

# COMBINED TREATMENT WITH OMEGA-3 FATTY ACID AND CHOLECALCIFEROL INCREASES 1,25 DIHYDROXY VITAMIN D LEVEL BY INHIBITING 24 HYDROXYLASE OF KIDNEY AND LIVER IN 5/6 NEPHRECTOMY RATS

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## Background

- The 1 $\alpha$ -hydroxylase (CYP27B1) and 24-hydroxylase (CYP24) in renal proximal tubules primarily involve vitamin D metabolism [Jones G et al, Physiol Rev 1998;78:1193-1231]
- Increased activity of CYP24 contributes vitamin D catabolism in chronic kidney disease (CKD) [Helvig CF et al, Kidney International 2010 78,463-472]
- Recent reports showed that CYP27B1 was strongly expressed in monocytes developing into hepatic macrophages and omega-3 fatty acid (FA) elevated 1,25-dihydroxyvitamin D [1,25(OH)<sub>2</sub>D] level in dialysis patients with scanty renal function [Vuica A et al, Exp Gerontol. 2015;72:167-76] [An WS et al, Nutr Res 2012;32:495-502]
- This study aimed to investigate whether the effect of omega-3 FA and cholecalciferol on vitamin D metabolism are related with the activity of CYP27b1 and CYP24 in liver and kidney of 5/6 nephrectomy (Nx) rat model.

## Methods

- Male Sprague-Dawley rats were divided into five groups and treated for 6 weeks
  - Control group (n = 5) ; rats received saline (1mL/kg/day by gastric gavage)
  - 5/6 subtotal nephrectomy (Nx) (n = 6) → rats received saline (1mL/kg/day by gastric gavage)
  - 5/6 Nx treated with vitamin D (n = 6) → rats received cholecalciferol (3000 IU/kg/week by gastric gavage)
  - 5/6 Nx treated with Omega-3 FA group (n = 6) → rats received omega-3 FA (300 mg/kg/day by gastric gavage)
  - 5/6 Nx treated with Vitamin D and Omega-3 FA group (n = 6) → rats received both cholecalciferol (3000 IU/kg/week) and omega-3 FA

- Measurements
  - Expression of CYP27B1 and CYP24 were examined by using western blot
  - Serum 25-hydroxyvitamin D [25(OH)D] and 1,25(OH)<sub>2</sub>D levels were checked

## Results

- Serum BUN and creatinine levels were the lowest in 5/6 Nx group treated with omega-3 FA and vitamin D among other 5/6 Nx groups
- The levels of serum 25(OH)D and 1,25(OH)<sub>2</sub>D were the highest in 5/6 Nx group treated with omega-3 FA and vitamin D among other 5/6 Nx groups
- The expression of CYP24 was significantly increased in remnant kidney and liver of 5/6 Nx control compared to sham control
- Increased expression of CYP24 in remnant kidney and liver of 5/6 Nx control was significantly decreased by combined treatment with omega-3 FA and cholecalciferol
- The expression of CYP27B1 was significantly increased in remnant kidney and significantly decreased in liver of 5/6 Nx control compared to sham control
- The increased expression of CYP27B1 in remnant kidney and decreased expression of CYP27B1 in liver of 5/6 Nx control was nearly normalized by combined treatment with omega-3 FA and vitamin D

Table 1. Laboratory Data

	normal control	5/6 Nx	5/6 Nx with vitamin D	5/6 Nx with omega-3 FA	5/6 Nx with omega-3 FA and vitamin D	P value
BUN (mg/dL)	17.7±1.5	77.7±28.4*	75.3±22.1*	63.9±17.0*	51.3±8.7 <sup>ab</sup>	0.003
Creatinine (mg/dL)	0.4±0.0	1.3±0.6*	1.2±0.3*	1.0±0.3*	0.8±0.1 <sup>abc</sup>	0.002
Calcium (mg/dL)	6.8±0.3	6.9±0.7	6.5±0.4	7.2±1.0	6.6±0.7	0.502
Phosphorus (mg/dL)	8.4±0.4	9.7±4.0	8.1±0.8	8.2±0.6	7.8±0.5	0.261
25(OH)D	97.5±5.6	27.8±16.5*	50.4±41.7	61.6±38.5 <sup>a</sup>	111.1±37.1 <sup>abc</sup>	0.003
1,25(OH) <sub>2</sub> D	170.9±40.5	44.6±16.9*	58.0±41.0*	83.1±42.3*	107.9±43.3 <sup>ab</sup>	0.002

Figure 1. Renal Function and Serum level of Vitamin D

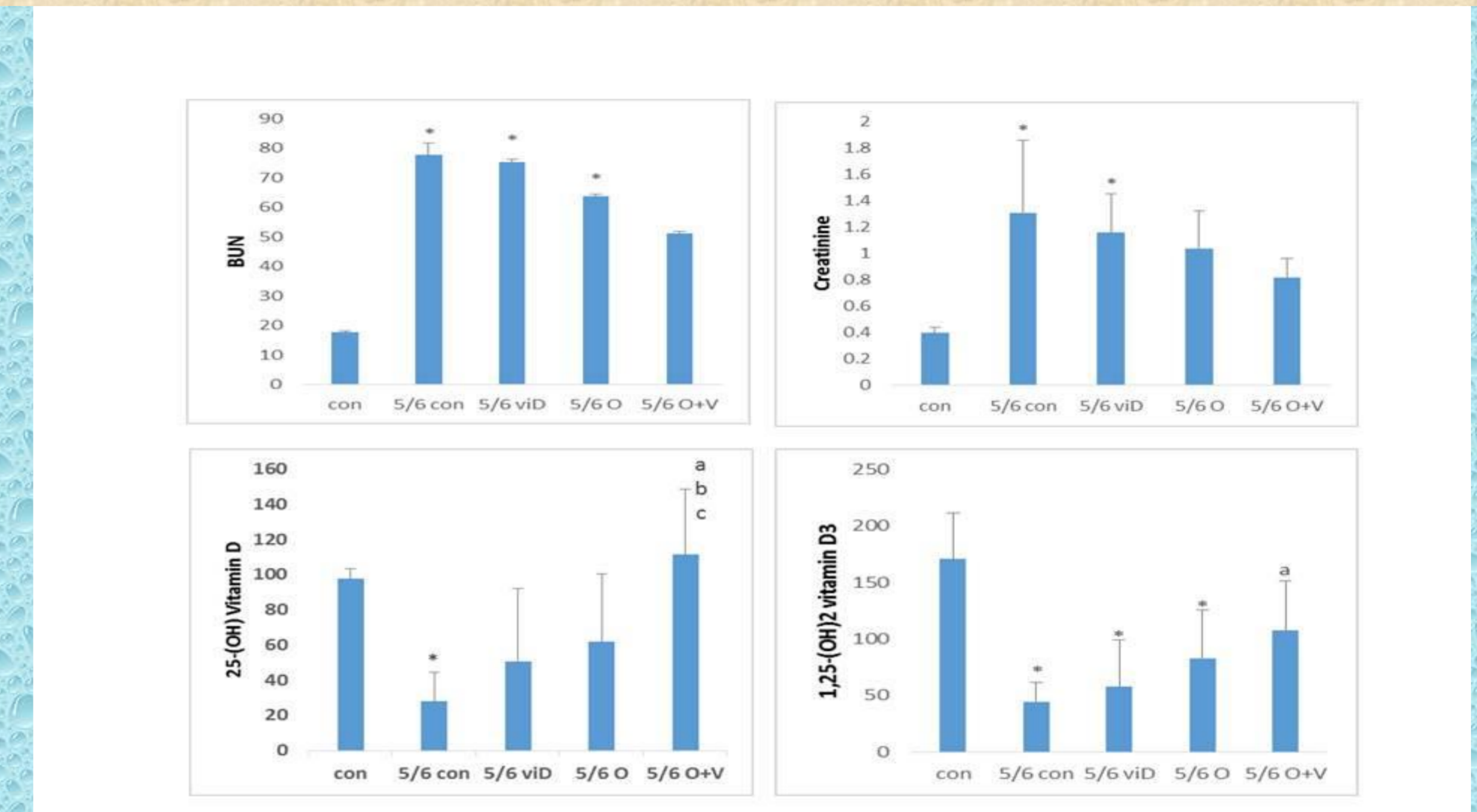


Figure 2. 24-hydroxylase (CYP24) and 1 $\alpha$ -hydroxylase (CYP27B1) expression in the remnant kidney

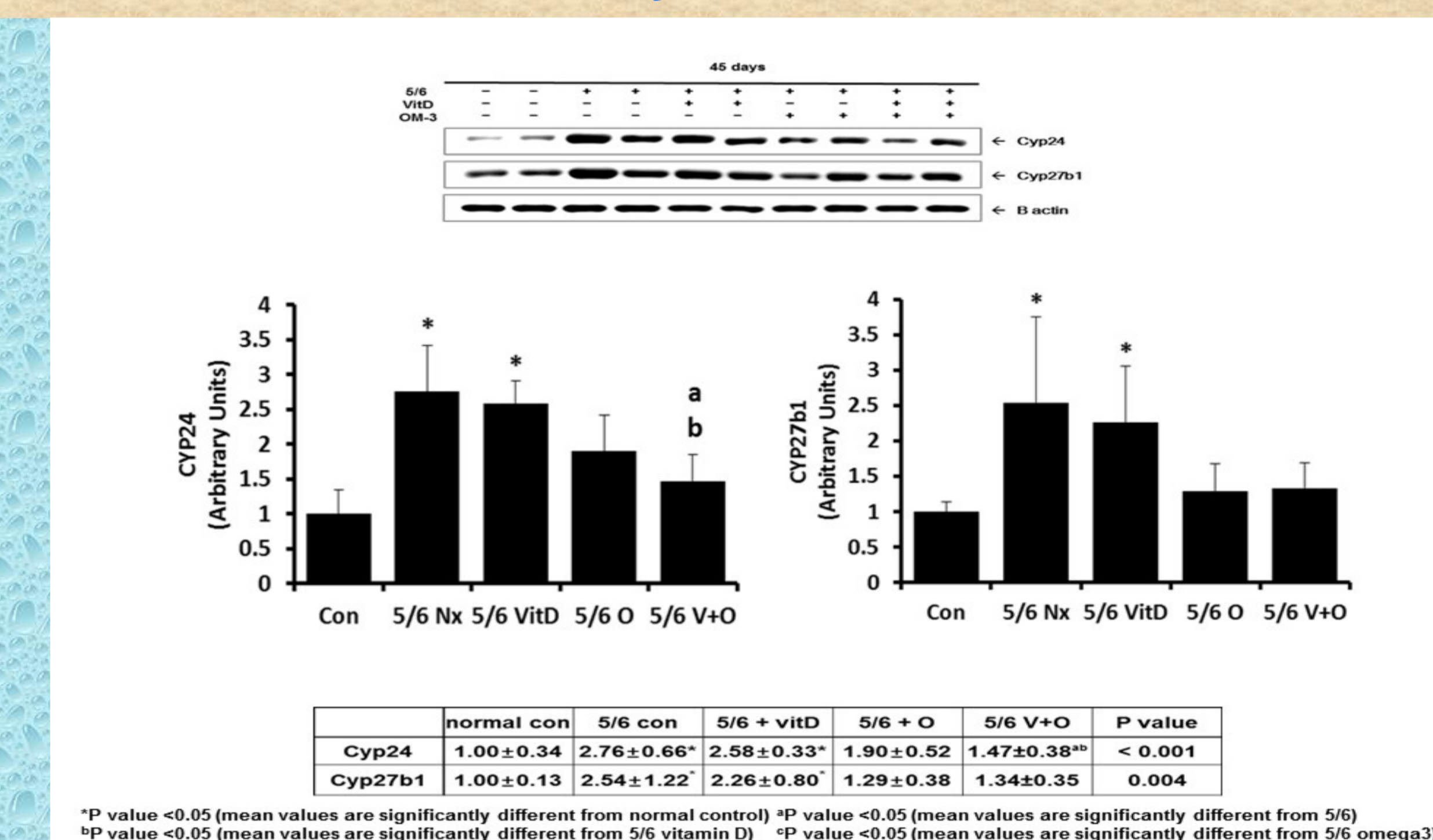
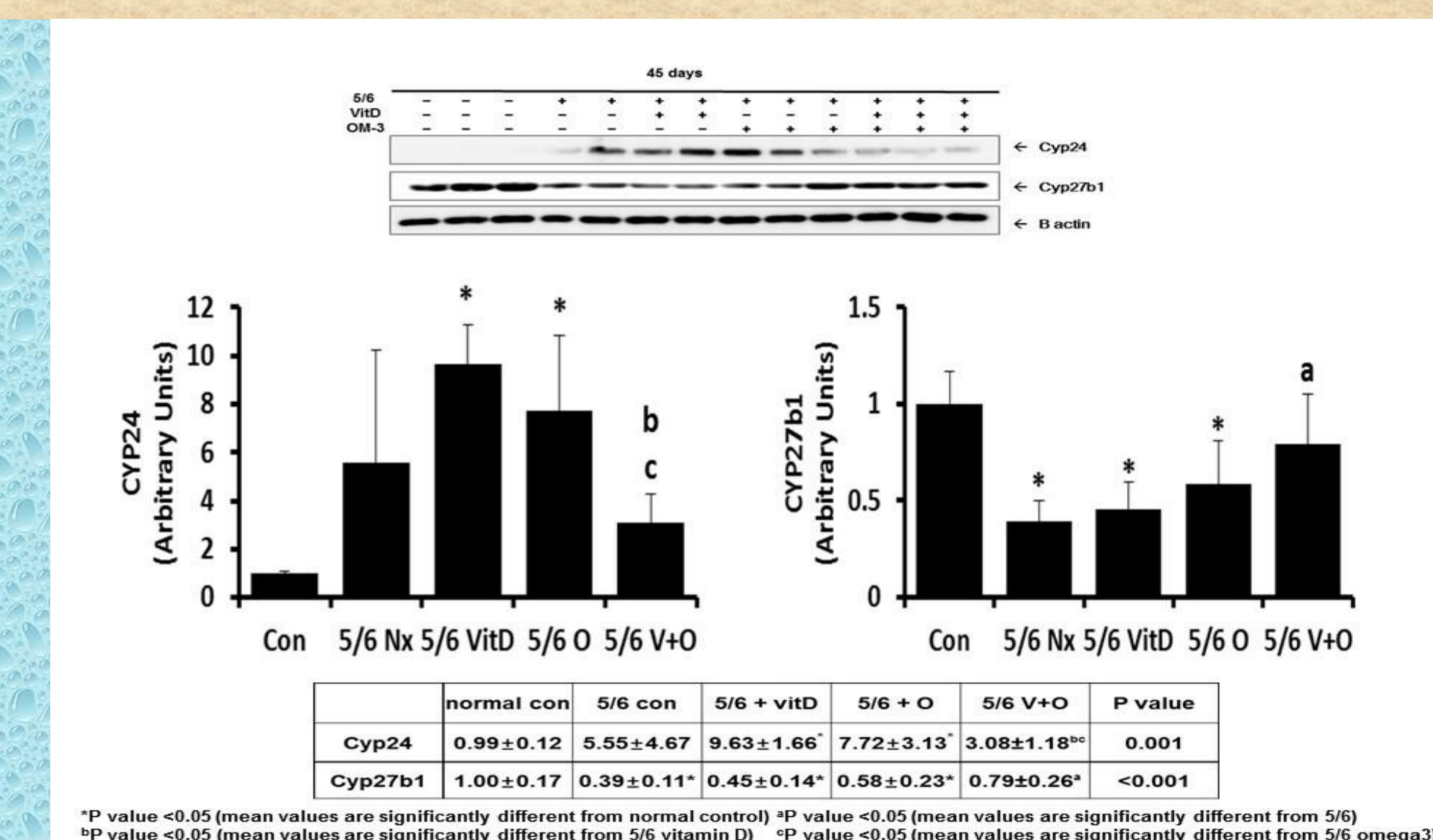
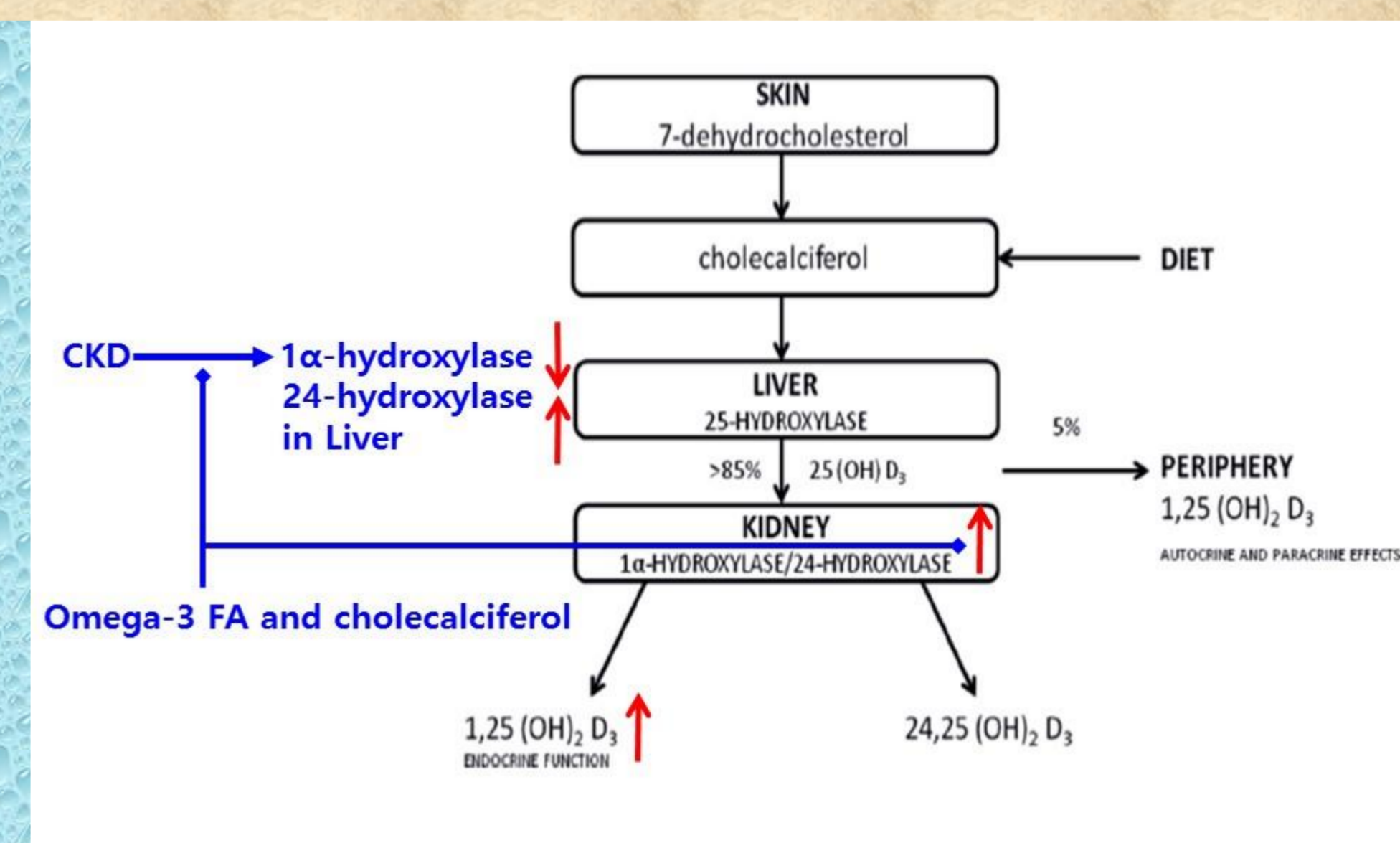


Figure 3. 24-hydroxylase (CYP24) and 1 $\alpha$ -hydroxylase (CYP27B1) expression in the liver



## Discussion

- To our knowledge, this is the first report to show decreased 1 $\alpha$ -hydroxylase expression in the liver of CKD model. Therefore, we pay attention to the role of liver in the aspect of vitamin D metabolism in CKD.
- The source of increased expression of 1 $\alpha$ -hydroxylase in remnant kidney and decreased expression of 1 $\alpha$ -hydroxylase in the liver should be elucidated in CKD model.



## Conclusions

- Combined treatment with omega-3 FA and cholecalciferol definitely increases 1,25(OH)<sub>2</sub>D level by inhibiting expression of CYP24 in remnant kidney and liver and activating expression of CYP27B1 in liver of 5/6 Nx rats