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Vitamin D Metabolites Affect Serum Calcium and Phosphate in Freshwater Catfish, *Heteropneustes fossilis*

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ABSTRACT—The effects of vitamin D₃, 24,25(OH)₂ vitamin D₃, 25(OH) vitamin D₃ and 1,25(OH)₂ vitamin D₃ were investigated on the serum calcium and phosphate levels of freshwater catfish, *Heteropneustes fossilis*. The fish were injected daily intraperitoneally with these secosteroids for 10 days. Blood samples were collected at day 1, 3, 5 and 10. Serum calcium and inorganic phosphate levels were elevated by all of the treatments except for 24,25(OH)₂ vitamin D₃.

INTRODUCTION

Bony fishes, particularly those inhabiting seawater, contain large hepatic stores of vitamin D (Copping, 1934; Urist, 1976; Takeuchi *et al.*, 1984). Vitamin D₃, which itself apparently lacks direct biological activity, produces a number of metabolites after sequential hydroxylations in liver and kidney (Norman *et al.*, 1982). Teleosts inhabiting freshwater and seawater are able to convert vitamin D₃ and 25(OH)D₃ to more polar metabolites (Hayes *et al.*, 1986; Takeuchi *et al.*, 1991). Moreover, fish contains circulating levels of vitamin D₃, 25(OH)D₃, 1,25(OH)₂D₃ and 24,25(OH)₂D₃ (Hay and Watson, 1976; Nahm *et al.*, 1979; Avioli *et al.*, 1981; Takeuchi *et al.*, 1991; Sundell *et al.*, 1992; Rao and Raghuramulu, 1995). Furthermore, specific binding proteins for 1,25(OH)₂D₃ have been demonstrated in tissues from European eel and Atlantic cod (Marcocci *et al.*, 1982; Sundell *et al.*, 1992). These studies suggest a physiological role for vitamin D₃ system in fishes.

The effects of vitamin D₃ and its metabolites on calcium homeostasis have been studied in a few freshwater teleosts (*Amphipnous cuchia*; Srivastav, 1983; *Anguilla rostrata*; Fenwick *et al.*, 1984; *Clarias batrachus*; Swarup and Srivastav, 1982; Swarup *et al.*, 1984; Srivastav and Srivastav, 1988; *Cyprinus carpio*; Swarup *et al.*, 1991; Srivastav *et al.*, 1993; *Carrasius auratus*; Fenwick, 1984; *Heteropneustes fossilis*; Srivastav and Singh, 1992) and a few marine species (*Gadus morhua*; Sundell *et al.*, 1993; *Pagothenia bernachii*; Fenwick *et al.*, 1994). Nevertheless there is still considerable controversy regarding the physiological role of this vitamin and its metabolites in teleosts as many of the previous reports are

contradictory. Administration of vitamin D or its metabolites has been reported to cause either (i) no significant change (Urist, 1962; MacIntyre *et al.*, 1976; Lopez *et al.*, 1977), (ii) increase (Srivastav, 1983; Fenwick, 1984; Swarup *et al.*, 1984, 1991; Fenwick *et al.*, 1984, 1994; Srivastav and Srivastav, 1988; Srivastav and Singh, 1992), or (iii) decrease (Sundell *et al.*, 1993) in the blood calcium content. Moreover, the effect of 24,25(OH)₂D₃ has been investigated only in *Sarotherodon mossambicus* (a freshwater species, Wendelaar Bonga *et al.*, 1983) and *Gadus morhua* (a marine species, Sundell *et al.*, 1993).

The present study was undertaken to investigate the effects of vitamin D and some of its major metabolites on serum calcium and phosphate of a freshwater catfish, *Heteropneustes fossilis*.

MATERIALS AND METHODS

Freshwater catfish, *H. fossilis* of both sexes were procured and acclimated to laboratory conditions at 27 ± 2°C for one week prior to the experiment. The fish weighed between 45–64 g and were not fed following their capture. Blood samples from six fish was taken prior to the start of the experiment (zero hour). The remaining fish were randomly divided into five groups of 24 fish each. These groups received daily intraperitoneal injections of either vehicle (95% ethanol; group A), vitamin D₃ (5 µg; group B), 24,25(OH)₂D₃ (2 µg; group C), 25(OH)D₃ (1 µg; group D), or 1,25(OH)₂D₃ (0.5 µg; group E). The doses indicated are per 100 g body wt of fish/0.5 ml. The doses of various vitamin D metabolites used in the present study correspond more or less to the doses used in other teleosts by previous investigators (Wendelaar Bonga *et al.*, 1983; Sundell *et al.*, 1993).

Six fish from each group were anesthetized with MS222 and blood samples were collected 4 hr after the last injection (by a syringe from the caudal vessels) after 1, 3, 5 and 10 days of treatment. Sera were separated by centrifugation and total calcium and phosphate were measured according to Trinder (1960) and Fiske and Subbarow (1925).

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methods, respectively. Calcium from serum was precipitated as an insoluble orange red complex by an alkaline solution of naphtholhydroxamic acid. After centrifugation the precipitate was dissolved in alkaline disodium ethylenediamine tetraacetate, then treated with ferric nitrate and the resultant amber colour was measured colorimetrically. For phosphate, the serum was deproteinized by adding trichloroacetic acid. To the filtrate, ammonium molybdate was added followed by 1,2,4-aminonaphtholsulfonic acid. The resultant blue colour was measured colorimetrically.

Student's *t* test was used to determine statistical significance. In all cases, the experimental group was compared with the vehicle-injected group sampled at the same time. The data were also subjected to two-way ANOVA.

RESULTS

Serum calcium levels of fish treated with various vitamin D analogs are shown in Fig. 1. Both vitamin D₃ and 25(OH)D₃ increased the serum calcium levels at day 3 and day 5. No changes were observed in calcium concentrations following 24,25(OH)₂D₃ treatment. The serum calcium level of 1,25-(OH)₂D₃ treated fish increased more rapidly and showed a significant increase on day 1 which progressively increased till day 5. All groups were normocalcemic by day 10.

Serum phosphate levels were unaffected through day 3 except for the 1,25(OH)₂D₃ treated fish which were hyperphosphatemic. By day 5, all the treated groups were hyperphosphatemic with the exception of the 24,25(OH)₂D₃ treated group (Fig. 2). Unlike the situation with calcium which return to normal values by day 10, the hyperphosphatemic effect, when stimulated, remained so up to day 10.

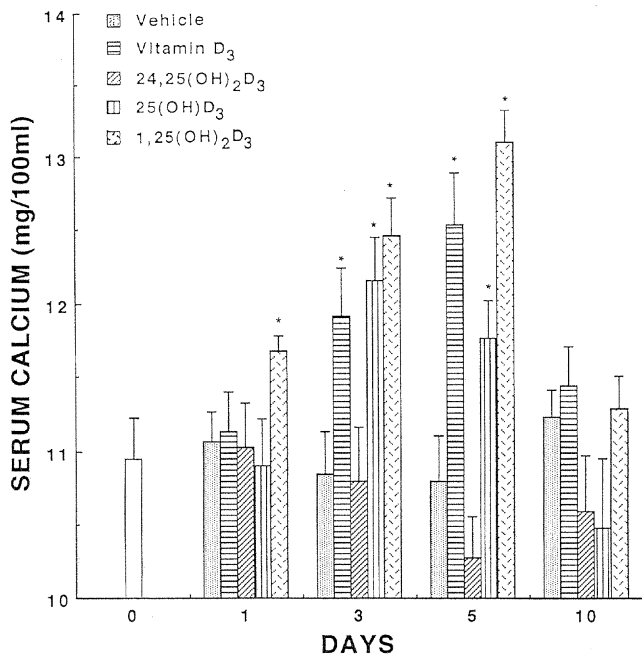


Fig. 1. Serum calcium levels of *H. fossilis* treated either with vehicle, vitamin D₃, 24,25(OH)₂D₃, 25(OH)D₃ or 1,25(OH)₂D₃. Values are mean \pm SE of six specimens. Asterisk values are significantly different ($P < 0.05$) as compared to the vehicle-injected group.

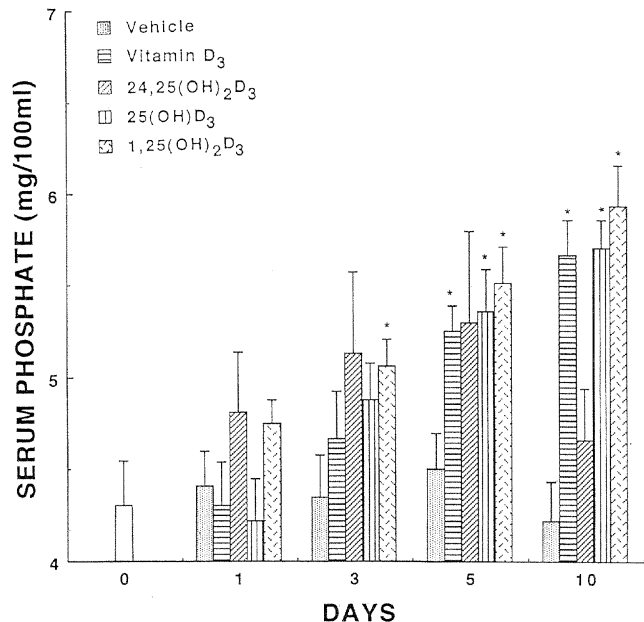


Fig. 2. Serum inorganic phosphate levels of *H. fossilis* treated either with vehicle, vitamin D₃, 24,25(OH)₂D₃, 25(OH)D₃ or 1,25(OH)₂D₃. Values are mean \pm SE of six specimens. Asterisk values are significantly different ($P < 0.05$) as compared to the vehicle-injected group.

Comparing (two-way ANOVA) serum calcium and phosphate levels of *H. fossilis* treated with various vitamin D metabolites, it has been observed that these electrolytes differed significantly between the exposure period (for calcium $F = 4.581$ and $P < 0.01$; for phosphate $F = 3.465$ and $P < 0.04$), whereas between the various treatments used in this study, only phosphate levels differed significantly (for calcium $F = 2.002$ and $P < 0.16$, not significant; for phosphate $F = 4.323$ and $P < 0.02$).

DISCUSSION

The data shows that vitamin D₃, 25(OH)D₃ and 1,25(OH)₂D₃ affect calcium homeostasis in *H. fossilis*. These observations are in accord with the results of other investigations in which administration of vitamin D₃ and these metabolites elevated the serum/plasma calcium (total) concentrations in other fishes (Swarup and Srivastav, 1982; Srivastav, 1983; Fenwick, 1984; Swarup *et al.*, 1984, 1991; Srivastav and Srivastav, 1988; Srivastav and Singh, 1992; Fenwick *et al.*, 1984, 1994). Administration of 1,25(OH)₂D₃ to marine fishes has been reported either to increase (*Gadus morhua*; Sundell *et al.*, 1993) or decrease (*Pagothernia bernachii*; Fenwick *et al.*, 1994) the ionized calcium concentration without altering the total plasma calcium levels. In contrast to the present study, daily injections (for seven days) of 25(OH)D₃ to Atlantic cod lowered the total calcium levels (Sundell *et al.*, 1993). 25(OH)D₃ treatment produced no significant effect on either ionized or total calcium concentration of *Pagothernia bernachii* (Fenwick *et al.*, 1994).

24,25(OH)₂D₃ injections to *H. fossilis* did not affect serum calcium levels and this agrees with the studies of Sundell *et al.*

al. (1993) who have also noticed no change in calcium contents of 24,25(OH)₂D₃ treated Atlantic cod.

In fishes vitamin D₃ and 1,25(OH)₂D₃ increased calcium uptake (Chartier *et al.*, 1979; Flik *et al.*, 1982; Fenwick, 1984; Fenwick *et al.*, 1984). In Atlantic cod 25(OH)D₃ stimulated intestinal calcium absorption whereas vitamin D₃ and 1,25(OH)₂D₃ did not affect the calcium influx across the intestinal mucosa (Sundell and Björnsson, 1990). The observed hypercalcemia in *H. fossilis* may be explained by mobilization of calcium from internal stores and/or increased renal retention of calcium. Indeed, 1,25(OH)₂D₃ was shown to increase bone demineralization in teleosts (Lopez *et al.*, 1977; Wendelaar Bonga *et al.*, 1983). Moreover, an increased calcium uptake by the gills from the environment after treatment with these metabolites can not be ruled out.

The hyperphosphatemia evoked by the administration of vitamin D₃, 25(OH)D₃ and 1,25(OH)₂D₃ to *H. fossilis* is similar to that reported previously (MacIntyre *et al.*, 1976; Fenwick *et al.*, 1984; Swarup *et al.*, 1984, 1991; Srivastav and Singh, 1992). In contrast, Sundell *et al.* (1993) and Fenwick *et al.* (1994) have found no effect of these secosteroids on plasma phosphate content. It is of interest to note that in *H. fossilis* 24,25(OH)₂D₃ produced elevated phosphate levels although this increase was not statistically significant. The hyperphosphatemic response of vitamin D₃ and its metabolites in *H. fossilis* suggests that the nondietary phosphorus, possibly from the bone and/or from the soft tissues, can be mobilized. The increased renal retention of phosphate also can not be ruled out.

The different outcomes in the calcium and phosphate levels of *H. fossilis* at some time intervals in response to vitamin D₃ and its metabolites administration may be due to reported differences in the mechanism of actions of these metabolites—a slow genome-mediated and a rapid nongenome-mediated transcaltachic response (Larsson *et al.*, 1995).

In the present study serum calcium levels returned to normal at day 10 whereas phosphate levels were still increased. The recovery of serum calcium may be attributed to increased release of the hypocalcemic factor stanniocalcin from the corpuscles of Stannius after continuous hypercalcemic challenge induced by vitamin D₃ and its metabolites. Stanniocalcin has been reported to inhibit branchial Ca²⁺ influx (Lafeber *et al.*, 1988; Verbost and Fenwick, 1995). An increased activity of corpuscles of Stannius after vitamin D₃/1,25(OH)₂D₃ has been reported in a freshwater catfish (*Clarias batrachus*) (Srivastav *et al.*, 1985; Srivastav and Srivastav, 1988). The persisting increased serum phosphate levels at day 10 could be ascribed to the possible renal retention of phosphate by enhanced secretion of stanniocalcin which has been shown to stimulate the net renal phosphate reabsorption (Renfro *et al.*, 1996).

The present study concludes that vitamin D₃ and two of its prime metabolites, 25(OH)D₃ and 1,25(OH)₂D₃ can affect both calcium and phosphate metabolism in a freshwater teleost, *H. fossilis*. We also do not feel it unreasonable to speculate that vitamin D₃ and 25(OH)D₃ have to be converted to a

more active form, probably 1,25(OH)₂D₃ as these secosteroids produced an effect only on day 3 whereas 1,25(OH)₂D₃ produced an effect in one day. The present results together with those of previous report (Sundell and Björnsson, 1990) suggest that there exists different functional aspects in the actions of vitamin D₃ and its metabolites in freshwater teleosts (freshwater environment is hypotonic in relation to the blood—where vitamin D₃ analogs affect calcium homeostasis) and marine teleosts (sea water is hypertonic in relation to the blood—where vitamin D₃ and 1,25(OH)₂D₃ produced contradictory effects; Sundell *et al.*, 1993; Fenwick *et al.*, 1994).

REFERENCES

- Avioli LV, Sonn Y, Jo D, Nahn TH, Haussler MR, Chandler JS (1981) 1, 25 dihydroxyvitamin D in male, nonspawning female, and spawning female trout. *Proc Soc Exp Biol Med* 166: 291–293
- Chartier MM, Milet C, Martelly E, Lopez E, Warrot S (1979) Stimulation par la vitamin D₃ et le 1, 25-dihydroxyvitamine D₃ de l'absorption intestinale du calcium chez l'anguille (*Anguilla anguilla* L.). *J Physiologie, Paris* 75: 275–282
- Copping AM (1934) Origin of vitamin D in cod-liver oil: vitamin D content of zooplankton. *The Biochemical Journal* 28: 1516–1520
- Fenwick JC (1984) Effect of vitamin D₃ (cholecalciferol) on plasma calcium and intestinal ⁴⁵calcium absorption in goldfish, *Carrasius auratus* L. *Canadian J Zool* 62: 34–36
- Fenwick JC, Smith K, Smith J, Flik G (1984) Effect of various vitamin D analogs on plasma calcium and phosphorus and intestinal calcium absorption in fed and unfed American eels, *Anguilla rostrata*. *Gen Comp Endocrinol* 55: 398–404
- Fenwick JC, Davidson W, Forster ME (1994) *In vivo* calcitropic effect of some vitamin D compounds in the marine Antarctic teleost, *Pagothenia bernachii*. *Fish Physiol Biochem* 12: 479–484
- Fiske CH, Subbarow Y (1925) The colorimetric determination of phosphorus. *J Biol Chem* 66: 375–400
- Flik G, Reijnens FMJ, Stikkelbroek J, Fenwick JC (1982) 1, 25-vitamin D₃ and calcium transport in the gut of tilapia (*Sarotherodon mossambicus*). *J Endocrinol* 94: 40
- Hay AWM, Watson G (1976) The plasma transport proteins of 25-hydroxycholecalciferol in mammals. *Comp Biochem Physiol B53*: 163–166
- Hayes ME, Guillard-Culling DF, Russell RGG, Henderson IW (1986) Metabolism of 25-hydroxycholecalciferol in a teleost fish, the rainbow trout (*Salmo gairdneri*). *Gen Comp Endocrinol* 64: 143–150
- Lafeber FPJG, Flik G, Wendelaar Bonga SE, Perry SF (1988) Hypocalcin from Stannius corpuscles inhibits gill calcium uptake in trout. *Am J Physiol* 254: R891–R896
- Larsson D, Björnsson BTh, Sundell K (1995) Physiological concentrations of 24,25-dihydroxyvitamin D₃ rapidly decrease the *in vitro* intestinal calcium uptake in the Atlantic cod, *Gadus morhua*. *Gen Comp Endocrinol* 100: 211–217
- Lopez E, Peignoux-Deville J, Lallier F, Colston KW, MacIntyre I (1977) Responses of bone metabolism in the eel (*Anguilla anguilla*) to injections of 1,25-dihydroxyvitamin D₃. *Calcif Tissue Res* 22: 19–23
- MacIntyre I, Colston KW, Ivans IMA, Lopez E, MacAuley SJ, Peignoux-Deville J, Spanos E, Szelke M (1976) Regulation of vitamin D: An evolutionary view. *Clin Endocrinol (Suppl)* 5: 85–95
- Marcocci C, Freaque HC, Iwasaki J, Lopez E, MacIntyre I (1982) Demonstration and organ distribution of the 1,25-dihydroxyvitamin D₃-binding protein in fish (*A. anguilla*). *Endocrinology* 110: 1347–1354
- Nahn TH, Lee SW, Fausto A, Sonn Y, Avioli LV (1979) 25OHD, a circulating vitamin D metabolite in fish. *Biochem Res Commun*

- 82: 396–402
- Norman AW, Roth J, Orci L (1982) The vitamin D endocrine system: steroid metabolism, hormone receptor, and biological response (calcium binding proteins). *Endocrine Rev* 3: 331–366
- Rao DS, Raghuramulu N (1995) Vitamin D and its related parameters in freshwater wild fishes. *Comp Biochem Physiol* 111A: 191–198
- Renfro JL, Lu M, Wagner GF, Swanson P (1996) Hormonal regulation of renal inorganic phosphate transport in the winter flounder. 3rd International Symposium on Fish Endocrinology, Hokkaido, Japan, p 38
- Srivastav Ajai K (1983) Calcemic responses in the freshwater mud eel, *Amphipneus cuchia*, to vitamin D₃ administration. *J Fish Biol* 23: 301–303
- Srivastav Ajai K, Singh S (1992) Effect of vitamin D₃ on serum calcium and inorganic phosphate levels of the freshwater catfish, *Heteropneustes fossilis*, maintained in artificial freshwater, calcium-rich freshwater, and calcium-deficient freshwater. *Gen Comp Endocrinol* 87: 63–70
- Srivastav Ajai K, Srivastav SP (1988) Corpuscles of Stannius of *Clarias batrachus* in response to 1,25 dihydroxyvitamin D₃ administration. *Zool Sci* 5: 197–200
- Srivastav SK, Jaiswal R, Srivastav Ajai K (1993) Response of serum calcium to administration of 1,25-dihydroxyvitamin D₃ in the freshwater carp *Cyprinus carpio* maintained either in artificial freshwater, calcium-rich freshwater or calcium-deficient freshwater. *Acta Physiologica Hungarica* 81: 269–275
- Srivastav SP, Swarup K, Srivastav Ajai K (1985) Structure and behaviour of Stannius corpuscles in relation to vitamin D₃-induced hypercalcemia in male *Clarias batrachus*. *Cellular Molecular Biol* 31: 1–5
- Sundell K, Björnsson BTh (1990) Effects of vitamin D₃, 25(OH) vitamin D₃, 24,25(OH)₂ vitamin D₃, and 1, 25(OH)₂ vitamin D₃ on the *in vitro* intestinal absorption in the marine teleost, Atlantic cod (*Gadus morhua*). *Gen Comp Endocrinol* 78: 74–79
- Sundell K, Bishop JE, Björnsson BTh, Norman AW (1992) 1,25 dihydroxyvitamin D₃ in the Atlantic cod: Plasma levels, a plasma binding component, and organ distribution of a high affinity receptor. *Endocrinology* 131: 2279–2286
- Sundell K, Norman AW, Björnsson BTh (1993) 1,25(OH)₂D₃ increases ionized plasma calcium concentrations in the immature Atlantic cod *Gadus morhua*. *Gen Comp Endocrinol* 91: 344–351
- Swarup K, Srivastav SP (1982) Vitamin D₃-induced hypercalcemia in male catfish, *Clarias batrachus*. *Gen Comp Endocrinol* 46: 271–274
- Swarup K, Norman AW, Srivastav Ajai K, Srivastav SP (1984) Dose-dependent vitamin D₃ and 1,25-dihydroxyvitamin D₃-induced hypercalcemia and hyperphosphatemia in male catfish, *Clarias batrachus*. *Comp Biochem Physiol* 78B: 553–555
- Swarup K, Das VK, Norman AW (1991) Dose-dependent vitamin D₃ and 1,25-dihydroxyvitamin D₃-induced hypercalcemia and hyperphosphatemia in male cyprinoid *Cyprinus carpio*. *Comp Biochem Physiol* 100A: 445–447
- Takeuchi A, Okano T, Ayame M, Yoshikawa H, Teraoka S, Murakami Y, Kobayashi T (1984) High-performance liquid chromatographic determination of vitamin D₃ in fish liver oils and eel body oils. *J Nutr Sci Vitaminol* 30: 421–430
- Takeuchi A, Okano T, Kobayashi T (1991) The existence of 25-hydroxyvitamin D₃-1-hydroxylase in the liver of carp and bastard halibut. *Life Sci* 48: 275–282
- Trinder P (1960) Colorimetric microdetermination of calcium in serum. *Analyst* 85: 889–894
- Urist MR (1962) The bone-body fluid continuum: calcium and phosphorus in the skeleton and blood of extinct and living vertebrates. *Perspect Biol Med* 6: 75–115
- Urist MR (1976) Biogenesis of bone: Calcium and phosphorus in the skeleton and blood in vertebrate evolution. In "Handbook of Physiology Vol 7" Ed by GD Aurbach, American Physiological Society, Washington DC, pp 183–213
- Verboost PM, Fenwick JC (1995) N-terminal and C-terminal fragments of the hormone stanniocalcin show differential effects in eels. *Gen Comp Endocrinol* 98: 185–192
- Wendelaar Bonga SE, Lammers PI, van der Meij JCA (1983) Effects of 1, 25- and 24, 25-dihydroxyvitamin D₃ on bone formation in the chichlid teleost *Sarotherodon mossambicus*. *Cell Tissue Res* 228: 117–126

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