

# COMBINATION OF OMEGA-3 FATTY ACID AND MENAQUINONE-7 PREVENTS PROGRESSION OF AORTIC CALCIFICATION IN ADENINE AND LOW PROTEIN DIET INDUCED RAT MODEL

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## Background

◆ Vascular calcification is common and progressing in chronic kidney disease and dialysis patients.  
[Blacher J et al, Hypertension 2001;38:938-942]

◆ Diet with high-dose menaquinone-7 (MK-7) (100 µg/g diet) inhibited the development of cardiovascular calcification in 5/6 nephrectomy rat combined with high phosphate diet.  
[Scheiber D et al, Nutrients 2015;7:6991-7011]

◆ Eicosapentaenoic acid (1 g/kg/day), one of omega-3 fatty acid (FA), attenuates arterial medical calcification induced by warfarin.  
[Kanai S et al, Atherosclerosis 2011;215:43-51]

◆ This study aimed to investigate whether the effect of omega-3 FA and NK-7 on aortic calcification in adenine and low protein diet induced vascular calcification rat model.

## Methods

◆ Male Sprague-Dawley rats were fed with the diets containing 0.75% adenine and 2.5% protein for 3 weeks.

◆ After 3 weeks, 4 rats were sacrificed for calcification evaluation of thoracic aorta.

◆ Thirty-two rats were randomly divided into four groups, which were treated and fed the diets containing 2.5% protein for 4 weeks.

- ✓ Adenine control group (n = 8) ; rats received saline (1mL/kg/day by gastric gavage)
- ✓ Adenine group treated with omega-3 FA (n = 8) → rats received omega-3 FA (300 mg/kg/day by gastric gavage)
- ✓ Adenine group treated with MK-7 (n = 8) → rats received MK-7 (50 µg/kg/day by gastric gavage)
- ✓ Adenine group treated with omega-3 FA and MK-7 (n = 8) → rats received both omega-3 FA and MK-7

◆ Normal control rats (n = 4) were fed the diets containing 2.5% protein for 7 weeks.

◆ For quantitative assessment of aortic calcification, von Kossa stain of aorta was done and calcium contents were measured with calcium colorimetric kit .

## Results

◆ Serum creatinine and BUN of adenine group treated with omega-3 FA and MK-7 was lower than adenine control group.

◆ Serum calcium were not significantly different between adenine group with treatment and without treatment.

◆ All treated groups and group without treatment were exposed to higher serum phosphorus level.

◆ Two rats among 4 rats showed aortic calcification at 3 weeks.

◆ After 4 weeks, aortic calcification was progressed in adenine group without treatment on von Kossa stain and calcium contents analysis of aorta.

◆ Aortic calcification on von Kossa stain and calcium contents was the least progressed in adenine control group treated with combination of omega-3 FA and MK-7 compared to omega-3 FA or MK-7 single therapy

Table 1 Laboratory data

	normal control	adenine control	Adenine with omega-3 FA	Adenine with MK-7	Adenine with omega-3 FA and MK-7	P value
BUN (mg/dL)	10.2±2.0	224.5±66.7*	80.9±33.3	83.5±31.5	70.8±18.5 <sup>a</sup>	0.008
Creatinine (mg/dL)	0.46±0.02	5.66±0.73*	3.99±0.33*	3.94±0.40*	3.85±0.36 <sup>a</sup>	<0.001
Calcium (mg/dL)	11.4±0.3	8.1±1.2	8.6±0.6	8.4±1.2	9.2±0.6	0.183
Phosphorus (mg/dL)	9.4±0.5	35.0±5.4*	23.8±1.9*	23.1±2.8 <sup>a</sup>	23.4±2.0 <sup>a</sup>	<0.001
Osteocalcin (ng/mL)	15.3±4.3	459.2±57.1*	475.2±18.6*	447.8±49.2*	469.9±29.8*	<0.001

Data are expressed as means ± SD

\*P value <0.05 (mean values are significantly different from normal control)

<sup>a</sup>P value <0.05 (mean values are significantly different from adenine control group)

Table 2 Quantitative assessment of aortic calcification

	normal Control	adenine control	Adenine with omega-3 FA	Adenine with MK-7	Adenine with omega-3 FA and MK-7	P value
Calcification scoring using Von kossa stain (%)	0.00±0.00	82.9±8.1*	37.5±15.8	36.3±14.0	23.5±10.8 <sup>a</sup>	0.004
Calcium concentration (µg/µL)	0.04±0.04	2.4±0.3*	1.4±0.4	1.2±0.3	1.1±0.3 <sup>a</sup>	0.001

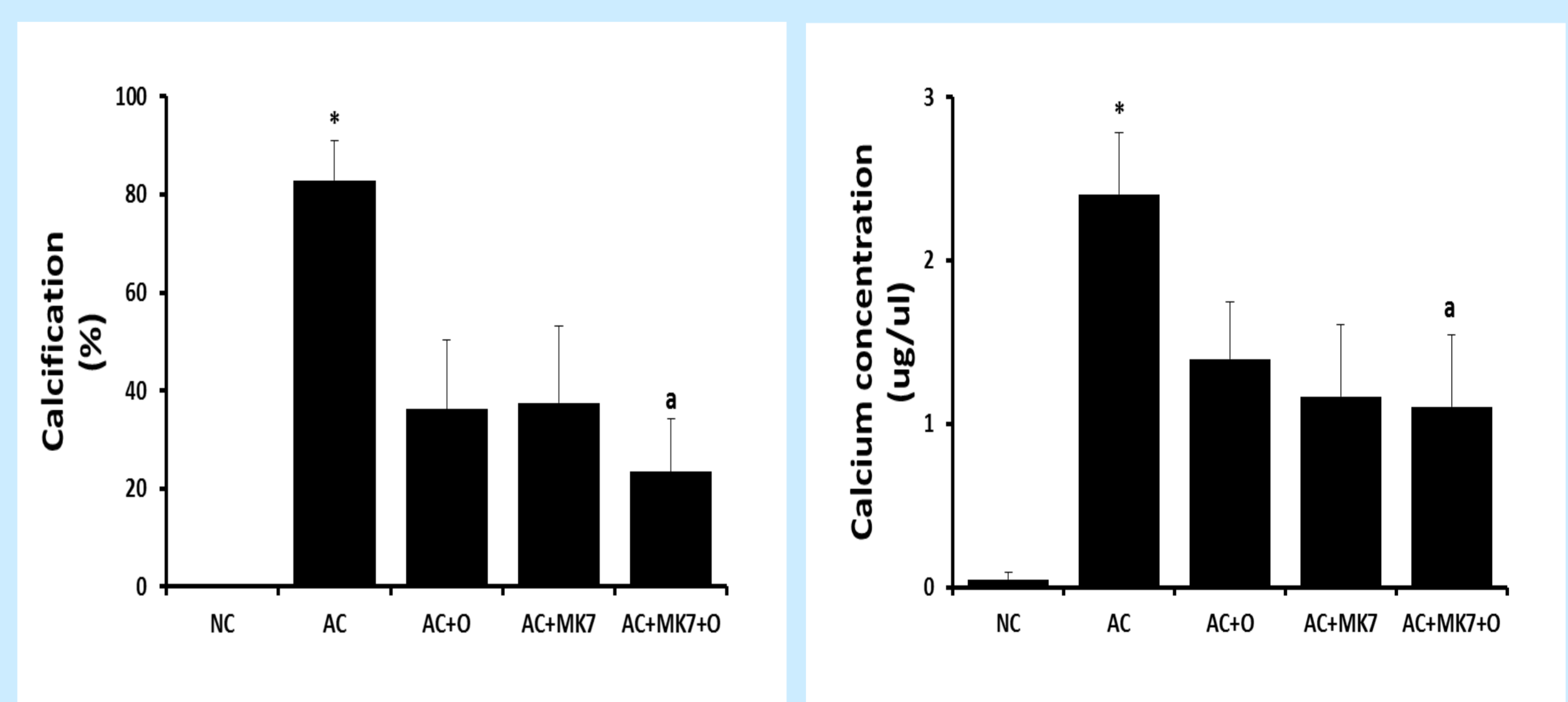
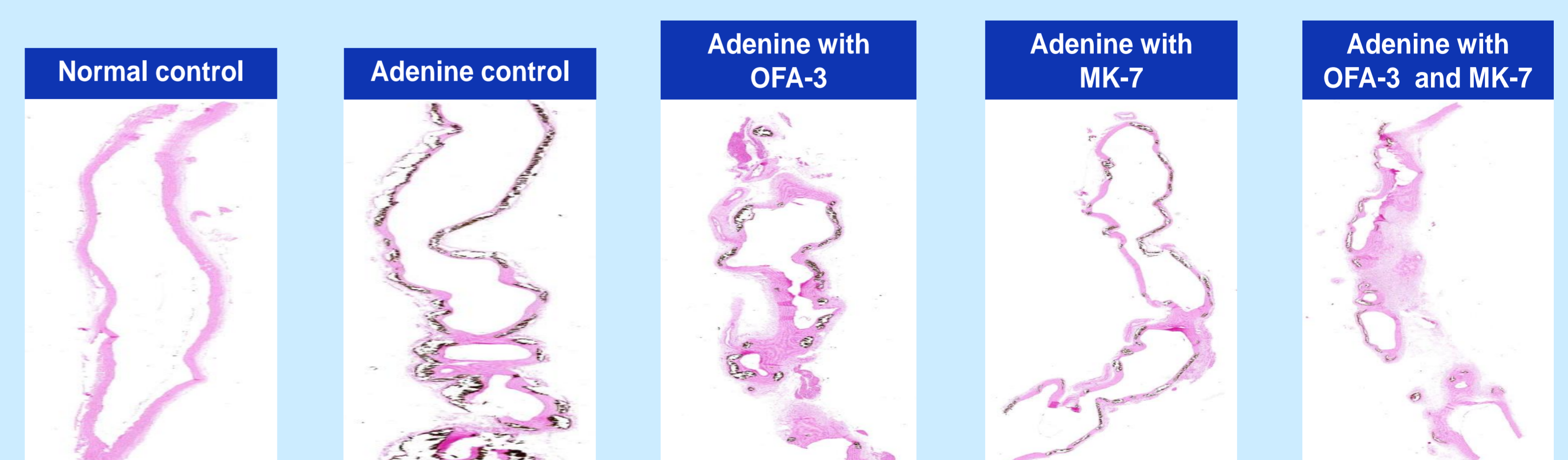


Figure 1 Aortic calcification on Von kossa in normal control, adenine control rats, adenine with omega-3 FA, adenine with MK-7, and both omega-3 FA and MK-7.



## Conclusions

◆ Combined treatment with omega-3 FA and MK-7 definitely prevents progression of aortic calcification compared to rat without treatment in adenine and low protein diet induced vascular calcification rat model