Role of G-protein-coupled Estrogen Receptor-1 (GPER-1) in Enhancing Chondrocyte Proliferation through PTHrP/Ihh Signaling during Pubertal Bone Growth in Male and Female Mice

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ABSTRACT INTRODUCTION:
Long-bone elongation depends on endochondral bone formation at the growth plate from the fetal stage to puberty. In both males and females, endochondral ossification is regulated by several endocrine factors, including estrogen, which acts via estrogen receptors (ERs). G protein-coupled estrogen receptor-1 (GPER-1) regulates several physiological processes in females and males. In our previous study, GPER-1 mediated longitudinal bone growth in female mice during early puberty; however, the underlying mechanisms and sex differences remain unclear. This study focused on exploring the impact of the GPER-1 on the regulation of growth plate development.

METHODS:
In this study, male and female C57BL/6 mice (n=8/group) were approved by the Kaohsiung Medical University Animal Care and Use Committee (107157). A GPER-1 agonist (G1), and GPER-1 antagonist (G15) were used to investigate the role of GPER-1 in growth plate chondrocytes. The effects of GPER-1 activation or inhibition on the tibial growth plate and bone growth, including changes in proliferation and hypertrophy, and the expression of parathyroid hormone-related peptide (PTHrP), Indian hedgehog (Ihh), and their ratio (PTHrP/Ihh) were investigated.

RESULTS SECTION:
GPER-1 activation increased tibial growth plate thickness, proliferative zone thickness, and chondrocyte proliferation in mice by G1 treatment during puberty. The hypertrophic zone thickness and type X collagen-stained area decreased in four-week-old G1-treated mice compared with the control group. GPER-1 activation increased the PTHrP/Ihh ratio in the growth plates of four- and eight-week-old mice. In contrast, blocking GPER-1 decreased the long bone elongation, proliferative zones of the growth plate, proliferative chondrocytes, and PTHrP/Ihh.

DISCUSSION:
GPER-1 is expressed in both young male and female growth plates, suggesting that GPER-1 regulates growth plate chondrocyte function in both sexes. In this study, we found that GPER-1 regulates bone growth processes in females and males. We focused on early intervention with a selective GPER-1 agonist, G1, to specifically activate GPER-1 before the physiologically abundant release of natural ligands during pre-puberty, resulting in increased growth plate thickness, chondrocyte proliferation, and PTHrP/Ihh in the growth plates of both male and female mice. Notably, this effect was similar in both males and females, suggesting that the GPER-1-mediated effects on growth plates are sex-independent.

SIGNIFICANCE:
Both male and female mice, GPER-1 plays an important role in promoting growth plate chondrocyte proliferation but suppresses hypertrophy, which in turn slows down terminal differentiation and results in increased long-bone elongation at puberty. This effect is mediated by PTHrP/Ihh upregulation. These findings provide novel insights into the biological mechanisms underlying estrogen-mediated long-bone growth during puberty.

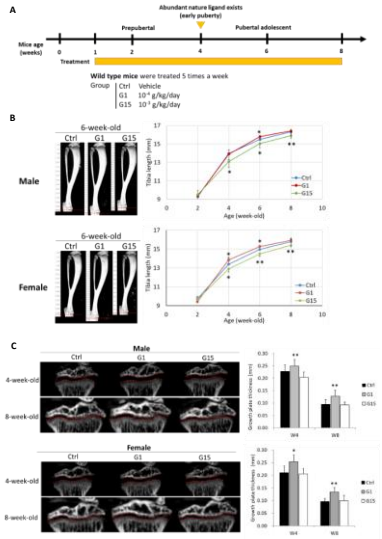

Fig 1. Effects of G1 and G15 on phenotype change in tibiae of growing mice. (A) Experimental timeline of treatment. (B) The representative phenotype of 6-week-old tibia and the quantitation of tibial length of 2- to 8-week-old. (C) Thicknesses of growth plate of 4- and 8-week-old mice were measured by μ-CT analysis.

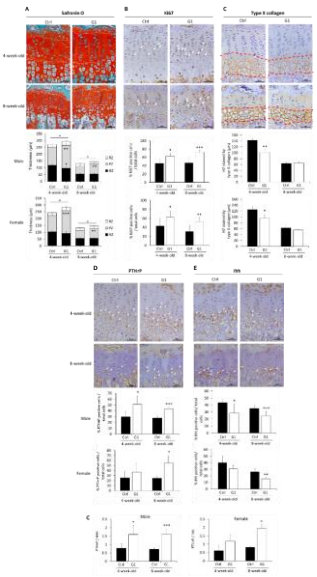

Fig 2. Effects of G1 on tibia growth plate development in 4- and 8-week-old mice. (A) The representative microslides and quantification of thicknesses in resting zone (RZ), proliferation zone (PZ) and hyperopic zone (HZ) by Safranin O staining. The representative microslides and quantification of (B) Ki67, (C) type X collagen, (D) PTHrP- and (E) Ihh-labeled chondrocytes by IHC staining.

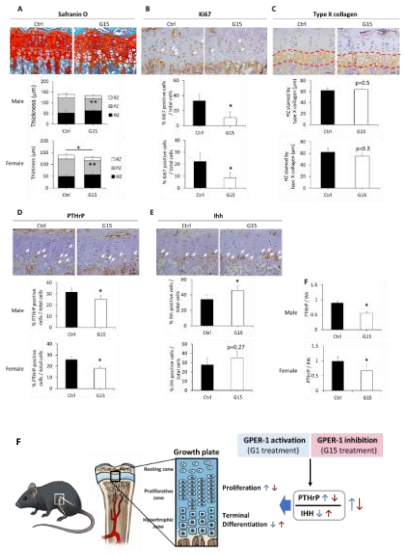

Fig 3. Effects of G15 on tibia growth plate development in 8-week-old mice. (A) The representative microslides and quantification of thicknesses in RZ, PZ and HZ by Safranin O staining. The representative microslides and quantification of (B) Ki67, (C) type X collagen, (D) PTHrP- and (E) Ihh-labeled chondrocytes by IHC staining. (F) Diagram illustrating the effect of GPER-1-mediated PTHrP/IHH, which subsequently regulates the proliferation and onset of chondrocyte hypertrophy.