Final Report

Title of Project:

Calcium and Magnesium Metabolism in Neonatal Calves Camel.

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1-Introduction

Maternal calcium homeostasis during pregnancy is strained due to foetal requirements for bone mineralization. In most animal species the mother adjust to mineral requirement of the foetus with alterations in her metabolism of vitamin D. This includes a decrease in 25-hydroxy vitamin D levels and an increase in circulating levels of the hormone 1,25-dihydroxy vitamin D which is active in maintaining calcium levels. The main factors involved in the control of plasma magnesium levels are absorption on one hand and excretion in the other hand, hence the maternal demands for the growing foetus were mostly met by dietary magnesium. The absorption of calcium from the intestinal tract is far greater than the absorption of magnesium despite the fact that they share a common pathway for absorption. Maternal calcium homeostasis is also under the control of the parathyroid hormone (PTH). Calcitonin also plays a part in calcium metabolism in the adult animal especially when calcium levels are higher than the physiological levels. The foetus during the last stage of gestation maintains itself hypercalcaemic and hypermagnasaemic relative to its mother by calcium and jmagnesium pumps that pumps calcium and magnesium from the maternal circulation to the foetal circulation. The situation in the neonate and young calf camel is unknown and needs to be investigated. The objective of this study is to investigate the following:-

1. The effect of increasing age on neonatal calcaemi and magnesaemia.
2. The role of the parathyroid gland in the neonatal and young calf camel calcium and magnesium metabolism.
3. Comparative study between the adult serum calcium and magnesium concentration and the young calf camel serum calcium and magnesium concentration.

4. The response of the young calf camel to induced hypocalcaemia and hypercalcaemia and its effects on serum magnesium concentration.

2- Literature Review:

Calcium has many vital functions in vertebrate animals, including muscle contraction, blood coagulaion, enzyme activity, neural excitability, hormone secretion, and cell adhesion as well as being an essential structural component of the skeleton (Capen and ROSOL, 1993b). To maintain a constant concentration of calcium, despite variations in intake and excretion, endocrine control mechanisms have evolved that primarily consist of interactions of three major hormones. Although direct roles of parathyroid hormone (PTH), calcitonin (CT), and vitamin D are emphasized in the control of blood calcium, other hormones such as adrenal corticosteriods, estrogens, thyroxine and parathyroid hormone related protein (PTHrP), may contribute in the maintenance of calcium homeostasis under certain conditions (Capen, 1989; Capen and ROSOL, 1993b).

Magnesium (Mg) is an essential dietary element for animals (Speich and Bousquet, 1991; McDowell, 1992 b). Green plants are an excellent dietary source of Mg for animals because of the presence of Mg^{2+} in chlorophyll (Wilkinson et al., 1990). Magnesium metabolism has been studied most extensively in cattle and sheep, because clinical disorders related to magnesium deficiency occur most commonly in those species (Birch, 1990). Serum magnesium concentration is less well controlled than that of Ca, and less is known concerning the regulation of serum Mg. There is a reciprocal relationship between Mg and Ca in the serum. Insufficient dietary Mg will lead to hypomanesaemia. Mg homeostasis is a result of balance between intestinal absorption and renal excretion with additional regulation by adrenals, thyroids and parathyroid glands. However, no endocrine gland exerts a primary regulatory role on plasma Mg concentration. In an immature animal the skeleton is a partially labile source of Mg, whereas in the adult the skeleton is largely inert relation to Mg mobilization.

Magnesium is essentially an intracellular caution and functions as an activator or a catalyst for more than 300 enzymes in the body, including phosphatases and enzymes that involve (Heaton, 1990). Mg plays vital role in muscle contraction, protein, fat and carbohydrate metabolism, methyl group transfer,oxidative phosphorylation, functional properties and stabilization of membranes, cell division, and immune responses. Magnesium regulates ribosomal RNA and DNA structure, thereby affecting cell growth and membrane stgructure (Gunther, 1990). Magnesium is required for maintenance of normal cellular potassium (Ryan, 1993), and Mg deficiency can lead to intracellular potassium depletion and excessive K excretion (Abbott and Rude, 1993). Magnesium regulates mitochondrial membrane permeability.

Phosphate in the mammalian body is present predominantly (90%) as hydroxyapatite $[Ca_{16}\ (PO_4)_6\ (Oh)_2]$ in the mineralized matrix of bone, with the remaining 10% occurring intracellularly in soft tissues. Phosphate is the major intracellular anion existing in organic (phospholipids, nucleic acids, phosphoproteins, AT) or inorganic forms and plays an integral role in many metabolic processes such as energy metabolism, delivery of 0_2 to tissues, muscle contraction and skeletal integrity (Dennis, 1996). In nonruminant animals on normal diets with adequate amounts of vitamin D, the kidneys are the major regulators of serum phosphate concentration (Yanagawa and Lee, 1992).

Parathroid hormone (PTH) is the principal hormone involved in the regulation of blood calcium in mammals. It exerts its biological actions by directly influencing the function of target cells primarily in bone and kidney and indirectly in the intestine to maintain plasma calcium at normal physiological levels. In general the most important biological effects of PTH are to elevate the blood calcium concentration, decrease the blood phosphorus concentration, increase the urinary excretion of phosphate by

decreasing tubular reabsorption of calcium, increase the rate of skeletal remodeling and the net rate of bone resorption, accelerate the formation of the principal active vitamin D metabolite (1,25 – dihydroxycholecalciferol) by the kidney through a trophic effect on the 1-x-hydroxylase in the kidney. Parathyroid hormone-related protein (PTHrP) is a 139-173 amino acid peptide originally isolated from human and animal tumors associated with humoral hypercalcaemia of malignancy (ROSOL and Capen, 1992) PTHrP shares 70% sequence homology with PTH in its first 13 amino acids. The N-terminal region of PTHrP (amino acids 1-34) binds and stimulates PTH receptors in bone and kidney cells with affinity equal to that of PTH and results in PTHrP functioning similarly to PTH in patients with humoral hypercalcaemia of malignancy (Orloff et al., 1994).

On the other hand, calcitonin secretion results in the development of varying degrees of hypocalcaemia and hypophosphataemia. These effects on plasma calcium and phosphorus are most evident in young or older animals with increased rates of skeletal turnover. The action of calcitonin on inhibiting bone resorption stimulated by PTH and other factors is from blockage of osteoclastic osteolysis. Specific structural alterations are produced in osteoclasts by calcitonin (Chambers and Moore, 1983). Calcitonin and parathyroid hormones acting in concert provide a dual negative feedback control mechanism to maintain the concentration of calcium in extracellular fluids within narrow limits. Present evidence suggests that parathyroid hormone is the major factor concerned with the minute-to-minute regulation of blood calcium under normal conditions. Calcitonin functions more as an emergency hormone to prevent the development of physiological hypercalcaemia and to protect against excessive loss of calcium and phosphorus from maternal skeleton during pregnancy.

Vitamin D and its active metabolites function to increase the absorption of calcium and phosphorus from the intestine, thereby maintaining adequate levels of these electrolytes in the extracellular fluids in order to permit the appropriate mineralization of bone matrix. A small amount of vitamin D is needed to permit PTH to exert its action on bone. The target tissue for the active form of vitamin D (1,25 – dihydroxy cholecalciferol) is the mucosa of the small intestine. Free 1,25 $(OH)_2D$ penetrates the plasma membrane of target cells and initially binds to cytoplasmic receptor in cells of the intestine (Walters, 1992). Subsequently the hormone-receptor complex is transferred to the nucleus and 1,25 – $(OH)_2D$ binds to specific receptors in the nuclear chromatin, where it stimulates gene expression leading to increased synthesis of vitamin D-dependent proteins such as calcium-binding protein by intestinal cells (Christakos et al., 1989) across which significant amounts of calcium are transported. The active metabolites of cholecalciferol also act on bone (High et al., 1981 b; Finkelman and Butler, 1985).

In mammals the foetus is maintained hypercalcaemic relative to its mother by means of a placental calcium pump which is driven by some unknown mechanism (Fisher, 1986). Thyroparathyroidectomy (TXPTX) (with thyroxine replacement) of the ovine foetus resulted in the loss of the transplacental calcium gradient, the foetal plasma a calcium fell below that of the mother and the lambs were borne rachitic (Care, Caple and Pickard, 1985; Aaron, Makins, Caple, Abbas, Pickard and Care, 1989). This suggested that the parathyroid gland was responsible for maintenance of the placental calcium gradient which provides maternal calcium for the needs of the growing foetal skeleton. The putative placental pump that maintains the foetus hypercalcaemic relative to its mother may exert a similar action on magnesium.

In the sheep during the last trimester of pregnancy, the foetal plasma magnesium concentration was shown to be higher than the maternal magnesium concentration (Mellor and Matheson, 1977). The situation after parturition for both calcium and magnesium in the neonate and young growing camel is unknown. The factors involved in the regulation of calcium and magnesium metabolism in the neonatal and young calf camel needs to be investigated.

3- Materials and Methods

a- Sampling:

Blood samples were collected from camels located at the premises of the camel research center (King Faisal University) and from slaughtered animals (Hofuf Slaughter House). Blood samples were collected into clean plain silcon coated vacutainer tubes and the centrifuged sera were kept in clean vials at 4^{o}C until analysis.

Matched blood samples were collected from female camels and their off springs at regular intervals for comparative studies of their calcaemia and magnesaemia.

Kidney tissues were collected from the slaughter house, kept in ice, then frozen until they were analysed for 1-x-hydroxylase enzyme activity.

Experimental work:

b- Induction of Hypercalcaemia:-

Two young camels one month old were used in this experiment. Each animal was given 400 ml of 40% calcium borogluconate intravenously and 400 ml of 20% calcium borogluconate subcutaneously. Blood samples were collected before and after calcium administration at appropriate intervals (0, 30, 60 and 90 minutes)

c- Induction of hypocalcaemia:-

Hypocalcaemia was induced into two young calf camels one month old using ethylene diamine tetra-acetic acid (EDTA) at a dose rate of 1 gm / 15 kg body weight given as 2% intravenously. Blood samples were taken regularly every 30 minutes for almost 12 hours. Sera were separated and kept at 4^{o}C until analysis.

d- Surgical removal of the parathyroid glands (PTX).

Surgical approach:-

Two young calf camels 1-2 month old were used in this experiment. Each animal was operated and a partial parathyroidectomy was performed and the removed glands were kept in 10% formal saline for histological processes. Blood samples were collected prior to parathroidectomy and at regular intervals (30, 60, 90, … 148 hrs) afterwards. Separated sera were frozen until being analysed.

Sedation of the animals was done and the dose of the anaethetic used was at a rate of 0.2 mg/kg body weight (1 ml xylazine; Seton 2% intravenously). Local infiltration was done using 2% lignocaine (Bomac Laboratories, New Zealand).

Surgical technique:-

All parathyroidectomy operations were carried out by Professor Ramadan Omer Ramadan at the Veterinary Teaching Hospital, College of Veterinary Medicine and Animal Resources, King Faisal University. The operated animal was casted on its right side and a 7 cm mid-ventral incision was made caudal to the larynx. Blunt dissection between the two sterothyroid muscles was performed. The parathyroids were identified as seed-like structure embedded within the thyroid tissue beneath the thyroid capsule, then it was removed and kept in 10% formal saline until used for histological examination. Hemostasis was achieved at a routine base and the subcutaneous tissues were coaptated using 4 metric polyglactin 910 (coated vicryl). (Bankhead Avenue, Edinbrough EH 11, 4HE, U.K.). The skin was closed with non absorbable material in an interrupted mattress pattern. The sutures were removed in 10 days. Antibiotics were given systematically (Oxytetracycline 20%) at a dose rate of 1 ml/kg (Kela Laboratoria, Belgium).

e- Histological technique:-

The parathyroid were fixed into 10% formal saline, processed by routine histological techniques, then embedded in paraffin wax and cut (7 µ) thickness by a microtome and stained with Haemotoxylin and Eosin and examined under light microscope to confirm the removal of the parathyroids.

Biochemical methods:

f- Serum calcium determination:-

Serum calcium was measured according to the method described by Willis (1960) using atomic absorption spectroscopy (Pye-Unicam SP 90, spectrophotometer, Unicam instruments, Ltd., Cambridge, England) and an air / acetylene flame. The standards and samples were prepared in 0.78% EDTA solution to reduce phosphate interference.

g- Serum magnesium determination:-

Serum magnesium was measured according to the method described by Willis (1959) using an atomic absorption spectrophotometer. The standards and samples were prepared in 0.78% EDTA solution to reduce phosphate interference.

h- Inorganic phosphorus determination:-

Serum inorganic phosphate was determined by the method described by Varley (1967) which was based on removal of protein from the serum by trichloro acetic acid with consequent treatment of filtrate with acid molybdate. This will react with inorganic phosphate giving phosphomolybdic acid; the hexvalent molybdenum of the latter was reduced by the metal giving a blue measurable compound.

4- Results

1. Effect of age on calcium and magnesium concentrations in the young calf camel:

The results of part of the study are summarized in Table 1 and Figure 4, whereas Figure 1 compares the overall adult mean serum calcium (Ca) with the young camel mean serum calcium concentration. It is evident that within 12 months the maternal or adult calcium concentration is greater than the serum calcium concentration of the young camel. Likewise, Figure 3 shows that the overall mean of adult serum magnesium is greater than that of the young calf 12 months of age.

Figure 2, compares the serum calcium concentration of the adult camel with the different ages of the young calf camel. It is evident from Figure 2, that the mean serum calcium concentration of the young calf camel at the age of 1-4 months is non-significant greater than the adult or maternal values ($P > 0.05$), (See Table 1). Figure 4, shows that serum magnesium concentration of the young calf camel at the age of 1-4 months is non-significantly greater than the adult ones ($P > 0.05$). With the advancement of age both cautions decline to value below the adult ones.

2. young camel: The effect of administration of EDTA on calcium and magnesium concentration in the young camel:

Figures 5 and 6, show the effect of administrating EDTA intravenously on young calf camels and its effects on their serum calcium and magnesium concentrations. In both animals injected intravenously with EDTA, the serum Ca concentration tended to decrease within 30-60 minutes from the commencement of dosing. Serum Mg concentration was not affected by EDTA infusion.

3. The effect of administrating calcium borogluconate on calcium and magnesium concentrations in the young camel:

Figures 7 and 8 show the effect of administrating calcium borogluconate intravenously on young calf camels and its effects on their serum Ca and Mg concentration. Within 30-60 minutes from dosing serum calcium concentration tended to rise then declined and remained constant till the end of the experiment. This treatment had no effect on serum Mg, although a slight increase in serum Mg concentration was observed in animal (2), 30 minutes after dosing and continued to be like this for 120 minute, then declined to pre-injection levels.

4. The effects of partial parathyroidectomy on serum calcium and serum magnesium in the young calf camel:

Figures 9 and 10 disclose the effect of partial parathyroidectomy (PTX) on young calves serum Ca and Mg concentrations. PTX resulted in a slight decrease in serum calcium and serum magnesium concentrations. After 24 hours post surgery the serum calcium and serum magnesium concentration were almost normal and comparable to pre-surgical levels. In animal No. 3 within 96 hrs post-surgery, serum magnesium started to rise sharply as calcium was stabilized.

5- Discussion

Calcium and magnesium metabolism in the pregnant camel, foetus, neonate and young calf camel is complex with many interrelated components. In humans, the placenta transmits calcium ions from mother to foetus against a concentration gradient, making the foetus in late pregnancy hypercalcaemic with respect to its mother (Schauberger and Pitkin, 1979). Magnesium and phosphorus, two other ions involved in calcium metabolism, are also in higher concentration in foetal than in maternal serum. With birth, the transplacental calcium source disappears and the new born's serum calcium level falls for the first 24-48 hours after birth, then stablize and rises slightly to reach adult levels by 1 week of age. The hypercalcaemia in sheep and cattle may persist until plasma calcium concentration has declined to adult level during the two weeks after birth, whereas in the pig and human, adult levels of calcium concentration are rapidly attained after birth (Care et al., 1982). The plasma magnesium concentration in human umbilical cord is similar to that in maternal plasma (Bogden, Thind, Kemp and Caterini, 1978), but in the sheep during the last trimester of pregnancy, the foetal plasma magnesium concentration was shown to be higher than the maternal magnesium concentration (Mellor and Matheson, 1977). The situation in the foetal camel and the young calf camel is unclear. The results of the current study have shown that the mean serum calcium concentration and the mean magnesium concentration of the calf camel at the age of 1-4 month is greater than the adult calcium and magnesium concentration (Fig. 2) then it started to decline to levels below the adult values at the age of five month. The result emphasizes the importance of calcium and magnesium for the growing calf camel to ensure more bone mineralization. However, this observation needs to be confirmed and more research should be carried out to determine the exact age at which serum Ca and Mg decline to adult values. The results

presented in Figure 5 and Figure 6 did show a slight decrease in calcium concentration but no effect on magnesium. This is probably due to the chelating effect of EDTA, however, this insignificant decrease may have been due to the small dose of EDTA used in this experiment or the parathyroids of the calf camel may have responded to this chelating effect producing more parathyroid hormone (PTH) which has resulted in increased calcium absorption from the small intestine thus a buffering effect was created which has resisted any decline in calcium concentration. It is evident from the resuls presented in Fig. 5 and Fig. 6 that EDTA infusion has no any effect on serum magnesium concentration. The results presented in (Fig. 7) and (Fig. 8) have shown a small rise in serum calcium concentration 30-60 minutes after administrating calcium borogluconate then the levels tended to decline to normal values. This slight increase is insignificant ($P > 0.05$) and it might have been resisted by the secretion of calcitonin by the parathyroid glands. The administration of calcium borogluconate has no effect on serum magnesium concentration. Fig. 9 and Fig. 10 show the effect of partial parathyroidectomy on serum Ca and Serum magnesium in the young calf camel. There was a decrease in both Ca and Mg within 30-60 minutes post-removal and this could be explained to reduced PTH levels and therefore reduced intestinal Ca and Mg absorption leading to decreased serum levels. Although this reduction in serum Ca and Mg is insignificant ($P > 0.05$) but this highlights a fact that the parathyroid gland may have a role in calcium and magnesium metabolism in the young calf camel through the action of PTH on intestinal absorption of Ca and Mg. Also, the insignificant decrease in Ca and Mg could be explained by the fact that this surgical approach is only partial and still a functioning pair of parathyroids is still intact and producing parathyroid hormones. It could be concluded from these experiments that:-

1. Ca and Mg concentrations in the blood of the growing calf camel are affected greatly by increasing age and that adult values are attained up to 4 months of age.
2. The higher values for Ca and Mg during the first 4 months suggested that the foetal camel like other mammals is maintained hypercalcaemic and hypermagnesamic during intra-uterine life.
3. The mild effect on serum Ca and serum Mg due to the infusion of EDTA and calcium borogluconate emphasizes a role for the calf camel parathyroids in Ca and Mg metabolism.
4. The results of partial parathyroidectomy are inconclusive perhaps due to the presence of another pair of parathyroids embedded in the thyroid gland.

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