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**FasL is a catabolic factor in alveolar bone homeostasis**

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**Abstract**

**Aim:** Fas ligand (FasL) belongs to the tumor necrosis factor (TNF) superfamily regulating bone turnover, inflammation, and apoptosis. The appendicular and axial skeleton phenotype of mature Fasl$^{gld}$ mice was reported. The impact of FasL on the alveolar bone providing support for the teeth at mature stages under healthy and induced inflammatory conditions remains unknown.

**Materials and methods:** We performed a phenotypical analysis of mice carrying the homozygous Fasl$^{gld}$ mutation and wild-type (WT) mice (C57BL/6) under healthy conditions and upon ligature-induced periodontitis. After 12 days, µCT analysis revealed the distance between the cement enamel junction and the alveolar bone crest (CEJ-ABC). Additional structural parameters like the bone volume fraction (BV/TV) and the periodontal ligament space volume (PLS.V) were measured. Histological analyses were performed to visualize the catabolic changes at the defect site.

**Results:** We report that healthy Fasl$^{gld}$ mice have more periodontal bone than wild-type littermates. Fasl$^{gld}$ had no significant effect on inflammatory osteolysis compared to WT controls with ligatures. Histology revealed eroded surfaces at the root and in the interproximal bone in both strains.

**Conclusions:** These findings suggest that FasL is a catabolic factor in alveolar bone homeostasis, however, FasL does not affect the inflammatory osteolysis.

**Keywords:** FasL, Fasl$^{gld}$ mouse, periodontitis, periodontal structure, micro-CT, histology, mouse model.

**Clinical Relevance**

*Scientific rationale for the study*: Periodontal disease challenges the scientific community to seek a molecular strategy that aims to reduce inflammatory osteolysis. Targeting FasL might become promissory as a therapeutic approach.

*Principal findings:* FasL plays a catabolic role in alveolar bone homeostasis in our mouse model. There was however no statistical effect of FasL under inflammatory conditions.

*Practical implications:* Targeting FasL may support periodontal bone homeostasis but is not a key target to protect the bone from inflammatory osteolysis.

**Introduction**

FasL (CD178; CD95L; APO1L) a transmembrane protein member of the tumor necrosis factor (TNF) family which interacts with Fas (CD95; APO-1; TNFRSF6) receptor plays an important role in the regulation of cell death (Suda et al., 1993). The FasL/Fas pathway ignites the extrinsic apoptotic machinery of immune cells (Griffith et al., 1995; Nagata, 1997; Tsutsui et al., 1996) but may extend toward affecting bone. There is evidence for a catabolic function as mice carrying the Fasl$^{gld}$ mutation causing a generalized lymphoproliferative disorder (Ramsdell et al., 1994), have an increased femoral bone mass compared to the WT littermates (Katavic, Lukic, et al., 2003). Further support for a catabolic role of FasL is based on the alveolar bone of 24-day-old Fasl$^{gld}$ mice having increased bone volume and trabecular thickness (Svandova et al., 2019). Thus, the Fasl$^{gld}$ mutation causes a positive remodeling shift culminating in an increased bone mass. Contrasting findings showed that Fasl$^{gld}$ had reduced bone structural parameters in long bones and vertebrae (Kim et al., 2020; Wang et al., 2015), and lower bone formation following tooth extraction (Apaza Alccayhuaman et al., 2021). Consistently, conditional knockout of FasL in osteoblasts results in reduced femoral bone mass (Wang et al., 2015). There is thus a controversial picture regarding the impact of FasL on affecting bone mass that may also affect the alveolar bone in periodontal health and disease.

Periodontitis, a chronic inflammatory disease affecting almost half of the adult population (Eke et al., 2012), is considered a major cause of tooth loss (Pihlstrom et al., 2005). Soluble FasL is higher in gingiva crevicular fluid obtained from patients with chronic periodontitis than in healthy controls (Dabiri et al., 2016) and the jawbone of human fetuses revealed the expression of Fas on both osteoblasts and osteoclasts, and of Fas ligand on osteoblasts (Hatakeyama et al., 2000). *P. gingivalis* can induce epithelial cell apoptosis through Fas-FasL activation of caspases (Brozovic et al., 2006) even though FasL polymorphism was not associated with severe chronic periodontitis (Asgari et al., 2018; Wohlfahrt et al., 2006). Considering that FasL affects the immune system (Griffith et al., 1995; Nagata, 1997; Tsutsui et al., 1996), the question arises if FasL affects inflammatory osteolysis. Inflammatory osteolysis is an umbrella term that integrates all molecular and cellular events that culminate into bone loss (Gruber, 2019; Mbalaviele et al., 2017). Our aim is to discover the role of FasL during periodontitis-mediated inflammatory osteolysis.

Mouse models allow us to study periodontal disease's pathogenesis and test new therapeutic approaches (Lin et al., 2021). The intended purpose of the ligature-induced periodontitis model is to increase the local accumulation of microbial plaque and thereby enhance microbial-mediated inflammation and bone loss (Lin et al., 2021; Marchesan et al., 2018). Bone loss is the hallmark of periodontal disease progression increasing the distance between the cementum enamel junction (CEJ) and the alveolar bone crest (ABC) (Hienz et al., 2015). Harnessing the potential of micro-computed

tomography (µCT), 3D evaluation of the bone changes can be measured (Catunda et al., 2021; Wilensky et al., 2005; Wu et al., 2020). Histological analysis can help to describe and quantify alveolar bone osteolysis (de Molon et al., 2018; Semenoff et al., 2008). Implementing and refining established methods, the present study aimed to explore the periodontium of mice carrying the Fasl$^{gld}$ mutation and the respective WT mice under physiological conditions and upon ligature-induced periodontitis.

## Material and Methods

*Study design*

The Medical University of Vienna ethical review board for animal research approved the study protocol (GZ BMWFW-66.009/0359-V/3b/2018). The study was performed at the Department of Biomedical Research of the Medical University of Vienna following the ARRIVE guidelines. Female and Male Mice homozygous for the FasL *gld* mutation (B6Smn.C3-Fasl$^{gld}$/J) were purchased from The Jackson Laboratory (Bar Harbor, ME). C57BL/6 served as wild-type control. Animals were housed in the Medical University of Vienna, Institute of Biomedical Research under specific-pathogen-free conditions. Fasl$^{gld}$ mice and wild-type littermates underwent ligature placement at around 10-12 weeks. According to the animal welfare guidelines, the animals were maintained with free access to water and a cereal grain based diet (Kilkenny et al., 2010).

*Ligature-induced periodontitis model*

Ligature-induced periodontitis was performed according to (Marchesan et al., 2018). All animals received ketamine 100 mg/kg (AniMedica, Senden, Erlangen, Germany) and xylazine hydrochloride 5mg/kg (Bayer Austria, Vienna, Austria) by intramuscular injection. Mice were stabilized on a printed mouse bed to keep the mouth open (Marchesan et al., 2018). Using a printed ligature holder, a dual knotted 4-0 silk ligature (Ethicon, Somerville, New Jersey) of 2.5 mm length was placed between the upper first (M1) and second molar (M2). Based on the 10–20% predicted ligature loss, the ligature was placed bilaterally (Marchesan et al., 2018). Mice were monitored and kept warm until they recovered from the anesthesia. For pain relief, buprenorphine 0.06 mg/kg s.c. (Temgesic®, Reckitt and Colman Pharm., Hull, UK) and piritramide in drinking water *ad libitum* were administered. For the first 72 hours after surgery a soft food consisting of pellets soaked in water was provided. Mice were euthanized on day twelve and only jaws where the ligature remained in situ were considered for analysis.

*Micro-CT analysis*

The heads were fixed in 4% phosphate-buffered formalin (Roti-Histofix, Carl Roth, Karlsruhe, Germany). Micro-CT scans (Scanco µCT 50; Scanco Medical AG, Bruttisellen, Switzerland) were taken at 90 kV/200 µA with an isotropic resolution of 8 µm and an integration time of 500 ms. Volume rendering and segmentation of the bone and teeth was done (Amira 6.1.1; Thermo Fisher Scientific, Waltham, USA) (Supplemental video). For linear measurements of the CEJ-ABC, a set of B-splines (equidistant knots along the CEJ and ABC) was placed at the buccal and palatal sites of molars (M1-M3). Using linear regression, the distance was measured every 50 µm perpendicular to a plane fitted to the CEJ. The mesial, central, and distal part of the teeth were distinguished (Figure 1A). For volumetric analysis (Definiens Developer XD 2.7; Definiens AG, Munich, Germany), the segmented files were aligned using Fiji software (Schindelin et al., 2012). The volume of interest (VOI) for measurement of the bone was set

between M1 and M2. A virtual plane was created from the tip of the roots of M1 to M3. The bone VOI began 0.2 mm coronal of this plane and extended to the alveolar crest. In buccal-palatal direction, the VOI was limited to the tooth width. In mesial-distal direction, the VOI is constrained by surface extension, which limits the VOI to the space between the roots (Figure 1B, red bone). Bone volume fraction (BV/TV) was measured in this VOI. The periodontal ligament space volume (PLS.V) was measured in a region of 2.5 mm length in mesial-distal direction, centered on the gap between M1 and M2 and extending 0.6 mm coronal of the virtual plane at the root tips (Figure 1B, magenta line). Due to the heavy resorption of bone in the ligature group, a measurement of PLS.V in the same VOI was not possible. Instead, the PLS.V was measured in the bone VOI for those samples. The tooth volumes were measured (M1.V and M2.V) not limited by any VOI.

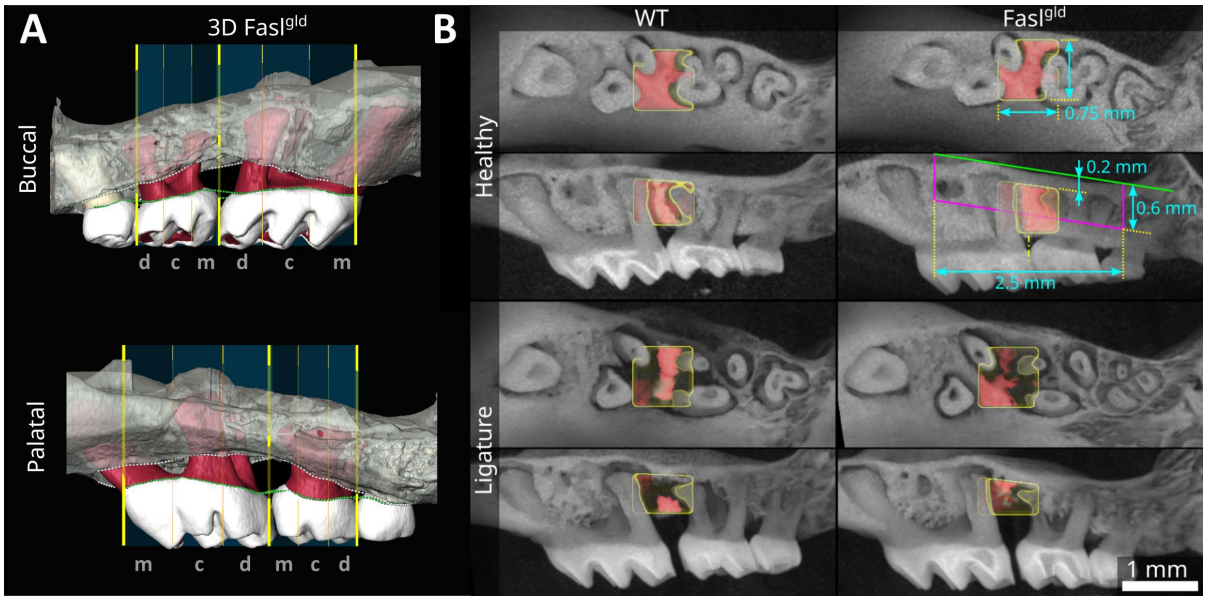


*Figure 1. Linear and volumetric measurement in the region of interest. (A) The distance between cement enamel junction (CEJ) the alveolar bone crest (ABC) was measured by point-based image registration. In the three-dimensional (3D) isosurface of the WT and Fasl$^{gld}$ mice, B-splines were placed connecting the CEJ (green knots) and the alveolar bone crest (white knots) on the buccal and palatal side. The first and second molars were divided into three parts: mesial (m), central (c), distal (d). (B) An equally sized volume of interest (VOI) was set based on the ligature position between M1 and M2, for the healthy and the ligature-induced periodontitis group with their respective WT and Fasl$^{gld}$. Periodontal ligament space volume in healthy samples was measured in a 2.5 mm wide region centered on the gap between M1 and M2 in the most apical 0.6 mm relative to the tip of the roots of M1 and M3.*

*Histological analysis*

The samples were dehydrated with ascending alcohol grades and embedded in light-curing resin

(Technovit 7200 VLC + BPO; Kulzer & Co., Wehrheim, Germany). The cutting plane was planned by µCT. Blocks were processed using a cutting and grinding equipment (Exakt Apparatebau, Norderstedt, Germany). Thin-ground sections were stained with Levai–Laczko dye (Morphisto GmbH, Frankfurt, Germany) (Donath & Breuner, 1982). The sections were scanned using an Olympus BX61VS digital virtual microscopy system (DotSlide 2.4, Olympus, Tokyo, Japan) with a 20x magnification resulting in 0.32 µm per pixel. Using a 25 µm grid, the eroded bone and root surface between M1 and M2 was quantified. The distance between the CEJ and the interproximal alveolar bone was also measured.

*Statistical analysis*

Statistical analysis was based on the multilevel data obtained from the µCT analysis. Descriptive statistics, unpaired Student's t-test, and plotting of the data were performed by Prism 9 (GraphPad Software, San Diego, CA, USA). Significance was set at $p<0.05$. To study the potential influence of FasL on the healthy and ligature induced periodontitis at the tooth level, a linear mixed regression model was used, where the strains (WT or Fasl$^{gld}$), location (buccal or palatal), teeth (M1 or M2), and position (mesial, central, or distal) were fitted as fixed effects. The random effect was the mouse ID. This analysis respects the dependence structure of the data and was performed using R version 4.0.2 (Team, 2022). The sample size calculation was performed using G*power 4 (Düsseldorf, Germany) based on data from previous studies (Marchesan et al., 2018). Considering a bone loss of 0.49 mm with a SD of 0.08 and expecting a 30% of variation between the wild-type and the Fasl$^{gld}$ group, with a 90% power and type I error rate = 5%, we estimated a sample of 16 mice per strain. The primary outcome of the ligature model was the distance between the CEJ and the alveolar bone crest.

**Results**

*Alveolar bone crest level under healthy conditions and ligature-induced periodontitis*

To understand how FasL affects the maxillary bone under healthy conditions the distance between the CEJ-ABC was measured in the WT (n=6) and Fasl$^{gld}$ (n=6) female mice. The alveolar crest level was significantly higher in the Fasl$^{gld}$ than in the WT mice (Figure 2 A, B), particularly in the buccal M1 distal position (*p=0.0003*), the M2 mesial (p=0.0009) and the M2 central and distal part (*p=<0.0001*) (Figure3, healthy). In the palatal side, this effect is less pronounced (Figure 3, Palatal). There were no clinically relevant differences between locations (left or right). (Supplementary Table 1).

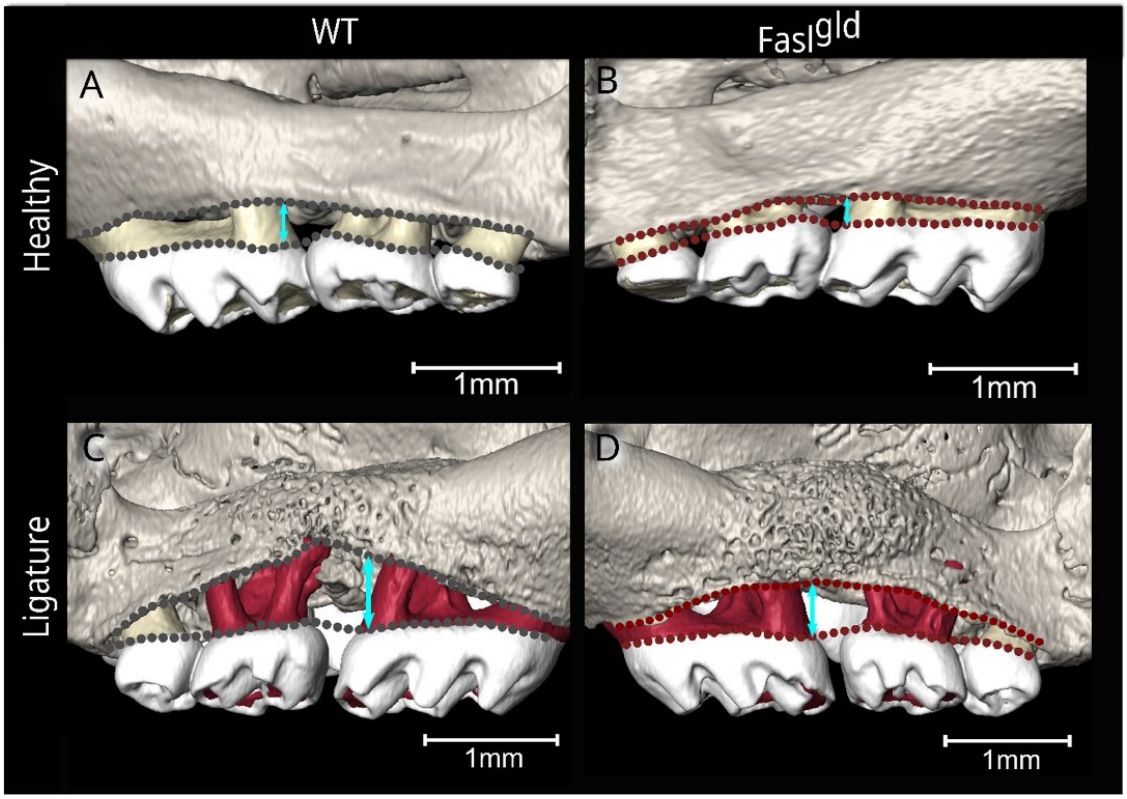


*Figure 2. Linear measurements of the CEJ-ABC. Representative 3D images of the buccal side showing a higher CEJ-ABC distance in the WT (A) compared to the Fasl$^{gld}$ (B) under healthy conditions. After 12 days of ligature-induced periodontitis, the WT (C) and Fasl$^{gld}$ mice (D) presented substantial bone loss (representative double arrows show the distance between the the CEJ and ABC (b-splines) and the porous structure of the compensatory fast-growing woven bone as revealed by histology of Figure 6.*

Under healthy conditions the alveolar crest level was significantly influenced by the lack of FasL (*p=0.0107*). Nevertheless, under inflammatory osteolysis, the CEJ-ABC distance in the WT (n=14) and Fasl$^{gld}$ (n=8) mice (Figure 3, Ligature), the statistical analysis revealed no impact of the mouse phenotype on CEJ-ABC. However, considering the original ABC level, we have calculated the average net loss by subtracting the median CEJ-ABC of mice with and without ligatures. Fasl$^{gld}$ mice lost only marginally more buccal bone (0.20 and 0.23 mm; M1 distal and M2 mesial) than WT animals with 0.17 and 0.20 mm; respectively). Also, in the palatal aspect, Fasl$^{gld}$ lost only slightly more bone (0.17 mm; M1 distal) than WT animals (0.12 mm; M1 distal; Supplementary Table 2). Descriptive statistics show no differences in CEJ-ABC regarding gender and location (Supplementary Table 3, 4).

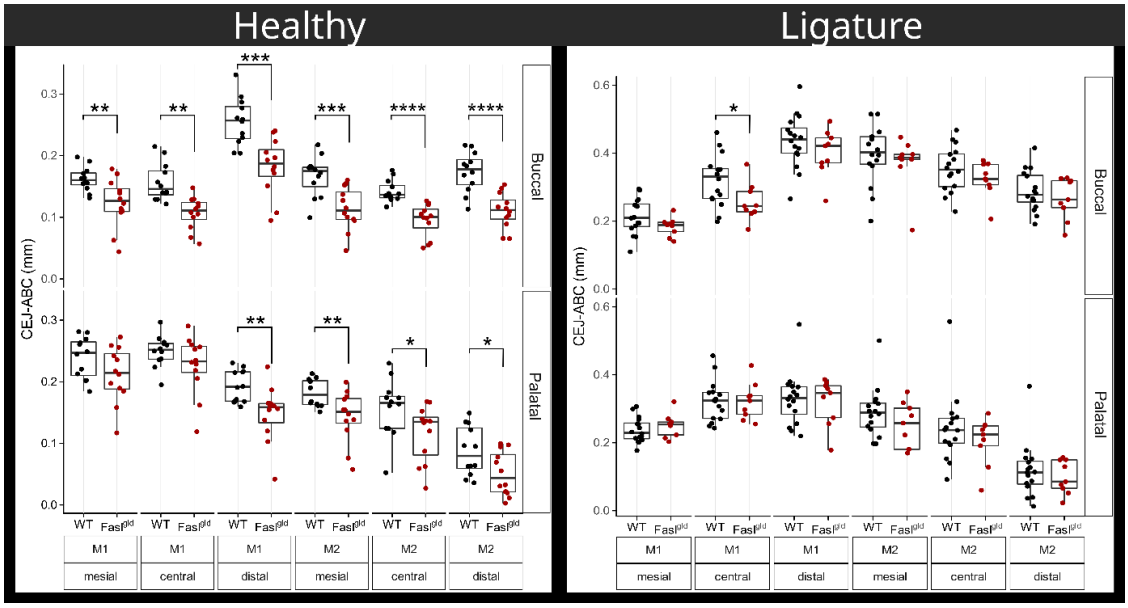


*Figure 3. Linear measurements of the CEJ-ABC. Under he*althy condition, the CEJ-ABC was smaller in the Fasl$^{gld}$ compared to the WT mice in the buccal and the palatal aspect. After the ligature-induced periodontitis, CEJ-ABC failed to reach the level of significance when comparing WT and Fasl$^{gld}$ mice. *The strain (WT/ Fasl$^{gld}$), teeth (M1/M2), and position (mesial, central, and distal) are displayed on the x-axis. For the healthy animals, each dot represents the left and right side of the maxilla for WT (n=12) and Fasl$^{gld}$ (n=12). For the ligature-induced periodontitis, each dot represents the location with remaining ligatures in WT (n=16) and Fasl$^{gld}$ (n=9). *P < 0.05, **P < 0.01; ***P < 0.001, ****P < 0.0001 WT vs. Fasl$^{gld}$.*

*Alveolar bone volume fraction under healthy conditions and ligature-induced periodontitis*

Next, the BV/TV was analyzed. Under healthy conditions, the BV/TV of the WT (58.17±4.3%) was significantly lower than in Fasl$^{gld}$ (63.67±2.7%) mice (p=0.001, Figure 4, Healthy row). Ligature-induced periodontitis caused a strong bone loss in the WT (20.25±9.7%) and Faslgld (24.56±14.09%) mice which failed to reach the statistical significance (p=0.37, Figure 4, row ligature). The calculated loss of the bone volume was comparable between both mouse strains. Taken together, Fasl$^{gld}$ display more alveolar bone mass under healthy conditions than WT mice, however the inflammatory osteolysis is similarly affected in both strains.

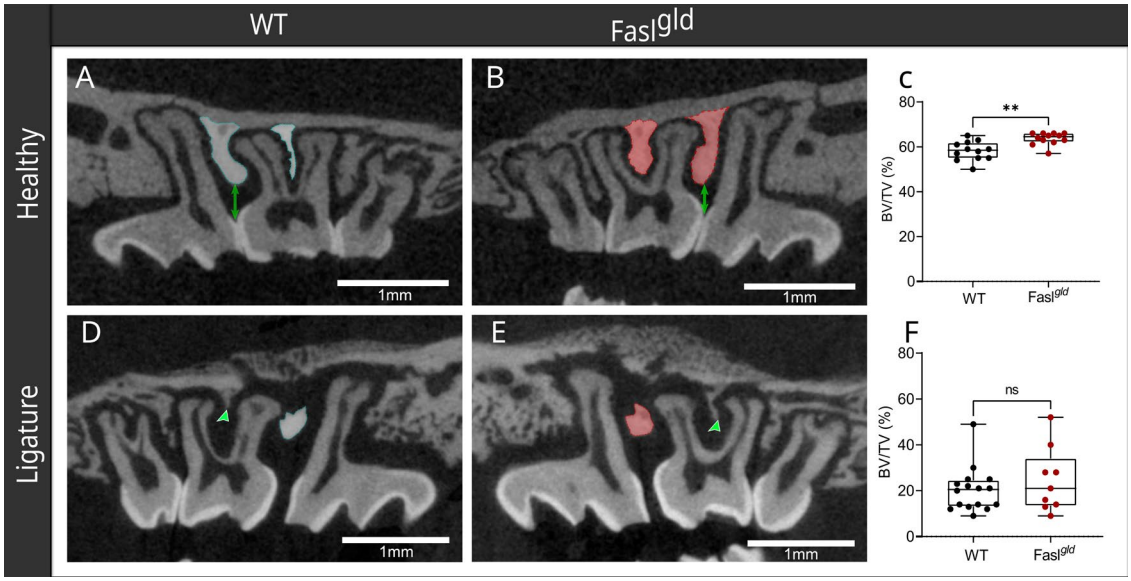


*Figure 4. Volumetric measurements of the region of interest. Under healthy conditions, representative µCT images revealed that WT (A, cyan outline) have less interproximal bone (between M1-M2) and the interradicular septum of the M2 compared to Fasl$^{gld}$ (B, red outline).* The green double arrows show the distance between the ABC and the contact point. *The respective difference in BV/TV is showed in the boxplots with* **P < 0.01 *(C). Under the ligature-induced periodontitis, almost the entire interproximal (cyan and red outline) and the interradicular bone (green arrows) were lost in WT (D) and Fasl$^{gld}$ (E) mice.* There were no significant (ns) differences regarding the BV/TV (F).

*The volume of the periodontal ligament under healthy and ligature-induced periodontitis*

The periodontal ligament volume (PLS.V) under healthy conditions in WT (0.11±0.008 $mm^3$) mice was higher than in $Fasl^{gld}$ (0.10±0.009 $mm^3$) mice (*p=0.003*; Figure 5, Healthy row). After ligature-induced periodontitis, there was no statistically significant difference (*p=0.82,* Figure 5, Ligature row) between the WT (0.17±0.04 $mm^3$) and the $Fasl^{gld}$ (0.17±0.03 $mm^3$) mice. The tooth volume was not affected in $Fasl^{gld}$ mice compared to their WT littermates. (Supplement Figure 1). Furthermore, after ligature placement, the interdental space became wider in the WT (0.07±0.06 mm) and in the $Fasl^{gld}$ (0.05±0.06 mm) mice; however there was no significant difference (*P=0.5*) between the strains.

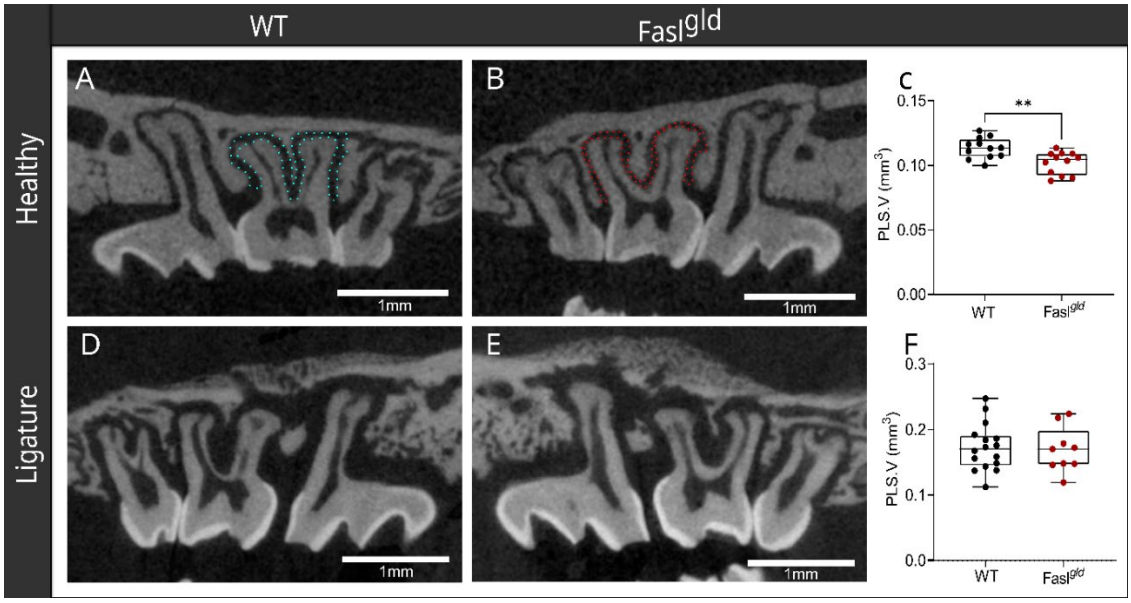


*Figure 5. Volumetric measurements of the periodontal ligament space. Under healthy conditions, representative µCT images revealed that WT (A, cyan outline) have a higher periodontal ligament volume (PLS.V*) compared to $Fasl^{gld}$ (B, red outline) that was confirmed by the quantitative measures with ***P < 0.01 (C). Under the ligature-induced periodontitis the PLS.V gets considerably expanded in both strains at the interproximal bone and interradicular septum (D and E). The boxplots did not show significant difference (ns) for the WT and $Fasl^{gld}$ (F).*

*Histological analyses*

Finally, we assess the morphological features of inflammatory osteolysis by histological analysis. Signs of bone resorption came along with fast-growing woven bone originating from the periosteum in both strains (arrows pointing in Figure 6, A and B, see also Supplemental Figure 2S). Resorption of dentin and cementum was present in WT and Fasl$^{gld}$ mice (arrows pointing in figure 6, C and D). Surface erosion was comparable between WT (42.23±18.7%) and Fasl$^{gld}$ (48.02±22.8%) mice (Figure 6, E and H). To complement the µCT measurements, the CEJ-ABC was determined at the interproximal bone of M1 and M2. Again, there was no significant differences between WT (0.38±0.17; 0.31±0.17 mm) and Fasl$^{gld}$ (0.35±0.16; 0.28±0.16 mm) mice (Figure 6, K). On a descriptive level, the junctional epithelium of the mesial part of M1 was not obviously affected by the Fasl$^{gld}$ mutation (Supplement Figure 3S).

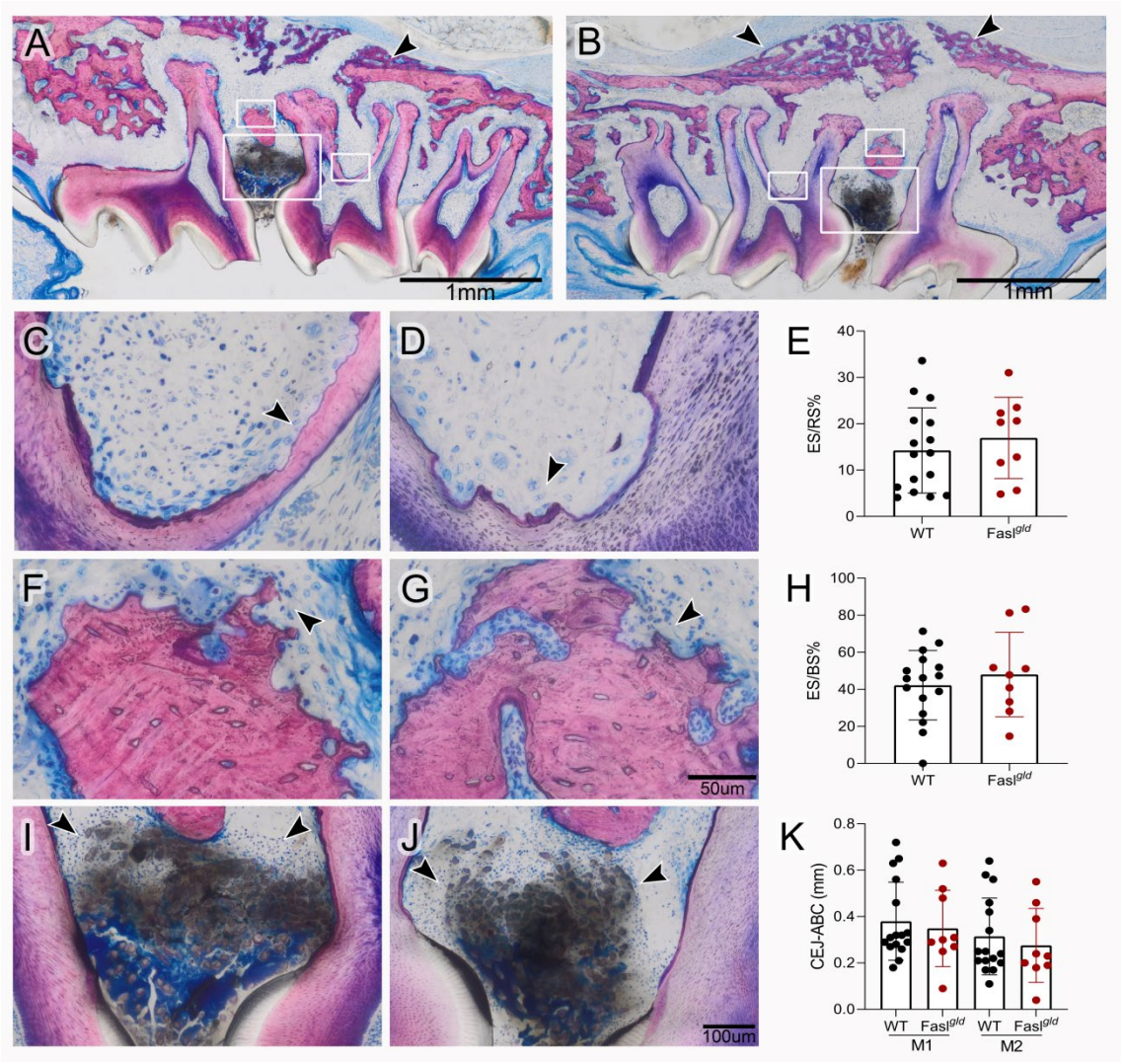


*Figure 6. Representative histological images of WT and Fasl$^{gld}$ mice. Low magnification overviews revealed the morphological features of inflammatory osteolysis caused by the ligatures that are visible between M1 and M2 in WT (A) and Fasl$^{gld}$ (B) mice. Arrows pointing towards the signs of bone formation*

*originating from the periosteum to compensate the severe bone resorption caused by the inflammatory osteolysis. The higher magnification images display the eroded root surface in the furcation in WT (C) and Fasl$^{gld}$ mice (D). Also severe bone resorption of the ABC in the WT (F) and Fasl$^{gld}$ (G) is clearly visible. The silk ligature provoked an obvious inflammatory infiltrate (arrows pointing) for the WT (I) and Fasl$^{gld}$ mice (J). There was no difference between the strains regarding the percentage of the eroded root surface (ER/RS%; E) and at the eroded bone surface (ER/BS%) (H). The CEJ to the interproximal ABC length was measured at the M1 and M2 also showing no significance between the two strains (K).*

**Discussion**

The present study supports existing evidence that Fasl$^{gld}$ mice experience denser bones, lesser bone loss after ovariectomy, and more trabecular bone after bone marrow ablation than WT controls (Katavic, Lukic, et al., 2003). Moreover, in Fasl$^{gld}$ mice more ectopic bone and cartilage formation was induced upon injection of natural bone morphogenetic protein (BMP) and recombinant BMP-2 compared to WT controls (Katavic, Grcevic, et al., 2003; Mori et al., 1997). These findings not necessarily reflect the cranial tissues because of the type of ossification (Berendsen & Olsen, 2015) and the biomechanical situation (McElhaney et al., 1970). Nevertheless, jawbone of human fetuses revealed the expression of Fas on both osteoblasts and osteoclasts, and of Fas ligand on osteoblasts (Hatakeyama et al., 2000). To understand the impact of FasL on the periodontal tissues, we have analyzed the alveolar bone in Fasl$^{gld}$ mice and the respective WT controls under healthy conditions and ligature-induced periodontitis.

Our first main finding was that Fasl$^{gld}$ mice display a significantly higher alveolar bone level and bone volume compared to the WT mice under healthy conditions. The difference was somewhat stronger on the buccal compared to the palatal side. Particularly, we found a substantial difference at the distal part of M1 and M2 based on advanced image processing and algorithms for tissue segmentation. Consistently, the volume of the periodontal ligament was lower in Fasl$^{gld}$ mice than in WT mice. Indirect support for our observations comes from 24-day old Fasl$^{gld}$ mice, which displayed increased bone volume and trabecular thickness (Svandova et al., 2019). The underlying cellular mechanism may involve an anabolic shift where osteoblastic bone formation exceeds osteoclastic bone resorption (Katavic, Lukic, et al., 2003). Future research could therefore consider lineage-specific knock-out mice where Fasl$^{gld}$ is exclusively expressed in osteoblast, osteocytes, or osteoclast. Moreover, contrasting findings that FasL is required to reach adequate bone mass in the axial and appendicular skeleton leave us with uncertainty (Kim et al., 2020; Wang et al., 2015).

Knowing why healthy Fasl$^{gld}$ mice have more periodontal bone than WT littermates might involve the effect of lymphocytes – all of which are known to modulate the osteoblast and osteoclast formation, activity, and death (Gruber, 2019; Mbalaviele et al., 2017). Thus, the generalized lymphoproliferative

disorder caused by the Fasl$^{gld}$ mutation could indirectly affect bone turnover. At the level of the oral cavity, the lymphoproliferative disorder can occur in various ways, most commonly as lymphoid lesions with extranodal externalization (Castellarin et al., 2010), nothing similar we have observed in the oral histology of our mice. Fasl$^{gld}$ mice failed to generate oral diseases that can be linked to the lymphoproliferative disorder. It can thus be speculated that the possible impact of the lymphoproliferative disorder in Fasl$^{gld}$ mutations on the periodontium was bone anabolic.

Contrasting to our Fasl$^{gld}$ mice, in vitro studies showing that FasL reduced the WNT-antagonist sclerostin in IDG-SW3 osteocytic cells suggest an anabolic activity of the ligand (Kratochvilova et al., 2021). Nevertheless, the WNT pathways plays a role in regulating the stem cell niche at the interface between tooth and oral epithelia (Yuan et al., 2020), and activation of WNT signaling promotes FasL expression in periodontal ligament cells (Yu et al., 2019). In general, however, the evidence for a functional link between FasL and WNT signaling is low. Considering the importance of TGF-β in bone biology (Crane & Cao, 2014), TGF-β1 and FasL can synergize in dendritic cell activation (Qiu et al., 2017). Moreover, the FasL-mediated apoptosis pathway is regulated by TGF-β3 signaling being essential for palatal fusion during craniofacial development (Huang et al., 2011). TGF-β1 can also affect the sensitivity of lung epithelial cells to FasL mediated apoptosis (Bai et al., 2011). Thus, it is possible that in our model, impaired FasL signaling increases the responsiveness of bone cells to growth factors and other signaling molecules.

The second main finding was that upon ligature-induced inflammatory osteolysis the CEJ-ABC distance and the volume of the remaining alveolar bone was similar in WT and Fasl$^{gld}$ mice. It can be concluded that Fasl$^{gld}$ does not substantially prevent or enhance inflammatory osteolysis, also when considering that healthy Fasl$^{gld}$ originally had more alveolar bone than the WT mice. These findings are in line with other mouse models showing no effects of FasL on induced pneumococcal meningitis (Paul et al., 2004) and chemically induced colitis (Reardon et al., 2008). Moreover, there is controversial in vitro evidence that osteoclasts are targets of FasL-stimulated apoptosis (Wu et al., 2005) while recombinant FasL enhanced osteoclast differentiation involving the expression of inflammatory cytokines (Park et al., 2005). In general, FasL can reduce inflammation by triggering apoptosis of inflammatory cells but exhibits a proinflammatory activity independent of its ability to mediate immune privilege (O'Connell, 2000). The effects of FasL are thus heterogenous and we must carefully interpret the role of FasL to modulate periodontal inflammatory osteolysis.

The clinical relevance of our findings remains at the level of speculation, but we can at least conclude that a nonfunctional FasL is beneficial for the alveolar bone in a healthy periodontium and that FasL is not the main factor when it comes to the catabolic events driving inflammatory osteolysis. It seems that

FasL is weak if at all a candidate molecule to be targeted by pharmacological therapy (Risso et al., 2022). Moreover and even though FasL was higher in gingiva crevicular fluid obtained from patients with chronic periodontitis than in healthy patients (Dabiri et al., 2016) our mouse model suggests that the higher expression of FasL is presumably a consequence of the periodontitis but not a major driver of inflammatory osteolysis. What needs to be tested in future studies is the possibility that pharmacological blocking of FasL supports bone regeneration, although our recent tooth extraction model on Fasl$^{gld}$ mice points in another direction (Apaza Alccayhuaman et al., 2021).

The present study has various limitations. The major limitation is that the present study is descriptive, and the molecular and cellular mechanisms to explain the higher bone mass displayed in the Fasl$^{gld}$ remains unclear. Future studies may consider a specific knock out mouse to discover the mechanisms involved and consider the bone remodeling parameters regarding the osteoblast-osteoclast differentiation, activity, and cell fate. Moreover, a DMP1-FasL$^{gld}$ conditional knockout mice would show us the importance of osteocytes in regulating bone remodeling and inflammatory osteolysis. Another limitation was that the ligature slipped off before the expected period; thus the animals were excluded from the analysis and we remain with an unbalanced sample requiring a mixed regression model. The histology from the healthy periodontium is not shown here but high-resolution µCT images reflects the healthy phenotype. Also, the Technovit 7200 embedding is not ideal to perform a phenotyping of neutrophils and other cells of the innate immunity, as well as staining for the osteoclast marker tartrate-resistant acid phosphatase. We can, however, identify the resorption lacunae and occasionally we see multinucleated cells that are most likely osteoclasts. In conclusion, we have discovered that in healthy conditions Fasl$^{gld}$ mice have more alveolar bone compared to the WT littermates. Concerning inflammatory osteolysis, Fasl$^{gld}$ and the WT mice showed similar levels of remaining alveolar bone suggesting that FasL is not critically involved in inflammatory osteolysis.

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**Conflict of Interest**

The authors declare no conflicts of interest related to this study.

**Supporting Information**

Supplemental tables and figures are provided.

Supplemental video (AVI). Volume rendering of the periodontal structure under physiological and inflammatory condition.

**Data availability statement**

The data of the present study are available upon reasonable request.

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