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Dietary additions of lactose, casein and soy protein exerted only moderate effects on calcium homeostasis in calcitriol deficient piglets☆

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Abstract

It has been reported from rats and mice that blood and bone calcium can be normalised in the absence of Vitamin D hormone or its receptor by dietary means. It was the aim of this study to test, whether a similar result can be obtained with pigs. Piglets with inherited calcitriol deficiency were fed with high calcium and Pi diets and supplemented with soy protein or casein and lactose or corn starch, which have been shown to normalise plasma and bone calcium in Vitamin D deficient rats and in mice. In the calcitriol deficient piglets none of the diets was capable to prevent the development of hypocalcemia. However, additions of lactose and soy protein improved somewhat plasma calcium ($P < 0.001$). Feeding of soy protein also had a significant positive effect on plasma phosphate concentration ($P < 0.001$). The study shows that in contrast to rats, calcitriol is essential for maintaining a normal plasma and bone calcium status. Responses of this type, when obtained with rats or mice can probably not directly be transferred to pigs and perhaps also not to humans.

Keywords: PDDR-I; Calcitriol deficiency; Lactose; Bone-mineral-content; Soya; Isoflavones

1. Introduction

The effects of lactose and casein on intestinal calcium absorption and bone mineral content were investigated already before the term functional food was introduced. Most controlled nutritional studies of this kind were carried out with rats. In non-rodent mammalian species including man the nutritional effects of lactose and casein are less well defined. The same is true for the effect of soy protein on calcium metabolism in these species.

Intestinal calcium absorption, whether by active transport or by passive diffusion depends on the concentration of free calcium in the digesta. Casein phosphopeptides (CPP), digestive products of casein, interact with calcium and prevent by this mechanism the formation of insoluble calcium phosphate [1]. CPP have been shown to increase in vitro paracellular passive flux of calcium across the distal small intestine in rats [2]. In aged ovariectomized rats it was shown that a diet that contained calcium bound to CPP [3] or soy bean protein [4,5] was superior in preventing bone loss and in improving intestinal calcium transport [6] compared to diets without CPP or soy protein but with the same mineral content. Lactose has been shown to increase calcium absorption in the small intestine in vivo [7] and in pieces of isolated small and large intestine [8]. Continuous feeding of lactose improved intestinal calcium absorption in germfree as compared to conventional rats [9] and bioavailability of calcium in vivo in 7–100 days old rats [10]. Moreover, feeding a high calcium (2%) high phosphate (1.25%) diet with 20% lactose to Vitamin D deficient rats was capable of preventing hypocalcemia, hypophosphatemia and hyperparathyroidism compared to a similar diet without lactose [11]. The isoflavones of soy bean genistein and diadzein, are regarded to be the active principle which improves calcium balance. Both are selective estrogen receptor modulators and bind to the estrogen receptor β. In early postmenopausal women genistein was capable to prevent bone loss compared to placebo [12]. Whether soy protein may improve intestinal calcium uptake in non-rodent species is not known. In human Caco-2 cells for example, isoflavones from soy bean and estrogenes did not improve calcium uptake, but calcitriol did [13]. Isoflavones were also ineffective in menstruating young women to improve bone mineral content [14]. Casein had an advantage over

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soy protein with respect to solubility of calcium in the fluid fraction of the ingesta compared to soy protein in piglets [15].

The regulatory mechanisms of calcium and phosphate and the dependency on Vitamin D might differ between the nocturnal rat and pigs or humans. This could also be one reason for the different outcomes of this type of feeding experiments between rats and non-rodent species. An interesting observation in this context is that in Vitamin D deficient rats normal calcium homeostasis can be maintained only by feeding a high calcium and phosphate diet without Vitamin D [11]. This has never been shown for pigs or humans. Pigs are regarded a suitable model for the study of problems related to human nutrition. In a porcine animal model with inherited calcitriol deficiency [16] we have investigated whether high calcium and phosphate diets with casein as a protein source and a high lactose content, similar to those used in rats [11] can normalize calcium metabolism in calcitriol deficient pigs.

2. Material and methods

2.1. Animals and diets

The experiments were carried out in a porcine animal model with an inherited defect of the renal 25(OH)D_3-1-hydroxylase. These piglets spontaneously develop severe hypocalcemia and hypophosphatemia [16]. The clinical symptoms are similar to those seen in humans with pseudo Vitamin D deficiency rickets, type I (PDDR-I). A total number of 109 PDDR-I piglets with an age of 4 weeks weighing 3.76 ± 0.71 kg were divided into four experimental groups and were fed with four semisynthetic diets (Table 1). All the diets contained 2.1% Ca and 1.2% Pi. Another group of 50 piglets served as controls and was fed a commercial piglet diet with 0.9% Ca and 0.6% Pi (Club VMK, Club Kraftfutterwerke, Hamburg, Germany). Twelve of these control piglets were PDDR-I piglets and 38 had a normal Vitamin D status. The animals were held in groups of four to eight on concrete floor with straw bedding.

2.2. Experimental setup

The piglets were weaned at an age of three weeks. The experimental period lasted for 6 weeks. Venous blood was collected at weekly intervals and heparinized plasma was stored. Ionized calcium (Ca^{2+}) was measured with an ion sensitive electrode (ISE Analyzer 987-S, AVL, Bad Homburg, Germany) and Pi was photometrically determined as vanadate–molybdate complex. The animals were sacrificed at an age of 9 weeks. The left tibiae were removed from which the muscles, periost, and cartilage were stripped off. The bones were dried at 85 °C, defatted in acetone, dried again, grinded, and ashed in an oven at 620 °C for 7 h. Chyme was collected from the stomach, jejunum, caecum and colon ascendens and was centrifuged at $4000 \times g$ for 20 min at 4 °C. Ca^{2+} and pH were measured in the supernatant fraction.

2.3. Statistics

Differences in Ca^{2+} and Pi in blood plasma between the four experimental groups were tested after a three way ANOVA using a Tukey test. Differences in BMC and Ca^{2+} content, pH in chyme were tested after calculating a one way ANOVA and a one way ANOVA on ranks. Significance testing was made by use of the Student–Newman–Keuls test and Dunn's test, respectively.

3. Results

At the beginning of the feeding period the Ca^{2+} concentration in the PDDR-I piglets was already significantly lower than in healthy control piglets and continued to decline until the end of the experiment (Fig. 1). Inorganic phosphate in plasma stayed permanently 29.6–40.3% lower than in the normal control piglets (Fig. 1).

In the four experimental groups of piglets replacement of the commercial piglet starter diet by either one of the four experimental diets could not prevent the decrease in Ca^{2+} ($P < 0.01$).

Table 1
Composition of the four experimental semisynthetic diets

Dietary component	Diet 1 soy protein, lactose	Diet 2 soy protein, corn starch	Diet 3 casein, lactose	Diet 4 casein, corn starch
Lactose	400.0	–	400.0	–
Corn starch	–	400.0	–	400.0
Soyamin®,[a]	296.0	296.0		
Casein	–	–	311.4	311.4
Barley	230.8	229.1	213.9	212.3
$CaCO_3$	7.6	3.4	10.8	6.6
$CaHPO_4$	35.6	41.5	33.9	39.7
Vitamin–mineral-premix	30.0	30.0	30.0	30.0
Number of animals	30	25	29	25

[a] Lyophylized soy protein isolate, isoflavone content per kg: diadzein >167 mg; genistein > 498 mg.

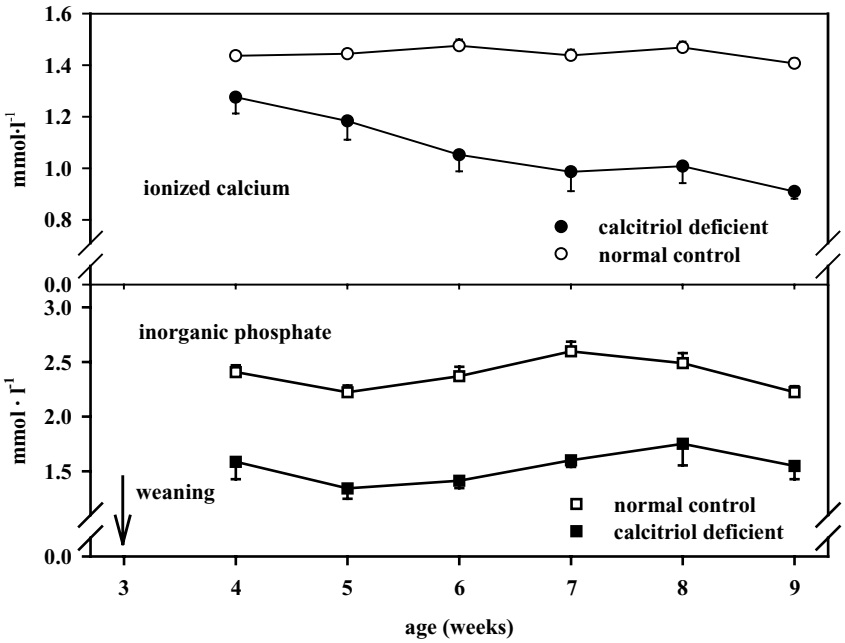

Fig. 1. Time course of ionized Ca and inorganic phosphate in blood plasma of calcitriol deficient and normal piglets from 3 to 9 weeks of age receiving a commercial piglet starter diet (mean ± S.E.M.).

With none of the four diets the piglets could maintain a normal Ca^{2+} concentration in blood plasma ($P < 0.001$) (Fig. 2). The decline was less in piglets receiving soy protein than in piglets consuming casein ($P < 0.001$) and was less with consumption of lactose than with uptake of corn starch ($P < 0.014$). Piglets receiving soy protein had higher Pi concentrations in plasma than those eating casein ($P < 0.001$) (Fig. 3) but consumption of lactose appeared to show no beneficial effect on plasma Pi when compared to the uptake of corn starch. At the end of the experiment Pi concentrations in plasma were about the same in the four experimental PDDR-I groups and in the normal control piglets.

Feeding the experimental diets could not maintain the BMC of the calcitriol deficient piglets in the normal range (Fig. 4).

At the end of the feeding period the BMC of the dry fat free tibiae was significantly 19% lower than in the normal control piglets ($P < 0.05$), but were higher than in the control PDDR-I piglets consuming the control piglet starter diet ($P < 0.05$) (Fig. 4).

No significant effects of the diets on Ca^{2+} and pH in chyme was observed in this study (Table 2). Ca^{2+} was generally higher in chyme than in blood plasma.

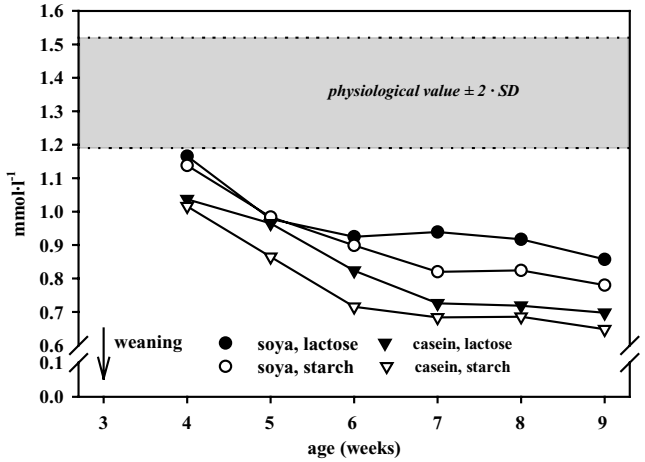

Fig. 2. Time course of ionized calcium from 3 to 9 weeks of age in blood plasma of piglets with inherited calcitriol deficiency receiving four different experimental diets (mean).

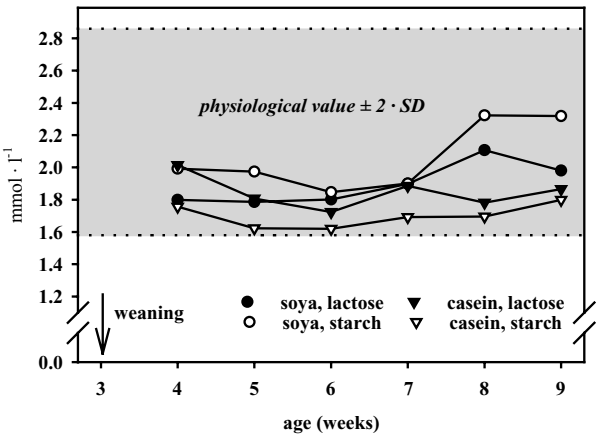

Fig. 3. Time course of inorganic phosphate from 3 to 9 weeks of age in blood plasma of piglets with inherited calcitriol deficiency receiving four different experimental diets (mean).

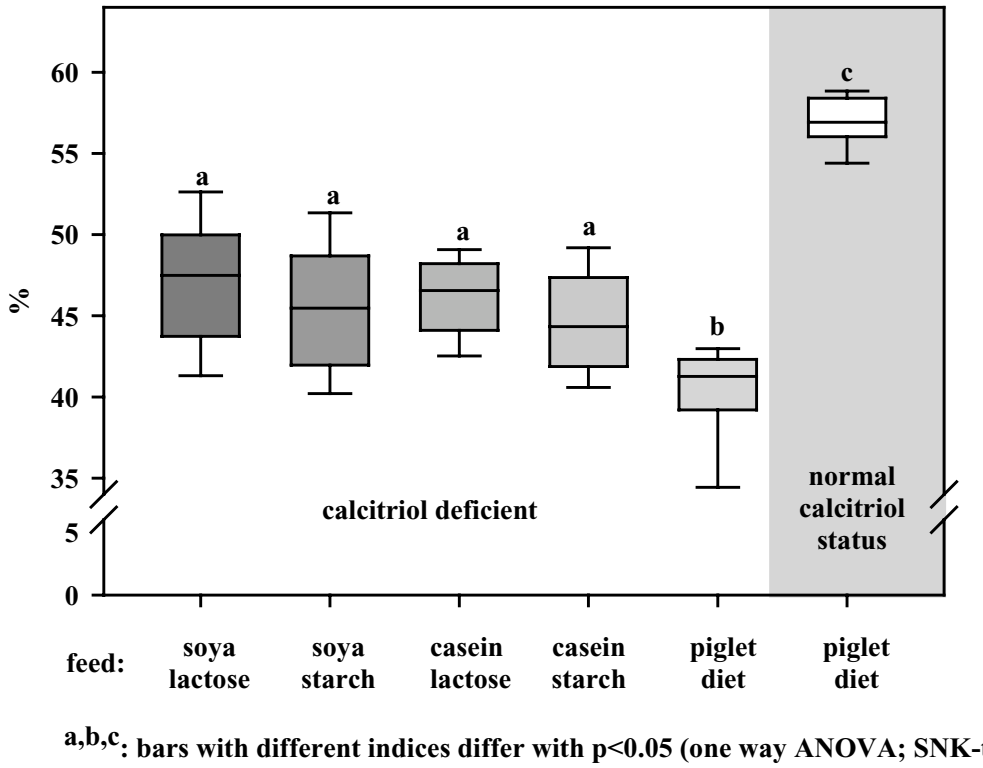

Fig. 4. BMC in the fat free tibiae of piglets with inherited calcitriol deficiency receiving four different experimental diets and of control piglets fed commercial piglet diet (median; 10; 25; 75 and 90% percentiles).

Table 2
Concentrations of Ca^{2+} (mmol l^{-1}) and pH in the gastrointestinal content of piglets fed five different diets from 3 to 9 weeks of age

Diet, additions	Stomach		Mid jejunum		Caecum		Colon ascendens	
	Ca^{2+}	pH	Ca^{2+}	pH	Ca^{2+}	pH	Ca^{2+}	pH
Soya lactose, $N = 6$	22.1 ± 10.9	3.3 ± 0.4	6.9 ± 3.1	6.9 ± 0.1	8.2 ± 3.3	6.8 ± 0.1	11.6 ± 5.2	6.7 ± 0.1
Soya corn starch, $N = 11$	26.7 ± 14.2	4.3 ± 0.4	4.6 ± 1.5	6.8 ± 0.2	6.4 ± 2.3	6.4 ± 0.1	3.2 ± 0.7	6.4 ± 0.1
Casein lactose, $N = 10$	16.4 ± 9.3	4.3 ± 0.5	4.0 ± 1.4	7.1 ± 0.2	4.7 ± 1.9	6.8 ± 0.1	2.3 ± 0.8	6.9 ± 0.1
Casein corn starch, $N = 10$	39.3 ± 19.2	3.6 ± 0.4	2.7 ± 0.5	7.3 ± 0.2	6.2 ± 2.3	6.9 ± 0.1	3.8 ± 1.5	6.9 ± 0.1
Commercial piglet diet, $N = 10$	8.4 ± 2.9	4.4 ± 0.2	5.0 ± 1.2	6.8 ± 0.2	12.3 ± 3.4	5.9 ± 0.1	17.8 ± 5.3	6.08 ± 0.2

The four experimental diets contained 2.1% calcium and 1.2% phosphate, the commercial piglet starter diet contained 0.9% calcium and 0.6% phosphate (mean ± S.E.M.).

4. Discussion

The observed improvement of Ca^{2+} and Pi in blood plasma and the higher BMC in the piglets receiving the experimental diets compared the PDDR-I control piglets are perhaps calcitriol independent effects. The possibility remains that isoflavones in the soy protein supplemented diets act through the Vitamin D system. From rat studies it is known that VDR is up [17] and downregulated [18] with increasing and decreasing estrogene concentrations in plasma. In the rat model estrogenes seem to be involved in intestinal calcium absorption [19,20] and also SERMS like isoflovones from soya [6] stimulated intestinal active calcium transport in rats. Whether this mechanism is also operative in other species like pigs and humans remains to be investigated. A two months estrogen treatment did not elevate intestinal calcium absorption of postmenopausal women [21].

The present study showed that in piglets in contrast to rats in the absence of calcitriol a normal blood and bone calcium status could not be maintained by dietary means alone. Vitamin D appeared to be essential to achieve this goal. Dietary additions of soy protein or casein or lactose in combination with high Ca and high Pi could not fully compensate for the lack of Vitamin D hormone. On the other hand, the plasma Ca^{2+} and Pi concentrations and the BMC showed some improvements in piglets consuming the experimental diets compared to the PDDR-I piglets. In this respect soy protein was more effective than casein and lactose was better than corn starch. Such differences were only apparent in

blood Ca^{2+} and partly in Pi. No corresponding differences related to diet composition were present in the BMC.

The different behavior of pigs and rats has probably some implication for the use of rats as an animal model for the study of hormonal and dietary effects on bone.

Our study showed that results obtained from in vivo studies with rats concerning the calcium and phosphate regulation should be used with care when they are transferred to non-rodent species like pigs and probably humans. This applies also to the discussion about the relative proportions of active and passive intestinal Ca^{2+} fluxes. [22,23].

From the findings of our study it appears useful to keep these species differences in mind. Comparative studies in other species, non-human primates for example appear to be useful to elucidate this problem.

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