

**Abstract Submission No.: A-1167**

## **Synergistic Effects of Folate and Riboflavin on Vascular Calcification Amelioration in Uremic Rats**

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**Objectives :** Vascular calcification (VC) is a serious complication in chronic kidney disease (CKD) associated with increased morbidity and mortality. Elevated homocysteine levels have been linked to the development of VC. We investigated whether lowering homocysteine levels through folate and riboflavin supplementation affects vascular calcification in an animal model.

**Methods :** We divided rats into five groups: a control group and four CKD groups induced by adenine. Each CKD group received a specific diet for 8 weeks: restricted folate and riboflavin, restricted folate, restricted riboflavin, or a combination of folate and riboflavin. After 8 weeks, the rats were sacrificed, and blood, urine, aorta, kidney, and heart samples were collected.

**Results :** After 8 weeks, CKD rats developed azotemia, hyperphosphatemia, and hyperhomocysteinemia. Homocysteine and creatinine levels were highest in the restricted folate and riboflavin group, followed by the folate-restricted, riboflavin-restricted, and combined folate and riboflavin groups. VC was assessed using Von Kossa staining and immunohistochemical staining for osteocalcin on aorta sections. Compared to the control group, the study groups exhibited severe VC. Calcium content in the aorta, kidney, and heart was highest in the restricted folate and riboflavin group and lowest in the combined folate and riboflavin group. Western blot analysis showed similar RUNX2 and BMP2 protein expression levels. To investigate oxidative stress association, Smad 2/3 expression and NADPH oxidase mRNA levels were measured, revealing the highest levels in the restricted folate and riboflavin group and the lowest levels in the group with both folate and riboflavin intake.

**Conclusions :** Our study demonstrates that folate and riboflavin supplementation mitigates vascular calcification by reducing hyperhomocysteinemia in CKD animal models, possibly through a reduction in oxidative stress.

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