Serum concentrations of 1,25-dihydroxycholecalciferol and 25-hydroxycholecalciferol in clinically normal dogs and dogs with acute and chronic renal failure

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Objective—To compare serum concentrations of 1,25-dihydroxycholecalciferol (1,25-(OH)\(_2\)D\(_3\)) and 25-hydroxycholecalciferol (25-(OH)D\(_3\)) in healthy control dogs and dogs with naturally occurring acute renal failure (ARF) and chronic renal failure (CRF).

Animals—24 control dogs, 10 dogs with ARF, and 40 dogs with CRF.

Procedure—Serum concentrations of 1,25-(OH)\(_2\)D\(_3\) were measured by use of a quantitative radioimmunoassay, and serum concentrations of 25-(OH)D\(_3\) were measured by use of a protein-binding assay.

Results—Mean ± SD serum concentration of 1,25-(OH)\(_2\)D\(_3\) was 153 ± 50 pmol/L in control dogs, 75 ± 25 pmol/L in dogs with ARF, and 83 ± 66 pmol/L in dogs with CRF. The concentration of 1,25-(OH)\(_2\)D\(_3\) did not differ significantly between dogs with ARF and those with CRF and was in the reference range in most dogs; however, the concentration was significantly lower in dogs with ARF or CRF, compared with the concentration in control dogs. Mean ± SD concentration of 25-(OH)D\(_3\) was 267 ± 97 nmol/L in control dogs, 130 ± 82 nmol/L in dogs with ARF, and 84 ± 60 nmol/L in dogs with CRF. The concentration of 25-(OH)D\(_3\) was significantly lower in dogs with ARF or CRF, compared with the concentration in control dogs.

Conclusions and Clinical Relevance—The concentration of 1,25-(OH)\(_2\)D\(_3\) was within the reference range in most dogs with renal failure. Increased serum concentrations of parathyroid hormone indicated a relative deficiency of 1,25-(OH)\(_2\)D\(_3\). A decrease in the serum concentration of 25-(OH)D\(_3\) in dogs with CRF appeared to be attributable to reduced intake and increased urinary loss. (Am J Vet Res 2003;64:1161-1166)

Metabolism of vitamin D is compromised in patients with renal failure. The active form of vitamin D is 1,25-dihydroxycholecalciferol (1,25-(OH)\(_2\)D\(_3\)), which is produced via 1α-hydroxylation of 25-hydroxycholecalciferol (25-(OH)D\(_3\)) in the proximal tubules of the kidneys. In patients with chronic renal failure (CRF), production of 1,25-(OH)\(_2\)D\(_3\) is impaired. It is not surprising that some authors have reported a significant difference in serum concentrations of 1,25-(OH)\(_2\)D\(_3\) between dogs with experimentally induced CRF and control dogs. However, it was found in another study that concentrations were not significantly lower in dogs with CRF; it is not known whether serum concentrations of 1,25-(OH)\(_2\)D\(_3\) in dogs with acute renal failure (ARF) are similar to those for dogs with CRF; however, in a study conducted in humans, 1,25-(OH)\(_2\)D\(_3\) was low in 59% of patients with ARF.

The glomerular filtration rate is lowest during the maintenance phase of ARF but improves during the recovery phase, and the serum concentration of creatinine decreases. In the maintenance phase, the serum concentration of 1,25-(OH)\(_2\)D\(_3\) correlates more with the extent of renal damage than with the concentration of creatinine, and the relative decrease of 1,25-(OH)\(_2\)D\(_3\) during ARF is less than the relative increase in the creatinine concentration. On the other hand, the half-life of 1,25-(OH)\(_2\)D\(_3\) is extremely short in humans, constituting only a few hours. Furthermore, a study in rats revealed that the production of 1,25-(OH)\(_2\)D\(_3\) is low during acute tubular necrosis. These findings imply that the serum concentration of 1,25-(OH)\(_2\)D\(_3\) would decrease quickly in patients with ARF.

Acidosis may also affect serum concentrations of 1,25-(OH)\(_2\)D\(_3\). Acute, but not chronic, acidosis in rats inhibited hydroxylation of 25-(OH)D\(_3\) such that concentrations of 1,25-(OH)\(_2\)D\(_3\) were lower in rats with ARF than in those with CRF. In another study, investigators documented that the serum concentration of 1,25-(OH)\(_2\)D\(_3\) was even higher than the reference range in humans with chronic metabolic acidosis, which was attributed to hypophosphatemia.

Vitamin D status of patients can be monitored by measuring serum concentrations of 25-(OH)D\(_3\), which has a half-life of approximately 3 weeks. Concentrations of 25-(OH)D\(_3\) in people may decrease because of increased loss in the urine or decreased uptake of vitamin D. Compared with the serum concentration of 25-(OH)D\(_3\) in patients with CRF, the serum concentration of 25-(OH)D\(_3\) in patients with ARF is expected to remain unchanged or be altered only slightly, because acute renal disease would not markedly alter 25-(OH)D\(_3\) reserves. Thus, the objective of the study reported here was to compare serum concentrations of 1,25-(OH)\(_2\)D\(_3\) and 25-(OH)D\(_3\) in healthy control dogs, dogs with ARF, and dogs with CRF.
Materials and Methods

Control dogs—Twenty-four healthy dogs that were between 1 and 13 years of age (median, 4 years) and ranged from 6.0 to 46.0 kg (median, 18.8 kg) served as control dogs in the study. Fifteen dogs were owned by clinic staff, and 4 dogs were owned by clients who had brought their dogs to the Clinic for Small Animal Internal Medicine at the University of Zürich for elective procedures. Permission was obtained from the clinical staff and clients to use the dogs in the study. The remaining 5 dogs were research dogs.

Control dogs comprised 9 males (8 sexually intact and 1 neutered) and 15 females (10 sexually intact and 5 spayed). Four dogs were mixed-breed dogs, and the remainder represented 13 breeds. Serum concentrations of urea, creatinine, calcium, phosphorus, and parathyroid hormone (PTH) were within the reference range in all control dogs. Diets of the dogs were not monitored.

Dogs with CRF—Forty dogs with CRF that were between 1 and 14 years of age (median, 6 years) and ranged from 3.2 to 44.0 kg (median, 21.2 kg) were used in the study. There were 22 males (16 sexually intact and 6 neutered) and 18 females (8 sexually intact and 10 spayed). Six were mixed-breed dogs, and the remainder represented 23 breeds.

A diagnosis of CRF was made in 22 dogs on the basis of histologic examination of samples of renal tissues. Tissue samples were obtained from 6 of these dogs during transcutaneous kidney biopsy and from the other 16 dogs during postmortem examination. Twelve of these 22 dogs also had a history of long-standing renal azotemia. In the remaining 18 dogs, a diagnosis of CRF was made on the basis of a history of long-standing renal azotemia. In 13 of these 18 dogs, ultrasonographic examination revealed chronic renal changes.

Nineteen dogs with CRF were examined because of acute crisis. All 19 dogs were anorectic and listless, 17 dogs were vomiting, 9 dogs had diarrhea, 1 dog was having seizures, and 1 dog had signs of acute depression.

The remaining 21 dogs with CRF were not in acute crisis. Of these dogs, 7 were examined because of polyuria and polydipsia, incontinence, or dysuria without any other history of disease; 6 were examined to evaluate the degree of control of CRF; and 8 dogs were examined because of nonspecific clinical signs, such as weight loss, inappetence, chronic intermittent vomiting, and diarrhea.

Dogs with ARF—Ten dogs with ARF that were between 1 and 12 years of age (median, 4 years) and ranged from 7.3 to 41.0 kg (median, 25.1 kg) were used in the study. There were 3 males (2 sexually intact and 1 neutered) and 7 females (4 sexually intact and 3 spayed). There were 2 mixed-breed dogs, and the remaining dogs represented 8 breeds.

All dogs had clinical signs of ARF. Lethargy, anorexia, and vomiting were reported in all dogs, and 3 dogs had diarrhea. On physical examination, all dogs were within anticipated reference ranges for body weight. The abdomen of 5 dogs was tense during palpation, and 3 dogs were icteric. One dog had oral ulcers. In 4 dogs, a diagnosis of acute nephropathy was made during postmortem examination, and leptospirosis was tentatively diagnosed in 1 of those dogs. In 4 other dogs, leptospirosis was definitively diagnosed on the basis of high antibody titers to Leptospira spp and rapid improvement after appropriate treatment for ARF. In 2 dogs, the cause of ARF was not determined, but azotemia resolved in both dogs after intensive long-term treatment.

Routine laboratory evaluations—Blood samples were obtained from all dogs before initiation of treatment. In most dogs, CBC and serum biochemical analysis, which included determination of bilirubin, glucose, urea, creatinine, total protein, albumin, cholesterol, sodium, potassium, chloride, calcium, and phosphorus concentrations and measurement of the activity of alkaline phosphatase, creatinine transaminase, aspartate transaminase, and amylase, were performed. In 1 dog with CRF, the PCV alone was measured instead of a CBC. Only urea and creatinine concentrations were measured in 1 dog with CRF, and in another dog with CRF, only urea, creatinine, calcium, and phosphorus concentrations were measured.

Urinalysis consisted of results of a urine test strip, microscopic examination of urine sediment, and determination of urine specific gravity. Urinalysis was performed on samples obtained from 36 dogs with CRF and 8 dogs with ARF. In 2 dogs with CRF, urinalysis was not performed, because it was known that these dogs had the disease for a long time. In the remaining 2 dogs with CRF and 1 dog with ARF, urine was not collected before IV administration of fluids was initiated. Urine could not be obtained from another dog with ARF, because the dog was anuric. The urine protein-to-urine creatinine ratio was only considered when urine sediment contained <4 leukocytes/hpf; however, it was evaluated for 26 dogs with CRF and 4 dogs with ARF.

Additional laboratory evaluations—Blood samples were collected from all dogs before initiation of treatment. Serum was harvested, placed in tubes that were protected from light, and immediately transported to our laboratory. A quantitative radioimmunoassay was performed to determine the concentration of 1,25-(OH) 2D 3 and validated for use in dogs.

Concentration of 25-(OH)D 3 was measured in serum by quantitative radioimmunoassay. Coefficient of variation (CV) for precision in a series of 10 repeated measurements of a single sample in the lower range of the assay was 4.8%, and the CV for 8 repeated measurements of a single sample in the mid-range of the assay was 11.3%. The between-day CV of 1 sample measured on each of 9 days was 21.9%, and the between-day CV of another sample measured on each of 8 days was 20.5%. Initial measurements were made on fresh serum; aliquots of serum were stored at −20°C for use in measurements 2 through 8. In comparison, intra-assay CV reported by the manufacturer for samples obtained from humans ranged from 5 to 9%, and interassay CV ranged from 10.8 to 16.8%. Accuracy of the assay was determined by assessing the linearity of serial-dilution results and measuring the concentration of a mixture of 2 samples of known concentrations. Comparison of actual values obtained for 2 samples diluted to 75, 50, 25, and 12.5% of the initial concentration with calculated values for those samples and dilutions yielded significant (P < 0.01) correlation coefficients of 0.996 and 0.998, respectively. Comparison of actual values obtained for 10 mixed samples with calculated values for those 10 samples yielded a significant (P < 0.01) correlation coefficient of 0.794.

Concentration of 25-(OH)D 3 was measured in serum by use of a modified protein-binding test, initially described elsewhere. The CV for the precision of a series of 10 repeated measurements of a single sample in the lower range was 5.3%, whereas the CV for 8 repeated measurements of a single sample in the mid-range was 6.5%. The between-day CV for 1 sample measured on each of 9 days was 13.2%, and the between-day CV for another sample measured on each of 8 days was 12.1%. In comparison, intra-assay CV reported by the manufacturer for samples obtained from humans ranges from 5.3 to 9.8%, and interassay CV ranges from 7.3 to 11.6%. Comparison of actual values for 2 samples diluted to 75, 50, 25, and 12.5% of the initial concentration with calculated values for those samples and dilutions yielded significant (P < 0.05) correlation coefficients of 0.935 and 0.978, respectively. Comparison of the actual values for 10 mixed samples with calculated values for those 10 samples yielded a significant (P < 0.01) correlation coefficient of 0.881.

Concentrations of PTH were measured by use of a
Chemiluminescence immunometric assay for intact PTH. The PTH concentration was not measured in 2 dogs with ARF.

Statistical analysis—Results were analyzed by use of a commercial computer program. Data were analyzed by use of an ANOVA and a χ² test. Post-hoc tests were performed by use of Bonferroni tests. Correlations were determined by use of the Pearson correlation coefficient. Each reference range was calculated as the mean ± 2 SD. Differences were considered significant for values of P < 0.05.

Results

Control dogs—Concentrations of 1,25-(OH)₂D₃ and 25-(OH)D₃ were both within the reference range in 7 of 10 dogs, but both were less than the reference range in 1 dog. In 1 dog, 1,25-(OH)₂D₃ was within the reference range, but 25-(OH)D₃ was less than the reference range; in the remaining dog, 1,25-(OH)₂D₃ was less than the reference range, but 25-(OH)D₃ was within the reference range. We did not detect significant differences in 1,25-(OH)₂D₃ and 25-(OH)D₃ concentrations among the 4 dogs with confirmed leptospirosis and a fifth dog with a tentative diagnosis of leptospirosis, compared with concentrations for the other 5 dogs.

Serum concentration of calcium was within the reference range in 4 dogs, higher than the reference range in 5 dogs. Serum concentration of phosphorus was within the reference range in 1 dog but higher than the reference range in 9 dogs. Serum concentration of PTH was higher than the reference range in all 8 dogs in which this variable was measured. The urine protein-to-urine creatinine ratio was increased in 2 dogs in which this variable was measured.

Dogs with ARF—Concentrations of 1,25-(OH)₂D₃ and 25-(OH)D₃ were both within the reference range in 7 of 10 dogs, but both were less than the reference range in 1 dog. In 1 dog, 1,25-(OH)₂D₃ was within the reference range, but 25-(OH)D₃ was less than the reference range; in the remaining dog, 1,25-(OH)₂D₃ was less than the reference range, but 25-(OH)D₃ was within the reference range. We did not detect significant differences in 1,25-(OH)₂D₃ and 25-(OH)D₃ concentrations among the 4 dogs with confirmed leptospirosis and a fifth dog with a tentative diagnosis of leptospirosis, compared with concentrations for the other 5 dogs.

Serum concentration of calcium was within the reference range in 4 dogs, higher than the reference range in 3 dogs, and less than the reference range in the remaining 3 dogs. Serum concentration of phosphorus was within the reference range in 1 dog but higher than the reference range in 9 dogs. Serum concentration of PTH was higher than the reference range in all 8 dogs in which this variable was measured. The urine protein-to-urine creatinine ratio was increased in 2 dogs (Table 2).

Dogs with CRF—For the 19 dogs with CRF that were in acute crisis, the concentration of 1,25-(OH)₂D₃ was within the reference range in 12 dogs and less than the reference range in 7 dogs. The concentration of 25-(OH)D₃ was within the reference range in 5 dogs and less than the reference range in 14 dogs. Serum concentration of calcium was within the reference range in 9 dogs, less than the reference range in 6 dogs, and higher than the reference range in 3 dogs; serum calcium concentration was not measured in 1 dog. Serum concentration of phosphorus was higher than the reference range in 18 dogs and was not measured in 1 dog. Serum concentration of PTH was higher than the respective reference range in all dogs. The urine protein-to-urine creatinine ratio was higher than the reference range in 11 dogs (Table 2).

For the 21 dogs with CRF that were not in acute crisis, the concentration of 1,25-(OH)₂D₃ was within the reference range in 15 dogs, less than the reference range in 4 dogs, and higher than the reference range in the remaining 2 dogs. The concentration of 25-(OH)D₃ was within the reference range in 11 dogs and less than the reference range in 10 dogs. Serum concentration of calcium was within the reference range in 14 dogs, less than the reference range in 3 dogs, and higher than the reference range in 4 dogs. Serum concentration of phosphorus was within the reference range in 2 dogs and higher than the reference range in 19 dogs. Serum concentration of PTH was higher than the reference range in all dogs. The urine protein-to-urine creatinine ratio was increased in 2 dogs in which this variable was measured.

### Table 1—Mean ± SD concentrations of 1,25-dihydroxycholecalciferol (1,25-(OH)₂D₃) and 25-hydroxycholecalciferol (25-(OH)D₃) in the serum of healthy control dogs, dogs with acute renal failure (ARF), and dogs with chronic renal failure (CRF)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Dogs with ARF (n = 10)</th>
<th>In acute crisis (n = 19)</th>
<th>Not in acute crisis (n = 21)</th>
<th>Control dogs (n = 24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,25-(OH)₂D₃ (pmol/L)</td>
<td>75 ± 25b</td>
<td>70 ± 46</td>
<td>114 ± 77</td>
<td>153 ± 50</td>
</tr>
<tr>
<td>25-(OH)D₃ (nmol/L)</td>
<td>130 ± 82*</td>
<td>88 ± 52</td>
<td>98 ± 64</td>
<td>267 ± 97</td>
</tr>
</tbody>
</table>

*Within a row, values with different superscript letters differ significantly (P < 0.05).

### Table 2—Mean ± SD values for several variables measured in the serum of dogs with ARF and CRF

<table>
<thead>
<tr>
<th>Variable</th>
<th>Dogs with ARF (n = 10)</th>
<th>In acute crisis (n = 19)</th>
<th>Not in acute crisis (n = 21)</th>
<th>Reference range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea (mmol/L)</td>
<td>53.4 ± 22.8</td>
<td>60.8 ± 29.2</td>
<td>53.4 ± 34.5</td>
<td>3.9–10.7</td>
</tr>
<tr>
<td>Creatinine (µmol/L)</td>
<td>806 ± 225</td>
<td>607 ± 215</td>
<td>542 ± 331*</td>
<td>48–90</td>
</tr>
<tr>
<td>Calcium (mmol/L)</td>
<td>2.8 ± 0.9</td>
<td>2.5 ± 0.7 (18)</td>
<td>2.7 ± 0.5</td>
<td>2.3–3.0</td>
</tr>
<tr>
<td>Phosphorus (mmol/L)</td>
<td>4.2 ± 1.5</td>
<td>5.6 ± 2.4 (18)</td>
<td>3.9 ± 2.0</td>
<td>1.0–1.6</td>
</tr>
<tr>
<td>PTH (µg/mL)</td>
<td>223 ± 133 (8)</td>
<td>450 ± 305</td>
<td>414 ± 360</td>
<td>8–45</td>
</tr>
<tr>
<td>UPC</td>
<td>1.2 ± 1.3 (4)</td>
<td>4.3 ± 7.2 (13)</td>
<td>1.7±2.0 (13)</td>
<td>&lt; 1</td>
</tr>
</tbody>
</table>

Values reported represent results for all dogs, unless indicated otherwise in parentheses.

*Value is significantly (P < 0.05) different from value for dogs with ARF.

PTH = Parathyroid hormone. UPC = Urine protein-to-urine creatinine ratio.
Concentration of 1,25-(OH)2D3 for the subgroup of dogs with CRF that were in acute crisis was significantly lower, but not significantly (P = 0.09), in dogs with CRF, compared with the concentration in dogs with ARF.

In our study, the concentration of 1,25-(OH)2D3 determined in the study reported here were higher than those in a number of other published reports.6,7,23 This difference is believed to be attributable to the type of laboratory method used to determine the concentration of 1,25-(OH)2D3, because our reference range was similar to that reported in another study (40 to 192 pmol/L)10 in which investigators used a test kit from the same manufacturer that was used in our study.

In humans, an increase in phosphorus concentration in the proximal tubules is believed to be responsible for inhibition of the hydroxylation of 25-(OH)D3.1 In our study, we had only a weak negative correlation (r = -0.35) between the serum concentrations of phosphorus and 1,25-(OH)2D3, but there was a significantly higher phosphorus concentration in the subgroup of dogs with CRF that were in acute crisis, compared with dogs with CRF that were not in acute crisis; the concentration of 1,25-(OH)2D3 was lower, but not significantly (P = 0.09), in that subgroup of dogs.

Theoretically, a decrease in dietary phosphorus uptake should result in an increase in the serum concentration of 1,25-(OH)2D3. However, in a study7 that involved the use of dogs with renal failure, no such increase was observed after restricting phosphorus intake, and it was believed that failure to increase production of 1,25-(OH)2D3 in those dogs was attributable to severe renal atrophy.

In our study, the concentration of 1,25-(OH)2D3 did not differ significantly between dogs with ARF and dogs with CRF. Experimental reduction of renal mass in rats failed to reveal a difference between immediate and long-term decreases in the concentration of 1,25-(OH)2D3.10 The authors of that study concluded that the production of 1,25-(OH)2D3 was dependent mainly on the nephron mass. Reduction of functional renal mass results in a decrease in the number of proximal tubules that are available for the hydroxylation of 25-(OH)D3.1

Serum concentration of creatinine was higher in dogs with ARF than in dogs with CRF that were not in acute crisis. Patients with ARF frequently have prerenal azotemia because of fluid loss associated with acute disease. In our study, blood samples were collected before administration of fluids, which may explain the difference in creatinine concentrations in the 2 groups of dogs. In rats, the concentration of creatinine is increased in the acute phase of renal failure, followed by normal values in the chronic stage, whereas the con-
centration of 1,25-(OH)\textsubscript{2}D\textsubscript{3} remains unchanged in both stages of the disease.\textsuperscript{10} The explanation for this phenomenon is that after an insult, the kidneys are capable of adjusting excretory function without affecting the production of 1,25-(OH)\textsubscript{2}D\textsubscript{3}.\textsuperscript{10}

The reference range calculated for 25-(OH)D\textsubscript{3} in the study reported here was similar to that in another study,\textsuperscript{4} but it was higher than the reference range reported in 2 other studies.\textsuperscript{7,23} These differences are likely attributable to the method of measurement and indicate that each laboratory should establish its own reference values for vitamin D metabolites.\textsuperscript{22}

The serum concentration of 25-(OH)D\textsubscript{3} is approximately 1,000 times higher than that of 1,25-(OH)\textsubscript{2}D\textsubscript{3} and is only minimally influenced by hydroxylation. The concentration of 25-OH-D3 reflects the vitamin D status of a patient.\textsuperscript{11} In humans with CRF, neither resorption nor hepatic transformation of vitamin D to 25-(OH)D\textsubscript{3} is affected, whereas the uptake of vitamin D is impaired.\textsuperscript{12,13} The same mechanisms may have applied to our dogs, considering that food intake is often decreased for extended periods in dogs with CRF but not in dogs with ARF. It could also explain the reason that dogs with ARF had a higher concentration of 25-(OH)D\textsubscript{3} than dogs with CRF; as well as accounting for the fact that 8 of 10 dogs with ARF had concentrations of 25-(OH)D\textsubscript{3} that were within the reference range. In addition, 25-(OH)D\textsubscript{3} is excreted in the urine along with vitamin D-binding protein, which, in humans, has a molecular weight similar to that of albumin.\textsuperscript{14,15}

In our study, the urine protein-to-creatinine ratio was higher than the reference range in 21 of 30 dogs. One dog had a urine protein-to-urine creatinine ratio of 27.5 and the second lowest serum concentration of 25-(OH)D\textsubscript{3} (ie, 11 nmol/L). In 16 dogs with CRF, the serum concentration of 25-(OH)D\textsubscript{3} was within the reference range; thus, it was assumed that the dietary intake must have provided adequate amounts of vitamin D in these dogs. Furthermore, renal loss of protein was probably not substantial in these dogs, because there was only a mild increase of the urine protein-to-urine creatinine ratio in most dogs.

In the study reported here, the serum concentration of 1,25-(OH)\textsubscript{2}D\textsubscript{3} was significantly lower in dogs with ARF or CRF, compared with concentrations in healthy control dogs; however, there was not a significant difference in the concentrations between dogs with ARF and those with CRF. In the majority of dogs with renal failure, the concentration of 1,25-(OH)\textsubscript{2}D\textsubscript{3} was within the reference range. This raises the question of whether administration of calcitriol would be justified in these dogs. However, serum concentrations of PTH were increased in these dogs, which indicated a relative deficiency of 1,25-(OH)\textsubscript{2}D\textsubscript{3}. Similar to the concentration of 1,25-(OH)\textsubscript{2}D\textsubscript{3}, the concentration of 25-(OH)D\textsubscript{3} was significantly lower in dogs with ARF or CRF, compared with the concentration in control dogs; however, it was within the reference range for most of the dogs with ARF.

\textsuperscript{c}Combust-test, Roche Diagnostics GmbH, Mannheim, Germany.
\textsuperscript{d}25 Dihydroxyvitamin D, radioimmunoassay kit, Nichols Institute Diagnostika GmbH, Bad Nauheim, Germany.

\textsuperscript{References}


Vitamin D3 screen, protein binding assay, Buhlmann Laboratories AG, Allschwil, Switzerland.

1. 25 Dihydroxyvitamin D, radioimmunoassay kit, Nichols Institute Diagnostika GmbH, Bad Nauheim, Germany.
2. PTH-parathormone hormone, Nichols Institute Diagnostic, San Juan Capistrano, Calif.
3. SPSS 8.0 for Windows, SPSS Inc, Chicago, Ill.


