FGF23 and x-linked hypophosphatemic rickets

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1. Introduction

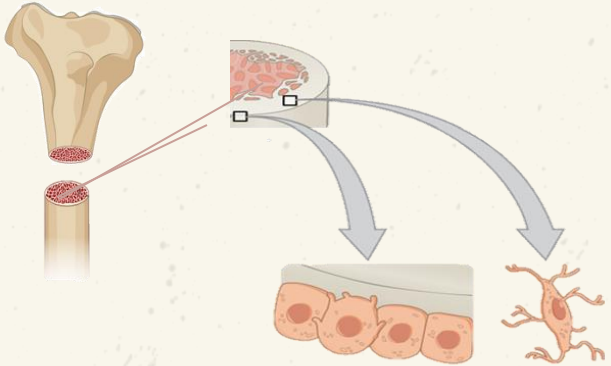
- **Fibroblast growth factor number 24 (FGF23)** is a protein secreted by bone cells.
- **Function:** regulation of phosphorus metabolism, promoting its elimination by urine through the kidney.
- **Disorders associated with increased expression of FGF23:** X-linked hypophosphatemic rickets, an inherited disease caused by mutations in the PHEX gene, which encodes regulatory enzymes of proteins such as FGF23.
- **Hypothesis:** increased levels of FGF23 in mutant mice with X-linked hypophosphatemic rickets will lead to increased phosphorus and calcium excretion.

2.Objectives

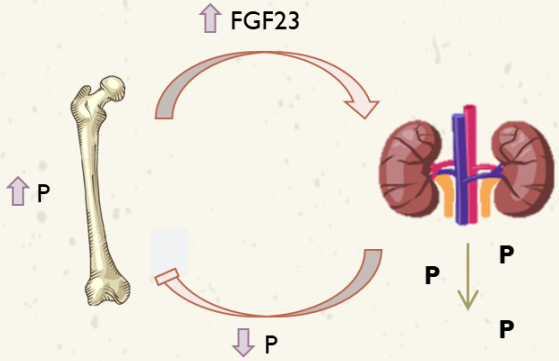
1	To determine whether or not the mice in the study present the mutation in the PHEX gene, using PCR techniques, to understand its relationship with the development of X-linked hypophosphatemic rickets.
2	To compare the elimination of phosphorus and calcium in urine between mice with and without the mutation, in order to analyze how the genetic alteration affects mineral metabolism.
3	To measure the levels of FGF23 in the different groups of mice, to evaluate its role in the regulation of phosphorus and calcium and its possible impact on the disease.

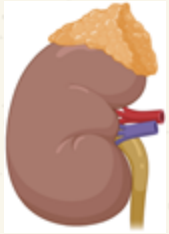
3. Theoretical framenwork

FGF23 (Fibroblast Growth Factor 23)



Mature osteoblast Osteocyte

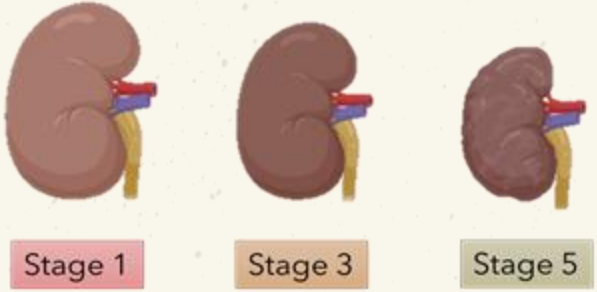


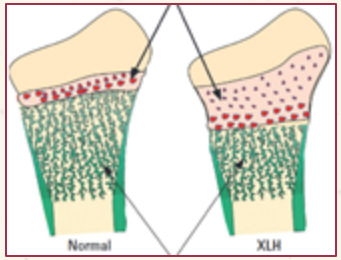


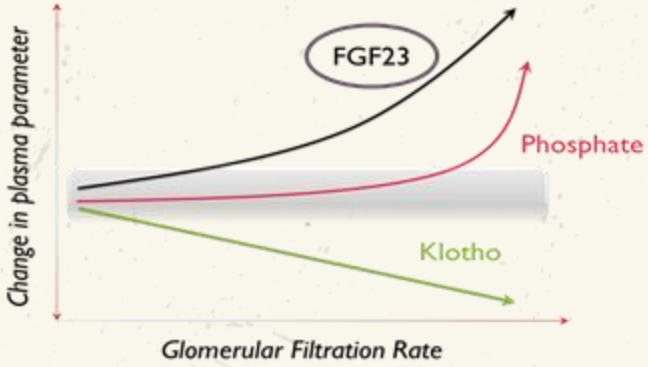
↑ **C**alcium reabsorption

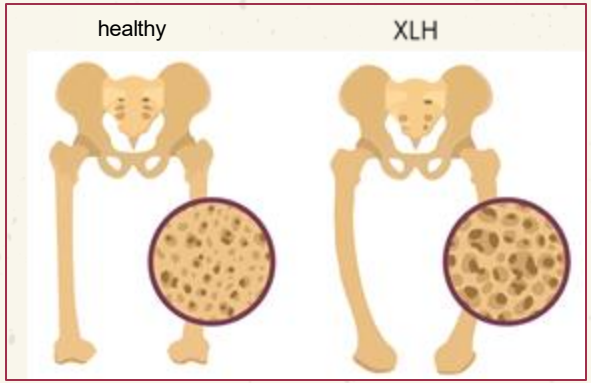
↑ **P**hosphate excretion

Chronic kidney disease









4. Materials and methods

Independent variable

Presence or absence of the PHEX gene mutation, determinant of X-linked hypophosphatemic rickets in mice..

Dependent variable

Concentration (mg/dl/24h) of phosphorus and calcium in the urine of mice, as this reflects the impact of the mutation on mineral metabolism.

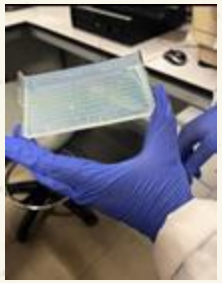
Materials

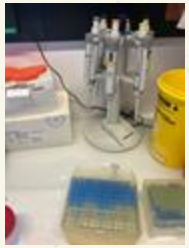
Primers and genotyping kit

For detecting and amplifying DNA

Micropipettes, agarose gel and electrophoresis system

For separation of DNA fragments





Thermocycler

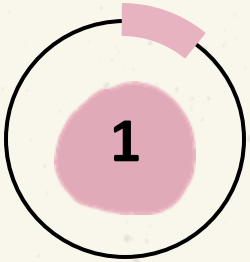
Bio-Rad Gel Doc

For electrophoresis documentation

Spectrophotometer and measuring kits

For phosphate and calcium quantification

Design of the Laboratory Work

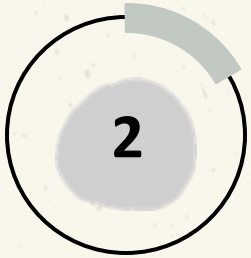


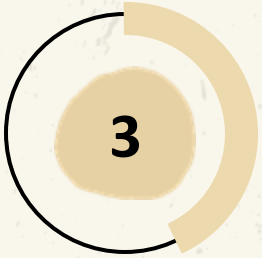
PCR genotyping

Identification of the PHEX mutation in mice by analysis of specific DNA fragments

Collection of urinary samples

Use of metabolic cages for 24 hours to obtain urine samples without contamination





Phosphorus and calcium quantification.

Spectrophotometric measurement with standardized dilutions and absorbance analysis.

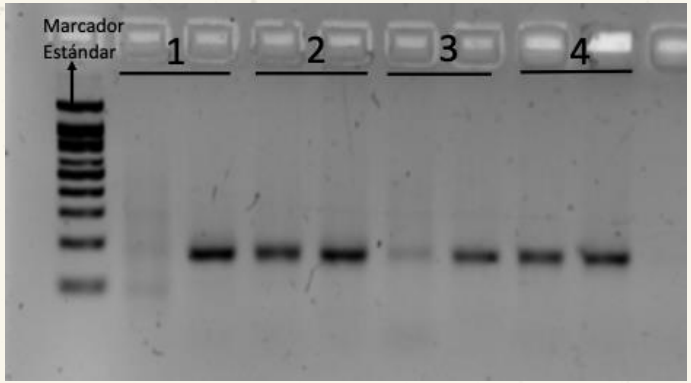
Statistical analysis

Comparison of data between WT and HYP groups using GraphPad Prism, applying Student's t-test ($p<0.05$).

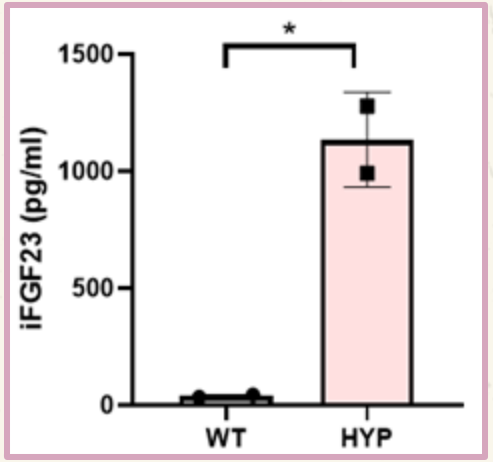
5. Results

Parameters to test the genotype between WT and HYP mice.

A)



B)

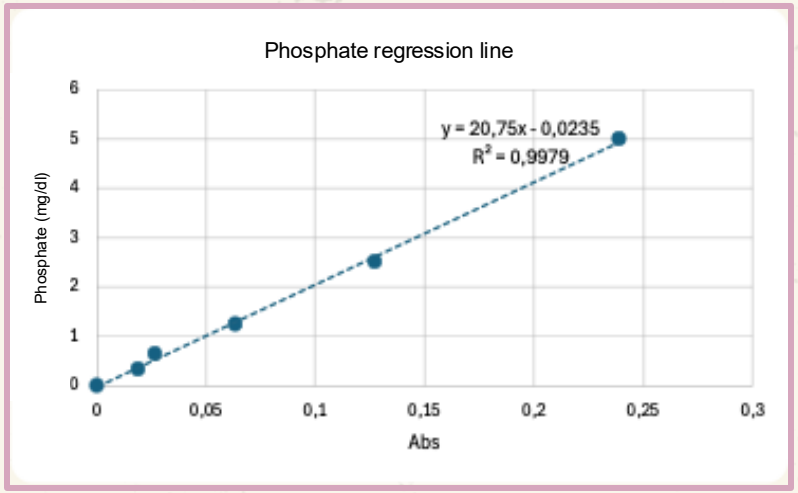


A) Genotyping of WT and HYP mice. Mice 2 and 4 present band for alleles 6 and 22 of the PHEX gene, indicating that they are WT. Mice 1 and 3 show banding for 6 and absence of allele 22, indicating that they are HYP.

B) Determination of intact FGF23. Previous results of the group show how HYP mice present a significant increase of FGF23 ($p<0.05$ *) in plasma.

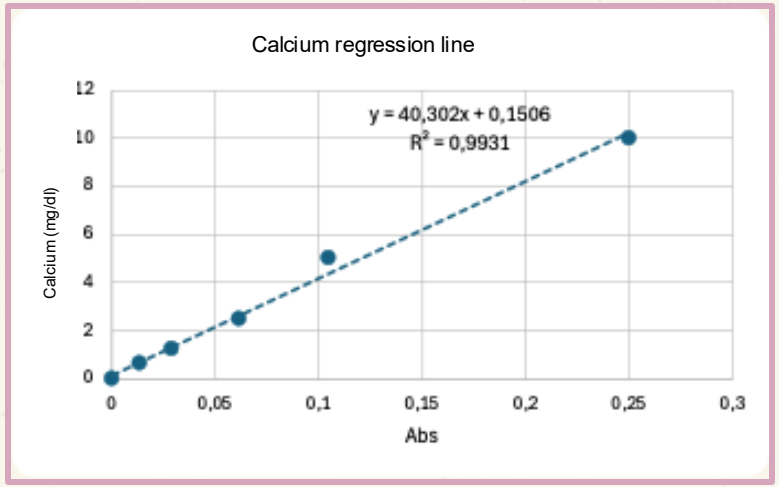
Analysis for the determination of phosphorus and calcium concentration in urine

A)



A) Phosphate regression line. The analysis shows a value of R2 = 0.99.

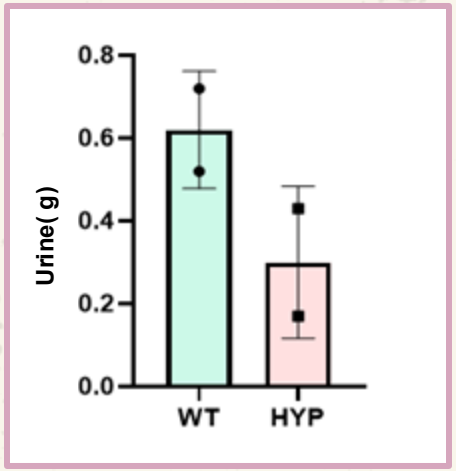
B)



B) Calcium regression line. The analysis shows a value of R2 = 0.99.

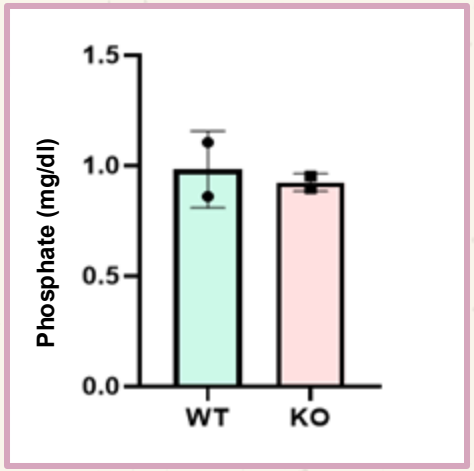
Analysis of urine mineral metabolism parameters

A)



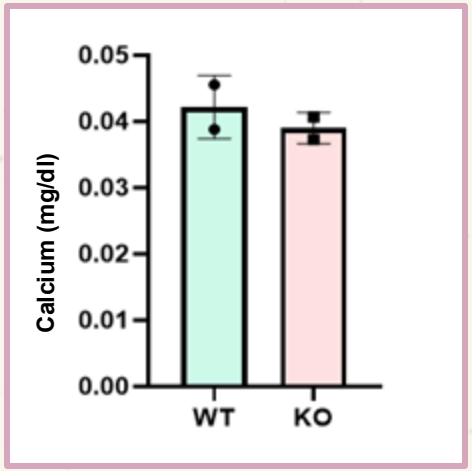
A) Urine volume between WT and HYP mice

B)



B) Phosphate in urine. Determination of phosphate concentration in 24h.

C)



C) Calcium in urine. Determination of calcium concentration in 24h.

These data show (B and C) how the amount of phosphorus and calcium in urine is very similar in both WT and HYP mice. We entered the calcium and phosphorus data obtained after analysis, and we obtained that the difference between WT and HYP is not significant in the mice evaluated, but we did find a significant difference in the amount of urine during 24 h, the mice with the mutation expel less urine than normal mice (A).

7. Conclusions

Increased FGF23 in HYP mice due to mutation in PHEX.

1.- Mutant mice with X-linked hypophosphatemic rickets (HYP) show a significant increase in plasma FGF23 at 10 weeks of age, confirming the initial hypothesis that the mutation in the PHEX gene causes this increase in the hormone.

Phosphate and calcium excretion in HYP and WT mice

Despite the increase in FGF23, no significant difference in urinary phosphorus and calcium excretion was observed between HYP and WT mice. This indicates that, although the mutation generates an increase in FGF23 levels, the excretion of these minerals is not directly affected in these animals at 10 weeks.

Possible mechanisms of mineral metabolism regulation in HYP mice

At 10 weeks, no impact is observed in the urine, suggesting that mechanisms such as intestinal absorption or renal reabsorption regulate phosphorus and calcium levels in HYP mice.

8.Acknowledgments

- We would like to thank the researcher **Raquel María García Sá**ez for helping us to carry out this project and making it a comfortable experience and a total learning experience.

- We thank Professor **Elena León Rodríguez**, who coordinated the project, for making us have a significant learning experience and for guiding us.

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THANKS FOR YOUR ATTENTION

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