

## Supplemental Data (Tables S1-S2 and Figures S1-S2)

### A High Fat Diet Induces Bone Loss in *Alox5* Null Mice

**Phuong Le<sup>1</sup>, Masanobu Kawai<sup>1,2</sup>, Sheila Bornstein<sup>1</sup>, Victoria E DeMambro<sup>1</sup>, Mark C Horowitz<sup>3</sup>, Clifford J Rosen<sup>1</sup>**

1. Center for Clinical and Translational Research, Maine Medical Center Research Institute, Scarborough, Maine 04074, USA

2. Department of Bone and Mineral Research, Osaka Medical Center and Research Institute for Maternal and Child Health, Izumi, Osaka, 594-1101 Japan.

3. Department of Orthopaedics and Rehabilitation, Yale University School of Medicine, New Haven, Connecticut 06520, USA.

**Running head:** Diet regulation of bone mass in *Alox5*<sup>-/-</sup> mice

**Address correspondence and requests for reprints to;**

Dr. Clifford J Rosen

Maine Medical Center Research Institute, 81 Research Drive, Scarborough, ME, 04074-7205, USA

TEL: +1-207-885-8100

FAX: +1-207-885-8174

E-mail: [ROSENC@mmc.org](mailto:ROSENC@mmc.org)

**Key words;** *Alox5*, high fat diet, bone loss

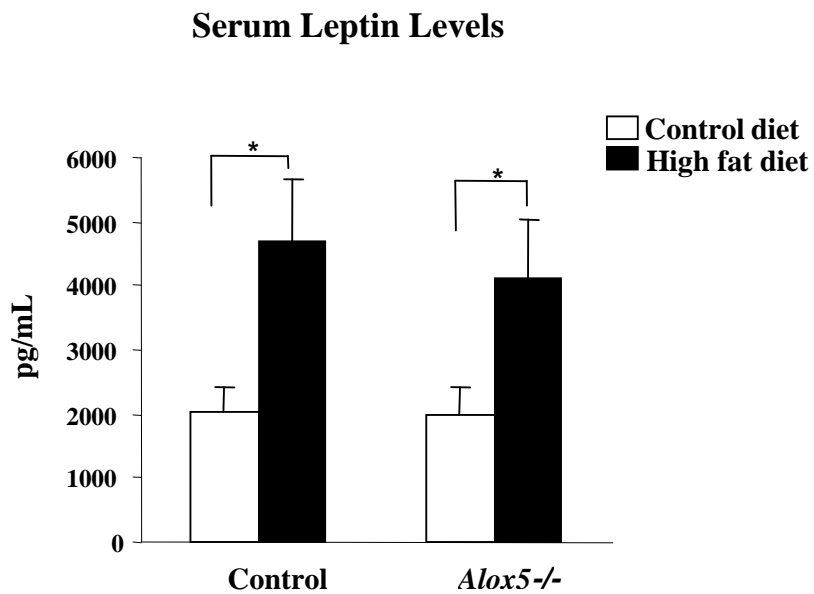
**Grant Support;** This work is supported by US National Institutes of Health grants: NIH AR 56404 (to CJR and MCH)

**Disclosure;** None

Supplemental Table S1

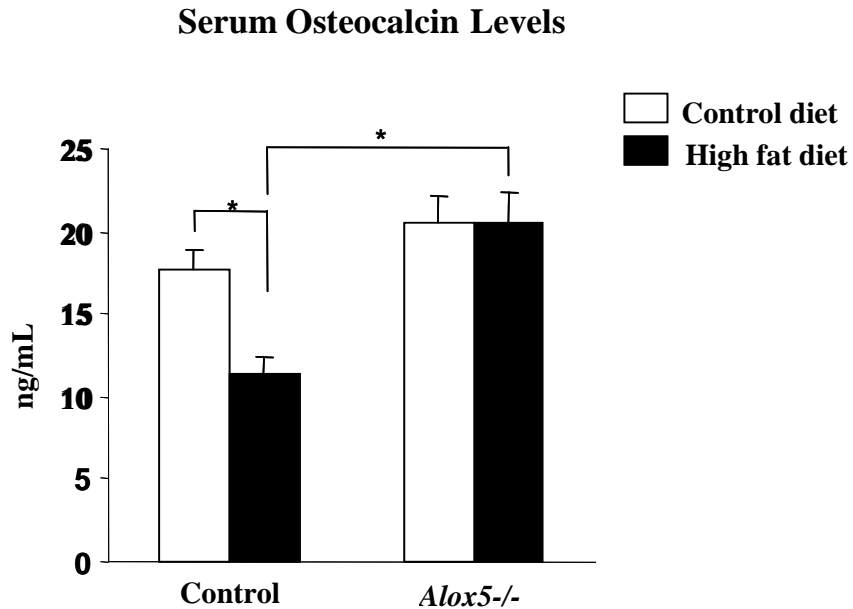
<b>Gene</b>	<b>Forward (5'-3')</b>	<b>Reverse (5'-3')</b>
<i>GAPDH</i>	TGA ACG GGA AGC TCA CTG G	TCC ACC ACC CTG TTG CTG TA
<i>Pparg<math>\gamma</math>2</i>	AAA CTC TGG GAG ATT CTC CTG TTG	GAA GTG CTC ATA GGC AGT GCA
<i>Pparg<math>\gamma</math></i>	TCA TCT CAG AGG GCC AAG GA	CAC CAA AGG GCT TCC GC
<i>Col1<math>\alpha</math></i>	GCT CCT CTT AGG GGG CAC	CCA CGT CTC ACC ATT GGG G
<i>RankL</i>	TGT TCC TGT ACT TTC GAG CG	GCA GGA GTC AGG TAG TGT G
<i>Opg</i>	TCC GGC GTG GTG CAA G	AGA ACC CAT CTG GAC ATT TTT TG
<i>Noc</i>	ACC AGC CAG ACA TAC TGT GC	CTT GGG GAA AAA CGT GCC T

Supplemental Figure S1



**Supplemental Figure S1. Serum leptin levels were comparable between controls and *Alox5* null mice.** *Alox5*<sup>-/-</sup> mice were fed a control diet (10% fat by kcal) or a high-fat diet (45% fat by kcal) from 3 weeks of age for 13 weeks. Serum was collected at 16 weeks of age and serum concentration of leptin was measured using ELISA (n=5-10). Values are expressed as the mean  $\pm$  SEM. \*p<0.05.

Supplemental Figure S2



**Supplemental Figure S2. Serum osteocalcin levels were significantly decreased in control mice on high fat diet but were unchanged in the *Alox5*<sup>-/-</sup> high fat diet.**

*Alox5*<sup>-/-</sup> mice were fed a control diet (10% fat by kcal) or a high-fat diet (45% fat by kcal) from 3 weeks of age for 13 weeks. Serum was collected at 16 weeks of age and serum concentration of osteocalcin was measured using ELISA (n=5-10). Values are expressed as the mean  $\pm$  SEM. \*p<0.05.

Supplemental Table S2

Histomorphometric analysis of B6 and *Alox5*<sup>-/-</sup>

	B6		<i>Alox5</i> <sup>-/-</sup>	
	Control diet	High-fat diet	Control diet	High-fat diet
MS/BS	35.28 ± 2.22	32.03 ± 2.45	34.56 ± 2.35	37.72 ± 3.19
MAR	2.01 ± 0.12	2.20 ± 0.15	2.06 ± 0.19	2.16 ± 0.18
BFR/BS	256 ± 0.91	253 ± 12.55	255 ± 11.80	303 ± 46
BFR/BV	1417 ± 158	1339 ± 115	1137 ± 103	1638 ± 218

Values are expressed as the mean ± SEM (n=5).

MS/BS; mineral surface/bone surface, MAR; mineral apposition rate, BFR/BS; bone formation rate/bone surface, BFR/BV; bone formation rate/bone volume