

# MEASURING THE AMOUNT OF VITAMIN K IN THE BODY FROM PRECISE QUANTIFICATION OF VITAMIN K<sub>3</sub> IN URINE

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## Background

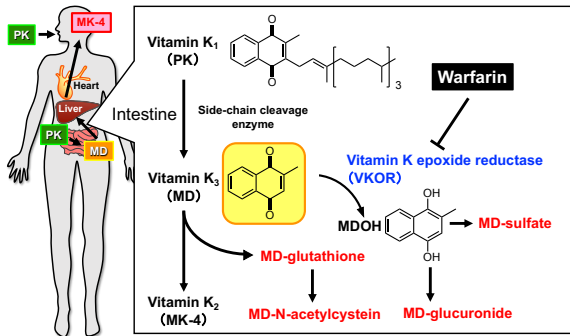


Fig.1 Mechanism of vitamin K metabolism

Vitamin K plays important role in blood coagulation and bone formation. Vitamin K is classified into different forms based on its chemical structure, specifically on the length and saturation of its side chain. Vitamin K<sub>1</sub> (Phylloquinone: PK) has a phytyl side chain, while Vitamin K<sub>2</sub> (Menaquinones: MK-n) has an isoprene side chain with a different number of repeating units and saturation level. Vitamin K<sub>3</sub> (Menadione: MD) doesn't have a side chain, and is a synthetic form of vitamin K (Fig.1). MD is not commonly found in the body, and is mostly in a conjugated form. Measuring vitamin K levels in the body can help identify vitamin K-related diseases, and we are working on finding non-invasive ways to measure vitamin K levels, such as by quantifying urinary MD levels.

## Methods and Results

### Determination of unconjugated MD by CA-derivatization

### Quantification of MD from urine in human

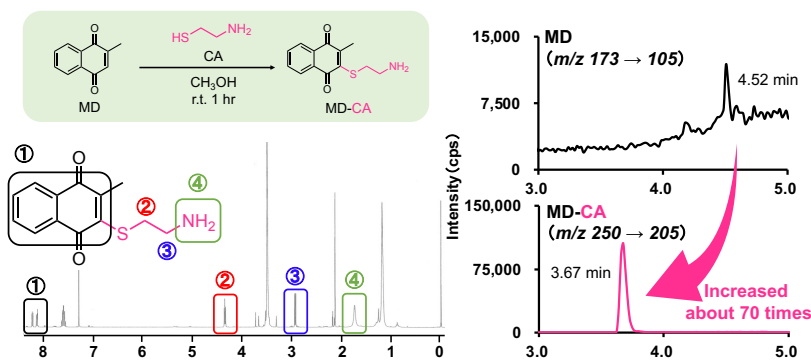


Fig.2 Structure of MD-CA and MS/MS spectrum of MD-CA

Unconjugated MD derivatized by CA (MD-CA) was measured by NMR, and the signals of the naphthoquinone ring and CA were detected. CA is bound to the 3-position of the naphthoquinone ring. The MD-CA was measured by UPLC-ESI-MS/MS. The detection sensitivity of MD-CA was increased about 70 times compared with unconjugated MD (Fig.2). These results indicated that the derivatization method enables precise quantification of unconjugated MD.

### Urinary MD

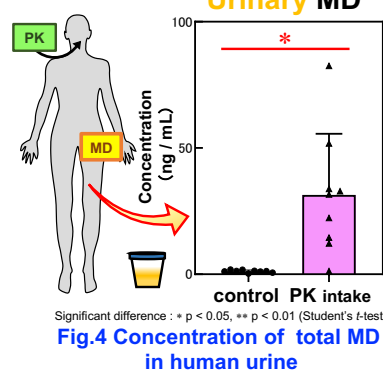


Fig.4 Concentration of total MD in human urine

The study collected urine samples from individuals who had taken 20 mg of PK before bedtime and from individuals who had not taken PK. This is done to compare the levels of MD in the urine of individuals who have taken PK and those who have not. The results of the study showed that the levels of MD in the urine increased with the amount of PK ingested (Fig.4).

### Synthesis of conjugated MD

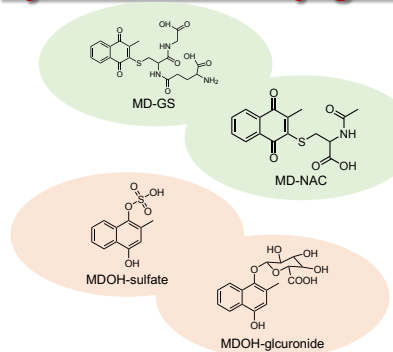


Fig.5 Synthesized conjugates MD and their structures

In order to measure the conjugate MD in the body, a standard of the expected conjugate MD was synthesized. The MD-glutathione (MD-GS) and MD-N-acetylcysteine (MD-NAC) were organically synthesized by reacting with the substrate compounds. The MDOH-glucuronide and MDOH-sulfate were synthesized by biological reaction using yeast-expressing metabolic enzymes. The structures of these conjugate MDs were determined by NMR and MS (Fig.5).

## Conclusion

We established a quantification method of MD. And, we found that urinary unconjugated MD derived from PK is increased than basal MD excretion after oral administration of PK. We have successfully synthesized four conjugate MDs. In the future, comprehensive quantitative analysis of conjugated MD in biological samples will contribute to a new understanding of the mechanism of vitamin K conjugation metabolism by clarifying the disposition of MD from conjugation to excretion (Fig.6).

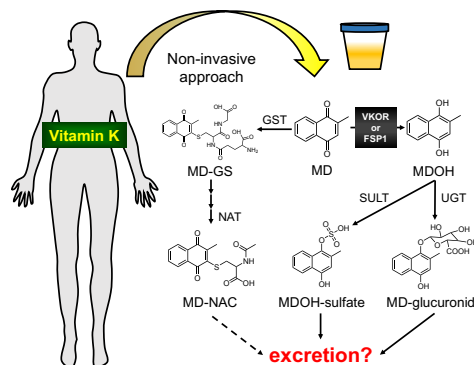


Fig.6 MD measurement from urine

### Quantification of unconjugated MD from urine in mice

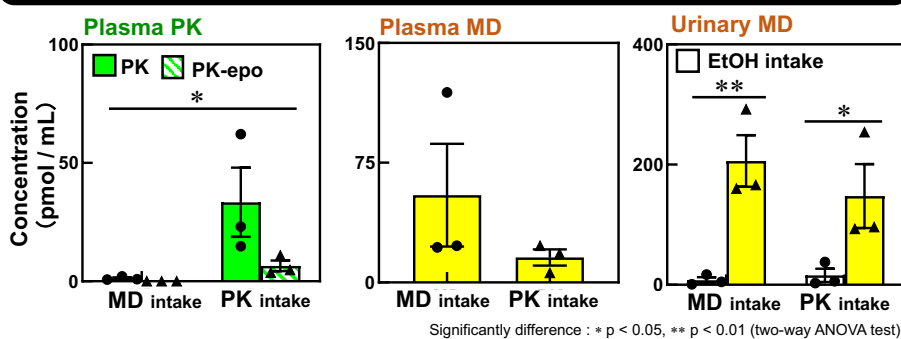
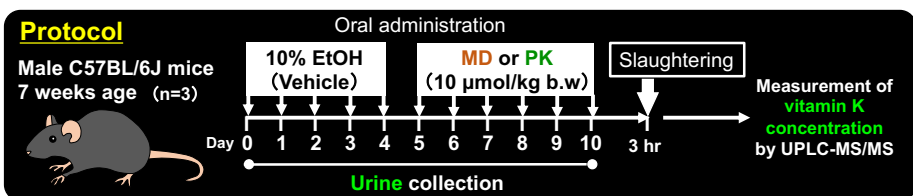


Fig.3 Concentration of unconjugated MD in mice urine

8-week-old C57BL/6J mice were used for the experiment and treated with MD or PK at 10 μmol/kg for 5 days. Plasma was obtained by slaughter 3 hours after administration. Mice were kept in metabolic cages, and urine was collected. Plasma vitamin K concentrations were measured, and it was confirmed that the administered MD and PK were transferred into the blood (Fig.3).

## Reference

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