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# Paracellular Calcium Absorption, Femur Mineralization and Biomechanics in Rats Fed Selected Dietary Proteins*

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*ABSTRACT*

*The absorption of $^{45}Ca$ from the distal section of the lower small intestine, and its subsequent deposition in the femur were examined in male Wistar rats fed diets containing 20% casein (C), whey protein (W), a milk protein concentrate (MPC), and a soy protein isolate (SPI), respectively. These experiments were performed along with in-vitro studies designed to estimate the relative digestibilities of the dietary protein sources. In the animal experiments, all diets contained an adequate calcium level (0·7%). Individual rats were meal-fed for 10 weeks, after which calcium absorption was measured from the disappearance of $^{45}Ca$ from the ligated ileal loop; as well, femoral deposition of $^{45}Ca$ and mineral composition were examined. Estimates of calcium utilization were made from femur and tibia biomechanical measurements. A markedly lower digestibility of the soy and whey proteins was observed compared to the casein and milk protein concentrate sources, respectively. There were no differences in the daily body weight gain, and plasma calcium levels. The absorption of $^{45}Ca$ from the ileum was significantly ($p = 0·043$) lower in SPI-fed rats, compared to C-, W- and MPC-fed animals. There was no treatment effect on femoral $^{45}Ca$ deposition, total Ca, Mg or $Ca/PO_4$ ratio. A significant correlation between femur calcium content and bone strength was determined; however, there was no treatment effect on either femur or tibia biomechanical properties. These results indicate a similar utilization of calcium in animals fed dietary proteins that may differ in digestibility.*

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## INTRODUCTION

The role of dietary, as well as supplemental calcium, in maintaining overall skeletal integrity and as a prophylactic in osteoporosis is now well recognized. In recent studies, calcium supplements have been shown to increase bone-mass in postmenopausal patients (Shih *et al.*, 1988). In addition to the calcium available to the individual through the diet, there has been considerable study into the role of dietary constituents, such as protein, on intestinal calcium absorption and urinary calcium excretion (Linkswiler *et al.*, 1981). Taken together, these two factors will affect the calcium balance of the individual.

In order to be absorbed from the intestine, dietary calcium must be present in a soluble and ionized form (Pak & Avioli, 1988). The primary milk proteins, namely $\beta$-casein and $\alpha_{s1}$-casein, have been shown to yield phosphorylated peptides (CPP) upon tryptic digestion (Naito *et al.*, 1972). These peptides have been shown to complex ionized calcium in the intestinal milieu. This action maintains the calcium in a soluble, bioavailable form by inhibiting its precipitation as calcium phosphate (Mykkanen & Wasserman, 1980). In other studies, amino acids, such as L-arginine and L-lysine, have also been shown to enhance calcium absorption in the small intestine (Wasserman *et al.*, 1956). In both of these examples, the relative rate of protein digestibility and protein composition will influence the number of peptides, or amino acids released during digestion. This effect would be expected, since a reduction in protein nutritional value can be largely explained by decreased digestibility and availability of amino acids (Mauron, 1973). Moreover, other studies have indicated that dietary proteins with varying digestibilities can alter the digestive functions of the pancreas relative to the nutritional value of the protein administered (Reboud *et al.*, 1966; Percival & Schneeman, 1979). The quality of dietary protein will therefore influence the proteolytic activity of the intestinal contents, by regulating pancreatic exocrine secretion (Meyer & Grossman, 1972), or rate of turnover of proteolytic enzymes. In the latter case, a protein with a high nutritional value may cause an increase in the rate of enzyme activation and, consequently, protein digestion. Since paracellular calcium absorption is dependent to a large extent on the peptide or amino acid mixtures of protein post-digestion products, the relative digestibility of the protein could represent a limiting factor for dietary calcium bioavailability.

Other studies have further demonstrated a direct association between the protein content of the diet and the degree of calciuria (Allen *et al.*, 1979). In rodent models, the calciuretic effect of different dietary proteins is believed to be related to the sulfur amino acid content (Whiting & Draper, 1981). These observations suggest that the source of dietary protein may have a bearing

on, not only the absorption of calcium, but also its utilization in bone metabolism or biomechanical function.

The purpose of the present study was to evaluate the relationship between protein digestibility, estimated *in vitro* by enzymatic hydrolysis, and calcium absorption from the small intestine, estimated *in situ* using a ligated ileal loop. Further, both the femur and tibia were used as endpoint determinants of calcium utilization, as indexed by bone mineral composition and biomechanical properties.

## MATERIALS AND METHODS

### In-vitro evaluation of protein digestibility

An in-vitro two-step proteolysis method using pepsin, followed by pancreatin enzymatic hydrolysis was used according to the method of Jacques *et al.* (1986). Casein, soy protein isolate (Sigma), milk protein concentrate and whey protein concentrate (Bariatrix International) were suspended in distilled water, respectively. Solutions were adjusted to pH 1·9 with dilute HCl and incubated with pepsin (porcine stomach mucosa 1:10 000) at 37°C in a shaking water bath. Following a 30-min pepsin digestion, samples were incubated once more with pancreatin (porcine pancreas; Grade II, Sigma). Aliquots were removed at frequent intervals, deproteinized with 20% TCA and reacted with TNBS (Kwan *et al.*, 1983). The initial slope of protein digestion was obtained by linear regression analysis (Maga *et al.*, 1973) and used to compare the initial proteolysis rate between proteins.

### Animals and diets

Thirty-two 5 week old male Wistar rats, matched for age and sex were purchased from Charles River, Canada. Animals were individually housed in stainless steel cages with controlled temperature and lighting (14:10 cycle). Animals were segregated into four dietary groups with respect to dietary protein: casein, whey protein concentrate, milk protein concentrate and soy protein isolate (Table 1). Animals were fed diets *ad libitum* until 100 g body weight was reached, whereupon mealfeeding was started. Animals were trained to consume their diets within a 6 h period daily. Deionized water was provided *ad libitum* to the animals. Daily feed intakes and weekly body weights were recorded.

Intestinal calcium absorption was measured by the in-situ ligated ileal loop technique based on the procedure of Lee *et al.* (1983) when the animals

**TABLE 1**
Experimental Diet Compositions Fed to Rats (g/100 g)

| *Diet composition* | *Casein* | *SPI* | *MPC* | *Whey* |
|---|---|---|---|---|
| Casein | 20·0 | — | — | — |
| SPI[a] | — | 20·0 | — | — |
| MPC[b] | — | — | 23·5 | — |
| Whey protein | — | — | — | 23·5 |
| D.L. Met | 0·3 | 0·3 | 0·3 | 0·3 |
| Cornstarch | 11·35 | 11·35 | 11·35 | 11·35 |
| Sucrose | 53·16 | 53·23 | 50·0 | 50·29 |
| Vegetable oil | 5·0 | 5·0 | 4·8 | 4·8 |
| Mineral mix[c] | 3·5 | 3·5 | 3·5 | 3·5 |
| $CaCO_3$ | 0·49 | 0·42 | 0·35 | 0·06 |
| Vitamin mix | 1·0 | 1·0 | 1·0 | 1·0 |
| Choline bitartrate | 0·2 | 0·2 | 0·2 | 0·2 |
| Fibre | 5·0 | 5·0 | 5·0 | 5·0 |

[a] Soy protein isolate.
[b] Skim milk protein concentrate.
[c] Ca free mineral mix.

were 14 weeks of age. On the final day of the experiment, animals were first allowed access to their diets for a 1·5 h period. Animals were anaesthetized by an intraperitoneal injection of pentobarbital (50 mg/kg body weight) 1·5 h after food withdrawal. A central longitudinal abdominal incision was made to expose the small intestine, and an ileal loop made by two ligations at points 8 cm and 20 cm from the ileocecal junction to make a closed sac leaving the intestinal contents intact. A dose (0·3 ml) of $^{45}Ca$ ($^{45}CaCl_2$, 18·2 mCi/mg Ca, ICN Biomedical Inc., Irvine, CA) in 0·9% saline was injected intraluminally into the loop (total dose was 5·4 $\mu$Ci). The loop was replaced inside the abdominal cavity and the tissue and skin layers closed. Animals were placed on a warm surface and allowed to rest undisturbed. Absorption of calcium was estimated from the amount of administered $^{45}Ca$ remaining in the loop after 1 h.

## Analyses

Intestinal lumen contents were flushed out with 5 ml of saline and homogenized (Polytron homogenizer). A portion was removed and digested with NCS tissue solubilizer (Amersham, Oakville, Ontario). The digest was mixed with ACS scintillation cocktail (Amersham) and radioactivity measured (LKB-1215 Scintillation counter) to determine the amount remaining intraluminally. $^{40}Ca$ in the homogenate was measured following wet ashing with an

$HCl/HNO_3$ mixture as described by Mauer (1977). After ashing, samples were diluted to appropriate volumes with 0·5% $LaCl_3$ for calcium determination by atomic absorption spectrophotometry (Perkin–Elmer-306 atomic absorption spectrophotometer).

Blood samples were obtained by cardiac puncture immediately prior to flushing out the intestinal contents. Samples were centrifuged in a heparinized test tube to separate plasma. Plasma minerals (calcium, magnesium, sodium and potassium) were determined by atomic absorption spectrophotometry. Plasma ionized calcium was calculated by the method of Zeisler (1954), from the total calcium and protein concentrations: $Ca^{2+} = (6Ca - P/3)/(P + 6)$ where both $Ca^{2+}$ and Ca are mg/dl and $P$ is protein concentration, g/dl.

**Bone biomechanics**

After euthanasia, bone samples, femur and tibia, were excised by blunt dissection, cleansed of adhering soft tissue, and the epiphyses removed. Bone biomechanical parameters were assessed using an Instron Universal Testing Machine (Model 1122, Instron Corp., Canton, MA). The femora were subjected to a single-blade shear test while the tibias underwent a 3-point bending analysis. In the single-blade shear test, the femur was placed unsupported on the load platform but resting on the greater trochanter, so that the blade (3 mm) would shear the sample 4 mm from the distal articular surface. Two indices of bone quality were quantified, bone strength and bone hardness (Segars & Kapsalis, 1987). Bone strength is defined as the force applied at the point of failure, or rupture, divided by the original cross-sectional area of the bone expressed as Newtons per square millimetre ($N/mm^2$). Bone hardness is defined as the work, or energy (area under the force-deformation curve) necessary to achieve fracture of the bone, expressed as Joules (J).

In 3-point bending, each tibia was bent until failure occurred, by lowering a centrally placed point at a constant speed. This test allows two whole bone properties to be determined, bending failure energy and maximum bending stress ($\sigma$). Bending failure energy is described as the work, or energy (area under the force–deformation curve) necessary to achieve failure of the bone in bending, expressed as Joules (J). The maximum bending stress is a calculated value that takes into consideration bone size:

$$\sigma = \frac{8 \times \text{Maximum Bending Load } (L - 1)D}{(D^4 - d^4)}$$

where $L$ is the distance between the supporting points (13 mm); $D$ and $d$ are the outer and inner diameters of the bone (mm).

### Bone mineral content analyses

Bone mineral analyses were performed on the femora and tibias used in the shearing and 3-point bending procedures. Samples were dried at 110°C for 3 days, then ashed at 550°C for 24 h. The ash was solubilized with concentrated HCl for determination of $^{45}Ca$ by liquid scintillation counting, $^{40}Ca$ and Mg by atomic absorption spectrophotometry and $PO_4$ by the method of Chen *et al.* (1956). $^{45}Ca$ deposition in bone was expressed as the per cent dose per g ash (Mykkanen & Wasserman, 1980).

### Statistical analyses

All the data are expressed as mean ± SEM. Differences between treatments were tested for by one-way analysis of variance. The Student–Newman–Keuls multiple range test was used to identify the source of the differences at the $p < 0.05$ level.

## RESULTS

### In-vitro study

The initial time courses in pepsin–pancreatin digestion of casein, milk protein concentrate, whey protein concentrate, and soy protein isolate are shown in Fig. 1. No attempt was made to remove the products of digestion, which are known to accumulate and ultimately inhibit the process of enzymatic digestion (Robbins, 1978). However, the use of the initial slope method overcomes this disadvantage. Notably slower rates of proteolysis were recorded for both soy protein isolate and the whey protein concentrate, compared to casein and the milk protein concentrate.

### In-vivo study

All animals exhibited good eating behaviour following meal-feeding training. There were no significant differences between experimental groups in body weight gained or dry matter intake (Table 2). In addition, feed efficiency ratios during the course of the experimental period were not significantly ($p < 0.05$) different between treatments (Table 2).

Plasma minerals were not different between dietary groups. Furthermore, neither the total plasma calcium (range 8·47 ± 0·08 to 8·72 ± 0·13 mg/dl), nor the calculated ionized plasma calcium (range 3·82 ± 0·10 to 4·04 ± 0·05 mg/dl) were found to be affected by the various treatments.

The percent of $^{45}Ca$ absorbed from the ligated ileal loop in casein-, whey protein- and MPC-fed rats was found to be significantly ($p < 0.05$) greater

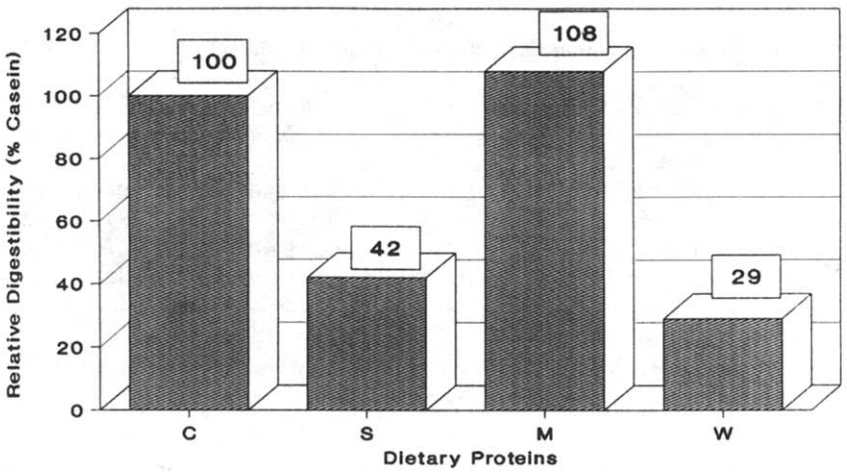


**Fig. 1.** Pepsin–pancreatic digestion estimates of casein (C), soy protein isolate (S), milk protein concentrate (M), and whey protein (W) calculated from the initial reaction rates (0–10 min). Respective slopes were: $C = 15{\cdot}7 \times 10^{-3}$; $S = 6{\cdot}6 \times 10^{-3}$; $M = 16{\cdot}9 \times 10^{-3}$; $W = 4{\cdot}5 \times 10^{-3}$; regression range = $0{\cdot}999 \geq r \leq 0{\cdot}968$.

than for animals fed soy protein isolate (Table 3). With the exception of whey protein-fed animals, intestinal specific activities were not significantly different between dietary groups. The specific activity of calcium present in the intestinal loop of whey protein-fed rats was significantly ($p < 0{\cdot}05$) higher. This observation was further reflected in the $^{45}Ca$ deposition to the bone (Table 3). The specific activity of the femora from the whey protein-fed animals was significantly ($p < 0{\cdot}05$) greater than that of animals fed casein, MPC, and soy protein isolate. The significantly ($p < 0{\cdot}05$) lower amount of $^{45}Ca$ absorbed from the ileal loop in soy-fed animals, however, was not reflected in either bone $^{45}Ca$ deposition, or bone specific activity (Table 3).

**TABLE 2**
Diet Efficiency of Rats Fed Experimental Diets[a]

| *Diet*[b] | *Body weight gain* (*g*) | *Dry matter intake* (*g*) | *Feed efficiency ratio* |
|---|---|---|---|
| C | 143·0 ± 9·6 | 528·9 ± 23·8 | 0·269 ± 0·009 |
| S | 132·8 ± 4·0 | 497·0 ± 12·7 | 0·268 ± 0·007 |
| M | 136·4 ± 9·9 | 504·0 ± 20·6 | 0·269 ± 0·012 |
| W | 120·8 ± 5·5 | 470·9 ± 16·3 | 0·256 ± 0·007 |

[a] Data are expressed as mean ± SEM.
[b] C, casein; S, soy protein isolate; M, milk protein concentrate; W, whey protein isolate.

**TABLE 3**
Intestinal Absorption and Femur Deposition of $^{45}$Calcium in Rats Fed Experimental Diets[a]

| *Diet*[b] | *Absorbed $^{45}$Ca (% dose)* | *Intestinal SA ($dpm \times 10^3/g^{40}Ca$)* | *Bone $^{45}$Ca (% dose/g Ash)* | *Bone SA ($dpm/mg^{40}Ca$)* |
|---|---|---|---|---|
| C | 36·53 ± 2·82* | 2·91 ± 0·35* | 0·857 ± 0·117* | 204·3 ± 19·9* |
| S | 22·69 ± 3·17** | 3·65 ± 0·48* | 0·706 ± 0·114* | 187·2 ± 19·4* |
| M | 35·19 ± 4·72* | 2·37 ± 0·28* | 0·654 ± 0·114* | 161·1 ± 26·1* |
| W | 31·70 ± 1·04*·** | 5·04 ± 0·52** | 1·505 ± 0·187** | 342·4 ± 33·8** |

[a] Data are expressed as mean ± SEM.
[b] C, casein; S, soy protein isolate; M, milk protein concentrate; W, whey protein isolate. Different superscripts are significantly ($p < 0·05$) different.

The physical dimensions, mineral contents, and biomechanical parameters of the femora and tibias of the animals from the different dietary groups are summarized in Tables 4, 5 and 6. Despite the enhanced intestinal absorption of calcium in rats fed the casein and MPC diets, compared to those fed the soy protein isolate and whey protein diets, no significant differences in either bone mineralization or bone biomechanical properties of these particular animals were observed. Significant correlations were found to exist between femur calcium content (mg Ca/g Ash) and bone hardness ($F(1,42) = 9·38$, $p \leq 0·003$) and femur weight ($F(1,42) = 10·43$, $p \leq 0·0024$).

## DISCUSSION

The absorption of calcium from the ileum, as well as its subsequent utilization for bone mineralization and biomechanics were examined in animals fed different dietary proteins. Dairy foods, considered to be

**TABLE 4**
Femur Mineral Composition in Rats Fed Experimental Diets[a]

| *Diet*[b] | *Ash wt (g/femur)* | *Calcium (mg/g Ash)* | *Ca/P* | *Magnesium (mg/g Ash)* |
|---|---|---|---|---|
| C | 0·270 ± 0·009 | 362 ± 5·3 | 2·01 ± 0·06 | 13·4 ± 0·421 |
| S | 0·274 ± 0·008 | 372 ± 9·1 | 2·10 ± 0·07 | 13·5 ± 1·068 |
| M | 0·277 ± 0·004 | 361 ± 9·1 | 2·05 ± 0·06 | 13·5 ± 0·580 |
| W | 0·256 ± 0·013 | 369 ± 4·8 | 2·08 ± 0·05 | 9·95 ± 0·315 |

[a] Data are expressed as mean ± SEM.
[b] C, casein; S, soy protein isolate; M, milk protein concentrate; W, whey protein isolate.

**TABLE 5**
Femur Physical Dimensions and Biomechanical Parameters in Rats Fed Experimental Diets[a]

| *Diet*[b] | *Femur wt* (*g*) | *Bone length* (*mm*) | *Bone strength* ($N/mm^2$) | *Bone hardness* ($\times 10^{-3} J$) |
|---|---|---|---|---|
| C | 0·646 ± 0·030 | 32·57 ± 0·23 | 1·312 ± 0·069 | 180·7 ± 19·1 |
| S | 0·655 ± 0·036 | 31·68 ± 0·36 | 1·315 ± 0·078 | 188·6 ± 16·9 |
| M | 0·684 ± 0·017 | 31·99 ± 0·14 | 1·232 ± 0·034 | 182·5 ± 16·6 |
| W | 0·626 ± 0·020 | 32·35 ± 0·40 | 1·289 ± 0·058 | 135·5 ± 8·7 |

[a] Data are expressed as mean ± SEM.
[b] C, casein; S, soy protein isolate; M, milk protein concentrate; W, whey protein isolate.

excellent sources of calcium, were examined to determine the protein–calcium interaction as applied to dietary calcium bioavailability. Dairy proteins; namely, casein, whey proteins and a combination of both in a milk protein concentrate were compared to soy protein, a plant protein source. The presence of casein, whey protein, and MPC in the respective diets was shown to result in a higher ileal absorption of $^{45}Ca$ compared to the soy protein diet. In the case of whey protein-fed animals, however, a lower level of $^{40}Ca$ in the intestinal lumen resulted in a higher specific activity for calcium in the ligated loop, and possibly an overestimation in the amount of $^{45}Ca$ absorbed. This result may be explained on the basis of the lower digestibility of whey proteins observed herein as compared with the in-vitro study. The poor digestibility of whey proteins is attributed to one of its constituent proteins, in particular α-lactalbumin, which has recently been shown to exhibit a lower mean true digestibility in animal feeding studies (Keith & Bell, 1988). The low digestibility of whey proteins could feasibly

**TABLE 6**
Tibia Calcium Content and Biomechanical Parameters of Three-Point Bending Analysis in Rats Fed Experimental Diets[a]

| *Diet*[b] | *Tibia wt* (*g*) | *Ash wt* (*g/tibia*) | *Calcium* (*mg/g Ash*) | *Bending failure energy* ($\times 10^{-3} J$) | *Maximum bending stress* ($N/mm^2$) |
|---|---|---|---|---|---|
| C | 0·375 ± 0·023 | 0·186 ± 0·007 | 406·0 ± 17·8 | 53·94 ± 6·31 | 60·44 ± 3·01 |
| S | 0·407 ± 0·021 | 0·207 ± 0·011 | 418·6 ± 25·4 | 53·61 ± 3·95 | 57·60 ± 2·31 |
| M | 0·395 ± 0·005 | 0·191 ± 0·004 | 421·0 ± 19·8 | 62·76 ± 4·56 | 56·18 ± 1·25 |
| W | 0·420 ± 0·016 | 0·190 ± 0·012 | 408·5 ± 23·7 | 63·85 ± 5·64 | 67·05 ± 2·00 |

[a] Data are expressed as mean ± SEM.
[b] C, casein; S, soy protein isolate; M, milk protein concentrate; W, whey protein isolate.

increase the intestinal transit time which would result in a lesser amount of $^{40}Ca$ being present in the ileal loop at any given time. Accordingly, the lower relative digestibility of soy protein observed in this study should have also increased the specific activity of $^{45}Ca$ in the intestinal loop, but this was not the case. The lower absorption of $^{45}Ca$ in rats fed the soy protein diet was therefore not related to the digestibility of this protein. Soy proteins are highly structured proteins and, consequently, are more resistant to enzymatic attack. The result is that the yields of peptide and amino acid products derived from soy protein digestions are considerably different from those of casein (Raghunath & Narasinga Rao, 1984).

In previous studies, it was considered that the amino acid profile of intestinal contents is virtually constant, regardless of the proteins digested (Nasset & Ju, 1961). However, recent in-vitro studies have clearly shown a markedly higher L-arginine and lower L-lysine content from digestion products derived from soy protein compared to casein (Jacques *et al.*, 1986). Moreover, Wasserman and coworkers (1956) reported that L-arginine enhances ileal calcium uptake by modifying the nonsaturable, paracellular pathway of calcium absorption. The fact that a lower relative absorption of $^{45}Ca$ was observed in the ligated ileal loop of animals fed soy, compared to both casein and the MPC-fed animals, strongly suggests that the digestion products of soy protein had little effect in enhancing calcium absorption. This observation would also negate any significance of the lower digestibility of soy products in eliciting this response.

On the other hand, phosphopeptides derived from casein (CPP) have been shown to increase the availability of ionic calcium, by maintaining it in a soluble form in the lower small intestine (Sato *et al.*, 1983). Lee *et al.* (1983) reported that rats fed a casein diet had a significantly greater amount of soluble calcium and decreased insoluble calcium in the lower intestine as compared to animals fed diets consisting of an amino acid mixture simulating casein or egg albumen, respectively. This observation has also recently been confirmed in our laboratory (Kitts *et al.*, 1989). Therefore, the enhanced intestinal absorption of calcium observed in both the casein and MPC-fed animals supports the hypothesis that casein phosphopeptides, produced from tryptic digestion of casein, will facilitate the absorption of calcium from the distal small intestine. The relative importance of this action to the overall calcium balance of the individual has not yet been ascertained.

In this study we also examined bone deposition of $^{45}Ca$, bone mineralization and biomechanics as indices of calcium utilization from rats fed the different dietary protein sources. It is noteworthy that the higher intestinal specific activity of calcium in the whey protein-fed animals corresponded to a greater $^{45}Ca$ deposition and specific activity in the femora

of these animals. This result indicates that the femoral $^{45}Ca$ deposition resulting from a particular dietary treatment can be a useful measurement of dietary calcium utilization. This method, however, is subject to overestimation when the intestinal calcium specific activity is artificially elevated due to associated factors characteristic of the diet. On the other hand, bone mineralization and biomechanical properties are measurements of calcium utilization which are not susceptible to similar sources of overestimation. In the present study, forces were applied in deformation to imitate a particular action in the single blade shear test. Data generated in this test were useful in comparing a series of samples tested under identical conditions. The magnitude of the values obtained had little importance from an absolute point of view, however, since they were highly dependent on test conditions (Segars & Kapsalis, 1987). In addition, a well defined engineering test involving 3-point bending was used. The application of a pure model of deformation to the sample was employed and a formula was used to calculate the maximum bending stress that models the bending strength of tubes that are circular in cross-section and possess uniform wall thickness (Ortoff & Oxlund, 1988). In the present study, significant correlations were obtained between femoral hardness and femur weight and calcium content, respectively. These results corroborate the postulation that bone strength may be associated with bone mineral content (Kusy *et al.*, 1987), and may be a useful functional test for assessing calcium utilization (Kusy *et al.*, 1987; Ortoff & Oxlund, 1988).

Despite the apparent increased calcium bioavailability from casein-containing diets, no enhancement of either plasma total and ionized calcium, or bone mineralization and biomechanical properties in the femur and tibia samples, were observed. Other workers have shown a marked increase in the urinary excretion rate of calcium in rats fed a high protein diet, albeit that no effect on intestinal absorption was observed (Allen *et al.*, 1979). This hypercalciuria has been attributed to a decrease in fractional renal tubular reabsorption and an increase in glomerular filtration of calcium (Schuette *et al.*, 1981). The findings of Whiting and Draper (1981), which showed protein sulfur amino acid content to be related to a calciuretic action, further demonstrated that the source of dietary protein, in addition to the quantity fed, can adversely affect calcium balance. In this study, diets were isonitrogenous and contained calcium at a level adequate for calcium homeostasis. Thus, the enhanced intestinal absorption of calcium due to the presence of CPP, or certain amino acids that may enhance either paracellular movement, or urinary excretion of this mineral, had little physiological significance in regard to its utilization for bone metabolism. Further studies are required to determine if this is the case in calcium deficient subjects.

## CONCLUSION

The absorption of calcium in the ileum was significantly enhanced in rats fed milk protein diets containing casein. The increase in absorbed calcium was shown to have little physiological significance in bone mineralization and biomechanics when animals were fed a diet adequate in dietary calcium.

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