Dietary potassium regulates vascular calcification and arterial stiffness

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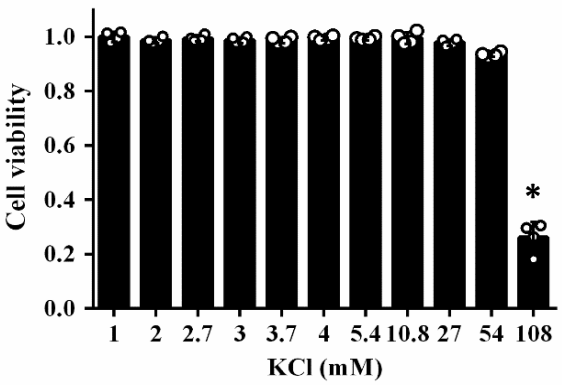
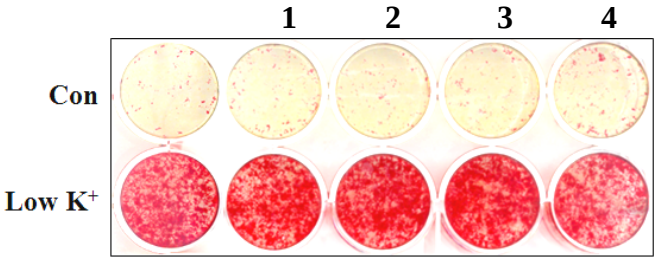
Running Title: Potassium regulates VSMC calcification

SUPPLEMENTAL FIGURE I



Supplemental Figure I. Effects of extracellular potassium on the viability vascular smooth muscle cells (VSMC). VSMC were cultured in osteogenic media with increased amounts of potassium, 1 to 108 mM, for 3 days and cell viability was determined by MTS assay. Results from four independent experiments performed in duplicate are shown. Bar values are means ± SD (n=4, **p*<0.05 compared with potassium at 5.4 mM). Statistical analysis was performed by one-way ANOVA followed by a Student-Newman-Keuls test.

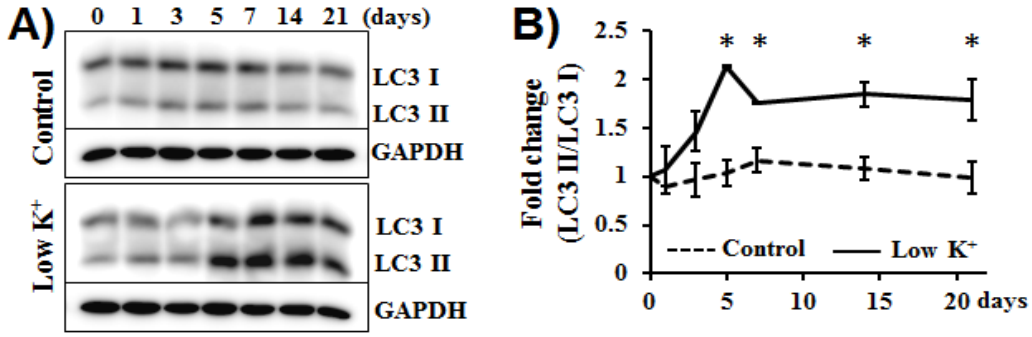
SUPPLEMENTAL FIGURE II



1) Glibenclamide — K_{ATP} inhibitor
2) 4-aminopyridine — K_V inhibitor
3) TRAM34 — K_{Ca} inhibitor
4) Charybdotoxin — K_{Ca} inhibitor

Supplemental Figure II. Inhibition of K_{ATP}, K_V, and K_{Ca} signaling did not affect low potassium-induced VSMC calcification. VSMC were cultured in osteogenic media containing 5.4 mM (Con) or 3.7 mM (Low K^+) potassium with the indicated potassium channel inhibitors: 1) glibenclamide (5 µM), 2) 4-aminopyridine (0.5 mM), 3) TRAM34 (5 nM) or 4) charybdotoxin (10 µM) for 3 weeks. Calcification was determined by Alizarin Red staining. Representative images from three independent experiments are shown.

SUPPLEMENTAL FIGURE III



Supplemental Figure III. Low potassium enhanced autophagy in VSMC. VSMC were cultured in osteogenic media with control or low potassium up to 3 weeks. **A)** Western blot analysis of the expression of autophagic marker, the microtubule-associated protein 1 light chain 3 (LC3), in the cytoplasmic form (LC3 I) and conjugated form (LC3 II). The expression of GAPDH was used as a loading control. Representative blots from three independent experiments are shown. **B)** Quantitative analysis of the LC3 II/LC3 I ratio, determined by densitometry and analyzed using ImageJ software (NIH Bethesda, MD) (n=3, *p<0.05 compared with potassium at 5.4 mM). Statistical analysis was performed by one-way ANOVA followed by a Student-Newman-Keuls test.

Supplemental Table 1. Serum electrolytes and other biochemical parameters in control and low dietary potassium-fed mice (*p<0.05, compared with control group)

	Unit	Low K^+		Control		High K^+	
		mean	SD	mean	SD	mean	SD
Na^+	(mEq/L)	152.2	2.1	151.0	1.7	155	2.4
K^+	(mEq/L)	3.70*	0.21	4.27	0.23	4.73*	0.15
Cl^-	(mEq/L)	111.4	0.9	111.3	2.1	116.3	3.6
Ca^{2+}	(mg/dL)	9.78	0.76	9.10	0.26	11.7	3.1
Mg^{2+}	(mg/dL)	2.96	0.34	2.73	0.15	3.53	1.24
BUN	(mg/dL)	29.0	4.3	28.0	1.73	34.3	9.6
Creatinine	(mg/dL)	0.23	0.15	0.25	0.07	0.36	0.11