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Brief Report

Circulating Levels of Leptin and Lipocalin-2 in Patients With X-Linked Hypophosphatemia

Christine A. Simpson,[1] Anna Maria Santoro,[1] Thomas O. Carpenter,[2] Yanhong Deng,[3] Stephen Parziale,[3] and Karl L. Insogna[1]

[1]Department of Internal Medicine, Yale University, New Haven, CT 06510, USA
[2]Department of Pediatrics, Yale University, New Haven, CT 06510, USA
[3]School of Public Health, Yale University, New Haven, CT 06510, USA

Correspondence: Karl L. Insogna, MD, FACP, Department of Internal Medicine, Yale University, 333 Cedar Street, New Haven, CT 06510, USA.
Email: karl.insogna@yale.edu.

Abstract

Individuals with X-linked hypophosphatemia (XLH) are at greater risk for being overweight or obese. Whether there are underlying metabolic abnormalities that put patients with XLH at greater risk for excessive weight gain is largely unknown. Lipocalin-2 (LCN2) has recently received attention as a factor regulating energy consumption and specifically is postulated to be anorexigenic and to improve insulin sensitivity. In a retrospective study, circulating levels of LCN2, leptin, and insulin were measured in 32 patients with XLH, ages 2-60 years, all of whom were being treated with burosumab, and 38 control subjects. Control subjects were chosen who were close in age to those with XLH, with a similar number of participants of each sex. Subjects were analyzed in 3 age cohorts, 2-10 years, 11-18 years, and 20-60 years. In all age groups LCN2 levels were lower in the patients with XLH than in controls but when adjusted for weight class (normal, overweight, obese) the differences were not significant. In contrast, serum leptin levels were significantly lower in children with XLH compared to controls in the 2-10 years age cohort. Serum levels of insulin were also significantly lower in the 2-10-year-old children with XLH when compared with controls.

We conclude that changes in expression of lipocalin-2 in children and adolescents with XLH is unlikely to contribute to their risk for obesity in adulthood. It is unclear if lower circulating levels of leptin in these children plays a role in the higher prevalence of obesity among adults with XLH.

Key Words: lipocalin-2, insulin, leptin, obesity, XLH

Abbreviations: BMI, body mass index; FGF23, fibroblast growth factor 23; LCN2, lipocalin-2; LS, least square; XLH, X-linked hypophosphatemia.

Individuals with X-linked hypophosphatemia (XLH) are at greater risk for being overweight or obese. It is generally assumed that the primary reason for this is impaired mobility due to accelerated osteoarthritis, abnormal biomechanics of ambulation, pseudofractures, and enthesopathy. These known complications limit the ability of patients with XLH to engage in regular aerobic exercise [1]. Two published studies have confirmed that patients with XLH, especially children between the ages of 11 and 18 years, have a predisposition to obesity [2, 3]. Evidence suggests that elevated circulating levels of fibroblast growth factor 23 (FGF23) are associated with an increase in fat mass and dyslipidemia in elderly normal individuals [4]. Whether the elevated levels of FGF23 in XLH play a direct pathogenic role in the risk for excessive weight gain in XLH is unclear. Lipocalin-2 (LCN2) has recently received attention as a factor regulating energy consumption and specifically is postulated to be anorexigenic and to improve insulin sensitivity. Silencing LCN2 in vivo exacerbates diet-induced obesity in mice while increasing circulating levels of the hormone improves insulin sensitivity [5, 6]. Leptin is another anorexigenic hormone that has a crucial role in maintaining body weight. We wondered if there were differences in circulating levels of LCN2 or leptin in individuals with XLH, compared with nonaffected individuals, that might contribute to the high prevalence of excessive weight gain and obesity in that disorder.

Methods

Population and Samples

This study was approved by the Institutional Review Board of the Yale Human Research Protection Program. We used single donor serum samples from 32 patients with XLH from the clinical practices of 2 authors that had been previously used for measuring vitamin D metabolites and 38 previously analyzed single donor control samples from individuals chosen to match as close as possible the age and sex of the individuals with XLH. Subjects were defined as normal weight, overweight, or obese, based on calculated body mass index (BMI) for adults and BMI z-score for children using pediatric standards [7]. Samples were de-identified, aliquoted, and stored at minus 20°C until analyzed. The de-identified samples received a separate numerical identifier in accordance with Yale University Human Investigation Committee regulations.

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Assays

Commercially available assays were used to measure lipocalin-2, leptin, and insulin. The assays used were Human Lipocalin-2/NGAL: R&D Systems, Inc., Minneapolis, MN, RRID:AB_2894833 (coefficient of variation (%CV): inter-assay 6.5%, intra-assay 3.7%); Human Leptin: R&D Systems, Inc., Minneapolis, MN, RRID:AB_2783014 (%CV: inter-assay: 4.4%, intra-assay 3.2%); and Insulin ELISA, ALPCO, Salem, NH, RRID:AB_2801438 (%CV: inter-assay 10.6%, intra-assay 6.2%).

Statistical Analyses

Descriptive statistics of patient demographics were generated by genotype (XLH vs Control) and age group. Since the primary outcome measures (LCN2, leptin, and insulin) were not normally distributed, all data were log-transformed before analysis. Univariate models were first created for each log-transformed outcome with genotype, sex, weight class, and age as univariate predictors. Interactions among age and genotype controlling for age class were included in the multivariate model. Pairwise differences of least square (LS) means for each outcome were estimated between age group and genotype. The comparison between genotype within each age group was performed and the LS means with 95% CIs were estimated. For the log-transformed outcomes, their LS means were back-transformed after being estimated from the model. LS mean values and their 95% CIs were then plotted for each outcome. *P* values less than .05 were deemed to be significantly different. Data analyses were conducted using SAS version 9.4 (SAS Institute, Cary, NC).

Results

Table 1 summarizes the demographic distribution of age, sex, and weight category in the 3 age groups (2-10 years, 11-18 years, and 20-60 years) separated by genotype. Individuals were categorized as Healthy Weight, Overweight, and Obese. Subject weights and BMI z-scores were classified based on published data for children and adults [7-11].

When the log-transformed data from each of the 3 age groups were combined and analyzed in the multivariate model, there was no significant interaction between genotype and age group, that is, the effect of genotype did not vary significantly by age group. Although LCN2 levels were numerically lower in subjects with XLH in each of the 3 age categories, these differences were not significant when controlling for weight class, although the difference in the 11-18 years age group approached significance (Fig 1A, $P = .06$).

When the same multivariate analysis was conducted for leptin (Fig. 1B), LS mean data for XLH subjects were found to be significantly lower in the 2-10 years age cohort among the children with XLH when compared with control subjects ($P < .0001$). Similarly, in a univariate sensitivity analysis, leptin levels were found to be highly significantly lower in the 2-10 age group for children with XLH compared with controls ($P < .0001$).

Surprisingly, in the multivariate analysis, serum levels of insulin were significantly lower in the 2-10 age group for children with XLH when compared with controls (Fig. 1C, $P = .008$). This difference was also seen in a univariate sensitivity analysis, where insulin was found to be significantly lower in the 2-10-year-old children with XLH compared with control children ($P = .001$).

Discussion

The 2 study cohorts were well matched for age and sex. Among the adults in the XLH cohort, 86% were either overweight or obese (Table 1), consistent with the findings of Lecoq et al [3], who reported an overweight prevalence of 35.5% and an obesity prevalence of 20.5% among patients with XLH. Although the mean BMI z-score in both children's

Table 1. Demographics

Children 2-10 years	n	Sex F/M	Mean age, years	BMI z-score	Weight Category n (%)			
					Healthy	Overweight	Obese	Missing
XLH	14	5/9	5.5 ± 0.6	0.8 ± 0.3	8 (57)	3 (21)	3 (21)	0 (0)
Control	14	5/9	5.8 ± 0.7	1.1 ± 0.3[a]	12 (86)	0 (0)	1 (7)	1 (7)
Children 11-18 years	**n**	**Sex F/M**	**Mean age, years**	**BMI z-score**	**Weight Category n (%)**			
					Healthy	Overweight	Obese	Missing
XLH	11	8/3	14 ± 0.6	1.0 ± 0.3[b]	6 (55)	2 (18)	2 (18)	1 (9)
Control	13	6/7	14 ± 0.6	1.0 ± 0.2	10 (77)	1 (8)	2 (15)	0 (0)
Adult 20-60 years	**n**	**Sex F/M**	**Mean age, years**	**BMI kg/m^2**	**Weight Category n (%)**			
					Healthy	Overweight	Obese	Missing
XLH	7	6/1	35 ± 6	34 ± 4	1 (14)	2 (29)	4 (57)	0 (0)
Control	11	10/1	41 ± 5	34 ± 2	1 (9)	2 (18)	8 (73)	0 (0)

Data are mean ± SEM. Pediatric age- and sex-specific BMI z-scores were calculated using the Children's Hospital of Philadelphia calculator, which is based on the CDC Growth Charts. Pediatric BMI z-score classifications are defined as: Healthy weight −1.6449-1.0364 (5th-85th percentile); Overweight 1.0364-1.6449 (85th-95th percentile); Obese >1.6449. Adult BMI classifications are defined as: Healthy weight 20-24.9; Overweight 30-34.9, Obese >35 [7-11].

[a]N = 13; data unavailable for calculation for one control subject.

[b]N = 10; data unavailable for calculation for one XLH subject.

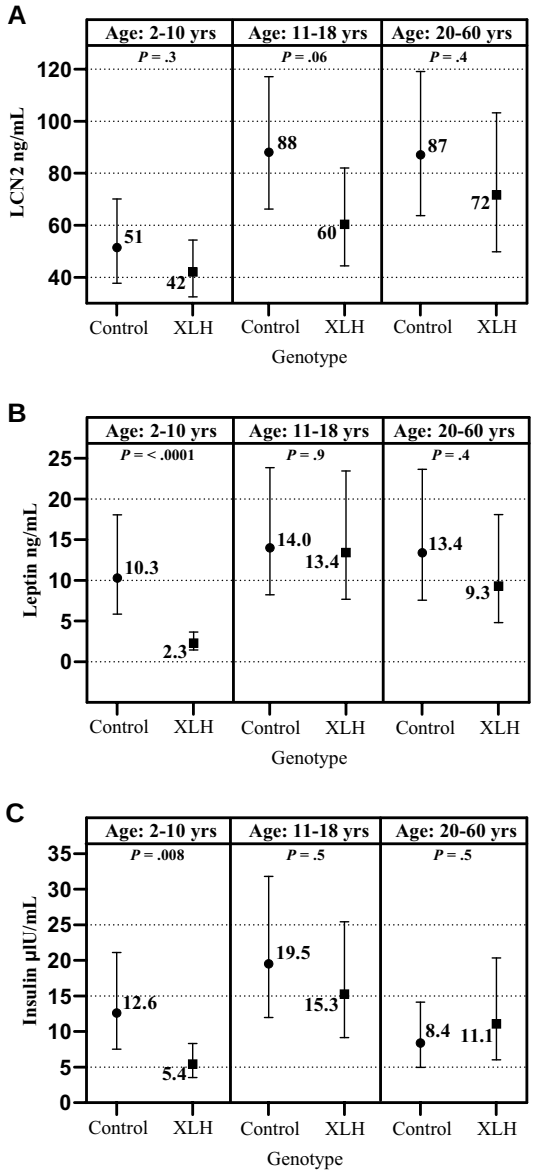

Figure 1. Back transformed mean values with 95% confidence intervals (CI) for LCN2 (A), leptin (B), and insulin (C) in three age categories separated by genotype. All *P* values are those generated from the multivariate model.

age groups with XLH was normal, overweight or obesity was present in 42% of the 2-10-year-old cohort and in 36% of the 11-18-year-old cohort. Similarly, Zhukouskaya et al [2] found that almost a third of children with XLH were either overweight or obese.

Since Petropoulou and Mosialou reported a high prevalence of either overweight or obesity among children and adolescents with XLH, the subjects in the current study were separated into 3 age categories, children aged 2-10 years, children aged 11-18 years, and adults 20-60 years of age. In all these groups, LCN2 was lower in the patients with XLH than in controls. However, when these data were analyzed using the multivariate model just described, there was no significant difference in any age group based on genotype. Leptin levels were found to be significantly lower in subjects with XLH in the 2-10-year-old category. This is surprising, since more of our subjects with XLH were overweight or obese compared with controls and generally, cross-sectional studies have found higher levels of leptin in children who are overweight compared with control children with normal weight [12]. As just noted, the sensitivity analysis demonstrated that leptin levels were significantly lower in children with XLH in the group aged 2-10 years.

Although there were a greater number of 2-10-year-old children with XLH who were overweight or obese when compared with controls, levels of insulin were significantly lower in the former group. Based on our limited data it would appear that hyperinsulinemia is less likely to be a predisposing factor for subsequent weight gain in children with XLH.

The relationship between circulating levels of leptin and the risk for weight gain is unclear and controversial. In one prospective study, lower baseline levels of leptin in young adults were associated with greater weight gain over the subsequent 2 years [13]. However, other studies have suggested that lower leptin levels predict greater weight gain in the intermediate term (2-3 years) but not over longer time frames [13-15].

Since all our XLH patients were treated with burosumab, it is unlikely that excess circulating levels of FGF23 acutely mediated the observed changes in serum levels of leptin. It has been postulated that there are endocrine circuits that exist between fat and bone [16] and whether dysregulation of the XLH osteoblast or osteocyte underpins the change in leptin is not addressed by this study.

Another important difference between individuals with XLH and overweight individuals without the XLH genotype is that elevated circulating levels of FGF23 are present from prenatal life in XLH. As noted, elevated levels of FGF23 are associated with obesity in the general population [17]. Further, recent studies have suggested that XLH is a state of chronic inflammation which may contribute to a predisposition to obesity [18].

Whether dysregulation of leptin has a pathogenic role in the predilection to obesity seen in XLH, separate and apart from the contributions of impaired mobility and other musculoskeletal complications, which likely have prominent roles in mediating this complication, is unclear. Since leptin levels were lower in the 2-10-year-old age group of children with XLH, it is possible that leptin contributes to an abnormal hormonal milieu that increases the likelihood of excessive weight gain in children with this disease.

Our study has several limitations. This was a retrospective study and, as such, could not account for all potential confounders. Further, since XLH is a rare disease, our samples sizes were perforce small, and as noted above, the data were not normally distributed. These data do not provide any insight into whether lifelong elevations in FGF23 play a role in mediating this change but since all the subjects with XLH were treated with burosumab for many months, it is unlikely that elevated FGF23 levels acutely suppress leptin. Leptin levels are generally reported to be elevated in individuals who are overweight or obese [19], so our finding that leptin levels are significantly lower in children with XLH warrants further study in larger numbers of patients with XLH.

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Disclosures

C.A.S., A.S., Y.D., S.P., and K.L.I. have nothing to disclose. T.O.C. is on the Advisory Board and is conducting clinical trials for Ultragenyx Pharmaceutical.

Data Availability

Original data generated and analyzed during this study are included in this published article.

References

1. Carpenter TO, Imel EA, Holm IA, Jan de Beur SM, Insogna KL. A clinician's guide to X-linked hypophosphatemia. *J Bone Miner Res*. 2011;26(7):1381-1388.
2. Zhukouskaya VV, Rothenbuhler A, Colao A, *et al.* Increased prevalence of overweight and obesity in children with X-linked hypophosphatemia. *Endocr Connect*. 2020;9(2):144-153.
3. Lecoq A-L, Trabado S, Schilbach K, *et al.* SUN-336, Obesity and impaired glucose metabolism in adult patients with X-linked hypophosphatemia. *J Endoc Society*. 2020;4(Suppl_1):SUN-336. https://doi.org/10.1210/jendso/bvaa046.1355
4. Mirza MA, Alsiö J, Hammarstedt A, *et al.* Circulating fibroblast growth factor-23 is associated with fat mass and dyslipidemia in two independent cohorts of elderly individuals. *Arterioscler Thromb Vasc Biol*. 2011;31(1):219-227.
5. Petropoulou PI, Mosialou I, Shikhel S, *et al.* Lipocalin-2 is an anorexigenic signal in primates. *Elife*. 2020;9:e58949.
6. Mosialou I, Shikhel S, Luo N, *et al.* Lipocalin-2 counteracts metabolic dysregulation in obesity and diabetes. *J Exp Med*. 2020;217(10):e20191261.
7. Childrens's Hospital of Philadelphia Research Institute BMI Z-score calculator. 2023. Accessed March 10, 2023. https://zscore.research.chop.edu/calcbmi.php
8. Ogden CL, Kuczmarski RJ, Flegal KM, *et al.* Centers for disease control and prevention 2000 growth charts for the United States: improvements to the 1977 National Center for Health Statistics version. *Pediatrics*. 2002;109(1):45-60.
9. Kuczmarski RJ, Ogden CL, Guo SS, *et al.* 2000 CDC growth charts for the United States: methods and development. *Vital Health Stat*. 2002;11(246):1-190.
10. National Center for Health Statistics. CDC Extended BMI-for age Growth Charts. 2022. Accessed June 28, 2023. https://www.cdc.gov/growthcharts/extended-bmi.htm
11. Centers for Disease Control and Prevention. Healthy Weight, Nutrition, and Physical Activity. Accessed June 28, 2023. https://www.cdc.gov/healthyweight/bmi/calculator.html
12. Ellis KJ, Nicolson M. Leptin levels and body fatness in children: effects of gender, ethnicity, and sexual development. *Pediatr Res*. 1997;42(4):484-488.
13. Allard C, Doyon M, Brown C, Carpentier AC, Langlois M-F, Hivert M-F. Lower leptin levels are associated with higher risk of weight gain over 2 years in healthy young adults. *Appl Physiol Nutr Metab*. 2013;38(3):280-285.
14. Ravussin E, Pratley RE, Maffei M, *et al.* Relatively low plasma leptin concentrations precede weight gain in Pima Indians. *Nat Med*. 1997;3(2):238-240.
15. Hivert MF, Langlois MF, Carpentier AC. The entero-insular axis and adipose tissue-related factors in the prediction of weight gain in humans. *Int J Obes (Lond)*. 2007;31(5):731-742.
16. Karsenty G, Ferron M. The contribution of bone to whole-organism physiology. *Nature*. 2012;481(7381):314-320.
17. Zaheer S, de Boer IH, Allison M, *et al.* Fibroblast growth factor 23, mineral metabolism, and adiposity in normal kidney function. *J Clin Endocrinol Metab*. 2017;102(4):1387-1395.
18. Meaux MN, Alioli C, Linglart A, *et al.* X-linked hypophosphatemia, not only a skeletal disease but also a chronic inflammatory state. *J Clin Endocrinol Metab*. 2022;107(12):3275-3286.
19. Considine RV, Sinha MK, Heiman ML, *et al.* Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *N Engl J Med*. 1996;334(5):292-295.