Volume 212, number 1, 138–140 FEB 04435 February 1987

Increased 25-hydroxyvitamin D levels in portal blood following cholecystokinin injection in the dog

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Received 4 December 1986

To establish whether an enterohepatic circulation of the metabolites of vitamin D exists, polyethylene catheters were cannulated into the portal vein of dogs. The dogs were then starved for 24 h and injected with cholecystokinin (CCK) to induce gall bladder contraction. At various time intervals thereafter blood samples were collected from the portal and the saphena veins, and sera prepared and analyzed for the metabolites of vitamin D. The serum levels of 25-hydroxyvitamin D [25(OH)D] were found to be significantly higher in the portal blood when compared with levels in peripheral blood following CCK injection. Since portal blood collects nutrients absorbed from the gut and as the dogs were starved for 24 h prior to blood collection, the only source of the increased concentrations of 25(OH)D in portal blood is likely to be bile. These findings support the notion that an enterohepatic circulation of 25(OH)D does exist under normal physiological conditions.

Vitamin D; Enterohepatic circulation

1. INTRODUCTION

Deficiency of vitamin D is a common complication in patients with bowel diseases and following surgical resection of the intestine [1,2]. The main source of vitamin D is the skin, in response to ultraviolet irradiation [3]. The apparent vitamin D deficiency in these patients is despite normal exposure to sunlight. Impaired enterohepatic circulation of the metabolites of vitamin D could be the main cause of the deficiency in these patients. However, the existence of an enterohepatic circulation of the metabolites of vitamin D is still a controversial issue, mainly due to the fact that very little of the biologically active metabolites of the vitamin could be detected in the bile [4,5]. In an attempt to clarify these controversies and to establish whether an enterohepatic circulation of the metabolites of vitamin D exists, we measured metabolites of vitamin D in the portal and peripheral circulations of dogs following an i.v. injection of CCK.

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2. MATERIALS AND METHODS

Three 1-year-old dogs weighing 15 kg and in good nutritional status were anesthetized with phenobarbiton, and a polyethylene catheter of 18 G size was cannulated into the portal vein. The catheter was connected to the skin in the interscapular region. 18 h later, following 24 h starvation, each dog was injected with 30 U CCK (Kabi Diagnostica, Studsrik, Sweden) in 3 ml saline and 3 ml blood samples were collected from the portal vein and the saphena vein at 0, 30, 45, 60, 75, 90, and 105 min. 3 ml saline without CCK were injected prior to the CCK injection into the same dogs to serve as controls.

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/87/$3.50

The blood samples were subjected to lipid extraction [6] and to analysis of the hydroxylated metabolites of vitamin D.

25(OH)D and 24,25-dihydroxyvitamin D [24,25$(OH)_2$D] were measured by the competitive binding assay [7] following their separation on Sephadex LH-20 columns and utilizing diluted rat serum (limit of detection 0.37 ng). All samples were analyzed simultaneously in one assay (inter-assay variation 5%). 1,25-Dihydroxyvitamin D [1,25$(OH)_2$D] was measured by the radioreceptor assay [8] utilizing intestinal receptor from rachitic chicks. Peaks of 1,25$(OH)_2$D eluted off the Sephadex LH-20 columns were further purified on HPLC and analyzed simultaneously (limit of detection 2 pg with inter-assay variation 5%). Internal standards of authentic ^{3}H-labelled metabolites were included in all plasma samples, for correction of recoveries during all the steps of the purification.

At the end of the experiment the intestines were observed to confirm the absence of any food in the intestinal lumen.

3. RESULTS AND DISCUSSION

As seen from fig.1 the levels of 25(OH)D in portal and peripheral blood of dogs are not significantly different. However, 60 min following the injection of CCK a significant rise in the level of 25(OH)D can be observed in the portal blood compared to the peripheral blood, as well, compared to time 0. Each time interval represents measurements of 3 blood samples obtained from each dog. Student's *t*-test was used to establish significance of difference. Differences in levels of 1,25$(OH)_2$D and 24,25$(OH)_2$D between portal and peripheral plasma were found to be insignificant. Injection of 3 ml saline without CCK had no effect on vitamin D metabolites in the portal blood.

Vitamin D derivatives are excreted in the bile following i.v. injection of vitamin D. Bile analysis would confirm that the bile is the source of the 25(OH)D in portal blood. However, the majority of the excreted derivatives appears to be more polar than the known hydroxylated metabolites [1,9–11], some of which are glucuronidated and therefore are not measurable [1,4]. The existence of an enterohepatic circulation of the metabolites of vitamin D is a controversial issue [4]. In an at-

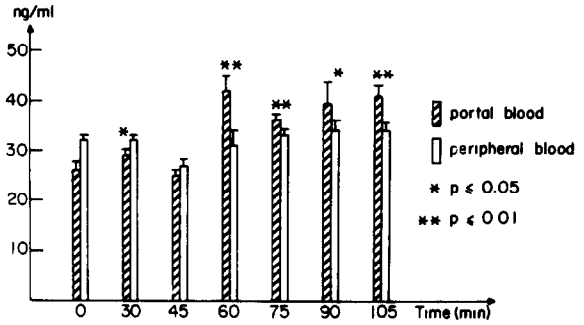

Fig.1. Concentrations of 25(OH)D in portal and peripheral blood of dogs following the injection of CCK. For further details see text.

tempt to establish whether such a circulation exists under normal physiological conditions, we tried to avoid in the present study any interventions, such as bile-duct cannulation, isotope injections, in order to eliminate any possible non-physiological stimuli. We therefore utilized normal dogs, and the only substance given was CCK in saline solution, thus causing gall bladder contraction known to occur after every meal [12]. We were able to demonstrate in the present study that following the injection of CCK into dogs the levels of 25(OH)D in portal blood are elevated. Since portal blood collects nutrients absorbed from the gut and since the dogs were starved for 24 h prior to blood collections, the only source of the increased concentrations of 25(OH)D is likely to be bile. Moreover, it is also known that 25(OH)D is absorbed in the gut in approximately equal amounts by both lymphatic and portal systems [13]. It is therefore possible that the amounts reabsorbed from the gut are even higher than those observed based on portal blood sampling, as we did not collect lymph.

It seems therefore that polar derivatives of vitamin D in bile are cleaved or transformed in the gut by intestinal flora or by intestinal cells back into 25(OH)D which in turn reabsorbed. This may explain why bile normally possesses poor antirachitic activity in spite of its large content of vitamin D derivatives. In summary, our findings of elevated levels of 25(OH)D in portal blood of fasting dogs following the administration of CCK support the notion that an enterohepatic circulation of 25(OH)D exists under normal physiological conditions. Thus the bile-intestine passage is essen-

tial in order to transform polar biliary derivatives of vitamin D back into active metabolites.

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