Chromosomal sex (XX vs XY) contributes to bone and muscle mass independently of the gonadal sex (ovaries vs testis) in the 4 core genotype mouse model

Lilian I Plotkin,[1,2,3] Matthew Arnett,[1] Dyann M Segvich,[4] Padmini Deosthale,[1] Chiebuka Okpara,[1] Julian Balanta Melo,[1,5] Joseph M. Wallace,[2,3,4]

[1]Department of Anatomy, Cell Biology & Physiology, Indiana University School of Medicine; [2]Roudebush Veterans Administration Medical Center; and [3]Indiana Center for Musculoskeletal Health, Indianapolis, IN, 46202, USA.

[4] Universidad del Valle School of Dentistry, Cali, 760043, Colombia

[5]Department of Biomedical Engineering, Indiana University-Purdue University Indianapolis, 46202, Indianapolis, IN

Email of Presenting Author: lplotkin@iu.edu

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INTRODUCTION: Vertebrate sexual dimorphism is ascribed to the presence of testis or ovaries, and, hence, to gonad-specific hormone production. However, sex differences also stem from the presence of sex chromosomes (XX or XY). To tease out the contribution of chromosome (CS) from gonad (GS) sex, the Sry gene that dictates testis formation was either deleted in XY mice, resulting in XY female (XYF) mice with ovaries or overexpressed in XX mice, resulting in XX male (XXM) mice with testis. These mice, together with XY males (M) and XX females (F) form the 4core genotype (FCG) model. It has been shown that levels of estrogen in XXF/XYF and androgen in XXM/XYM are similar.

METHODS: The skeletal phenotype of 2- and 4-month (m) FCG was analyzed. Mice were scanned for DXA, bones were collected, and tested by µCT and 3-point bending. GS/CS effects were assessed by 2-way ANOVA (Tukey post-hoc test).

RESULTS: GS influenced body weight (BW), and total/femur/spine BMD at 2m, and BW and total/femur BMD at 4m, all lower in gonadal females (**Table 1**). A GS-CS interaction was found in 2m mouse spine BMD, which was lower in XYF than XYM, but similar in XXF and XXM mice. At 4m, XY mice show lower BW and BMD at the 3 sites than XX mice, whereas % lean mass was higher in XYM and XYF than the respective XX mice, and % fat mass was lower in XYM vs XXM, indicating CS effects (**Table 2**). µCT showed GS and CS contribution to structural parameters and tissue mineral density (TMD) in femur mid-diaphysis -lower in XYF vs XXF, no different in males- whereas only GS affected marrow cavity and total tissue area and MOI at 4m (**Table 3**). Bone mechanical properties were overall lower in gonadal females (**Table 4**). CS only affected ultimate force - lower in XYF vs XXF, no different in males. GS affected all distal femur trabecular structural parameters, which were lower in gonadal females, but only partially lumbar vertebrae 5 (L5) vertebrae (**Table 3**). L5 TMD depended on CS and was higher only in XXF vs XYF.

DISCUSSION: While GS has a major role, CS is a so far unrecognized contributor to bone mass and bone strength.

SIGNIFICANCE/CLINICAL RELEVANCE: Understanding the basis for sex dimorphism in the skeleton could help develop gender-tailored treatments to improve bone mass and strength.

		2months					4months			
	measurement	**XXM**	**XXF**	**XYM**	**XYF**	**measurement**	**XXM**	**XXF**	**XYM**	**XYF**
Table 1. body weight (g) / bone mineral density (mg/cm^2)	body weight@	25.1±2.9	**20.6±1.9***	**24.8±1.6**	19.3±1.5*	body weight@ £	32.7±3.5	**24.8±2.4***	**29.0±1.7 #**	23.3±1.8*
	total@	49.8±4.0	**46.8±1.9***	**50.0±2.7**	45.3±1.4*	total@ £	52.5±3.5	52.0±1.6*	52.4±2.3	49.5±1.6*#
	femur@	69.4±7.2	**59.2±2.6***	**67.5±5.6**	56.2±3.1*	femur@ £	77.1±6.5	70.0±2.9*	73.8±5.1	63.4±3.2*#
	spine@	53.9±5.1	54.2±2.8	56.1±3.2	51.4±3.2*	spine£	58.9±5.6	58.0±3.5	54.6±3.4	56.7±3.3
Table 2. body composition (% body weight)	lean body mass	No significant differences				lean body mass£	76.6±5.0	**76.3±3.5**	**81.1±2.3#**	79.7±2.6#
	fat mass					fat mass£	21.9±5.2	**21.3±3.8**	**17.3±2.4#**	18.6±2.6[a]
Table 3. µCT - femur mid-diaphysis	BA/TA (%)	Not measured				BA/TA (%)@ £ &	49.6±2.8	**53.3±2.4***	**47.6±3.6**	48.2±2.4#
	CtTh (mm)					CtTh (mm)@ £	.25±.02	.24±.01	.24±.02	.21±.01*#
	tissue area (mm^2)					tissue area (mm^2)@	2.32±0.29	**1.83±0.08***	**2.28±0.21**	1.85±0.14*
	marrow area (mm^2)					marrow area (mm^2)@	1.18±0.19	**0.86±0.05***	**1.20±0.16**	0.86±0.05*
	Imax (mm^4)					Imax (mm^4)@ £	0.47±0.1	**0.31±0.04***	**0.43±0.07**	0.27±0.04*
	TMD ($mgHA/cm^3$)					TMD ($mgHA/cm^3$)@ £	1.18±0.03	**1.22±0.02[c]**	**1.16±0.04**	1.17±0.03#
Table 3. µCT - distal femur	BV/TV (%)					BV/TV (%) @	23.7±7.0	**5.71±1.6***	**22.07±4.7**	5.7±2.7*
	Tb.N (/mm)					Tb.N (/mm)@	2.95±0.60	**0.94±.0.24***	**2.97±0.55**	0.91±0.39*
	Tb.Th (mm)					Tb.Th (mm)@	.08±.01	**.06±.00***	**.08±.01**	.06±.01*
	Tb.Sp (mm)					Tb.Sp (mm)@	0.19±0.02	**0.28±0.02***	**0.20±0.02**	0.30±0.04*
	TMD ($mgHA/cm^3$)					TMD ($mgHA/cm^3$)@	.71±.03	**.67±.02***	**.70±.02**	.65±.03*
Table 3. µCT - L5	BV/TV (%)					BV/TV (%)@	32.6±6.3	**22.2±4.6***	**31.14±5.71**	21.10±4.82*
	Tb.N (/mm)					Tb.N (/mm)@	4.07±0.68	**2.88±0.58***	**4.06±0.52**	2.78±0.53*
	Tb.Sp (mm)					Tb.Sp (mm)@	.16±0.2	**.23±.03***	**.16±.02**	.24±.04*
	TMD ($mgHA/cm^3$)					TMD ($mgHA/cm^3$)£	.71±.03	.72±.01	.71±.03	.69±.02#
Table 4. biomechanical properties (3-point bending)	Ultimate force (N)					Ultimate force (N)@ £	20.58±4.94	17.31±1.72*	19.20±2.62	14.25±1.37*#
	Yield Force (N)					Yield Force (N)@	12.86±3.00	12.07±1.14	13.08±14.7	10.96±1.81*
	Stiffness (N/mm)					Stiffness (N/mm)@	114.8±23.0	110.4±9.3	118.1±17.8	99.7±16.3*
	Modulus (GPa)					Modulus (GPa)@	4.59±1.25	**6.35±0.64***	**4.96±0.86**	5.94±1.57
	Yield Stress (MPa)					Yield Stress (MPa)@	76.6±19.9	94.7±6.9[e]	81.8±13.5	89.6±20.8

Values indicate mean ± sd, only parameters that are significantly different at least for one comparison are shown by 2-way ANOVA: **@:** gonadal sex effect; **£:** chromosome sex effect; **&:** GS x CS interaction. Tukey post-hoc test: * p<0.05 vs same gonadal sex, # p< 0.05 vs same chromosome sex. Underlined bold corresponds to p<0.05 for gonadal female XX vs gonadal male XY. P values indicating tendencies towards significant differences: a: p=0.08 vs XXF; b: p=0.08 vs XXF; c: p=0.06 vs XXM; d: p=0.08 vs XXM; e: p=0.09 vs XXM.