L-lysine ameliorates vascular calcification in adenine-induced uremic rats

Akihiro Shimomura [1], Isao Matsui [1], Takayuki Hamano [2], Kazunori Inoue [1], Yasuo Kusunoki [1], Chikako Nakano [1], Yoshitsugu Obi [1], Yoshiharu Tsubakihara [2], Hiromi Rakugi [1], and Yoshitaka Isaka[1]

[1] Department of Geriatric Medicine and Nephrology, Osaka University Graduate School of Medicine
[2] Department of Comprehensive Kidney Disease Research, Osaka University Graduate School of Medicine

SP074

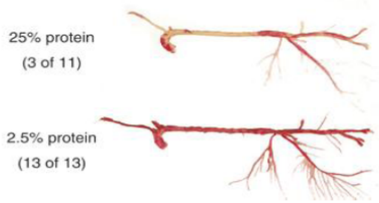
Introduction

Vascular calcification (VC) is one of the major problems in patients with chronic kidney disease (CKD) because satisfactory therapeutic strategies have not been established. Therefore, it is important to develop novel strategies.

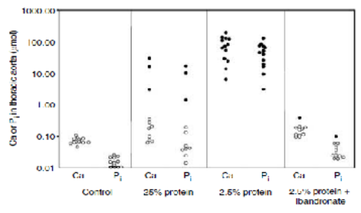
(Arterioscler Thromb Vasc Biol 1996;16:978–98)
(Nephrol Dial Transplant 2004;19:2387–2393)
(Nephrol Dial Transplant 2003;18:1731–1740)
(J Am Soc Nephrol 2009;20:1453-64)

Lowering the protein content of the diet dramatically exacerbated VC in adenine-induced uremic rats.

Alizarin red stain of the aorta



Mineral contents of thoracic aorta



(Kidney Int. 2006;70(9):1577-83)

Hypothesis

These results prompted us to hypothesize that supplementation of certain amino acid would ameliorate VC.

Amino acids (AAs) Essential / Non-essential

AAs with Hydrophobic Side Chains	AAs with Electrically Charged Side Chains (Positive)
Alanine (Ala)	Arginine (Arg)
Isoleucine (Ile)	Histidine (His)
Leucine (Leu)	Lysine (Lys)
Methionine (Met)	**AAs with Electrically Charged Side Chains (Negative)**
Phenylalanine (Phe)	Aspartic Acid (Asp)
Tryptophan (Trp)	Glutamic Acid (Glu)
Tyrosine (Tyr)	**AAs with Polar Uncharged Side Chains**
Valine (Val)	Serine (Ser)
Special Cases	Threonine (Thr)
Cysteine (Cys)	Asparagine (Asn)
Glycine (Gly)	Glutamine (Gln)
Proline (Pro)	

Among nine essential amino acids, L-Lys is the first-limiting amino acid in the most of cereal grains and has long been added to feed grains in order to improve the utility of feed proteins in the area of animal husbandry. Moreover, L-Lys supplementation has been reported to protect the bone from osteoporotic changes in humans. Because VC often coexists with bone loss, we focused on the therapeutic effects of L-Lys on VC in adenine-induced uremic rats.

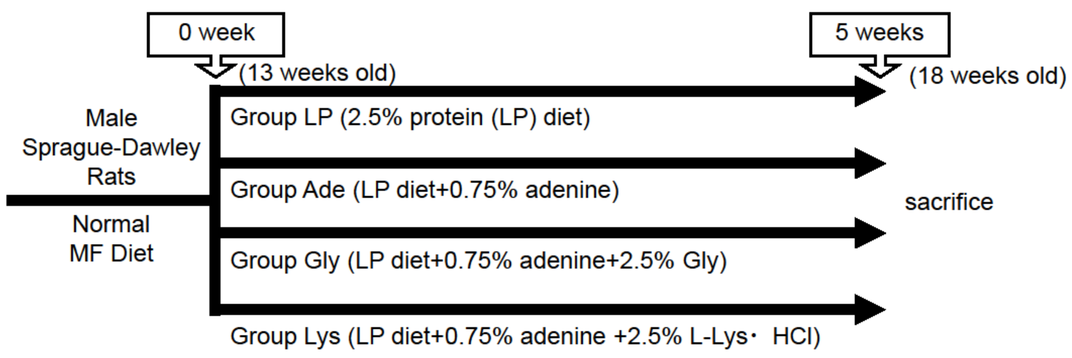
(Cereal Chemistry 1970;47:615-625)
(Journal of Animal Science 1974;38:941-946)
(Nutrition 1993;9:71-2)

Objective

To examine whether L-Lys supplementation ameliorates VC in adenine-induced uremic rats

Methods

Adenine-induced uremic rats preparation



Gly served as an amino-acid-control because it is the amino acid that has the simplest structure.

Calcium-phosphate precipitation assay

Although the mechanism of VC is multifactorial and incompletely understood, the passive deposition of apatite plays a role in the development of VC.

(Z Kardiol .2001; 90 suppl 3:116-124)

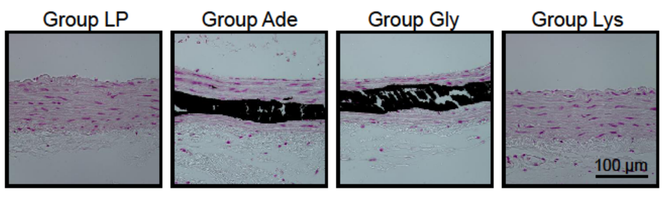
Because plasma levels of Ala, Pro, Arg, and homoarginine (Homo-Arg) in group Lys were significantly higher, we performed *in vitro* analyses using these amino acids.

500 mM HEPES (pH 7.4)
+ amino acids (pH7.4) 25 mM-100 mM
(Gly, L-Lys, L-Ala, L-Pro, L-Homo-Arg, L-Arg)
+ final 10 mM $CaCl_2$
+ final 10 mM phosphate buffer (pH 7.4)
↓ 10 min incubation at room temperature
Centrifuge 10 sec, 1,890 g
Quantification of pelleted calcium & phosphate

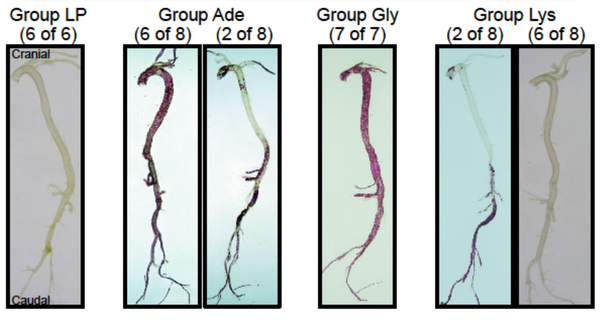
Results

L-Lys supplementation ameliorated VC without affecting renal function and physical parameters in adenine-induced uremic rats.

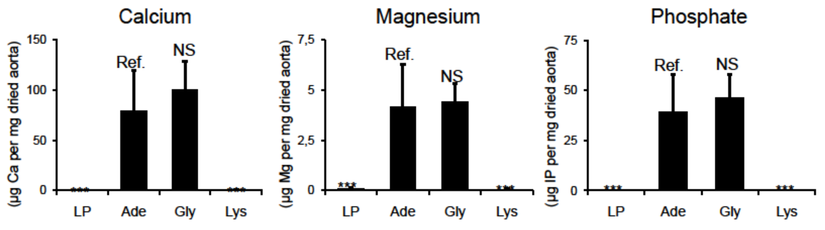
von Kossa-stained thoracic aorta (18 weeks old)



Alizarin red stain of the aorta (18 weeks old)

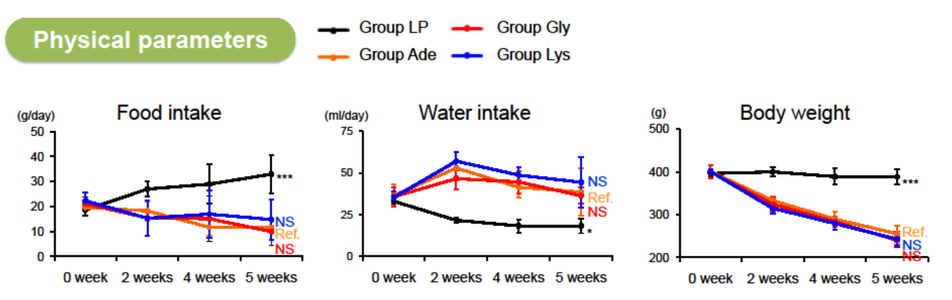


Mineral contents of the aorta (18 weeks old)



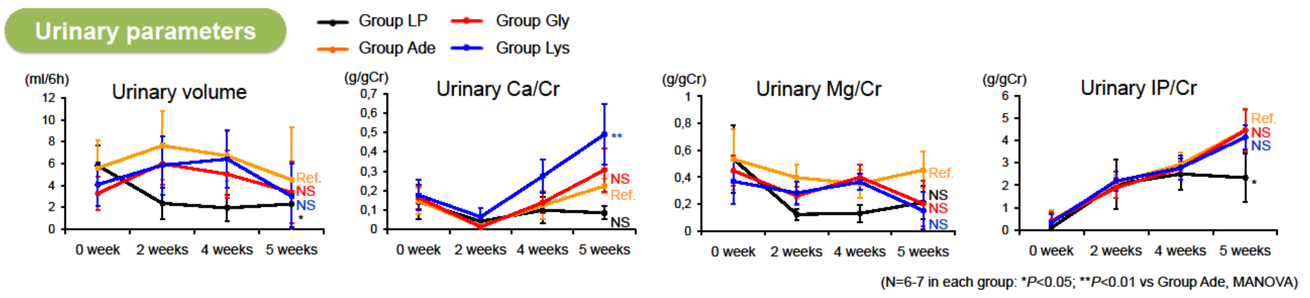
(N=6-8 in each group: ***P<0.001 vs Group Ade, Dunnett's test)

Physical parameters



(N=4-7 in each group: *P<0.05; ***P<0.001 vs Group Ade, MANOVA)

Urinary parameters

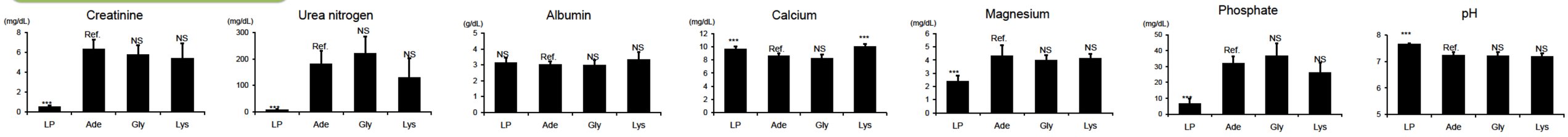


(N=6-7 in each group: *P<0.05; **P<0.01 vs Group Ade, MANOVA)

Serum Ca level in group Lys was slightly but significantly higher than those in groups Ade and Gly. Reflecting the high serum Ca, high urinary Ca/Cr ratio was observed in group Lys.

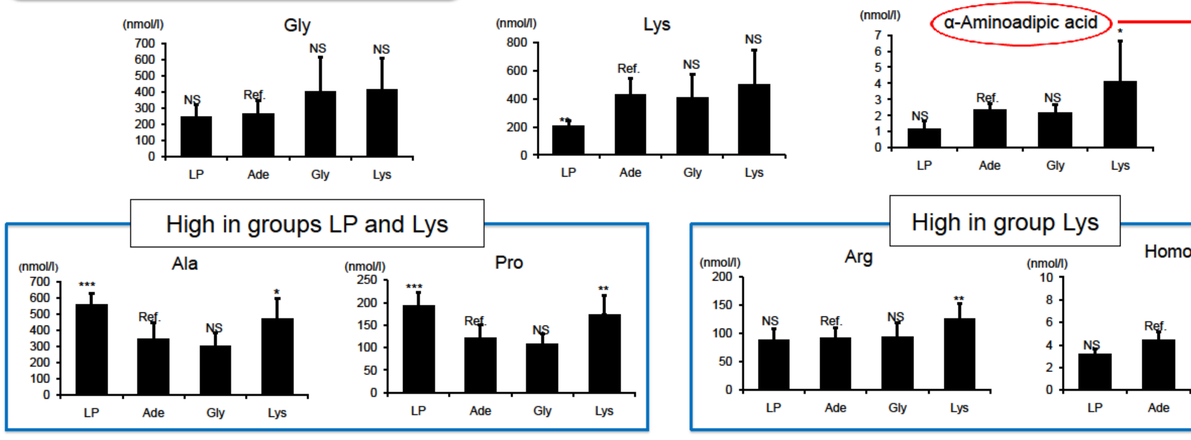
Serum parameters (18 weeks old)

(N=10 in each group: ***P<0.001 vs Group Ade, Dunnett's test)

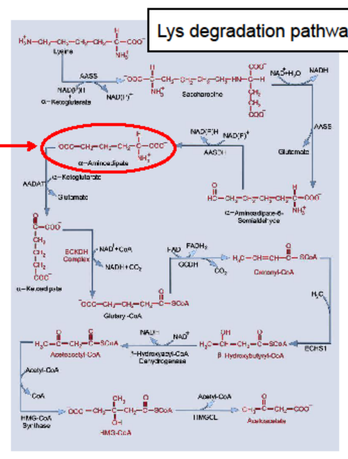


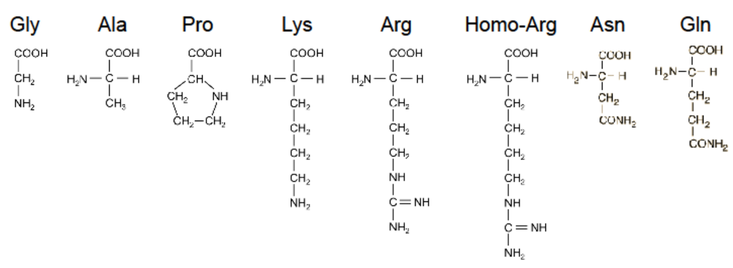
Plasma amino acids (18 weeks old)

(N=10 in each group: *P<0.05; **P<0.01; ***P<0.001 vs Group Ade, Dunnett's test)



Lys degradation pathway



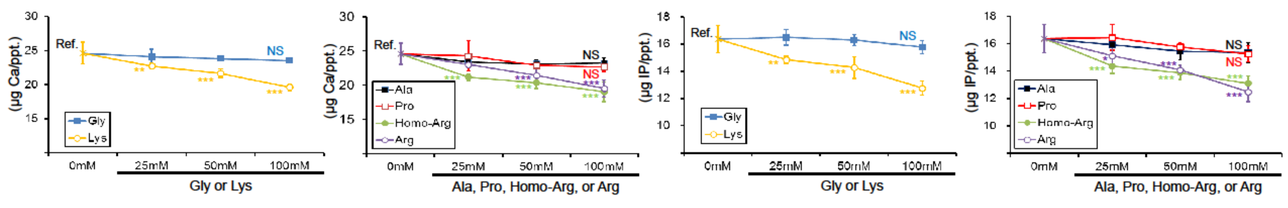


Although plasma Lys concentrations were not different among the adenine-loaded three groups, high plasma α-aminoadipic acid, a metabolite of Lys, in group Lys indicated that rats in group Lys really took L-Lys from their diet.

Calcium-phosphate precipitation assay

(N=5 in each group: *P<0.05; **P<0.01; ***P<0.001, Dunnett's test)

L-Lys, L-Arg, and L-Homo-Arg dose-dependently attenuated spontaneous precipitation of minerals in a solution of supersaturated Ca/P.



Conclusions

- L-Lys attenuated VC in adenine-induced uremic rats.
- In a solution of supersaturated Ca/P, Arg and Homo-Arg inhibited precipitation of minerals, thus suggesting that the elevation of plasma Arg and Homo-Arg explains the mechanism, at least in part, how VC was attenuated in group Lys.
- Our findings provide a novel approach for the treatment of VC in CKD.

OSAKA UNIVERSITY