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Determinants of circulating 1,25-dihydroxyvitamin D_3 levels: the role of renal synthesis and catabolism of vitamin D☆

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Abstract

Details of the molecular mechanisms determining levels of the secosteroid, 1,25-dihydroxyvitamin D_3 (1,25D) remain to be elucidated. The current paradigm for the control of serum 1,25D levels is the tight regulation of renal 25-hydroxyvitamin D-1α-hydroxlase (CYP27B1) activity by a number of physiological factors. 1,25D production is also regulated by the cytochrome P450 enzyme, 25-hydroxyvitamin D-24-hydroxylase (CYP24), which through side chain hydroxylation reactions, inactivates 1,25D. We have recently demonstrated that renal CYP27B1 and CYP24 expression contribute equally to regulating serum 1,25D levels. We now describe the contribution of renal Vitamin D receptor (VDR) expression in determining serum 1,25D levels. Serum 1,25D levels were decreased when the dietary calcium intake was increased. We measured mRNA levels for CYP27B1, CYP24 and VDR in kidney RNA extracts from animals fed diets containing different levels of calcium, ranging from 0.05 to 1%. Serum 1,25D levels were negatively correlated with renal CYP24 mRNA levels ($R^2 = 0.35$, $P < 0.01$) while renal VDR is positively correlated with renal CYP24 mRNA ($R^2 = 0.80$, $P < 0.001$). However, only renal VDR mRNA remained a significant determinant of renal CYP24 expression when both these variables were included in multiple linear regression analysis (multiple $R^2 = 0.89$, $P < 0.001$). These findings suggest that kidney CYP24 activity acts in concert with kidney CYP27B1 to control serum 1,25D levels and that serum 1,25D stimulates renal CYP24 expression by acting through the renal VDR.

Keywords: 25-Hydroxyvitamin D-1α-hydroxylase; 25-Hydroxyvitamin D-24-hydroxylase; Vitamin D receptor; Kidney; Quantitative real-time reverse transcriptase–polymerase chain reaction; Taqman™

1. Introduction

The biological activity of 1,25-dihydroxyvitamin D (1,25D) is determined by the regulation of three genes, two coding for the enzymes required for 1,25D synthesis and catabolism and a third gene for the Vitamin D receptor (VDR). The renal enzyme, 25-hydroxyvitamin D 1α-hydroxylase (CYP27B1), catalyses the synthesis of circulating 1,25D, while the catabolism of this metabolite is catalysed by the enzyme, 25-hydroxyvitamin D-24-hydroxylase (CYP24), which occurs in the kidney and in extra-renal tissues that contain the VDR [1]. The renal regulation of both CYP27B1 and CYP24 enzyme activities are effected by several physiological factors [1–3], which modulate 1,25D production.

1,25D plays a primary role to regulate blood calcium levels within a narrow range by modulating calcium fluxes such as intestinal absorption and renal tubular reabsorption of calcium [1]. In response to hypocalcemia, PTH strongly induces CYP27B1 activity [4] and inhibits CYP24 activity [5,6]. In response to hypercalcemia, calcitonin suppresses calcium resorption in bone [7]. Dietary calcium intake modulates serum 1,25D levels presumably through the actions of these calciotropic hormones where low dietary calcium increases serum 1,25D [8]. The renal levels of CYP27B1 and CYP24 mRNA levels are both significant determinants of serum 1,25D under these conditions while renal VDR mRNA levels are markedly increased at the higher dietary calcium intakes [8].

We now report the contribution of renal VDR mRNA in determining serum 1,25D levels under conditions of varying dietary calcium intake in a rat model.

2. Material and methods

Twenty four Sprague–Dawley female rats were allocated to four different dietary treatment groups. The AIN-93-VX

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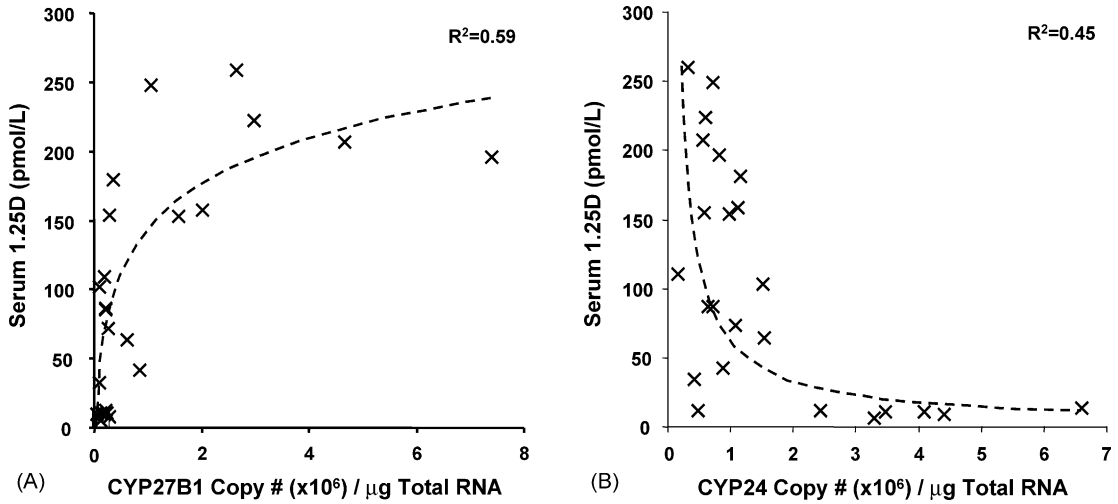

Fig. 1. Relationship between serum 1,25D (pmol/l) and kidney CYP27B1 mRNA levels (A) or kidney CYP24 mRNA levels (B) (copy numbers per microgram total RNA). The coefficient of determination (R^2) is shown for each regression analysis. 1,25D, 1,25-dihydroxyvitamin D_3; CYP27B1, 25-hydroxyvitamin D-1α-hydroxylase; CYP24, 25-hydroxyvitamin D-24-hydroxylase.

semi synthetic diets (ICN Biomedicals Australasia, Seven Hills, Australia) were prepared in the laboratory according to a standard formula. The amount of $CaCO_3$ added was varied to obtain either a 0.05, 0.2, 0.4 or a 1% calcium diet. Animals were housed in a 12 h light/dark cycle and water was provided ad libitum. Rats were sacrificed at the ages of 3, 6, 9, 12, 15, 26, 52, and 104 weeks of age.

2.1. Biochemical analysis

Non-fasting blood samples were collected at time of death. Serum 1,25D was measured by a ^{125}I radioimmunoassay (Immunodiagnostic Systems Ltd., Bolden, UK).

2.2. Messenger RNA analyses

The isolation of RNA from rat kidney was performed by a phenol/chloroform extraction method. First strand cDNA synthesis was performed as previously described [8]. Quantification of CYP27B1, CYP24, and VDR mRNA by real-time RT-PCR was performed using specific primers (Geneworks, Adelaide, Australia) and Taqman® fluorgenic probes (Perkin-Elmer Applied Biosystems, CA, USA), as previously described [8].

2.3. Data expression and statistical analyses

The effect of dietary calcium on biochemical markers, CYP27B1, CYP24, and VDR mRNA were statistically analysed with one-way analysis of variance. A Tukey's post-hoc test was used to identify the differences between age groups. Multiple linear regression analyses were used to determine the relationship between mRNA levels of specific target gene and two or more variables when each variable demonstrated a significant linear relationship.

3. Results

3.1. Determinants of serum 1,25D levels

Serum 1,25D levels correlated positively with kidney CYP27B1 mRNA levels ($R^2 = 0.38$, $P < 0.01$) and negatively with kidney CYP24 mRNA levels ($R^2 = 0.35$, $P < 0.01$) (Fig. 1). Renal CYP24 mRNA levels were strongly correlated with renal VDR mRNA ($R^2 = 0.80$, $P < 0.001$,

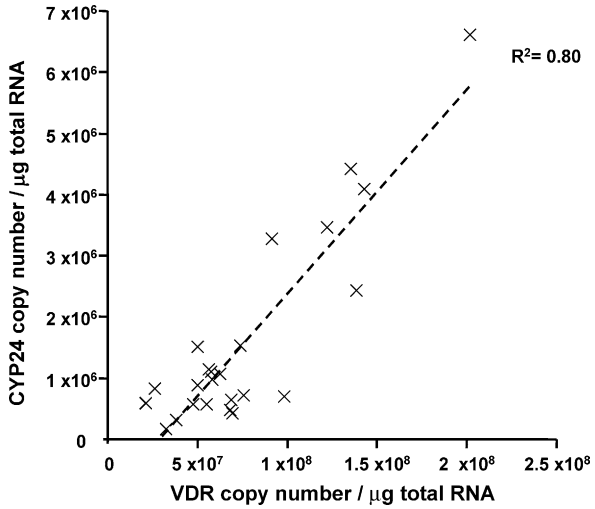

Fig. 2. Relationship between kidney CYP24 and kidney VDR mRNA expression (copy numbers per microgram total RNA). The coefficient of determination (R^2) is shown for the linear regression analysis. CYP24, 25-hydroxyvitamin D-24-hydroxylase; VDR, Vitamin D receptor.

Table 1
Single and multiple linear regression equations for serum 1,25D and renal VDR mRNA expression as determinants of CYP24 mRNA

Independent variable	Equation	R^2	P-value
1,25D	$CYP24 = -1.13 \times 10^{-4}$ (1,25D) $+ 2.8 \times 10^{6}$	0.35	0.002
VDR	$CYP24 = 3.3 \times 10^{-2}$ (VDR) $- 9.6 \times 10^{5}$	0.80	4.8×10^{-9}
1,25D+VDR	$CYP24 = 156.5$ (1,25D) $+3.4 \times 10^{-2}$ (VDR) $- 9.9 \times 10^{5}$	– Multiple $R^2 = 0.89$	0.95 1×10^{-6}

1,25D, 1,25 dihydroxyvitamin D_3; CYP24, 25-hydroxyvitamin D-24-hydroxylase; VDR vitamin D receptor. ($n = 19$).

Fig. 2). In a multiple linear regression analysis with serum 1,25D and renal VDR mRNA, only the renal VDR mRNA level remained a significant determinant of CYP24 mRNA levels (Table 1).

4. Discussion

1,25D is essential for the stimulation of intestinal calcium absorption [9,10]. Serum 1,25D levels fall with increasing dietary calcium and correlate positively with kidney CYP27B1 mRNA levels and negatively with kidney CYP24 mRNA levels. Multiple linear regression analysis indicates that the fall in serum 1,25D levels is associated with both a reduction in CYP27B1 mRNA and an increase in CYP24 mRNA in the kidney [8]. These data suggest that the decrease in serum 1,25D with increased dietary calcium intake is the result the reduction in the synthesis of 1,25D by kidney CYP27B1 and to the increase in the catabolism of 1,25D by kidney CYP24. Both these enzymes appear to be equally important in the regulation of the metabolism of Vitamin D in the kidney. Renal VDR mRNA levels increase with dietary calcium intake [8]. In this study, CYP24 mRNA levels correlated positively with VDR mRNA levels. A concomitant rise in kidney VDR and CYP24 mRNA levels has been indicated previously by Johnson et al. [11]. They showed that mRNA and protein levels of renal VDR and CYP24 were both high in 18-month-old female Fischer 344 rats and both low in 6-month-old or younger rats. Liganded VDR can directly regulate CYP24 gene expression by binding to one of two distinct Vitamin D response elements (VDRE) which are located in the promoter region of the CYP24 gene [12]. Multiple linear regression analysis suggests that serum 1,25D acts to repress CYP24 mRNA expression only through the regulation of renal VDR mRNA.

When studying the metabolism of Vitamin D in the kidney, the regulation of CYP27B1 activity should not be the only focus of investigation. Equal importance should be attributed to the effects of the catabolising enzyme, CYP24. Since both enzymes appear to be regulated by factors such as 1,25D as well as other calciotropic hormones including PTH, calcium and calcitonin, future research should focus on the mechanisms by which these factors control Vitamin D synthesis and catabolism under different physiological conditions.

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