Puberty Blockade Via Leuprolide Acetate Delays Bone Mineral Accrual in an Adolescent Rodent Model

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INTRODUCTION: Adolescence is a critical window for skeletal development, during which bone accrual and remodeling are tightly regulated by hormonal signaling. Gonadotropin-releasing hormone (GnRH) agonists like leuprolide acetate (LA) are clinically prescribed to delay puberty in youth with central precocious puberty or as part of gender-affirming care. While these medications are known to suppress the hypothalamic-pituitary-gonadal (HPG) axis, little is understood about how temporary hormone suppression during adolescence affects skeletal development. This study aims to investigate how pharmacological puberty blockade influences bone maturation in adolescent rats, with the goal of informing care for children receiving GnRH agonist therapy.

METHODS: All animal procedures conformed to the NIH guidelines and the Institute of Laboratory Animal Resources, National Research Council, and were approved by the University of Wisconsin Animal Care and Use Committee. Juvenile male and female rats (postnatal day 23) were randomly assigned to receive a depot injection of LA (0.6 mg) or vehicle. After three weeks, rats were euthanized, and serum and skeletal tissues were collected. Serum levels of bone turnover markers and mineral hormones were quantified by ELISA. Bone architecture and mineral density were assessed using micro-CT on excised femora**.** Kidneys were processed for histology, and glomerular cell number was determined**.** Data were analyzed using non-parametric *t*-tests.

RESULTS: Leuprolide acetate caused sex-specific alterations in circulating bone biomarkers, bone density, and microarchitecture (**Table 1**). Micro-CT revealed structural deficits in trabecular bone among LA-treated females, with lower bone volume fraction (**Fig 1A**), bone mineral density (**Fig 1B**), and trabecular number (**Fig 1C**)**,** leading to greater trabecular spacing (**Fig 1D**) compared to controls. Cortical bone was unaffected by LA administration in females. Adolescent males administered LA had reduced cortical thickness and cortical bone area fraction, with thinner trabeculae. Histomorphometry revealed greater osteoid deposition and osteoblast activity in LA-treated female animals, with no change in osteoclast activity (**Table 1**). Male histomorphometry is ongoing. In females, serum P1NP was elevated in LA-treated animals compared to controls (**Fig 1F**), as was serum FGF23 (**Fig 1G**), with no change in PTH (**Fig 1H**). By contrast, LA-treated males had lower serum FGF23 than male controls, with no change in P1NP. Neither sex had any change in serum resorption markers (CTX-I, TRAcP5b), calcium, or phosphate (**Table 1**). In the female kidney, LA administration increased glomerular area, but not number of nuclei, resulting in a modestly lower number of nuclei per glomerulus (**Fig 1I, J**). Average glomerular area was not different between control and LA-treated males, but LA treatment increased the number of nuclei (**Table 1**).

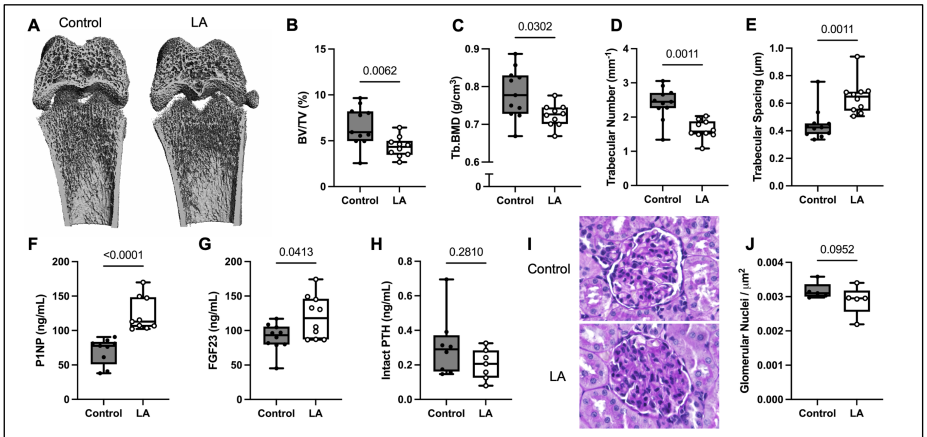

Figure 1. Puberty blockade using leuprolide acetate inhibits bone formation in juvenile rats. Female rats were administered a single LA depot from postnatal days 23 to 44. MicroCT reconstructions reveal lower bone volume fraction (**A, B**), trabecular BMD (**C**), and trabecular number (**D**), with more spaced trabeculae (**E**). Serum P1NP (**F**) and FGF23 (**G**) were elevated, with no change in serum PTH (**H**). In the kidney, LA administration reduced the number of nuclei per glomerulus (**I, J**).

DISCUSSION: Pharmacological suppression of the HPG axis during adolescence has sexually dimorphic effects on bone metabolism and structure in a rat model. In females, elevated P1NP and osteoblast activity and reduced trabecular bone volume and density indicates a potential mismatch between bone formation signaling and mineral accrual. This may reflect a dysregulated remodeling process in the absence of sex hormones. Conversely, the significant reduction in FGF23 in LA-treated males may indicate differential endocrine adaptation to GnRH agonism, possibly related to timing or sensitivity of skeletal development to sex hormones. These results underscore the importance of understanding how GnRH agonist therapy during adolescence, a period of rapid bone turnover, affects long-term bone health. Further studies will explore the cellular and molecular mechanisms underlying LA effects on juvenile bone and determine whether skeletal defects persist into adulthood.

Table 1. Puberty blockade has sexually dimorphic effects on mineral metabolism in rats (mean ± SD).

	Females			Males		
	Control (n≥10)	LA (n≥10)	P-value	Control (n≥10)	LA (n≥10)	P-value
Femur length (mm)	28.4 ± 0.8	28.6 ± 0.9	0.56	29.6 ± 0.9	28.4 ± 1.9	0.19
Bone volume fraction (%)	6.6 ± 2.1	4.3 ± 1.1	**0.006**	5.3 ± 2.9	3.4 ± 1.2	0.22
Bone mineral density (/cm^3)	0.78 ± 0.07	0.72 ± 0.03	**0.03**	0.73± 0.07	0.7 ± 0.04	0.47
Trabecular number (mm^{-1})	2.4 ± 0.5	1.6 ± 0.3	**0.001**	2.2 ± 0.8	1.6 ± 0.3	0.10
Trabecular thickness (µm)	54 ± 2.5	51 ± 2.2	0.07	52 ± 2.8	48 ± 4.0	**0.04**
Trabecular spacing (µm)	0.45 ± 0.12	0.64 ± 0.12	**0.001**	0.52 ± 0.2	0.66 ± 0.1	0.11
Cortical thickness (mm)	0.42 ± 0.04	0.41 ± 0.03	0.74	0.45±0.04	0.41±0.05	**0.05**
Tissue mineral density (g/cm^3)	3.04 ± 0.06	3.00 ± 0.06	0.13	2.98±0.07	2.96±0.06	0.43
Cortical bone area fraction (%)	47 ± 3.8	46 ± 2.3	0.43	48 ± 2.8	45 ± 3.4	**0.02**
Osteoid surface / BS (%)	28 ± 6.9	36 ± 3.9	**0.04**			
Osteoblast surface / BS (%)	38 ± 5.9	48 ± 5.0	**0.05**			
Osteoclast surface / BS (%)	24 ± 4.7	21 ± 4	0.24			
P1NP (ng/mL)	70 ± 19	124 ± 25	**<0.0001**	270 ± 158	225 ± 55	0.90
CTX-I (ng/mL)	43 ± 15	46 ± 14	0.70	55 ± 14	58 ± 18	0.68
TRAcP (U/L)	3.00 ± 1.03	3.04 ± 0.67	0.95	3.85 ± 1.5	4.12 ± 1.4	0.80
Calcium (mg/dL)	17.8 ± 2.6	18.1 ± 3.7	0.70	19.8 ± 2.7	20.3 ± 3.2	0.85
Phosphate (mg/dL)	0.26 ± 0.05	0.27 ± 0.06	0.85	0.31±0.06	0.3 ± 0.06	0.99
FGF23 (ng/mL)	90 ± 20	120 ± 31	**0.04**	151 ± 21	122 ± 19	**0.003**
PTH (ng/mL)	0.31 ± 0.18	0.20 ± 0.09	0.28	0.49 ± 0.4	0.55 ± 0.4	0.48
Avg glomerular area (µm^2 x 10^{-3})	26.5 ± 2.8	31.9	0.10	32.6 ± 7.5	29.6 ± 2.1	0.99
Avg nuclei / glomeruli (x10^{-3})	3.2 ± 0.2	2.9 ± 0.4	0.10	3.1 ± 0.3	3.5 ± 0.3	0.06

SIGNIFICANCE/CLINICAL RELEVANCE: These findings provide translational insight into the skeletal consequences of temporary puberty suppression. Because adolescence is the period of peak bone mineral accrual, alterations to bone structure and turnover during this window may have lasting effects on fracture risk and skeletal health in adulthood. Understanding the sex-specific effects of LA will inform clinical monitoring and long-term management strategies for children and adolescents receiving GnRH agonist therapy.