Nutrient sensing by Tas1R proteins is required for normal bone resorption

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Current therapies for diseases of low bone mass consist of inhibiting osteoclast activity or increasing the PTH or Wnt signaling pathways. While largely effective, these approaches have significant drawbacks that limit their use in specific patient populations and/or negatively impact patient compliance with therapy. Thus, there is a need to identify new therapeutic targets and, we contend, this requires diversifying our understanding of the mechanisms underlying postnatal bone remodeling by examining lesser-known signaling pathways. One such pathway is the taste receptor type 1 (TAS1R) family of heterotrimeric G protein-coupled receptors, which participates in monitoring energy and nutrient status. Simon *et al.* (2015) reported that global deletion of TAS1R member 3 (TAS1R3), which is a bi-functional protein that recognizes amino acids or sweet molecules when dimerized with TAS1R member 1 (TAS1R1) or TAS1R member 2 (TAS1R2), respectively, leads to increased cortical bone mass. But, the underlying cellular mechanisms leading to this phenotype remain unclear. Here, we independently corroborate the increased thickness of cortical bone in femurs of 20-week-old male *Tas1R3* knockout mice and confirm that *Tas1R3* is expressed in the bone environment. Quantification of serum bone turnover markers indicate that this phenotype is likely due to uncoupled bone remodeling, with levels of the bone resorption marker CTx being reduced greater than 60% in *Tas1R3* mutant mice; no changes were observed in levels of the bone formation marker PINP. Consistent with this, *Tas1R3* and its putative signaling partner *Tas1R2* are expressed in primary osteoclasts and RAW264.7 cells following RANKL-mediated differentiation. Moreover, the responsiveness of RAW264.7 cells to the TAS1R2:TAS1R3 ligand saccharin, as indicated by phosphorylation of ERK1/2 and S6 Kinase, is increased in RANKL-treated RAW264.7 cells. These findings suggest that osteoclast function and/or differentiation may be altered in the absence of *Tas1R3* expression. To test this, we quantified bone-specific expression of *Rankl* and determined the *Rankl:Opg* ratio; however, no differences were observed between control and *Tas1R3* knockout mice in these analyses. Studies involving in vitro functional assays is control versus *Tas1R3*-deficient osteoclasts are currently underway. Collectively, our findings provide the first demonstration that nutrient monitoring by TAS1R3 is essential for normal bone resorption *in vivo*.

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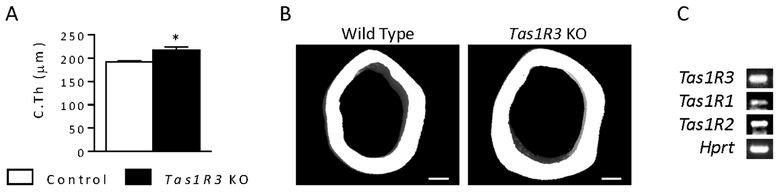

Fig. 1: Evaluation of bone mass in Tas1R3 mutant mice. A-B: A, Quantification of average thickness of the cortical bone (cortical thickness, C.Th) at the femoral mid-diaphysis of twenty-week-old male Tas1R3 knock-out (KO) as compared to wild types; data are expressed as mean±SEM, n=3 for each genotype and * indicates p<0.05 by unpaired t test. B, Images of mid-diaphyseal bone for wild type and Tas1R3 KO most representative of the genotype mean in A; scale bar is 200 μm. C: RT-PCR for TAS1R family members in nine-week-old marrow-free humeral diaphysis from wild type mouse; Hprt serves as loading control. Data are representative of five marrow-free humerii.

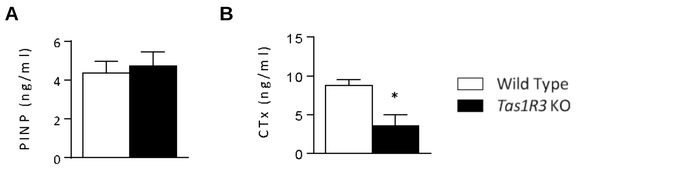

Fig. 2: A-B: Quantification of Procollagen Type I N-terminal Propeptide (PINP) (Fig, 2A) and CTx (2B) levels in serum from twenty-week-old male wild type and *Tas1R3* knock-out (KO) mice; data are expressed as mean±SEM. n≥5 for each group. *p*=0.7 by unpaired t test.

A

	Parameter	Wild Type	Tas1R3 KO	p value
Osteoclast	Cortical Osteoclast Number (Oc.N/B.Pm)	5.230±1.540	6.219±1.863	0.7035
	Cortical Osteoclast Surface (Oc.S/BS)	0.09106±0.026	0.1183±0.040	0.6314
	Trabecular Osteoclast Number (Oc.N/B.Pm)	7.621±0.9471	8.358±1.009	0.6226
	Trabecular Osteoclast Surface (Oc.S/BS)	16.03±2.427	15.88±2.127	0.9639
Osteoblast	Trabecular Osteoblast Number (Ob.N/B.Pm)	20.78±3.288	16.07±2.767	0.3344
	Trabecular Osteoblast Surface (Ob.S/BS)	14.8±3.523	12.88±2.921	0.6956

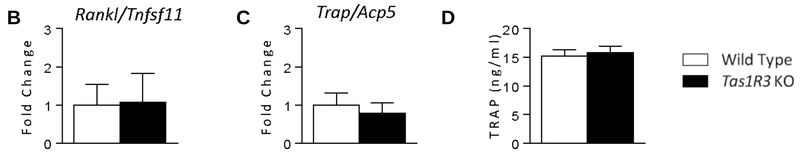

Fig. 3: A: Histomorphometric analyses of femora from twenty-week-old Tas1R3 mutant and wild type mice. **B:** Quantification of fold change in *RANKL* expression observed in twenty-week-old male mice huerii; data expressed as mean ±SEM. N=5 for each group. **C:** Quantification of Trap/Acp5 observed in twenty-week-old male humerii. **D:** Serum TRAP levels of twenty-week-old male humerii; n=8 for each group.

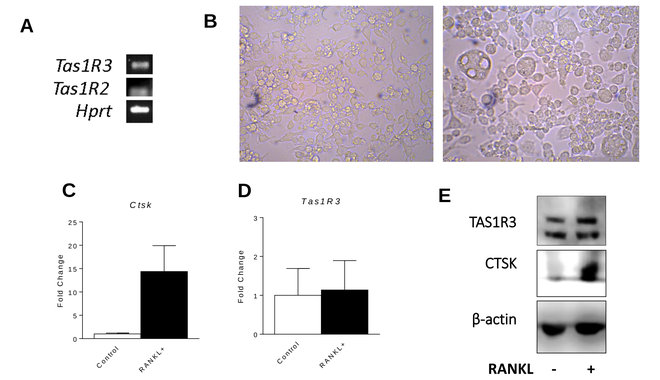

Fig. 4: A: RT-PCR expression of Tas1R3 and Tas1R2 in murine primary cells after RANKL differentiation. Hprt used as loading control. **B-E:** Differentiation assay using RAW264.7 osteoclast precursor cells treated with RANKL (B, right) for six days compared to control (B, left). Osteoclast differentiation confirmed by up-regulation of *Ctsk* mRNA (C) and protein (E) while *Tas1R3* expression is unchanged (D-E). β-actin used as loading control.

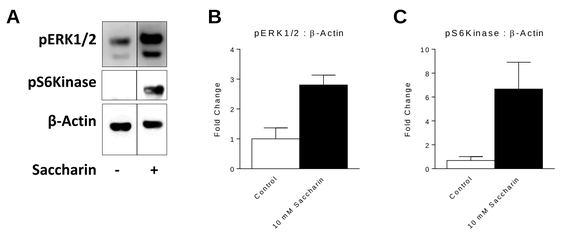

Figure 5: Western Blot(A) and densitometry (B-C) for phosphorylated isoforms of ERK1/2 and p70/S6 Kinase in response to activation of TAS1R3 with saccharin. β-actin used as loading control.

Conclusions

- Cortical bone mass is increased in *TAS1R3* knock out mice and is associated with decreased osteoclast activity with no observed defect in osteoblast parameters.
- RAW264.7 osteoclast precursor cells express Tas1R3 and are responsive to the ligand saccharin upon differentiation using RANKL.
- Future studies will attempt to inhibit TAS1R3 function using gurmarin, a known antagonist, and/or suppress TAS1R3 expression in order to determine the precise role this receptor plays in osteoclast function.