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ORIGINAL ARTICLE

Effects of casein, whey and soy proteins on volumetric bone density and bone strength in immunocompromised piglets ☆

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Summary
Background & aims: Bone-promoting effect of different proteins in early life, under immunocompromised conditions, is unknown. We investigated effects of milk- and plant-derived proteins on bone development in immunocompromised piglets.
Methods: Newborn, colostrum-deprived piglets were assigned to a formula based on either casein ($n = 11$), whey ($n = 11$) or soy ($n = 10$) as the protein source (each 55 g/L), and equal amounts of fat, carbohydrates, calcium and phosphorus.
Results & Conclusion: Despite efforts to sustain immuno-protection (sow serum and antibiotic injections), some piglets became sick and were early euthanised. After 6 days, bone density (peripheral quantitative computed tomography), bone mechanical strength (three-point bending test) and serum insulin-like growth factor-I (sIGF-I) (immunoassay) were measured in the surviving piglets (casein $n = 5$, whey $n = 9$, soy $n = 5$).

Abbreviations: BA, bone area; BMC, bone mineral content; BMD, bone mineral density; CPP, caseinophosphopeptides; FN, femoral neck; MF, midshaft femur; MT, midshaft tibia; pQCT, peripheral quantitative computed tomography; PT, proximal tibia; sIGF-I, serum insulin-like growth factor-I.

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Results: Baseline body weight was not significantly different between the groups and between early euthanised and surviving piglets. After 6 days, all piglets showed depressed growth and low sIGF-I levels. At trabecular sites, whey reduced bone mineral density compared with casein and soy ($P \leqslant 0.01$), and reduced mineral content compared with casein ($P \leqslant 0.01$). Whey decreased cortical thickness and bone strength compared with casein ($P \leqslant 0.04$).
Conclusion: Despite immunocompromised conditions, casein and soy protein may promote a better bone development in colostrum-deprived piglets.

Introduction

Osteoporosis, a disease characterised by decreased bone mass and deterioration of bone tissue, is estimated to cause 9 million fractures worldwide per year.[1] Maximising the amount of bone mass and optimising bone geometry have been recognised as preventive strategies for osteoporosis outbreak later in life.[2] Bone mineral density (BMD) increases by 160% during infancy,[2] demonstrating importance of this period for bone development.

Bone growth depends on the balance between the activity of bone forming osteoblasts and bone resorbing osteoclasts. Since this balance can be altered by the immune components, the immune system is an important regulator of bone homeostasis, particularly during immunocompromised conditions.[3]

Nutrition, including dietary protein, is essential for bone growth.[4] Increasing protein intake increases serum levels of insulin-like growth factor-I (sIGF-I),[4] a key regulator of bone metabolism.[5] In infant nutrition, the main sources of protein originate from milk-derived casein and whey, and from plant-derived soy. Milk-derived proteins contain several bioactive peptides thought to exhibit bone-enhancing properties.[6,7] Caseinophosphopeptides (CPP) obtained from a tryptic digestion of casein have been reported to increase intestinal calcium absorption in *in vitro* and in animal models,[6] indicating that CPP may stimulate mineral acquisition. Similarly, whey protein has been shown to exhibit bone-protective properties, including stimulation of osteoblasts[8] and suppression of osteoclast[9] *in vitro*. Furthermore, whey decreased estrogen-related bone loss in rats[10] and humans,[11] but no studies have investigated its effect on bone growth during early stages of skeletal development. Soy protein is a widely used alternative for feeding infants with cow-milk allergies. It has been hypothesised that low content of sulphur containing amino acids in soy protein[12] may decrease urinary calcium excretion and thereby benefit bone density. In contrast, soy-derived phytic acid inhibits mineral absorption and may be detrimental for skeletal development.[13]

The effect of dietary protein on bone development early in life, under immunocompromised conditions is unknown. We designed a model of colostrum-deprived piglets, to study the effect of milk-derived casein and whey, and plant-derived soy on neonatal bone growth, under immunocompromised conditions.

Materials and methods

Animals and study design

In total, 32 term piglets obtained from three sows (Duroc × [Yorkshire × Danish Landrace]) were delivered at term (115 ± 2 days of gestation), following an induction of labour by an intravulvar injection of 0.175 mg cloprostenol (Estrumat vet, Schering-Plough, Farum, Denmark). Immediately after birth, the piglets were anaesthetised with ketamin (Ketaminol, Intervet, Skovlunde, Denmark, 5 mL/kg body weight, i.m.) and medetomidin (Domitor, Orion Pharma, Espoo, Finland, 0.04 mg/kg body weight, i.m.) and fitted with orogastric feeding tube (infant feeding tube 6F, Pharmaplast, Roskilde, Denmark). Following surgery, the piglets were administered an antidote (Antisedan, Orion Pharma, 2.5 mL/kg body weight, i.m.) to minimise the length of anaesthesia.

Neonate piglets lack transplacental, passive immunisation and are entirely dependent on colostral immunoglobulins.[14] To induce immunocompromised conditions and to avoid the bias due to colostrum-derived casein and whey proteins on the outcome of the trial, the piglets were deprived of colostrum. However, to allow a minimal systemic immunoprotection,[14] two boluses of sow serum obtained from stock were slowly injected (5 mL/kg body weight, at 0 and 30 min) via the transected cord artery. To further minimise the risk of bacterial infection, antibiotic injections were given daily throughout the trial (Baytril, Bayer, Leverkusen, Germany, 2 mg/kg body weight, i.m.). The piglets were housed in groups of three after random allocation. The weigh and the clinical status were monitored daily and the piglets were euthanised when signs of discomfort (hyperventilation, distended abdomen, decreased responsiveness) occurred following colostrum deprivation.[14]

The piglets were assigned to either a casein- ($n = 11$), whey- ($n = 11$), or soy group ($n = 10$), so that the mean baseline body weighs were similar between the groups. The piglets were fed milk formulas, given at 15 mL/kg body weight every $1\frac{1}{2}$ h between 0730 and 1930, and every 3 h between 2000 and 0600, for 6 days. The formulas (Arla Foods Ingredients, Viby, Denmark) were designed to mimic the nutritional composition of sow's milk, and contained equal amounts of energy (4.1 MJ/L), fat (76 g/L; butter oil), protein hydrolysate (55 g/L), lactose (53 g/L; Variolac