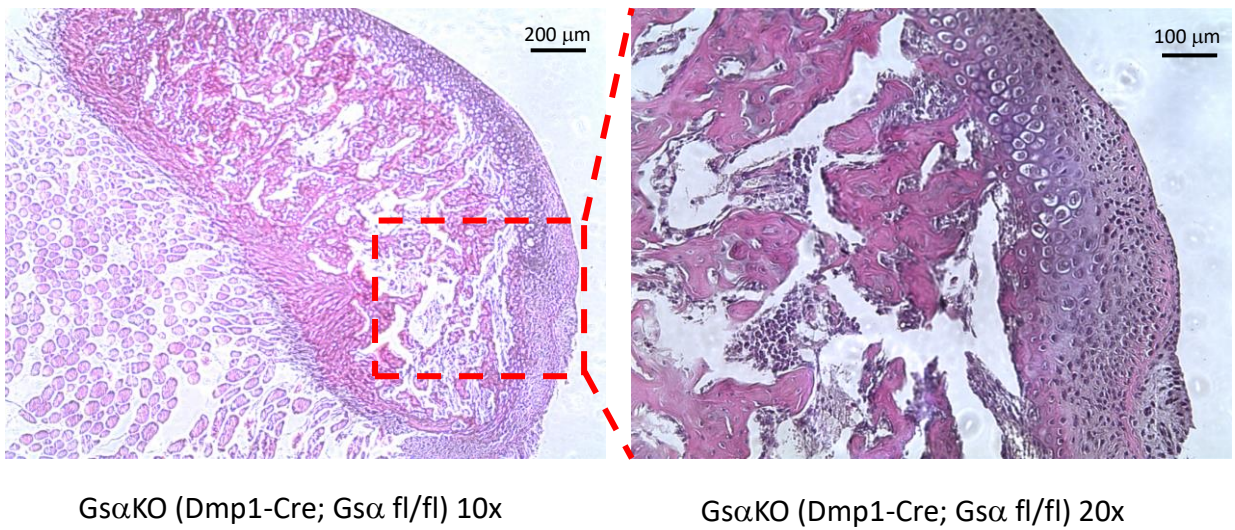
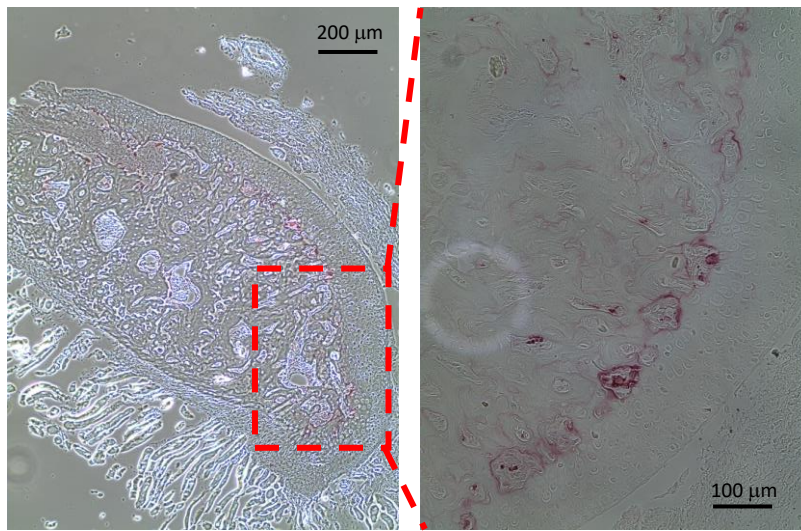
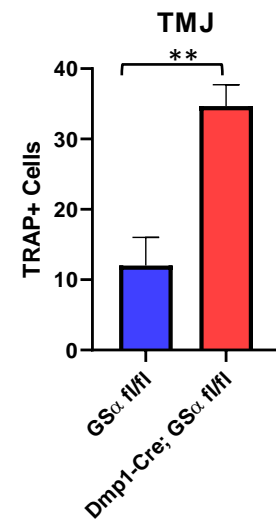


Figure 7. Degenerative changes in DMP1-GsαKO condyles. Condyles were analyzed by H&E staining for differences in histology. (A) GsαWT characterized by normal bone structure, in contrast to (B) DMP1-GsαKO condyles that exhibited loss of bone structure.

A

Gs α WT (Gs α fl/fl) 10xGs α WT (Gs α fl/fl) 20x

C



B

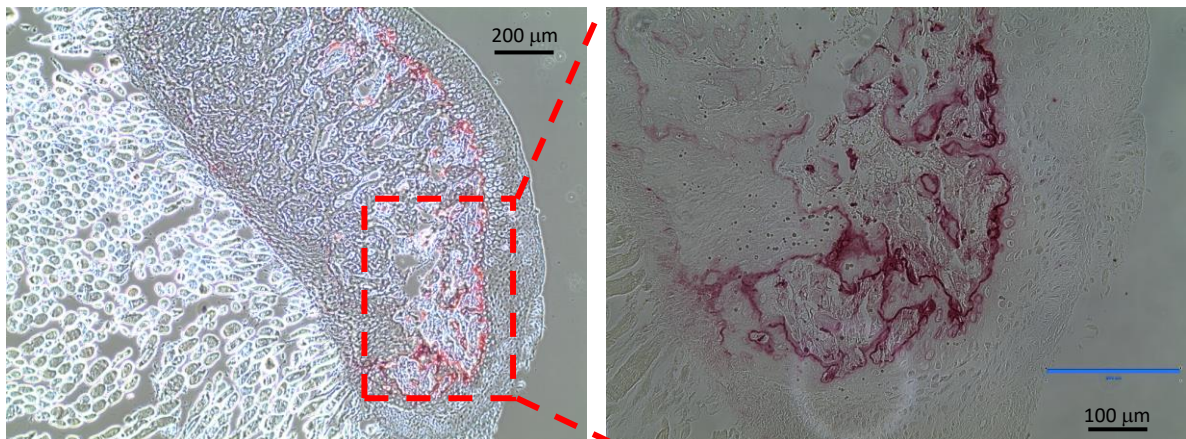
Gs α KO (Dmp1-Cre; Gs α fl/fl) 10xGs α KO (Dmp1-Cre; Gs α fl/fl) 20x

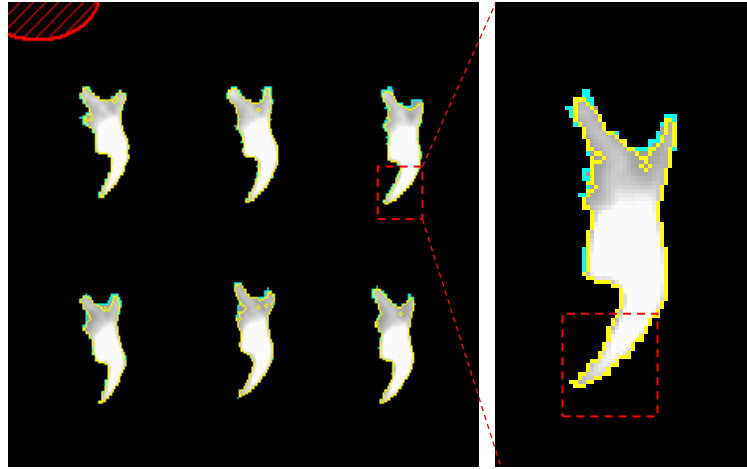
Figure 8. Degenerative changes in DMP1-Gs α KO condyles. Condyles were analyzed by tartrate-resistant acid phosphatase staining to identify osteoclasts. (A) Gs α WT was characterized by lower osteoclast count compared to (B) DMP1-Gs α KO condyles that exhibited more TRAP+ cells, suggesting a more osteoclastic activity. (C) Quantification of TRAP-positive osteoclastic cells (WT: N=3, KO: N=3). All data: shown as the mean (+SEM) (Unpaired t-test; **P < 0.005).

Effect of Gsa deficiency on tooth mineralization

Given that DMP1 plays a significant role in tooth development (5) , we asked if tooth mineralization is affected in the *Dmp1-Cre; Gsα fl/fl* mice model. We imaged samples by DEXA using a Lunar PIXImus II densitometer to measure incisors mineral content by identifying a region of interest that was drawn around the incisors, ROI 3, and it showed less mineral content in *Dmp1-Cre; Gsα fl/fl* mice. Therefore, a significant reduction in enamel mineral content was observed in DMP1-*Gsα*KO mice (Figure 9).

Furthermore, we used μ CT imaging to compare mineral content in both incisors and molars. It is more accurate to measure each region alone because tooth mineralization occurs at different timings (8). We contoured molars and incisors separately, as shown in (Figure 10A). We then measured the mineral content in young (6-week old) female animals. μ CT quantitative evaluation of both molars and incisors showed that the enamel mineralization content was decreased in the teeth of 6-week old DMP1-*Gsα*KO female mice compared to wild type (Figure 10B).

A



B

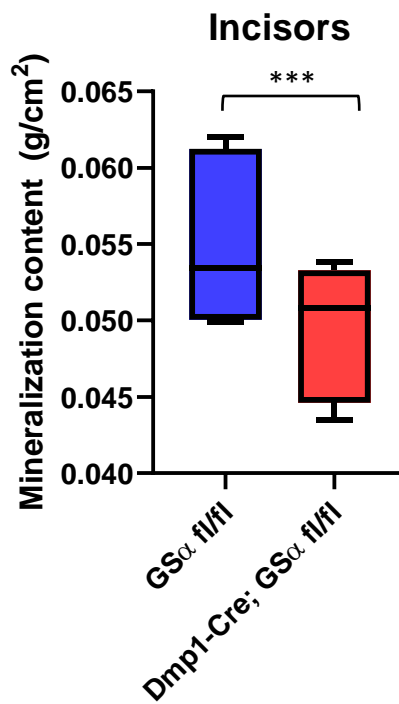
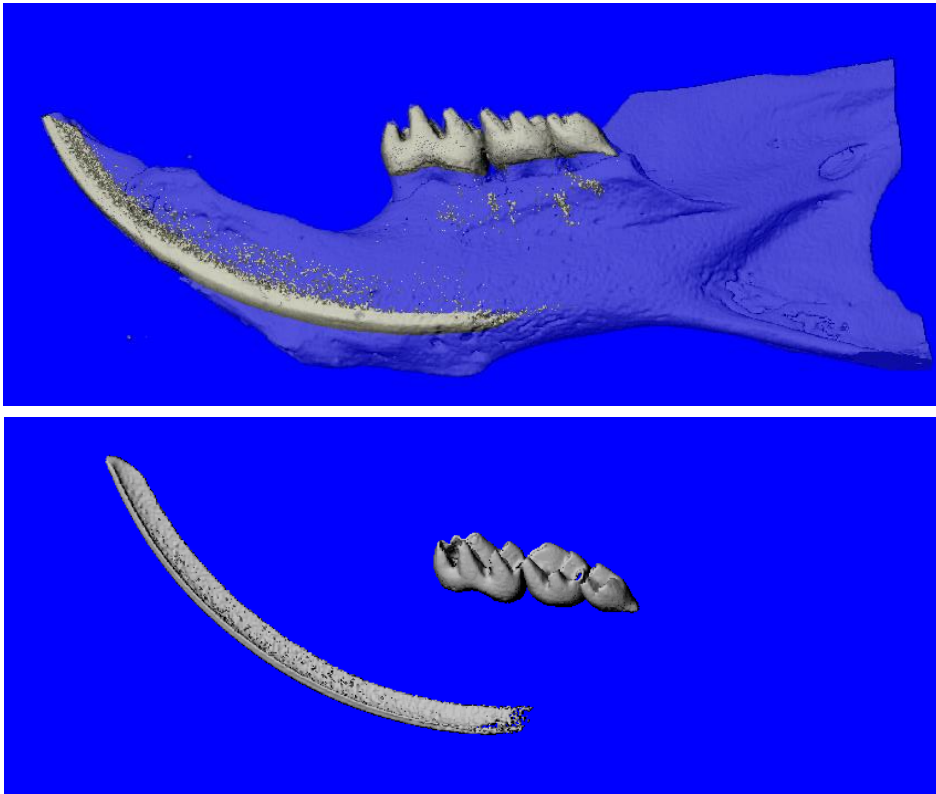


Figure 9. Effect of *Gsa* ablation on tooth mineralization. (A). Region of interest (ROI) 3 used to calculate the mineralization content for the incisors by DEXA scan. (B). Quantitative measurements for the incisors' mineralization content showed 6-week old female control and DMP1-*Gsa*KO mice (WT: N=8, KO: N=6). All data: shown as the mean (+SEM) (Unpaired t-test; *** P < 0.0005).

A



B

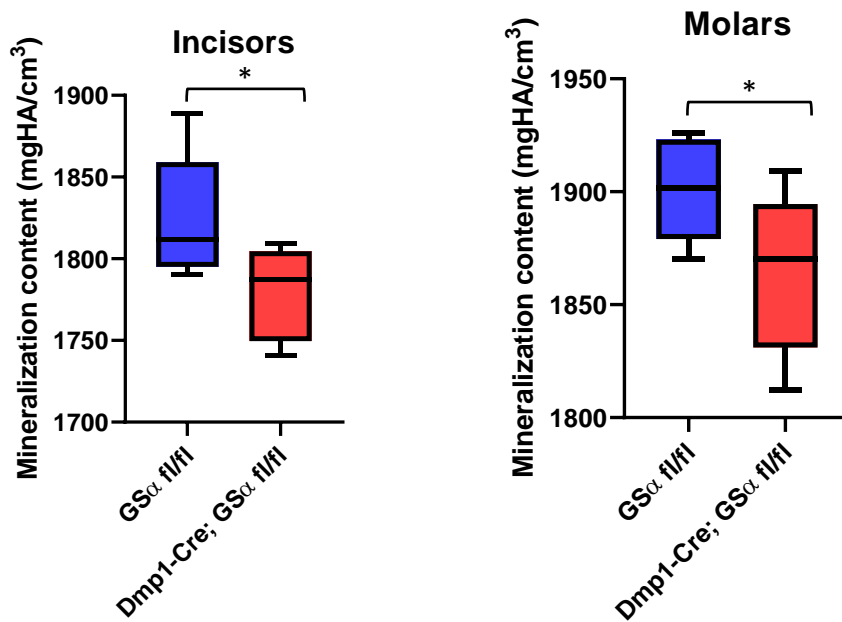


Figure 10. Effect of *Gsa* ablation on tooth mineralization. (A) Reconstruction of the mandible by μ CT scan showing the subtraction of enamel from all other structures in the mandible (i.e., bone, dentin, and cementum) (B) Quantitative measurements for the mineralization content in both incisors and molars, all teeth, for the 6-week old female control and DMP1-*Gsα*KO mice (WT: N=8, KO: N=6). All data: shown as the mean (+SEM) (Unpaired t-test; * $P < 0.05$).

HIGHLIGHTS

- Mice lacking the $G\alpha$ expression predominantly in osteocytes (DMP1- $G\alpha$ KO) develop severe osteopenia in the craniofacial bones and condyles specifically.
- $G\alpha$ signaling in osteocytes and odontoblasts is essential in maintaining normal bone and tooth homeostasis.
- In the absence of $G\alpha$ signaling in osteocytes and odontoblasts, there is a significant increase in osteoclasts.
- $G\alpha$ signaling affects the mineralization of enamel; thus, it might play an important role in tooth formation.
- $G\alpha$ signaling plays an integral part in condylar remodeling; thus, it might be essential in developing the temporomandibular joint.

DISCUSSION

In this study, we examined the craniofacial phenotype of mice lacking $Gs\alpha$ in osteocytes and some mature osteoblasts (DMP1- $Gs\alpha$ KO mice). We have previously reported that these mice have severe skeletal osteopenia in the femur and tibia (4). A similar phenotype was also present in mice lacking $Gs\alpha$ in both mature osteoblasts and osteocytes (Oc- $Gs\alpha$ KO mice), suggesting that phenotype is mostly driven by lack of $Gs\alpha$ in osteocytes. Initial studies demonstrated a significant increase of sclerostin in the serum of DMP1- $Gs\alpha$ KO animals. These animals express high sclerostin levels and have marked osteopenia. A recent study has identified the role of sclerostin in the bone phenotype of DMP1- $Gs\alpha$ KO mice and treated these animals with anti-sclerostin antibodies (4). They reported that sclerostin neutralizing antibody treatment was capable of restoring the number of endosteal osteoblasts to normal levels (4). They also found that high sclerostin levels reduce the numbers of newly formed osteocytes, and anti-sclerostin antibody treatment restores the osteoblast-to-osteocyte transition process (4). The sclerostin neutralizing antibody has a potent bone anabolic effect in mice and osteoporotic patients (37-39). The bone anabolic effect is due to increased osteoblast numbers and activity (37, 40).

Teeth movements in orthodontic treatment occur due to the coupling of bone formation and bone resorption (41) in which both jaws go through continuous remodeling. Remodeling is regulated by mechanical factors such as the force we apply to move teeth or molecular factors, i.e., hormones. Several hormones, including PTH, promote bone anabolism, by stimulating osteoblasts. In osteocytes, PTH exerts its bone-forming effect by suppressing SOST expression. Therefore, as an example of clinical implications, anti-sclerostin antibodies can be beneficial in orthodontics. On

many occasions, an absolute anchorage is needed when we move teeth, and currently, we use temporary anchorage devices (TADs) to gain anchorage. The most common type of TADs used is a mini-screw that is inserted in the cortical bone between adjacent roots. However, there are anatomical limitations to when we can use the mini-screws, so an alternative would be mini-plates inserted in the zygomatic process, which will require surgery. Therefore, a better alternative is to use a chemical anchorage concept by local injections of anti-sclerostin antibodies to the desired location/area. Although it's not the scope of this study, it's relevant to how we can clinically benefit from anti-sclerostin antibody in orthodontics.

The DMP1-Gs α KO mice showed osteopenia in both craniofacial (for example, the mandible) and the condyles. This is consistent with other studies showing dramatic osteopenia in the femur and tibia of DMP1-Gs α KO (4) as well as mice lacking Gs α in pre-osteoblasts (osterix-Gs α KO) and mature osteoblasts (Oc-Gs α KO) (17, 25, 29). Our results demonstrate that the condyles undergo more active osteoclastic activity, as shown by an increase in tartrate-resistant acid phosphatase staining, which overlapped with the bone resorption area we observed in H&E staining suggesting increased osteoclastic activity. A similar outcome has been reported with cartilage degenerations due to cross-talks between PTH and local factors such as BMPs, Wnts, and TGFB signaling pathway (14). The condyle is an important landmark in orthodontics, as in some cases, condylar resorption would cause open bites and non-functional bite (42). Therefore, understanding the molecular basis of condylar remodeling, and how to alter it, is critically important.

DMP1 is essential in regulating the mineralization process and thus plays a vital role in tooth formation, bone, and cartilage development (5, 6). Our results show that

Gs α 's knockout resulted in reduced mineralization content in both incisors and molars, as shown by DEXA using a Lunar PIXImus II densitometer and μ CT scans. In orthodontics, healthy enamel is required to place brackets for braces or attachments for clear aligner treatment to be able to proceed with tooth movement (43). Therefore, bonding is a very critical step in orthodontic mechanotherapy. We aim to find ways to intervene with enamel mineralization while teeth are forming through DMP1 and Gs α signaling.

While analyzing the axial view of the head of DMP1-Gs α KO and wild type mice, we noticed that mice lacking Gs α in osteocytes have a cleft palate. In a future study, we are planning to investigate cleft palate with Gs α . We also plan to assess the furcation area and the periodontal ligament (PDL) for any signs of inflammation and ongoing periodontal disease.

CONCLUSION

Here, we show Gs α 's importance and how it could affect the future of orthodontic treatment regarding anchorage requirements, craniofacial growth, and tooth mineralization. Our study identified Gs α signaling in osteocytes and odontoblasts as an essential factor in maintaining normal bone and tooth homeostasis and the condylar remodeling.

LIST OF ABBREVIATED JOURNAL TITLES

Adv Exp Med Biol	Advances in Experimental Medicine and Biology
Am J Orthod Dentofacial Orthop	The American Journal of Orthodontics and Dentofacial Orthopedics
Ann N Y Acad Sci	The Annals of the New York Academy of Sciences
Aust Dent J	Australian Dental Journal
Bone Rese	Bone Research
Curr Opin Pharmacol	Current Opinion in Pharmacology
Endocr Rev	Endocrine Reviews
Endocrinol Metab Clin North Am	Endocrinology and Metabolism Clinics of North America
FEBS Lett	FEBS Letters
Front Physiol	Frontiers in Physiology
Hum Mol Genet	Human Molecular Genetics
J Biol Chem	Journal of Biological Chemistry
J Bone Miner Res	Journal of Bone and Mineral Research
J Clin Invest	Journal of Clinical Investigation
J Dent Res	Journal of Dental Research
J Endocrinol	Journal of Endocrinology
J Indian Prosthodont Soc	Journal of Indian Prosthodontic Society
Kaohsiung J Med Sci	The Kaohsiung journal of medical sciences
N Engl J Med	The New England Journal of Medicine
Nat Genet	Nature Genetics

Nat Rev Endocrinol

Nature Reviews Endocrinology

Pharmacol Ther

Pharmacology & Therapeutics

Rev Bras Ortop

Revista Brasileira de Ortopedia

Sci Rep

Scientific Reports

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CURRICULUM VITAE

