High-salt diet modulates endocrine regulation between cortisol and FGF23

Matthias B. Moor[1,2], Klaudia Kopper[2,3], Antoinette Pechère-Bertschi[4,5], Michael S. Sagmeister[6], Rowan S Hardy[7,8], Eric Feraille[2,4,5], Daniel G. Fuster[1,2], Johannes Loffing[2,3], Ganesh Pathare[2,3,9,10]

[1]Department of Nephrology and Hypertension, Inselspital, Bern University Hospital, University of Bern, Bern, Switzerland.
[2]Swiss National Centre of Competence in Research "Kidney Control of Homeostasis", Switzerland
[3]Institute of Anatomy, University of Zurich, Zurich, Switzerland
[4]Service of Nephrology and Hypertension, University Hospital Geneva, Geneva, Switzerland.
[5]Department of Cell Physiology and Metabolism, University of Geneva, Geneva, Switzerland.
[6]Institute of Applied Health Research, College of Medicine and Health, University of Birmingham, Birmingham B15 2TT, UK.
[7]MRC Arthritis Research UK Centre for Musculoskeletal Ageing Research, University of Birmingham, Birmingham, B15 2TT, UK.
[8]Department of Biomedical Sciences, School of Infection, Inflammation & Immunology, College of Medicine and Health, University of Birmingham, Birmingham B15 2TT, UK.
[9]Institute of Veterinary Anatomy, University of Zurich, Zurich, Switzerland
[10]Bone-Kidney Axis and Regeneration Laboratory, Dept. of Infectious Diseases and Public Health, City University of Hong Kong, Hong Kong SAR

***Corresponding author**:
Ganesh Pathare, BVSc, PhD
Bone-Kidney Axis and Regeneration Laboratory
Dept. of Infectious Diseases and Public Health
Jockey Club College of Veterinary Medicine and Life Sciences
City University of Hong Kong, Hong Kong SAR
Email: gpathare@cityu.edu.hk

Keywords: High-salt-diet, cortisol (corticosterone), FGF23, bone and kidney

Word count: 2277

Competing Interests

The authors declare no potential conflicts of interest.

ABSTRACT

Excessive dietary salt intake is a global health concern, affecting cardiovascular, renal, and bone health. While the renin-angiotensin-aldosterone system (RAAS) is a known regulator of dietary salt-induced hormonal responses, the impact of adrenal cortisol remains unclear. Here, we performed a retrospective analysis in individuals (n=292) consuming a random diet. Dietary salt intake positively correlated with urinary cortisol and inversely correlated with plasma fibroblast growth factor 23 (FGF23), a bone-derived hormone regulating phosphate and vitamin D homeostasis. Controlled salt diets in healthy individuals confirmed a dose-dependent increase in urinary cortisol and suppression of plasma FGF23. In mice, oral corticosterone, a cortisol analogue, reduced circulating FGF23 levels. RNA-seq analysis of corticosterone-treated MC3T3 osteoblasts identified suppression of FGF23 via glucocorticoid receptor activation, anti-inflammatory pathways, and reduced osteoblast activity. Our findings reveal a novel endocrine cascade where high salt intake elevates cortisol and suppresses FGF23, with potential implications for bone, kidney, and cardiovascular health.

SIGNIFICANCE STATEMENT

Excessive dietary salt intake is a global health concern with poorly understood hormonal consequences beyond the renin-angiotensin-aldosterone system. Here, we identify a novel endocrine cascade in which high salt intake elevates cortisol signaling and suppresses fibroblast growth factor 23 (FGF23), a bone-derived hormone central to phosphate and vitamin D homeostasis. These findings are supported by a human cohort on random diets, a controlled dietary salt intervention, and corticosterone experiments in mice and osteoblasts. Mechanistically, cortisol suppresses FGF23 via glucocorticoid receptor activation, anti-inflammatory signaling, and repression of osteoblast activity. These findings have potential implications for bone, kidney, and cardiovascular health, and suggest that dietary salt intake may influence the clinical interpretation of cortisol and FGF23 measurements.

INTRODUCTION

Excessive dietary salt intake, a hallmark of the Western diet, is a global health concern associated with cardiovascular disease, bone loss, and autoimmune disorders, contributing significantly to morbidity and mortality[1–3]. Beyond its well-established effects on blood pressure, high salt intake disrupts metabolic signaling and hormonal responses[3]. The renin-angiotensin-aldosterone system (RAAS) is the primary hormonal pathway influenced by dietary salt[4]. Notably, adrenal-derived cortisol is known for its role in stress response and immunosuppression, but it may also contribute to salt homeostasis[5,6]. However, the mechanisms underlying this interaction and its impact on the pathophysiology of high salt intake are not yet fully understood.

Fibroblast growth factor 23 (FGF23), a bone-derived hormone, is central to phosphate and vitamin D homeostasis[7]. Elevated FGF23 is implicated in cardiovascular and kidney diseases, while its deficiency causes aging-like phenotypes in animal models[8–10]. Recent studies suggest a link between sodium homeostasis and circulatory FGF23 levels in humans and mice, but the mechanisms remain unclear[11–13.]. Given cortisol's emerging role in salt homeostasis, and its established effects on bone remodeling through glucocorticoid receptors (GR), it is crucial to investigate the relationship between dietary salt, cortisol, and FGF23.

FGF23 exerts its biological effects by binding to FGF receptors (FGFRs) in the presence of the co-receptor Klotho, a transmembrane protein predominantly expressed in the kidney[14]. Klotho abundance is regulated by physiological stimuli including osmotic stress and dehydration[15]. Distal tubular Klotho governs calcium reabsorption, while proximal tubular Klotho regulates phosphate homeostasis and prevents aging-like phenotypes in mice[14]. Sodium transport regulatory proteins along the nephron are closely linked to FGF23-Klotho axis, and disruption of tubular sodium handling consistently alters circulating FGF23 levels[11,16–19]. In addition, extracellular sodium directly regulates FGF23 formation in osteoblasts[20]. Whether dietary salt and cortisol influence this broader FGF23-Klotho regulatory network remains unknown.

Here, we hypothesize that dietary salt intake modulates endocrine regulation between cortisol and FGF23. To test this, we analyzed human cohort data and data from healthy individuals with and without dietary salt intervention and performed mechanistic experiments in mice and osteoblast cell lines. Our findings reveal a previously unrecognized endocrine cascade, suggesting that dietary salt elevates cortisol signaling, which may contribute to FGF23 suppression.

MATERIALS AND METHODS

Human cohort analysis

We retrospectively analyzed data from the BKSR[21], a single-center observational cohort consisting of individuals. Plasma C-terminal FGF23 (cFGF23) levels were quantified using ELISA. Steroids in urinary samples were quantified using mass spectrometric analysis on a gas chromatograph 7890A coupled to a mass-selective Hewlett-Packard 5975C detector. We analyzed data regarding

urinary and serum parameters from 292 individuals. Associations between urinary sodium, cortisol/cortisone, and plasma FGF23 levels were assessed using generalized linear models, after square-root or log transformation as appropriate based on residuals evaluation. Adjustments were made for age, sex, estimated glomerular filtration rate (creatinine CKD-EPI) and body mass index.

Dietary salt intervention study

Urine and serum samples were employed from previous prospective, crossover-controlled study comprising healthy male volunteers[22]. Participants underwent three 7-day dietary interventions: low-salt diet (LSD, 3 g NaCl/day), normal-salt diet (NSD, 6 g NaCl/day), and high-salt diet (HSD, 15 g NaCl/day), with constant potassium intake. Serum and 24-h urine samples were collected after each dietary phase. Concentrations of urinary free cortisol were measured using an ELISA (Diametra), whereas iFGF23 and cFGF23 levels were measured using an ELISA (Quidel). Changes in plasma hormone levels and urinary parameters across the dietary interventions were compared using paired t-tests (n=12).

Mouse experiments:

C57Bl6 male mice were housed in standard conditions with ad lib access to standard chow and water. All animal experiments were performed in accordance with the guidelines and regulations approved by the University of Birmingham. Drinking water was supplemented with either corticosterone (100 µg/mL, 0.6% ethanol), or vehicle (0.6% ethanol), with an average consumption of 1.25 mg per day. Mice were sacrificed at the end of 2 weeks' treatment. Plasma iFGF23 and cFGF23 levels were measured using ELISA (Quidel). Control mice received vehicle treatment. Data were analyzed using unpaired t-tests.

***In vitro* studies**

MC3T3-E1 subclone four mouse preosteoblast cells were cultured and differentiated as previously described[20]. Osteogenic differentiation was initiated by culturing the cells in a medium containing 50 µg/ml ascorbic acid and 10 mM β-glycerophosphate for 6 days. Upon differentiation, cells were treated with corticosterone or hydrocortisone (0–200 nM) for 24 hours as noted in results section. *Fgf23* mRNA levels were quantified using qPCR. FGF23 protein levels in the supernatant were measured by ELISA after concentrating the cell supernatant as described earlier[20]. For GR inhibition studies, cells were pre-treated with 50 nM RU-486 (Biotechne) for 1 hour, followed by corticosterone treatment. All experiments were performed in triplicate.

RNA-seq analysis

Differentiated MC3T3 cells were treated with 100 nM corticosterone for 24 hours (n=3 biological replicates). RNA was extracted, and sequencing was performed commercially at Novogene using the Illumina platform[20]. Differential gene expression (DEG) analysis was conducted using DESeq2, with adjusted p-values ($p_{adj} < 0.05$) and $\log_2$ fold-change > 1 considered significant. Gene ontology (GO) analysis was performed to identify enriched biological pathways as previously described[20].

187
188 **Statistical Analysis**
189 Data were presented as mean ± SEM. Statistical significance was determined using t-tests for two-
190 group comparisons. Correlation analyses were conducted using Pearson's correlation coefficient.
191 All analyses were performed using GraphPad Prism or RStudio.
192
193 **RESULTS**
194 **1. High salt intake in humans activates cortisol signaling and suppresses FGF23 formation.**
195 To investigate the association between dietary sodium intake and cortisol signaling, we reanalyzed
196 data from the thoroughly phenotyped population of the Bern Kidney Stone Registry (BKSR), a
197 single-center observational cohort of kidney stone formers[21]. We found that 24-hour urinary
198 sodium, a reliable surrogate marker of dietary sodium intake, was associated with 24-h urinary
199 cortisol (Fig. 1A). Further, urinary sodium was negatively associated with plasma FGF23 levels
200 (Fig. 1B). Notably, urinary cortisone and cortisol tended to show an inverse relationship with plasma
201 FGF23; however, only the association with urinary cortisone but not cortisol reached statistical
202 significance (Fig. 1C, D). After adjusting the models for renal function, age, sex and body mass
203 index in multivariable regression, the association with sodium remained whereas the association
204 with cortisone was attenuated and did not remain significant (p=0.12) (Figures 1E-F).
205
206 Next, we analyzed serum and urine samples from a prospective crossover-controlled study
207 involving healthy male volunteers[22]. These participants underwent three 7-day dietary
208 interventions: a low-salt diet (LSD, 3 g NaCl/day), a normal-salt diet (NSD, 6 g NaCl/day), and a
209 high-salt diet (HSD, 15 g NaCl/day), with constant potassium intake (Fig. 1G). Dietary sodium
210 intake dose-dependently increased both 24-hour urinary sodium excretion (Fig. 1H) and cortisol
211 levels (Fig. 1I). Interestingly, dietary sodium also dose-dependently suppressed both intact and
212 cFGF23 levels, with a more pronounced effect observed in assays measuring cFGF23 (Fig. 1J/K).
213 No significant differences in serum or urinary phosphate levels were observed across the dietary
214 interventions (Fig. 1L/M). This dissociation between FGF23 suppression and unchanged
215 phosphate levels suggests that the magnitude of FGF23 reduction may fall below the threshold
216 required to perturb phosphate homeostasis, or that compensatory mechanisms maintain
217 phosphate balance despite reduced FGF23.
218
219 **2. Corticosterone suppresses FGF23 formation by GR activation, anti-inflammation, and**
220 **repression of osteoblast activity**
221 C57Bl6 wild-type mice were administered corticosterone orally for 2 weeks. At the end of the
222 treatment period, blood samples were collected, and intact FGF23 (iFGF23) and C-terminal FGF23
223 (cFGF23) levels were measured. We found that corticosterone treatment significantly suppressed
224 both iFGF23 and cFGF23 levels in mice (Fig. 2A, B).
225

To investigate the mechanisms for FGF23 suppression by corticosterone, *in vitro* experiments were conducted using MC3T3 cells. Treatment with 100 nM corticosterone for 24 hours significantly reduced both cFGF23 levels in the cell supernatant and *Fgf23* mRNA expression in cell lysates (Fig. 2C, D). *Fgf23* mRNA expression was dose-dependently suppressed when cells were treated with 0–200 nM corticosterone (Fig. 2E). Treatment with hydrocortisone (100 nM), a synthetic cortisol analogue, also resulted in a reduction in *Fgf23* mRNA expression (Fig. 2F). Pretreatment with RU-486 (GR blocker) completely rescued the corticosterone-induced suppression of *Fgf23* mRNA, suggesting that the effects of corticosterone on *Fgf23* expression are mediated through GR activation (Fig. 2G).

RNA-seq analysis was performed on MC3T3 cells treated with 100 nM corticosterone for 24 hours. Differential gene expression analysis revealed 269 upregulated and 245 downregulated genes (padj < 0.05; $\log_2$FoldChange > 1), visualized in a volcano plot (Fig. 2H). Gene ontology (GO) analysis of biological processes revealed key pathways, including "ossification," "inflammatory response," "osteoblast differentiation," and "bone mineralization." (Fig. 2I). Further information is provided in the supplementary datasets 1 and 2 for DEGs and in the complete list of GO pathways. Genes associated with direct corticosteroid signaling, including *Sgk1*, *Fkbp5*, and *Per1*, were significantly upregulated, highlighting GR activation[23] (Fig. 2H/I). Anti-inflammatory pathways were prominently modulated, with a strong upregulation of classical corticosteroid-responsive genes such as *Hp* (Haptoglobin), *Orm1/2*, and *Serpine1* and a downregulation of *Ednra*[24] (Fig. 2H/I). Simultaneously, genes involved in osteoblast differentiation, such as *Runx2*, *Pth1r*, and *Ibsp*, were downregulated, indicating reduced osteoblast activity and extracellular matrix remodeling[25]. Suppression of *Bglap* (osteocalcin) indicates reduced osteoblast activity and impaired bone mineralization, while downregulation of *Tnfrsf11b* (OPG), an osteoblast-derived decoy receptor for RANKL, suggests a shift toward enhanced osteoclast-mediated bone resorption (Fig. 2H/I). These findings provide evidence that corticosteroids suppress FGF23 through combined GR signaling, anti-inflammatory effects, and suppression of osteoblast activity (Fig. 2H/I).

DISCUSSION

Our study identifies a potential endocrine cascade by which high salt intake elevates cortisol signaling and suppresses FGF23 levels, revealing non-classical pathophysiological consequences of high-salt diets on systemic health.

We measured 24-hour urinary cortisol for its accuracy in reflecting systemic cortisol secretion, overcoming the limitations of diurnal variation and inter-individual variability in serum levels[26]. Prior interventional studies with smaller sample sizes suggested that dietary salt intake modulates urinary cortisol levels[5,6]. While real-world dietary sodium variations are less extreme than those in interventional settings, our retrospective analysis showed that even among individuals on random diets, urinary cortisol positively correlates with dietary salt intake. Two possible mechanisms may

265 explain the cortisol rise with high salt intake. First, both aldosterone and cortisol are synthesized
266 in the adrenal cortex, share initial biosynthetic steps, and their levels are affected due to
267 competition for shared precursors and enzymes in the context of salt intake. Suppression of RAAS
268 activity by high salt reduces the demand for aldosterone synthesis, which depends on the enzyme
269 *CYP11B2*. This reduction may divert precursors such as 11-deoxycorticosterone toward cortisol
270 synthesis, potentially increasing cortisol levels[1,27]. Second, a recent study in mice suggest high-
271 salt intake elevates corticosterone levels due to activated hypothalamus-pituitary (HPA) axis and
272 stress response[28].
273
274 We found that high salt-mediated cortisol signaling was negatively associated with plasma FGF23.
275 In the retrospective analysis of individuals consuming random diets, urinary cortisone correlated
276 more strongly with FGF23 than cortisol, likely due to its higher stability. While the association did
277 not remain statistically significant after adjustment, potential residual confounding by renal function
278 cannot be excluded. Notably, in a controlled dietary salt intervention in young adults, high salt
279 intake consistently increased urinary cortisol and suppressed plasma FGF23, supporting this
280 relationship under experimental conditions. The more pronounced suppression of cFGF23
281 compared to iFGF23 under high-salt conditions may suggest that cortisol modulates not only
282 FGF23 production but also its proteolytic cleavage, though this warrants dedicated investigation.
283
284 FGF23 is primarily recognized for its role in phosphate and vitamin D homeostasis, but its
285 connection to salt metabolism is of emerging interest[11–13,16,17]. Aldosterone, a key hormone of the
286 RAAS, directly enhances FGF23 production in bone via mineralocorticoid receptors[11,12]. This was
287 linked to a potential mechanism for FGF23 regulation through dietary salt intervention in previous
288 studies[13]. Our findings suggest that elevated cortisol and reduced aldosterone may collectively
289 contribute to FGF23 suppression in response to high salt intake. However, our study provides
290 limited direct evidence for high salt-induced cortisol suppressing FGF23 levels and highlights the
291 need for further investigation. Furthermore, dedicated *in vivo* experiments are warranted to
292 determine the relative contributions of cortisol and aldosterone to FGF23 regulation under a high-
293 salt diet. Previous studies with potent glucocorticoids dexamethasone and prednisolone have
294 shown both stimulatory and inhibitory effects on FGF23 levels in mice[29,30]. In contrast, our results
295 indicate that the naturally occurring hormone corticosterone suppresses FGF23 production in mice
296 and osteoblast cells via GR activation, anti-inflammatory signaling pathways, and transcriptional
297 repression of osteoblast activity.
298
299 The interplay between dietary salt, cortisol signaling, and FGF23 may have important systemic
300 implications. Both high salt intake and elevated cortisol levels contribute to hypertension,
301 autoimmune diseases, inflammation and metabolic syndrome, bone loss and fracture risks,
302 therefore they may create a vicious cycle and amplify the burden of chronic diseases[1,3]. Future
303 studies are warranted to explore pharmacological strategies to counteract glucocorticoid-mediated
304 effects of high-salt diets on bone and cardiovascular health. Furthermore, both cortisol and FGF23

are used in many diagnostic tests[26,31]. Our results suggest that variations in dietary salt intake may influence the interpretations of these measurements.

In conclusion, our study uncovers an endocrine cascade wherein dietary salt intake elevates cortisol signaling, suppressing FGF23 levels. These findings suggest an unconventional role of dietary salt in systemic health and highlight the need for further study.

Conflict of Interest

The authors declare no potential conflicts of interest.

Funding

This work was supported by the Swiss National Science Foundation through the National Center of Competence in Research NCCR Kidney. C.H. [grant number N-403-03-55 (to GP)], City University of Hong Kong (to GP) funds from the Clinical Research Priority Program HYRENE of the University of Zurich (UZH) and intramural funding of the UZH (to JL). MSS received funding from the Medical Research Council (United Kingdom) as part of a Clinical Research Training Fellowship (MR/T008172/1).

Acknowledgments

The authors sincerely thank Monique Carrel for her excellent technical assistance. We are grateful to Prof. Franz Weber (Centre of Dental Medicine, University of Zurich) for providing the MC3T3-E1 cells, and to Prof. Mariusz P. Kowalewski (Institute of Veterinary Anatomy, University of Zurich) for critically reading the manuscript.

Author contributions

GP conceptualized research; MBM, APB, MSS, RSH, EF, DF and GP designed research; MBM, KK, APB, MS and GP performed research/reanalyzed human cohort data; JL contributed new reagents/analytic tools; MBM, KK, APB, MSS, RSH, EF, DF, JL and GP analyzed the data; GP wrote the manuscript; MBM, KK, APB, MSS, RSH, EF, DF and JL reviewed and edited the manuscript; GP and JL were responsible and funding acquisition; GP was responsible for supervision.

Data availability

The raw RNA-seq data used in this publication have been deposited in NCBI's Gene Expression Omnibus and are accessible through accession number GSE304562. All data are available from the authors upon request.

REFERENCES

1. Jobin, K., Müller, D. N., Jantsch, J. & Kurts, C. Sodium and its manifold impact on our immune system. *Trends Immunol.* **42**, 469–479 (2021).

2. Kawarazaki, W., Mizuno, R., Nishimoto, M., Ayuzawa, N., Hirohama, D., Ueda, K., Kawakami-Mori, F., Oba, S., Marumo, T. & Fujita, T. Salt causes aging-associated hypertension via vascular Wnt5a under Klotho deficiency. *J. Clin. Invest.* **130**, 4152–4166 (2020).

3. Hunter, R. W., Dhaun, N. & Bailey, M. A. The impact of excessive salt intake on human health. *Nature Reviews Nephrology 2022 18:5* **18**, 321–335 (2022).

4. Schweda, F. Salt feedback on the renin-angiotensin-aldosterone system. *Pflugers Arch.* **467**, 565–576 (2015).

5. Lewicka, S., Nowicki, M. & Vecsei, P. Effect of sodium restriction on urinary excretion of cortisol and its metabolites in humans. *Steroids* **63**, 401–405 (1998).

6. Wambach, G., Bleienheuft, C. & Bönner, G. Sodium loading raises urinary cortisol in man. *J. Endocrinol. Invest.* **9**, 257–259 (1986).

7. Kuro-o, M. & Moe, O. W. FGF23-αKlotho as a paradigm for a kidney-bone network. *Bone* **100**, 4–18 (2017).

8. Hu, M. C., Reneau, J. A., Shi, M., Takahashi, M., Chen, G., Mohammadi, M. & Moe, O. W. C-terminal fragment of fibroblast growth factor 23 improves heart function in murine models of high intact fibroblast growth factor 23. *Am. J. Physiol. Renal Physiol.* **326**, F584–F599 (2024).

9. Shimada, T., Kakitani, M., Yamazaki, Y., Hasegawa, H., Takeuchi, Y., Fujita, T., Fukumoto, S., Tomizuka, K. & Yamashita, T. Targeted ablation of Fgf23 demonstrates an essential physiological role of FGF23 in phosphate and vitamin D metabolism. *Journal of Clinical Investigation* **113**, 561–568 (2004).

10. Salas-Bastos, A., Bardet, C., Kopper, K., Jauze, L., Collaud, F., Francois, A., Moor, M. B., Chen, G., Mohammadi, M., Stockmann, C., Sommer, L., Loffing, J., Ronzitti, G. & Pathare, G. Chronically elevated FGF23 drives sustained renal ERK signaling and inflammatory transcriptional programs mitigated by cFGF23 gene therapy. *bioRxiv* 2024.01.18.576116 (2026) doi:10.1101/2024.01.18.576116.

11. Pathare, G., Anderegg, M., Albano, G., Lang, F. & Fuster, D. G. Elevated FGF23 Levels in Mice Lacking the Thiazide-Sensitive NaCl cotransporter (NCC). *Sci. Rep.* (2018) doi:10.1038/s41598-018-22041-1.

12. Zhang, B., Umbach, A. T., Chen, H., Yan, J., Fakhri, H., Fajol, A., Salker, M. S., Spichtig, D., Daryadel, A., Wagner, C. A., Föller, M. & Lang, F. Up-regulation of FGF23 release by aldosterone. *Biochem. Biophys. Res. Commun.* (2016) doi:10.1016/j.bbrc.2016.01.034.

13. Humalda, J. K., Yeung, S. M. H., Geleijnse, J. M., Gijsbers, L., Riphagen, I. J., Hoorn, E. J., Rotmans, J. I., Vogt, L., Navis, G., Bakker, S. J. L. & de Borst, M. H. Effects of Potassium or Sodium Supplementation on Mineral Homeostasis: A Controlled Dietary Intervention Study. *J. Clin. Endocrinol. Metab.* **105**, e3246–e3256 (2020).

14. Bourqui, L., Trnjanin, A., Kopper, K., Loffing-Cueni, D., Radvanyi, Z., Karpovich, A., Rahimi, T., Santos, R., Wengi, A., Pastor, J., Moe, O. W., Loffing, J. & Pathare, G. Klotho in the kidney distal convolution regulates urinary Klotho excretion and kidney calcium reabsorption, but not phosphate homeostasis. *Kidney Int.* (2026) doi:10.1016/J.KINT.2026.01.030.

15. Tang, C., Pathare, G., Michael, D., Fajol, A., Eichenmüller, M. & Lang, F. Downregulation of Klotho expression by dehydration. *Am. J. Physiol. Renal Physiol.* **301**, (2011).

16. Pathare, G., Föller, M., Michael, D., Walker, B., Hierlmeier, M., Mannheim, J. G., Pichler, B. J. & Lang, F. Enhanced FGF23 serum concentrations and phosphaturia in gene targeted mice expressing WNK-resistant spak. *Kidney Blood Press. Res.* (2012) doi:10.1159/000343393.

17. Pathare, G., Föller, M., Daryadel, A., Mutig, K., Bogatikov, E., Fajol, A., Almilaji, A., Michael, D., Stange, G., Voelkl, J., Wagner, C. A., Bachmann, S. & Lang, F. OSR1-sensitive renal tubular phosphate reabsorption. *Kidney Blood Press. Res.* **36**, (2012).

18. Andrukhova, O., Slavic, S., Smorodchenko, A., Zeitz, U., Shalhoub, V., Lanske, B., Pohl, E. E. & Erben, R. G. FGF23 regulates renal sodium handling and blood pressure. *EMBO Mol. Med.* **6**, 744–759 (2014).

19. Pathare, G., Hoenderop, J. G. J., Bindels, R. J. M. & San-Cristobal, P. A molecular update on pseudohypoaldosteronism type II. *Am. J. Physiol. Renal Physiol.* **305**, (2013).

20. Radvanyi, Z., Yoo, E. J., Kandasamy, P., Salas-Bastos, A., Monnerat, S., Refardt, J., Christ-Crain, M., Hayashi, H., Kondo, Y., Jantsch, J., Rubio-Aliaga, I., Sommer, L., Wagner, C. A., … Pathare, G. Extracellular sodium regulates fibroblast growth factor 23 (FGF23) formation. *J. Biol. Chem.* 105480 (2023) doi:10.1016/J.JBC.2023.105480.

21. Dhayat, N. A., Schneider, L., Popp, A. W., Lüthi, D., Mattmann, C., Vogt, B. & Fuster, D. G. Predictors of Bone Mineral Density in Kidney Stone Formers. *Kidney Int. Rep.* **7**, 558–567 (2021).

22. Pechère-Bertschi, A., Olivier, V., Burnier, M., Udwan, K., De Seigneux, S., Ponte, B., Maillard, M., Martin, P. Y. & Feraille, E. Dietary sodium intake does not alter renal potassium handling and blood pressure in healthy young males. *Nephrol. Dial. Transplant* **37**, 548–557 (2022).

23. Mifsud, K. R. & Reul, J. M. H. M. Acute stress enhances heterodimerization and binding of corticosteroid receptors at glucocorticoid target genes in the hippocampus. *Proc. Natl. Acad. Sci. U. S. A.* **113**, 11336–11341 (2016).

24. Barnes, P. J. How corticosteroids control inflammation: Quintiles Prize Lecture 2005. *Br. J. Pharmacol.* **148**, 245–254 (2006).

25. Ishida, Y. & Heersche, J. N. M. Glucocorticoid-Induced Osteoporosis: Both In Vivo and In Vitro Concentrations of Glucocorticoids Higher Than Physiological Levels Attenuate Osteoblast Differentiation. *Journal of Bone and Mineral Research* **13**, 1822–1826 (1998).

26. Nieman, L. K., Biller, B. M. K., Findling, J. W., Newell-Price, J., Savage, M. O., Stewart, P. M., Montori, V. M. & Edwards, H. The Diagnosis of Cushing's Syndrome: An Endocrine Society Clinical Practice Guideline. *J. Clin. Endocrinol. Metab.* **93**, 1526–1540 (2008).

27. Jobin, K., Stumpf, N. E., Schwab, S., Eichler, M., Neubert, P., Rauh, M., Adamowski, M., Babyak, O., Hinze, D., Sivalingam, S., Weisheit, C., Hochheiser, K., Schmidt, S. V., … Kurts, C. A high-salt diet compromises antibacterial neutrophil responses through hormonal perturbation. *Sci. Transl. Med.* **12**, (2020).

28. Costello, H. M., Krilis, G., Grenier, C., Severs, D., Czopek, A., Ivy, J. R., Nixon, M., Holmes, M. C., Livingstone, D. E. W., Hoorn, E. J., Dhaun, N. & Bailey, M. A. High salt intake activates the hypothalamic-pituitary-adrenal axis, amplifies the stress response, and alters tissue glucocorticoid exposure in mice. *Cardiovasc. Res.* **119**, 1740–1750 (2023).

29. Delucchi, Á., Toro, L., Alzamora, R., Barrientos, V., González, M., Andaur, R., León, P., Villanueva, F., Galindo, M., Las Heras, F., Montecino, M., Moena, D., Lazcano, A., … Michea, L. Glucocorticoids Decrease Longitudinal Bone Growth in Pediatric Kidney Transplant Recipients by Stimulating the FGF23/FGFR3 Signaling Pathway. *J. Bone Miner. Res.* **34**, 1851–1861 (2019).

30. Feger, M., Ewendt, F., Strotmann, J., Schäffler, H., Kempe-Teufel, D., Glosse, P., Stangl, G. I. & Föller, M. Glucocorticoids dexamethasone and prednisolone suppress fibroblast growth factor 23 (FGF23). *J. Mol. Med. (Berl).* **99**, 699–711 (2021).

31. Edmonston, D., Grabner, A. & Wolf, M. FGF23 and klotho at the intersection of kidney and cardiovascular disease. *Nature Reviews Cardiology 2023* 1–14 (2023) doi:10.1038/s41569-023-00903-0.

461 **FIGURE LEGENDS:**

462 **Fig. 1: High salt intake in humans elevates cortisol signaling and suppresses FGF23**
463 **formation**.
464 (A) Positive correlation between 24-hour urinary sodium excretion (a marker of dietary salt intake)
465 and urinary cortisol levels in the BKSR cohort. (B) Inverse correlation between 24-hour urinary
466 sodium excretion and plasma cFGF23 levels in the BKSR cohort. (C) Inverse correlation between
467 plasma cFGF23 levels and 24-hour urinary cortisone levels in the BKSR cohort. (D) Inverse
468 correlation between plasma cFGF23 levels and 24-hour urinary cortisol levels in the BKSR cohort.
469 The p-value indicates the statistical significance of the observed correlation. n= 292. (E)
470 Multivariable regression analysis showing the association between 24-hour urinary sodium
471 excretion and plasma cFGF23 levels after adjustment for age, sex, eGFR, and BMI in the BKSR
472 cohort. (F) Multivariable regression analysis showing the association between 24-hour urinary
473 cortisone and plasma cFGF23 levels after adjustment for age, sex, eGFR, and BMI in the BKSR
474 cohort (p=0.12). n=292 for panels A–F. (G) Schematic representation of the dietary salt intervention
475 study design. (H) Urinary sodium excretion; (I) urinary cortisol levels after dietary salt intervention
476 (low-salt, normal-salt, and high-salt diets). (J) Plasma iFGF23 levels; (K) plasma cFGF23 levels;
477 (L) plasma phosphate levels after dietary salt intervention. (M) Urinary phosphate excretion after
478 dietary salt intervention. Data are presented as mean ± SEM. Statistical significance is denoted
479 with p-value (paired t-test). n=12 per group.
480

481 **Fig. 2: Corticosterone suppresses FGF23 levels via GR activation and repressed**
482 **osteoblast activity.**
483 (A) Plasma iFGF23 levels and (B) cFGF23 levels in C57Bl6 wild-type mice treated with oral
484 corticosterone (n=12 per group). (C) *Fgf23* mRNA expression in cell lysates and (D) FGF23 protein
485 levels in cell supernatant after 100 nM corticosterone treatment for 24 hours in MC3T3 osteoblasts
486 (n=3 per group). (E) Dose-dependent suppression of *Fgf23* mRNA expression in MC3T3
487 osteoblasts treated with corticosterone for 24 hours (n=3 per group). (F) Suppression of *Fgf23*
488 mRNA expression by 100 nM hydrocortisone after 24-hour treatment (n=3 per group). (G) Rescue
489 of corticosterone (100 nM)-induced suppression of *Fgf23* mRNA expression by pretreatment with
490 RU-486, a glucocorticoid receptor antagonist (n=3 per group). (H) Volcano plot showing
491 differentially expressed genes (DEGs) in corticosterone-treated MC3T3 osteoblasts, highlighting
492 significantly upregulated and downregulated genes (padj < 0.05; |log2FoldChange| > 1). Key
493 genes implicated in GR activation, anti-inflammatory responses, and osteoblast activity are
494 indicated. (I) GO enrichment (biological processes) analysis of DEGs. Pathways related to bone
495 mineralization, inflammatory response, and osteoblast differentiation are highlighted in red. Data
496 are presented as mean ± SEM, with statistical significance denoted with p-value.
497
498

FIGURES

Fig. 1

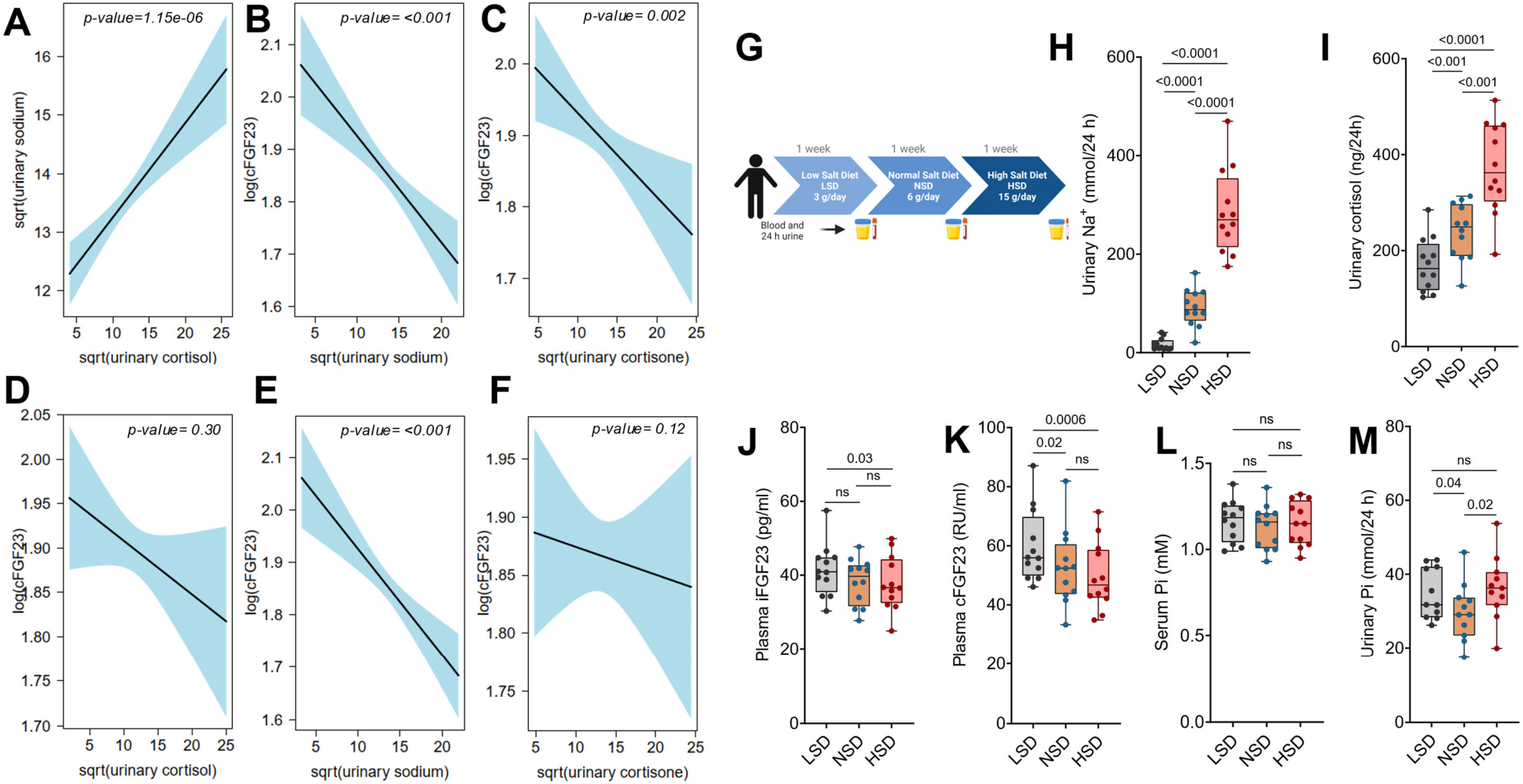
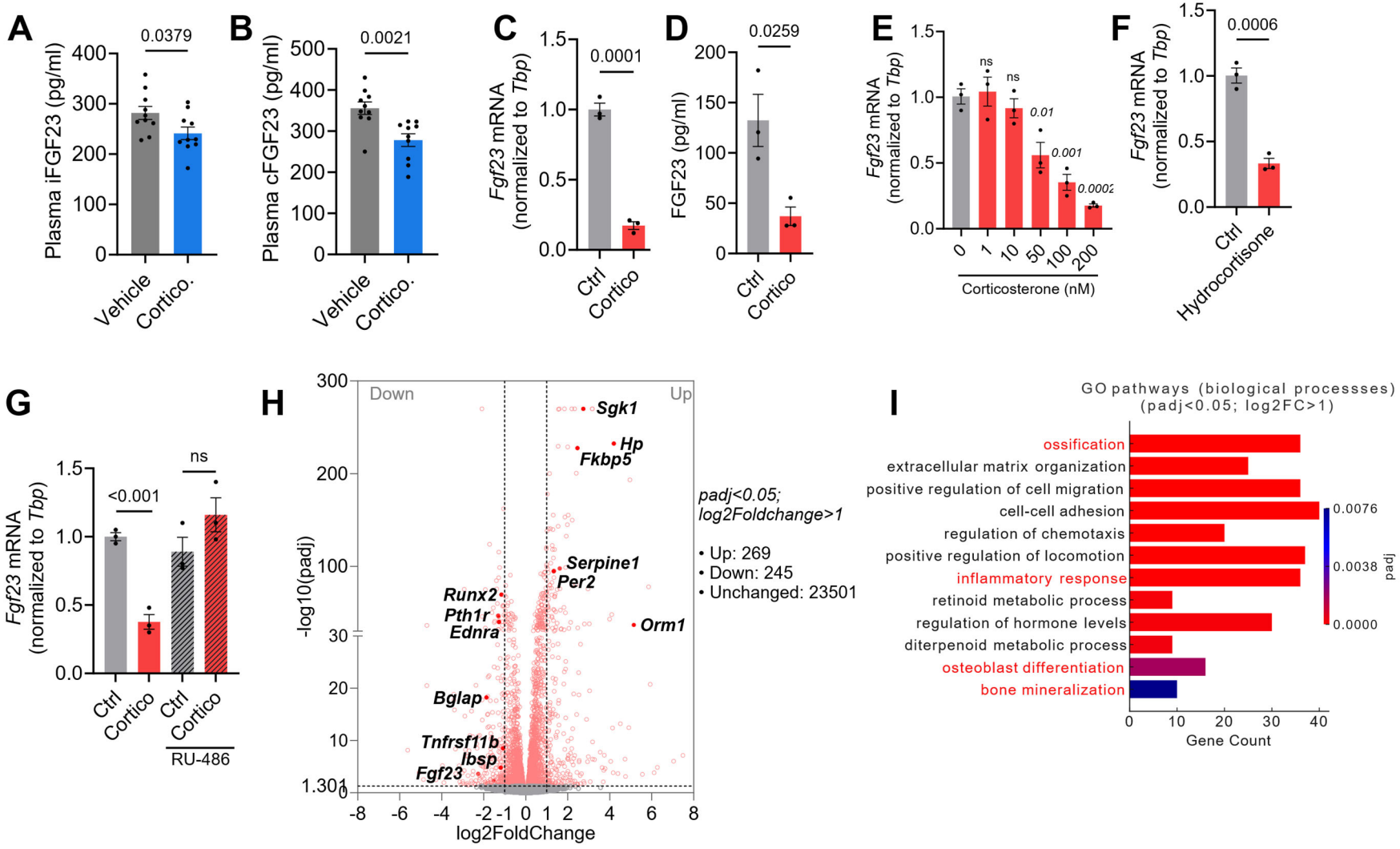


Fig. 2


A
Plasma iFGF23 (pg/ml)
0.0379
Vehicle
Cortico.
B
Plasma cFGF23 (pg/ml)
0.0021
Vehicle
Cortico.
C
Fgf23 mRNA (normalized to Tbp)
0.0001
Ctrl
Cortico
D
FGF23 (pg/ml)
0.0259
Ctrl
Cortico
E
Fgf23 mRNA (normalized to Tbp)
ns
ns
0.01
0.001
0.0002
0
1
10
50
100
200
Corticosterone (nM)
F
Fgf23 mRNA (normalized to Tbp)
0.0006
Ctrl
Hydrocortisone
G
Fgf23 mRNA (normalized to Tbp)
<0.001
ns
Ctrl
Cortico
Ctrl
Cortico
RU-486
H
Down
Up
Sgk1
Hp
Fkbp5
Serpine1
Per2
Orm1
Runx2
Pth1r
Ednra
Bglap
Tnfrsf11b
Ibsp
Fgf23
-log10(padj)
log2FoldChange
padj<0.05;
log2Foldchange>1
• Up: 269
• Down: 245
• Unchanged: 23501
I
GO pathways (biological processses)
(padj<0.05; log2FC>1)
ossification
extracellular matrix organization
positive regulation of cell migration
cell-cell adhesion
regulation of chemotaxis
positive regulation of locomotion
inflammatory response
retinoid metabolic process
regulation of hormone levels
diterpenoid metabolic process
osteoblast differentiation
bone mineralization
Gene Count
padj
0.0076
0.0038
0.0000