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Availability of calcium from skim milk, calcium sulfate and calcium carbonate for bone mineralization in pigs

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Abstract —Dairy products provide abundant, accessible calcium for humans, while some calcium sulfate-rich mineral waters could provide appreciable amounts of calcium. But there is little evidence that this calcium is as available as milk calcium for making bone. The availability of calcium was studied by monitoring bone parameters in 2-month-old pigs fed restricted amounts of calcium (70% RDA) for 2.5 months. The 3 main (≥ 50% Ca intake) Ca sources were either $CaCO_3$ or $CaSO_4$ or skim milk powder (29% of the diet). The bones of the pigs fed the "milk" diet had higher ($P < 0.01$) ash contents, breaking strength and density (DEXA) than those of the two others groups, in which the bone values were similar. Thus, the calcium provided by a diet containing milk appears to ensure better bone mineralization than do calcium salts included in a non-milk diet. The calcium restriction may have enhanced some milk properties to stimulate calcium absorption in these young, rapidly growing pigs.

calcium availability / calcium sulfate / milk calcium / bones / pigs

Résumé — Biodisponibilité comparée du calcium du lait, du sulfate et du carbonate de calcium pour la minéralisation osseuse: étude chez le porc. Le sulfate de calcium, dont certaines eaux minérales sont riches, est-il aussi bien utilisé pour la minéralisation osseuse que le calcium des produits laitiers ? Trois lots de 8 porcs, de 2 mois, recevaient pendant 2,5 mois, trois régimes dont au moins 50 % de l'apport calcique était fourni par $CaSO_4$, $CaCO_3$ ou la poudre de lait. Bien que différents dans leur composition, les trois régimes étaient formulés pour avoir la même valeur nutritionnelle et leur teneur en Ca était restreinte (70 % des recommandations). Avec le régime « lait », les cendres, la résistance à la rupture et la densité minérale des os étaient supérieures à celles des deux autres groupes, non différents l'un de l'autre. L'analyse des paramètres circulants liés au métabolisme osseux (en particulier au collagène), des 3 groupes, exclut l'hypothèse d'un effet direct de la source

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de calcium sur la formation ou la résorption osseuse. En revanche, la phosphatasémie plus élevée, avec le régime « lait » qu'avec les deux autres, est cohérente avec une stimulation éventuelle de l'accrétion osseuse liée à une disponibilité plus grande de Ca et P. La restriction calcique pourrait avoir exacerbé certaines propriétés des composantes du lait chez ces jeunes porcs à très forte vitesse de croissance.

calcium du lait / sulfate de calcium / os / modèle porc / biodisponibilité

1. INTRODUCTION

There is no doubt that milk contains large amounts of calcium. Many trials have been carried out over the past decade to compare the availability of calcium in milk with several other sources of calcium, such as salts, mineral waters, and plant products [26]. The studies on availability were carried out on men or women, using a variety of methods (fractional or apparent absorption, urinary calcium). None showed that the calcium in milk was more efficiently used than that from any other source. Carbonate, gluconate, citrate-malate (CCM), chloride, lactate, acetate, phosphate and citrate have all been tested [26]. Mineral waters containing calcium bicarbonate or sulfate were not any better [4, 5, 56].

The growing pig is a useful experimental model in which changes in bone and mineral metabolism caused by diets with varying mineral content may be investigated [8, 31, 37, 39, 45]. Clearly, it is much more feasible to work with young growing animals, whose calcium metabolism is very active, than to attempt such studies on children.

Our previous studies in pigs have provided no evidence that calcium is better absorbed from milk and milk products (casein phosphopeptides, skim milk or yogurt) than from mineral salts ($CaCO_3$, CCM, $CaHPO_4$) incorporated in cereal + soya diets [35, 36, 38]. However, the breaking strength of bones from pigs fed yogurt as a calcium source is better than that of bones from pigs that obtained their calcium mostly from inorganic salts [38].

Long term clinical studies on bone have not yet been done using calcium-rich mineral waters. Hence, there is, as yet, no evidence showing that the calcium from mineral water (providing calcium as sulfate or bicarbonate) is as effective as calcium from carbonate or milk. While several human studies indicate that calcium from these sources is as well absorbed as that from milk or calcium carbonate [5, 7, 56], only one study, that of Cepollaro et al. [4], reported a positive effect of consuming a high-calcium bicarbonate water on the bone density of 45 menopausal women, after 13 months of this form of supplementation. The control group (who drank a low-calcium water) was given no calcium supplement (calcium intake: supplemented, 1500 $mg \cdot day^{-1}$; unsupplemented, 949 $mg \cdot day^{-1}$). Apart from this trial, no data on the long-term effects of other high-calcium mineral waters on bone are available.

Only one study on rats found that increasing dietary calcium intake with calcium sulfate led to an increase in bone mineral content 4 weeks later. The same calcium intake from milk provided similar (ash as % dry matter), or better mineralization (Ca as a % of bone-dry matter) than calcium sulfate [41]. Lastly, milk and dairy products are not only excellent sources of calcium; they also provide an almost complete diet whose consumption provides a meal effect. This fosters the absorption of calcium and provides an intake of phosphorus that is essential for bone deposition [16]. Any other source of calcium, such as calcium supplements and mineral waters cannot provide these advantages. Thus, three calcium sources, milk-Ca, calcium sulfate (analogous to high

Ca-sulfate mineral waters) and calcium carbonate (as a reference source) were compared by a combination of direct and indirect (bone-markers) bone measurements in growing pigs.

2. MATERIALS AND METHODS

2.1. Animals and feeding

Twenty-four 2 month-old male crossbred pigs (Cepra, Vermenton, France) weighing 19.6 ± 0.4 kg were randomly assigned to one of three groups. Each of them was fed a diet in which one of the 3 Ca sources provided more than 50% of the total intake. The dietary Ca levels were approximately one third lower than the normal Ca dietary allowances of growing pigs (at the start of the experiment) [15] to emphasize any difference in the availability of calcium from the 3 sources.

The diets were initially formulated to provide 0.6% total calcium, of which 70% (0.42% Ca) was derived from milk ("milk" group), or from calcium sulfate ("sulfate" group), or from calcium carbonate (control "carbonate" group). The 0.42% Ca was obtained from milk by incorporating 29% skimmed milk powder into the diet, the basal ingredients provided 0.06% Ca and the 0.6% total Ca was reached by adding 0.372% Ca carbonate (providing 0.14% Ca). The 0.42% Ca for the calcium sulfate diet would have been obtained by incorporating 1.6% Ca SO_4. Since pigs fed such a "sulfate" diet suffered from diarrhea in a preliminary assay, the percent of Ca sulfate utilized was 1.23 (providing 0.33% Ca), basal ingredients provided 0.14% Ca and 0.44% calcium carbonate was added. The average Ca provided as sulfate was 50% of the total calcium in the "sulfate" group. The control diet contained 0.13% Ca provided by basal ingredients and 0.51% by calcium carbonate.

Milk, sulfate and control diets were not identical, but they were equivalent in terms of energy [14.8 (or 10.6) MJ digestible (or net) energy·kg^{-1} diet], protein, crude fibers, fat, vitamins and minerals. They were also equivalent in terms of phosphorus contents including phytic P: 2 (carbonate), 1.7 (milk) and 2 (sulfate diet) g·kg^{-1} (estimated according to [34]). Moreover, the three diets contained similar amounts of wheat (≥ 42%), a phytase-rich cereal which optimizes the plant phosphorus utilization by hydrolyzing phytates [34]. These diets (Tab. I) were formulated to meet the French recommendations for nutrient supply to growing pigs (except for Ca) [19]. The digestible amino acids and energy contents (including starch and soluble carbohydrates) of the three diets were carefully equalized using recent data obtained in pigs [20, 32, 47].

All the pigs were pair-fed. The pigs were housed individually (pens of 1×2.5 m, concrete floor and walls), and their growth was evaluated weekly for a total of 75 days. The animals were stunned by electronarcosis and killed by exsanguination. The study complied with the French regulations governing animal care (n° 88–123, 1988; n° 87–848, 1987).

2.2. Plasma parameters

Venous blood samples were monitored for calcium, inorganic P, total and bone alkaline phosphatase (ALP) isoenzyme activity, osteocalcin, 1,25-dihydroxycholecalciferol (calcitriol), immunoreactive parathyroid hormone (iPTH), carboxy-terminal propeptide of type I procollagen (PICP), and the carboxy-terminal cross-linked telopeptide of type I collagen (ICTP). The samples were collected from the anterior vena cava, 1 month before slaughter (day 45), or at slaughter during exsanguination.

Ca, inorganic phosphorus, PTH, calcitriol, ICTP and PICP were determined at slaughter only. Total and bone ALP (EC 3131), and osteocalcin were determined twice, at slaughter and one month before. Plasma ALP and osteocalcin were used as markers of osteoblast activity [6]. The plasma concentration of PICP was

considered to be a marker of the synthesis of bone collagen and ICTP as a marker of its breakdown [9]. ICTP is the carboxy-terminal telopeptide region of type I collagen, cross-linked via pyridinoline cross-links; it is liberated during the degradation of type I collagen, the only collagen type found in mineralized bone [43]. Plasma Ca, inorganic

Table I. Diet composition.

Diets	Carbonate	Milk	Sulfate
Ingredient(1), %			
Wheat	42.00	42.50	42.00
Barley	18.38	24.67	17.65
Soybean meal 48	13.50	–	13.70
Fish soluble	6.00	–	6.00
Skim milk powder	–	29.00	–
Saccharose	14.00	–	14.00
Maize oil	3.26	3.00	3.42
Ca carbonate	1.33	0.372	0.472
Ca sulfate	–	–	1.23
Mono Na phosphate	1.05	–	1.06
Mineral and vitamin mix(2)	0.10	0.10	0.10
L-lysine-HCl	0.231	–	0.228
DL-methionine	0.045	0.040	0.045
L-threonine	0.085	0.065	0.085
L-tryptophan	0.013	–	0.013
NaCl	–	0.25	–
Nutrients, %			
Mineral contents (analyzed)			
Calcium	0.64	0.62	0.65
Phosphorus	0.53	0.54	0.52
Calculated data(3)			
Phytic P	0.20	0.17	0.20
Starch	33.34	36.40	32.96
Soluble carbohydrates	16.80	16.78	16.80
Fat	5.42	4.56	5.57
Crude fiber	2.44	2.10	2.42
Neutral detergent fiber	8.83	8.15	8.74
Proteins	18.46	18.60	18.46
Digestible lysine	1.021	1.022	1.021
Digestible methionine	0.378	0.350	0.350
Digestible sulfur amino acids	0.614	0.614	0.613
Digestible threonine	0.664	0.664	0.664
Digestible tryptophan	0.210	0.189	0.189
Digestible energy, $MJ \cdot kg^{-1}$	14.82	14.79	14.81

(1) Wheat, barley and soybean meal 48 (i.e. 48% protein) from Bonaliment, Limours. Fish soluble: solubilized fish protein concentrate (CPSP 90) from Sopropêche, Boulogne-sur-Mer. Skim milk powder from Sofivo, Condé-sur-Vire. Added aminoacids from Rhône-Poulenc, Antony. Calcium carbonate, sodium chloride and monosodium phosphate from Prolabo, Bondoufle and calcium sulfate ($CaS0_4$, 1/2 H_20; 26.5% Ca) from Merck-France, Nogent-sur-Marne, France.
(2) Trace elements and vitamin mixtures adapted from [8].
(3) According to [19, 20, 32, 34].

P, total and bone ALP were measured as previously described [39, 40]. PICP was determined by radioimmunoassay (Orion Diagnostica, Espoo, Finland). The lowest detectable concentration was 1.2 $\mu g \cdot L^{-1}$ and the intra- and inter-assay coefficients of variation were 3% and 5%, respectively. ICTP was determined by radioimmunoassay (Orion Diagnostica, Espoo, Finland). The lowest detectable concentration was 0.5 $\mu g \cdot L^{-1}$ and the intra- and inter-assay coefficients of variation were both near 5%. A modified radioimmunoassay kit (Ostk-Pr, Oris, Gif- sur-Yvette, France) was used to determine osteocalcin, with purified bovine osteocalcin as the standard and tracer, rabbit antiserum to bovine osteocalcin, and an internal porcine standard [39]. Calcitriol was measured by a radioreceptor assay using a kit (Nichols Institute Diagnostics, San Juan Capistrano, USA) after extraction on modified $C_{18}OH$ columns. Immunoreactive PTH was measured by a two-site immunoradiometric assay (Allegro intact PTH kit, Nichols Institute, Mallinckrodt Diagnostica, Evry-Lisses, France) [8]. The sensitivity of the method was 0.1 $pmol \cdot L^{-1}$. The intra- and inter-assay coefficients of variation were 3.4% and 5.6%, respectively.

2.3. Urinary parameters

A sample of 15 pigs (5 per group) were kept in individual cages and fed the same amounts of feed (1.8 ± 0.0 $kg \cdot day^{-1}$) for the last 10 days immediately before slaughter. A 5% sample of each 24 h urine collection was taken and the samples were pooled for the 10 days. Calcium and deoxypyridinoline were determined on these urine samples [40]. The concentration of deoxypyridinoline was measured using a competitive enzyme immunoassay (PYRILINKS-D, Metra Biosystems from Behring Diagnostic, Rueil-Malmaison, France). This metabolite is a crosslink found mainly in the type I collagen of bone and released in the process of bone degradation. It is thus a marker of bone resorption [6].

2.4. Bone parameters

Bone density, breaking strength and ash contents were assessed on a total of 9 bones collected at slaughter and dissected free of soft tissues: 1 tibia (right), the first 2 lumbar vertebras (L1 and L2), 4 main (finger III, internal and IV, external) metatarsals (right and left hind-legs) and 2 main (finger III and IV) metacarpals (left foreleg).

2.4.1. Bending moments

The "three-point bending test" at fracture was used to determine the bending moment on the tibia and the 6 metatarsal and metacarpal bones [8]. The failure load, the force (Newtons [N]) applied to the midpoint of the bone until breaking, was determined with a universal testing machine (AP 4000 Erichsen, Villetaneuse, France). The bending moment was calculated according to the formula: bending moment = $F \times L/4$, where F is the failure load, and L the length (m) between the two fulcrum points supporting the bone. It is expressed in $N \times m$. This parameter was selected because of its great sensitivity to mineral nutrition in the pig [8, 35, 38, 39].

2.4.2. Ash contents

External, left and right, metarsal and left internal metacarpal bones were used to determine the volume (external metatarsals only), dry matter and ash contents [39]. Ash ($g \cdot 100\ g^{-1}$ dry matter) was evaluated for the whole bone, the diaphyses (to represent cortical bone), and the proximal and distal epiphyses (to represent spongy bone). Values for ash relative to apparent whole bone volume were calculated for the two external, left and right, metatarsals.

2.4.3. Bone mineral density

Bone mineral density (BMD), in grams per square centimeter, and bone mineral contents (BMC), in grams, were measured

using dual-energy X-ray absorptiometry (DEXA) with a Hologic QDR-1000 X-ray bone densitometer (Hologic France, Massy, France). BMD was measured in the whole bone and in three regions of the tibia and 2 metatarsal (left, internal and external) bones: 2 corresponding to the distal and proximal epiphyses which are rich in cancellous bone and one to the mid-diaphysis which consists of cortical bone. Total BMD was also determined on the 2 vertebrae. Lastly, the total ash content, obtained by mineralization of the whole bone, and the bone mineral content (BMC), evaluated by densitometry, of the external left metatarsal were compared.

2.5. Statistical methods

The data were analyzed using a statistical software (SuperANOVA, Abacus Concepts Berkeley, CA). Growth parameters, plasma minerals and hormones, ICTP and PICP, urinary bone-related markers were analyzed by one-way (diet) analysis of variance (ANOVA) and Newman-Keuls multiple mean comparison (MMC) as a post test [50]. Bone ash contents, densitometry and bending moments were analyzed by two-way (diet and bone) ANOVA and MMC. Plasma ALP, osteocalcin were analyzed by two-way (diet and time) ANOVA and MMC. Relationships between plasma and/or bone variables were assessed by linear correlation [50] with all individual data obtained at slaughter.

3. RESULTS

3.1. Performance

The performances of the 3 groups were similar. The body weights (kg) at slaughter did not differ: 71 ± 0.8 (carbonate), 73 ± 2 (milk) and 72 ± 1.3 (sulfate), nor did the average daily weight gains, 0.69, 0.71 and 0.69 (± 0.02) kg·day^{-1}, nor the feed-efficiency ratios (kg body weight gain·kg^{-1} diet consumed), 0.47, 0.49 and 0.47 (± 0.01). The total amount of calcium (g) consumed by each of the 3 groups during the whole experiment did not differ: 675 ± 14 (carbonate), 671 ± 7 (milk) and 699 ± 5 (sulfate).

3.2. Plasma minerals, calciotropic hormones and bone-related markers (Tab. II)

The diets had no effect on the plasma concentrations of minerals, calcitriol, iPTH, osteocalcin, or on the urinary or plasma markers of bone collagen synthesis (plasma PICP), or degradation (plasma ICTP and urinary deoxypyridinoline). The plasma concentrations of total and bone-specific alkaline phosphatase were greater ($P < 0.05$) in the milk group than in the two others, and these two parameters were highly correlated ($r = 0.77$, at 45 days and $r = 0.71$ at slaughter, $P < 0.01$). The urinary Ca excretion did not differ in the 3 groups.

3.3. Bone parameters

The fresh weights of the bones (g) for the 3 groups of pigs were similar: 21.5 ± 0.8 (carbonate), 23 ± 1 (milk) and 22.6 ± 1 (sulfate); average of 4 bones (3 metatarsal and 1 metacarpal bones).

The ash contents of the metacarpal and metatarsal bones (Tab. III) from the pigs fed the milk diet were generally greater than those fed the two other diets. This was significant ($P < 0.01$) for ash % dry matter in the spongy and whole bone, for total bone ash content and for ash/unit volume. This effect was less marked in the cortical bone, for which the difference between milk and carbonate values was not significant.

Bone bending moments (Tab. IV) in the milk group were also greater than those in the 2 other groups. This was significant for the overall metacarpal mean, for each pair (right and left) mean of metatarsals, for the

Table II. Plasma concentrations of minerals and calcium-regulating hormones, and plasma or urinary concentrations of bone markers(1).

Diet	Carbonate	Milk	Sulfate	Pooled SEM	Diet effect (P)
Plasma minerals and hormones					
Calcium, mmol·L^{-1}	2.57	2.67	2.60	0.05	NS
Phosphate, mmol·L^{-1}	2.16	2.10	2.29	0.05	NS
PTH, pmol·L^{-1}	0.51	0.60	0.79	0.13	NS
Calcitriol, pmol·L^{-1}	173	161	185	12	NS
Plasma bone-markers					
Alkaline phosphatase					
total IU·L^{-1}					
day 45	67	88	57		
slaughter	91	107	77		
average(2)	79^{b}	97^{a}	68^{b}	7.3	0.001
bone isoenzyme, U·L^{-1}					
day 45	75	102	75		
slaughter	72	92	65		
average(2)	74^{b}	97^{a}	70^{b}	10	0.014
Osteocalcin, ng·mL^{-1}					
day 45	257	19l	229		
slaughter	285	209	251		
average(2)	271	200	240	29	NS
PICP, µg·L^{-1}	6.3	6.1	6.1	0.1	NS
ICTP, µg·L^{-1}	11.4	11.7	12.2	0.9	NS
Urinary parameters, per mmol creatinine					
Deoxypyridinoline, nmol	11.4	11.6	10.9	1.4	NS
calcium, mmol	0.10	0.18	0.20	0.03	NS

(1) Values at slaughter, except for alkaline phosphatase and osteocalcin; one way (diet) ANOVA except for alkaline phosphatase and osteocalcin(2).
(2) Two-way, diet and time, ANOVA, significant time-effect (slaughter > day 45, $P < 0.05$) for plasma total alkaline phosphatase only; a > b, $P < 0.05$, multiple mean comparison post-ANOVA.
PTH: immunoreactive parathyroid hormone, PICP: type I procollagen C-terminal propeptide, ICTP : type I collagen C-terminal cross-linked telopeptide.

overall mean from the 4 metatarsals and, for the overall metacarpal + metatarsal mean. There was no significant difference between the 3 groups for the tibia values.

Bone densitometry (Tab. V). The BMD of the milk-fed pigs was greater than that of bones of pigs on the two other diets. This was significant ($P < 0.01$ or $P < 0.05$) for the total BMD of each of the 5 bones tested, and for the BMD of each cancellar bone area of the 3 bones tested. Again, this effect was less marked or even absent for the cortical bone samples from the tibia or the internal metatarsals. The whole BMD of the left external metatarsals was highly and significantly ($P < 0.01$) correlated with the other bone parameters, such as whole bone ash ($r = 0.87$), ash % dry matter of the whole bone ($r = 0.63$), ash relative to bone volume ($r = 0.71$), apparent bone density (ratio fresh weight without medulla/whole apparent volume measured according to Archimede's principle, $r = 0.63$) and, spongy BMD with ash % dry matter of the spongy

Table III. Ash contents of the metatarsal and metacarpal bones.

Diet	Carbonate	Milk	Sulfate	Pooled SEM	Diet effect (P)
Total ash per bone[1], g	5.1B	6.1A	5.5B	0.2	0.0001
Ash % bone dry matter[1]					
Spongy bone	35.6B	38.9A	37.2B	0.8	0.001
Cortical bone	52.1ab	53.6a	51.5b	1.1	0.04
Whole bone	40.5B	43.3A	41.7B	0.7	0.0001
Ash/volume, g·100 cm^{-3}[2]	27.1B	30.4A	27.4B	0.8	0.0003
Bone mineral content, g[3]					
densitometry	5.8a	6.9b	6.2a	0.2	0.001
mineralization	5.3a	6.4b	5.7a	0.2	0.002

[1] Average of 3 bones: external, left and right, metatarsals + left internal metacarpal, *P* value for two-way, bone and diet, ANOVA.
[2] Average of 2 bones: external, right and left, metarsals, *P* value for two-way, bone and diet, ANOVA.
[3] Values obtained either by densitometry (BMC, g) or mineralization (ash, g) on the same bone: external left metatarsals correlation between both measures: y (ash, g) = 0.953 × (BMC, g) –0.217; $r = 0.995$, $P < 0.001$.
Means with no letters in common differ; lower case, $P < 0.05$; upper case, $P < 0.01$; multiple mean comparison post-ANOVA.
No significant bone × diet interaction except for cortical bone, $P = 0.014$.

Table IV. Bending moments (N × m) of the metacarpal and metatarsal bones.

Diet	Carbonate	Milk	Sulfate	Pooled SEM	Diet effect (P)
Left metacarpals[1]					
Internal	6.5	7.5	6.5	0.4	0.10
External	4.6	5.2	4.7		
Metatarsals					
Right[1]					
Internal	4.8	5.5	5.1	0.3	0.013
External	4.7a	5.7b	4.9a		
Left[1]					
Internal	4.9	5.8	4.8	0.3	0.015
External	4.7	5.7	4.7		
Overall metatarsal mean[2]	4.8B	5.7A	4.9B	0.3	0.001
Overall, metatarsals + metacarpals, mean[3]	5.0B	5.9A	5.lB	0.3	0.001
Right tibia	39.7	44.7	44.3	2.3	NS

[1] Two-way, diet and bone (internal/external), ANOVA.
[2] Two-way, diet and bone (4 bones per diet), ANOVA.
[3] Two-way, diet and bone, ANOVA; 6 bones per diet: internal and external, left metacarpals and, left and right metatarsals, overall bone effect: internal metacarpals > all other bones ($P < 0.01$).
Means with no letters in common differ; lower case, $P < 0.05$, upper case, $P < 0.01$, multiple mean comparison post-ANOVA.
No significant treatment × bone interaction whatever the group of bones.

Table V. Bone mineral density (BMD) ($g \cdot cm^{-2}$) of tibia, metatarsals and vertaebrae. Measures on whole bones and on cortical and spongy subregions.

Diet	Carbonate	Milk	Sulfate	Pooled SEM	Diet effect (P)(*)
a. Measures on whole bone					
Tibia	0.45B	0.59A	0.43B	0.02	0.0001
Left metatarsals (MT)					
Internal	0.25b	0.27a	0.24b		0.04
External	0.25b	0.29a	0.25b		0.001
Mean int. + ext.(1)	0.25B	0.28A	0.25B	0.01	0.0001
Lombar vertebrae (L)					
Ll	0.27b	0.32a	0.29b		0.007
L2	0.31B	0.38A	0.33B		0.0001
mean Ll + L2(1)	0.29B	0.35A	0.31B	0.01	0.0001
Overall mean MT + L(2)	0.27B	0.32A	0.28B	0.01	0.0001
b. Measures on spongy or cortical bone					
Tibia(3)					
spongy area 1	0.49B	0.62A	0.46B		0.0005
spongy area 2	0.49B	0.69A	0.43B	0.03	0.0001
cortical	0.55	0.62	0.52	0.04	NS
Metatarsals(4)					
spongy					
Intemal	0.33b	0.36a	0.34b		0.02
External	0.31B	0.36A	0.31B		0.001
mean int. + ext.(1)	0.32B	0.36A	0.33B	0.01	0.0001
cortical					
Internal	0.30	0.31	0.30		NS
External	0.27b	0.31a	0.29b		0.005
mean int. + ext(1)	0.28b	0.31a	0.29ab	0.01	0.02

(*) *P* value for one-way (diet) or two-way (diet and bone) ANOVA.
(1) Two-way, diet and bone (external/internal or Ll/L2), ANOVA.
(2) Two way, diet and bone (4 bones) ANOVA.
Significant overall bone effect ($P < 0.0001$) with L2 > L1 > MT (int. or ext.), post-ANOVA multiple mean comparison ($P < 0.01$).
(3) Left tibia, spongy area 1 and 2: distal and proximal epiphysis respectively, cortical bone: diaphysis.
(4) Left metatarsals, spongy bone: mean value for distal and proximal epiphysis, cortical bone: diaphysis.
Means with no letters in common differ; lower case, $P < 0.01$; upper case, $P < 0.05$, post-ANOVA multiple mean comparison.
No bone × treatment interaction whatever the row.

bone ($r = 0.78$) and cortical BMD with bending moment ($r = 0.68$). There were high ($r \geq 0.8$), significant ($P < 0.01$) correlations between the overall bone mineral contents obtained by densitometry (BMC) and those obtained after mineralization (ash content) (Tab. III, for the left external metatarsal, $r = 0.995$).

4. DISCUSSION

The results clearly indicate that the diet in which the calcium was mainly provided by the skimmed milk powder led to bones with a greater mineral content, breaking strength and density. These bone changes cannot be due to better growth, since the growth

performance of the pigs and the bone weights were not affected by the diets. Consequently, the bone changes in the "milk" group must be due to less resorption or to greater bone formation, or a combination of the two, resulting in better mineralization. The plasma ICTP, urinary deoxypyridinoline (parameters reflecting bone collagen degradation) and PTH levels for the three dietary groups were similar. Modification of bone resorption can therefore be ruled out. The plasma PICP concentrations for the three groups were also similar, indicating that the synthesis of the bone matrix was not modified. But the circulating levels of bone and total ALP were elevated in the milk-fed pigs. This increase in markers of bone calcification [1] and bone mineral content suggest that the mineralization was modified. Hence, the calcium from the "milk diet" was more available to the pigs than was the calcium in the two other diets.

The studies carried out to date in humans have failed to demonstrate that milk calcium is more efficiently absorbed than calcium from carbonate [17, 27, 30, 42, 48, 49], or calcium from mineral waters, bicarbonate or sulfate [5, 7, 56]. Similarly, long-term studies in growing pigs [36–38] have provided no evidence that the calcium in milk and milk products (casein phosphopeptides, skim milk or yogurt) is better absorbed than is calcium from mineral salts.

However, pigs fed yogurt as a calcium source have greater bone mineralization (evaluated by breaking strength) than pigs receiving inorganic calcium sources ($CaHPO_4$ + $CaCO_3$) [35]. Partridge [33] has also shown greater calcium absorption in very young pigs fed milk than in those fed an isocalcium diet containing soybean meal. Similar results have been obtained in piglets by Matsui et al. [29].

There is also evidence that dietary factors present in milk, such as casein phosphopeptides and lactose, enhance mineral retention. Milk casein phosphopeptides have been shown to have a positive effect on the absorption of calcium, in vivo and in vitro in rats with an increase in the intestinal transfer of calcium and, on bone metabolism [12, 21, 23, 28, 46, 51, 55]. Other in vivo studies on rats have demonstrated that lactose stimulates calcium absorption or retention by acting directly on the intestine, and on the bone [3, 11, 13, 25, 44]. There is also indirect in vitro evidence of the effect of lactose on calcium absorption from studies with isolated intestinal loops [2, 10], where lactose was compared to another sugar and to a control without lactose [2]. Calcium is better absorbed by unweaned human babies fed normal mother's milk than when fed mother's milk from which the lactose has been removed [22].

Another potential benefit of milk as a source of calcium is its phosphorus content. The simultaneous presence of calcium and phosphorus may create favorable conditions to enhance bone mineral deposition, as demonstrated in pigs [37, 39]. Ingesting milk may also be considered to be a meal, which itself enhances calcium absorption [17]. It is therefore surprising that some of the studies on pigs and all those done on humans have failed to demonstrate that calcium from milk is more available than the calcium from inorganic sources.

The calcium provided by the diets in the present experiment represented only 70% of the French recommended dietary allowance for pigs of that age [15]. Furthermore, our young pigs grew very rapidly (0.7 $kg \cdot day^{-1}$), and so must have used the minerals efficiently to build bones. Under these conditions of restricted intake and increased demand, which occurs frequently in young adolescents, the "milk" diet has ensured a better utilization of calcium (and phosphorus) than the two other diets in our pig study. Therefore, some milk components may have facilitated calcium absorption, allowing better bone mineralization. This effect was not observed previously when the calcium intake was not restricted [35, 38].

The three groups of pigs grew identically, although the protein and energy components of the three diets were not the same. This is different from the results obtained for pigs fed milk or yogurt and pigs fed soybean protein based diets [33, 38, 52, 53]. The milk and yogurt diets provided better growth, possibly because of better utilization of milk proteins. The discrepancy may be due to the careful equalization of the digestible amino acid and energy contents of the three diets. This adjustment was based on recent data [20, 32, 47], but such adjustments cannot be done in human trials. It is also difficult to control the calcium intake, the age of the subjects and the compositions of the calcium-containing diets (energy, proteins, fiber and so on). All these factors influence calcium absorption and bone metabolism, so that any one of them may mask the greater availability of calcium from milk compared to calcium from calcium salts.

The lower calcium availability of the sulfate and carbonate diets might also be confounded with the presence of soybean meal, a phytate-rich product, instead of milk. Soybean may decrease calcium absorption as shown in humans [18] and pigs [29, 33]. However, this did not interfere in our pigs. Firstly, the three diets were formulated to provide very similar levels of phytates. Secondly, we used phytase-rich cereals (namely wheat and barley) especially to enhance phytate hydrolysis, thus reducing the risks of calcium-phytate formation [34]. In fact, we have reported almost identical calcium absorptions in pigs fed normocalcic diets containing either wheat + soybean or wheat + milk-derived products [35, 38]. Thirdly, the soybean meal was incorporated at a moderate concentration (less than half the milk %) and was not the unique source of protein in the sulfate and carbonate diets, which also contained fish soluble (Tab. I).

The pigs fed the $CaSO_4$-diet used their calcium to build bone as efficiently as did those on the $CaCO_3$-diet in the present experiment. There was also no increase in urinary calcium in the pigs fed $CaSO_4$. But, sulfates are believed to increase urinary calcium in humans [24, 54], and this could prevent the incorporation of calcium into bone, as demonstrated in sheep [14]. Calciuria was not significantly elevated by $CaSO_4$-rich mineral waters in short-term (36 h) studies on humans [5]. But calciuria plays a much smaller role in Ca excretion (around 1% of ingested calcium in the present and previous experiences [37, 39]) in pigs than it does in humans, where urinary calcium excretion is the most important component of the calcium balance. These differences in the metabolism of calcium between the two species emphasize the caution required in extrapolating our results to humans.

In summary, our data suggest that the calcium provided by milk is more efficiently used to build bones by growing subjects with a restricted calcium intake than is the calcium provided as sulfate or carbonate salts.

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