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Maillard reaction products consumption: Magnesium bioavailability and bone mineralization in rats

Cristina Delgado-Andrade, Isabel Seiquer *, María Pilar Navarro

Unidad de Nutrición, Estación Experimental del Zaidín, CSIC, 18100, Granada, Spain

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Abstract

The aim of this study was to analyze the influence of the consumption of Maillard reaction products (MRP), derived from glucose-lysine and glucose-methionine mixtures heated for 90 minutes, on magnesium bioavailability and tissue concentration. Magnesium balances were performed in rats. Subsequently, the animals were sacrificed and certain organs removed to analyze the magnesium contents. Glucose-methionine MRP induced increases in magnesium fractional absorption in the last week of the assay (69.2 ± 2.9%) compared with the control group (59.4 ± 1.3%), although no variations in magnesium bioavailability during the total period balance were found. The intake of both MRP assayed seemed to increase renal and hepatic magnesium, decrease calcium bone concentration and mask the positive effect of the free amino acids still present in the samples on bone magnesium. The results show that MRP dietary consumption could lead to changes in bone mineralization. The long-term effects should be more deeply studied to predict possible implications for bone health.
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Keywords: Magnesium; Bioavailability; Maillard reaction products; Tissue distribution; Bone

1. Introduction

Magnesium is involved in a wide range of fundamental biological reactions. It is essential for oxidative phosphorylation, metabolism of nutrients, muscle contraction, and the nervous and immune systems; approximately 60% of body magnesium is located in bone (Perelson & Ellenbogen, 2002; Berdanier, 2002; Navarro, 2003). Because of its important health implications, factors affecting magnesium availability in meals and involved in modifying its metabolism are of special interest.

Transformations undergone by foods during industrial and/or home processing could affect the nature of their components and the interactions between them. Heat treatment is frequently used during manufacturing, and this promotes the development of the Maillard reaction (MR), which occurs between amino acids and reducing sugars. Maillard reaction products (MRP) are responsible, to some extent, for changes in the chemical properties and nutrient bioavailability of foodstuffs (Abu-Dweih, Tukan, & Takruri, 2000). Some of these changes produce positive effects, such as the natural antioxidant activity attributed to some MRP. Some authors have reported the suitability of browning products for preventing lipid oxidation in fish and in cooked ground pork patties (Chiu, Tanaka, Nagashima, & Taguchi, 1991; Bedinghaus & Ockerman, 1995). However, consumption of these compounds has undesirable nutritional effects derived from the reduction in the protein quality (Sarriá, López-Fandino, & Vaquero, 2000) and from the capacity to form complexes with magnesium, calcium, copper, iron or zinc, thus affecting their absorption (O'Brien & Morrissey, 1997; Delgado-Andrade, Seiquer, Nieto, & Navarro, 2004). Some low molecular weight water-soluble MRP are absorbed from the intestinal tract (Erbersdobler & Faist, 2001), thereby improving the absorption of the chelated metals. However, more

* Corresponding author. Tel.: +34 958 572757; fax: +34 958 572753.
E-mail address: iseiquer@eez.csic.es (I. Seiquer).

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advanced compounds, that are of high molecular weight and are water-insoluble, may increase fecal metal excretion and decrease absorption of the bound metal, including magnesium (Andrieux & Saquet, 1984).

Little is known about the action of these products on magnesium absorption and metabolism, or of their effects on soft tissue and bone. Different results have been obtained, in diverse studies, concerning the oral administration of MRP. Some have reported a decrease in the faecal excretion of Mg after glucose-glutamic acid MRP intake in rats (O'Brien, Morrissey, & Flynn, 1994), while others, using glucose and glycine as reactants, have described no such effects, but on the other hand, an increase in magnesium absorption (Andrieux, Sacquet, & Guegnen, 1980; Andrieux & Saquet, 1984). The consumption of diets containing different MRP has been found to increase the urinary elimination of calcium and zinc in rats (O'Brien, Morrissey, & Flynn, 1986; Furniss, Vuichoid, Finot, & Hurrel, 1989; Fairweather-Tait, Portwood, Symss, Eagle, & Minski, 1989; Hurrell, 1990; Friedman, 1996; Navarro, Aspe, & Seiquer, 2000; Seiquer, Aspe, Vaquero, & Navarro, 2001), but the urinary excretion of magnesium seems to be less affected (Hurrell, 1990).

Given that MRP formed in processed foods are varied and since the characteristics and effects of MRP are known to vary, depending on the reactants and the reaction conditions (Delgado-Andrade et al., 2004), the sugars and amino acids that produce the MRP must be carefully considered. It seems logical to begin by studying the effect of these compounds on magnesium bioavailability in an amino acid-sugar model system, in which amino acids can be chosen and their effects identified separately. Thus, lysine was selected because of its frequent presence in foods and its high sensitivity to thermal treatment (Ashoor & Zent, 1984; Van Barneveld, Batterham, Skingle, & Norton, 1995). Methionine, present in less abundance, was chosen because its losses during the heating process could be of considerable importance, thus making it the limiting amino acid in the meal (Nielsen, De Weck, Finot, Liardon, & Hurrell, 1995).

The goal of the present work was to carry out a comparative study of the influence of MRP from glucose-lysine *vs.* glucose-methionine model systems on magnesium bioavailability and on tissue distribution in rats. Simultaneous assays were developed to separate the possible effects induced by the consumption of residual free amino acids added to the diets together with MRP, from those caused by the MRP *per se*.

2. Materials and methods

2.1. Sample preparation

Glucose (Merck, Darmstadt, Germany), lysine and methionine (Sigma Chemical Co., St. Louis, Mo., U.S.A.) were used to prepare the samples. Equimolar mixtures of glucose-lysine-HCl or glucose-DL-methionine (both 40% moisture) were heated in open recipients in an oven (Selecta 2000210, Barcelona, Spain) at 150 °C for 90 min to obtain the GL and GM samples, respectively. After heating, the reaction was stopped by cooling in an ice bath and the products were removed, frozen, lyophilised and stored at 4 °C, as described by Delgado-Andrade, Seiquer, and Navarro (2002), until they were used to prepare the diets.

The characterization of these samples was carried out in a previous work in which the pH in water solution, the development of the brown colour during heat treatment by measuring the absorbance at 420 nm (Friedman & Molnar-Perl, 1990), the free amino acid content and magnesium solubility assays in the presence of samples were studied (Delgado-Andrade et al., 2004).

2.2. HPLC analysis

Free lysine and methionine content in the samples was determined by high-performance liquid chromatography (HPLC) according to the Waters Pico Tag method (Cohen, Meys, & Tarvin, 1989) with pre-column derivatization with phenylisothiocianate using a Waters 2695 separation module (Waters Cromatografía, S. A., Madrid, Spain), without the hydrolysis step. A Millenium 32 chromatography manager system (Waters Cromatografía) was used for gradient control and data processing.

2.3. Diet preparation

The AIN-93 G purified diet for laboratory rodents (Reeves, Nielsen, & Fahey, 1993) was used for the control group (C). GL and GM samples were individually included in AIN-93 G at 3% level, resulting in the GL and GM diets, respectively.

To differentiate between the possible effects of the residual free amino acids still present in the samples and those from the MRP, diets containing the residual lysine and methionine were also prepared. The free amino acid contents present in the GL and GM samples, determined by HPLC, as stated before, were 118 mg lysine/g and 266 mg methionine/g, respectively. Thus, 30 g/kg diet of GL and GM samples provided 3.54 g/kg diet and 7.98 g/kg diet of free lysine and free methionine, respectively. These quantities were individually added to the AIN-93G diet to prepare the L and M diets, respectively.

Analysis of the individual diets revealed no significant differences in energy and nutrient content, as was expected since they were all basically the AIN-93G diet. The nitrogen and magnesium contents of the diets (mean $\pm$ SD) were as follows: nitrogen (g/100 g diet) $C = 2.85 \pm 0.04$, $GL = 2.89 \pm 0.08$, $L = 2.88 \pm 0.01$, $GM = 2.87 \pm 0.01$ and $M = 2.88 \pm 0.03$; magnesium (mg/100 g diet) $C = 56 \pm 4$, $GL = 54 \pm 3$, $L = 53 \pm 1$, $GM = 56 \pm 6$ and $M = 55 \pm 3$.

2.4. Biological assays

Sixty five weanling Wistar rats, weighing 41.7 ± 0.4 g (mean $\pm$ SE), were involved in the study. Fifty five animals

were randomly distributed into 5 groups of eleven rats. The remaining ten animals were sacrificed by anaesthesia overdose at day 0 to analyze their initial magnesium body contents. Each group was assigned to one of the dietary treatments, designated C (control), GL, GM, L and M, respectively. These rats were individually housed in metabolic cages in an environmentally controlled room which was kept at 20-22 °C with a 12 h light-dark cycle and 55-70% humidity. The animals belonging to the C, GL and GM groups had *ad libitum* access to their diets, while the L and M groups were pair-fed with the GL and GM rats, respectively, beginning the assay one day afterwards to enable the pair-feeding, after knowing the food consumed the day before by the corresponding group. All the animals had free access to demineralised water (Milli-Q Ultrapure Water System, Millipore Corp., Bedford, Mass., U.S.A.).

Two different balances were carried out. The magnesium balance for the entire experimental period (21 days), henceforth termed the "total period balance", was calculated from the difference between the final magnesium body content of each animal and the initial magnesium body content (mean $\pm$ SE: 13.4 ± 0.22 mg of Mg/rat or 0.31 ± 0.04 mg of Mg/g rat). Five animals from each group were sacrificed by anaesthesia overdose on day 21 to calculate their final magnesium body contents. None of their organs were extracted. Magnesium intake was monitored during this period.

In the last week of the experimental period (days 14-21) another magnesium balance was performed in all the animals. The test involved a preliminary 14-day period during which solid food intake and body weight changes were monitored, followed by a 7-day period in which, moreover, faeces and urine were individually collected on a daily basis, and stored separately as a 1-week pool. The faeces were weighed, lyophilized and homogenized. Urine was collected on 0.5% HCl (vol/vol), filtered (Whatman Filter Paper No. 40, ashless, Whatman, England) and diluted to an appropriate volume. To control for possible environmental contamination during the collection of urine and faeces, empty metabolic cages were manipulated in the same way as those used for the animals. Thus, minerals from possible environmental contamination were collected on 0.5% HCl (vol/vol) and taken it into account in the excreta magnesium analysis. On day 21, after an overnight fast, six animals from each group were anaesthetized with sodium pentobarbital (5 mg/100 g of body weight) (Abbott Laboratories, Granada, Spain) and terminal exsanguination was performed by cannulation of the carotid artery. Blood was drawn to obtain serum and the liver, right kidney, right femur and cecal content were removed, weighed and frozen at −20 °C until analysis. As mentioned above, the rest of the animals in each group (five in each one) were used to perform the total period balance.

The results of the total period balance provided information about magnesium retention during the assay. The magnesium balance performed between days 14 and 21 supplied data specifically related to the absorptive and metabolic process, and allowed an analysis of the magnesium content and concentration in the organs extracted after the animals were sacrificed.

Although balance studies are laborious and expensive, when performed at least one week after starting the diet they are the only method that provides real data on mineral absorbability and bioavailiability (Gueguen & Pointillart, 2000). Moreover, this method allows calculation of the mineral bioavailability for an average diet, unlike the isotope balance methods, which are more accurate but which consider the bioavailability of the source of mineral studied as representative of the diet.

All management and experimental procedures carried out in this study were performed in strict accordance with current European regulations (86/609 E.E.C.) regarding laboratory animals. In addition, the Committee of Bioethics for animal experimentation at our institution (CSIC) approved the study protocol.

2.5. Analytical techniques

The liver, right kidneys, right femur and caecal contents were dry-ashed in a muffle furnace (Selecta, Mod.366, Barcelona, Spain) at 450 °C and the white ashes obtained were dissolved with $HCl/HNO_3/H_2O$ (1:1:2) (Suprapur, Merck, Darmstadt, Germany). Aliquots of the remaining samples were completely digested by the addition of concentrated HNO_3, $HClO_4$ and by heating at high temperatures (180–220 °C) in a sand beaker. Previously, whole carcasses were subjected to acid hydrolysis treatment (6N HCl), heating at 80 °C for several days until complete disintegration. All samples were diluted with milli-Q water to an appropriate volume for measurement.

The magnesium analyses in all samples and the calcium analysis in bone were carried out with flame AAS in a Perkin-Elmer Analyst 700 Spectrophotometer (Norwalk, Conn., U.S.A.). Standard solutions were prepared from a stock Tritisol magnesium solution ($MgCl_2$ in 65% HCl, 1000 mg Mg, Merck) and a Tritisol solution of calcium ($CaCl_2$ in 6.5% HCl, 1000 mg Ca, Merck). For calcium analysis, lanthanum chloride (Merck) was added to samples and standards to reach a final concentration of 0.3%.

Pools of faeces, urine and diet were used as an internal control to assess precision. The inter-assay coefficient of variation was 2.15% in faeces, 2.38% in urine and 5.16% in the diet. Milk powder (certified reference material CRM 063; Community Bureau of Reference, Brussels, Belgium), which yielded a magnesium value of 1.29 ± 0.02 mg/g (mean $\pm$ SD; certified value: 1.26 ± 0.02 mg/g) and a calcium value of 13.5 ± 0.04 mg/g (mean $\pm$ SD; certified value: 13.49 ± 0.10 mg/g), was used to quantify accuracy.

All glassware and polyethylene sample bottles were washed with 10N nitric acid, and milli-Q water was used throughout the study.

Several indices were calculated, using the data for magnesium intake (I) and fecal (F) and urinary excretion (U)

obtained in the last week of the assay. These indices included: apparent absorption (A = I – F), apparent retention or balance (R = A – U) and fractional absorption (%A/I = A/I × 100).

The parameters calculated for the total period balance were global retention (final magnesium body content - initial magnesium body content) and global utilization (%R/I = global retention/ total magnesium intake × 100).

2.6. Statistical analyses

Values shown represent the means with their standard errors (SE). An analysis of variance (ANOVA) was used to compare data from each group of animals*vs.* the control group; significance was declared at $p < 0.05$. The same statistical treatment was applied to compare the MRP groups *vs.* their respective pair-fed groups (GL *vs.* L and GM *vs.* M). A two-way analysis of variance (ANOVA) was realized with the results from all animals (GL, GM, L and M), excluding the control group (C), to analyze the effects of the amino acid added to the diet (lysine or methionine, free or as a heated mixture), the effects of the addition or not of MRP (added as a glucose-amino acid heated mixture) and the interaction between the principal effects (factors). Duncan's multiple range tests were used to determine whether the mean values were statistically different between groups ($p < 0.05$).

Evaluation of the relationship between calcium content in the femur (dependent variable) and magnesium content in the femur (independent variable) was carried out by computing the relevant correlation coefficient (Pearson linear correlation) at the $p < 0.05$ confidence level. All statistical analyses were performed using Statgraphics Plus, version 5.1, 2001.

3. Results and discussion

3.1. Magnesium utilization

Magnesium intake, as a consequence of the reduced food intake, decreased in the M group compared with the control animals, probably as a product of the free methionine consumption (Tables 1 and 2). In this context, Benevenga and Steele (1984) reported that the first adverse effect of the consumption of high methionine diets is the diminution of the food intake. The decrease observed in the M group did not appear in the GM one, with which the former was pair-fed. Probably in the GM diet the effect of the free methionine on consumption could have been counteracted by the presence of MRP. It must be pointed out that improved food palatability, and therefore enhancement of the appetite, has been attributed to MRP (Friedman, 1996).

Compared with the controls, decreases in magnesium faecal excretion were observed in the groups fed diets including methionine, both in the case of the heated mixture and in that of free amino acid (Table 1). Some authors have reported decreases in faecal magnesium excretion after consumption of diets supplemented with methionine (Singh, Hussain, Gupta, Pendse, & Raj-Kiran, 1993). In the M group, the lower values of magnesium faecal excretion are related to the lower level of magnesium consumption, and thus, fractional absorption of the element did not change. In the glucose-methionine group, however, an additional effect, probably depending on MRP, occurred and consequently magnesium absorption was higher than with the pair-fed group. Thus, magnesium fractional absorption increased in the GM animals with respect to the controls. Although the two way-Anova test revealed that magnesium absorption was higher in the MRP groups compared with the groups fed diets supplemented with free amino acids, no increases in absorption or fractional absorption appeared specifically in the glucose-lysine group, which proves the great importance of the reactants employed to obtain MRP in the final properties and actions (Friedman, 1996).

The increase in the fractional absorption of magnesium observed after feeding MRP from methionine could be related, to some extent, to the formation of MRP-magnesium complexes. It is known that, although with less affinity than some other metals, MRP derived from methionine are capable of forming magnesium complexes (Delgado-Andrade et al., 2004). Magnesium could be absorbed in this chelated form or the complexes could even be later destroyed by intestinal microflora, with the magnesium being liberated and subsequently absorbed in the colon (Andrieux & Saquet, 1984), where magnesium absorption is significant in the rat (Shils, 1999).

The two way-Anova analysis performed in the total intake data showed the significant effects of both, the type of amino acid and the MRP added to the diet (Table 2) on the food ingested, that did not appear in the case of the last week balance (Table 1). This disappointment could be related to the fact that results come from different periods of time (21 days *vs* 7 days) and at the end of the assay (last week data) the animals were probably adapted to their corresponding dietetic treatment, as has been suggested by some authors (Benevenga & Steele, 1984).

Despite that, the type of amino acid included in the diet did not influence the magnesium content in the body (Table 2) and global retention remained unchanged throughout the experimental period among all groups.

The absence of effects on the magnesium balance observed in the GL group (Tables 1 and 2) agrees with results reported in previous studies by our research group when rats were fed diets containing casein, a protein very rich in lysine, heated with glucose and fructose (Aspe, 1992).

Few studies in the literature describe the effects of MRP on magnesium utilization and, to the best of our knowledge, decreases in magnesium fractional absorption have not been described. In accordance with the results observed in the GM group, some authors have found decreases in fecal magnesium excretion when rats were fed MRP from a glucose-glutamate model system (O'Brien et al., 1994) or marked increases in net absorption when animals were

Table 1
Magnesium last week Balance (14-21 day)[a]

Diets	Intake (mg/day)	Faecal (mg/day)	Urinary (mg/day)	Apparent absorption (mg/day)	Apparent retention (mg/day)	A/I (%)
C	7.0 ± 0.2	2.8 ± 0.1	2.0 ± 0.1	4.2 ± 0.1	2.2 ± 0.2	59.4 ± 1.3
GL	6.7 ± 0.4	2.5 ± 0.1	2.1 ± 0.2	4.2 ± 0.3	2.1 ± 0.2	62.7 ± 1.5
GM	6.9 ± 0.3	2.1 ± 0.2*	2.1 ± 0.2	4.8 ± 0.3#	2.6 ± 0.3	69.2 ± 2.9*
L	6.5 ± 0.1	2.4 ± 0.1	1.8 ± 0.2	4.0 ± 0.1	2.3 ± 0.1	62.4 ± 2.0
M	6.2 ± 0.4*	2.2 ± 0.3*	1.8 ± 0.2	3.9 ± 0.1	2.1 ± 0.2	65.0 ± 3.3
Two way-Anova						
Amino acid[b]	NS	NS	NS	NS	NS	NS
MRP addition[c]	NS	NS	NS	$p = 0.036$	NS	NS
Interaction[d]	NS	NS	NS	NS	NS	NS

[a] Data are means ± S.E. of eleven animals. Superscript * within the same column indicates significant difference from the C group (control) ($p < 0.05$). Superscript # within the same column indicates significant difference from the corresponding pair-fed group ($p < 0.05$).
[b] Amino acid: lysine or methionine. NS: no significant effect.
[c] MRP addition: presence or not of MRP (GL or GM samples) in the diet. NS: no significant effect.
[d] Interaction amino acid × MRP addition.NS: no significant differences.

Table 2
Magnesium total period balance[a]

Diets	Total intake (mg)	Body content (mg)	Global retention (mg)	Global retention (mg/day)	Global R/I (%)
C	119 ± 4	46 ± 2	33 ± 2	1.6 ± 0.1	28.3 ± 1.7
GL	119 ± 6	46 ± 1	32 ± 1	1.5 ± 0.1	27.1 ± 1.0
GM	110 ± 1	43 ± 1	30 ± 1	1.4 ± 0.1	27.0 ± 1.1
L	112 ± 2	45 ± 1	32 ± 1	1.5 ± 0.1	28.9 ± 1.2
M	95 ± 8*	44 ± 3	31 ± 3	1.5 ± 0.2	34.4 ± 6.9
Two way-Anova					
Amino acid[b]	$p = 0.030$	NS	NS	NS	NS
MRP addition[c]	$p = 0.048$	NS	NS	NS	NS
Interaction[d]	NS	NS	NS	NS	NS

[a] Data are means ± S.E. of five animals. Superscript * within the same column indicates significant difference from the C group (control) ($p < 0.05$). No significant differences were found between the MRP groups and their corresponding pair-fed groups ($p > 0.05$).
[b] Amino acid: lysine or methionine. NS: no significant effect.
[c] MRP addition: presence or not of MRP (GL or GM samples) in the diet. NS: no significant differences.
[d] Interaction amino acid × MRP addition. NS: no significant differences.

fed diets containing glucose-glycine derivates (Andrieux & Saquet, 1984). None of these authors specifically mention modifications in magnesium retention.

3.2. Effects on body weight of animals and magnesium distribution in some organs

In parallel with the changes described in food intake, only the animals in the M groups exhibited lower body weights than the controls (Table 3). As mentioned above, the methionine seemed to be responsible for this finding. The presence of an excess of this amino acid (≈8 g methionine/kg diet, provided by the sample), could contribute to the weight reduction observed, since it is known that consumption of excess methionine, 3- to 4-times higher than dietetic requirements (9.8 g/kg diet, NRC, 1995), can induce deterioration of growth (Benevenga, 1974). Since the group consuming glucose-methionine MRP did not manifest such a marked decrease, it could be deduced that the presence of those MRP counteracted, at least partly, the effects induced by the free residual methionine.

According to the body weight decreases, organ weights were also lower; indeed, these even exhibited a significant decrease in some cases in which the body weight did not (Table 3).

The most significant findings appeared in the bones. The femur weights of the animals belonging to the L and M groups decreased with respect to the control group and also with respect to the MRP groups ($p = 0.007$). The fall in the total magnesium femur content in the GL, GM and M groups may be related to a lower femoral weight only in the latter case. However, magnesium concentration among GL and GM animals did not show variations with respect to the control group, but only with respect to their corresponding pair-fed groups. Moreover, femurs of M and L groups had higher magnesium concentrations than the control group. The positive influence of amino acids in relation to bone mineralization has been demonstrated by several authors. It has been shown that lysine increases insulin-like growth factor-1 (IGF-1) production and collagen synthesis in cultured mouse osteoblastic cells (Chevalley, Rizzoli, Manen, Caverzasio, & Bonjour, 1998), and that pigs fed

Table 3
Magnesium concentration in different organs and tissues, calcium concentration in femur[a]

	Groups					Two-way ANOVA		
	MRP added			Amino acid added		Amino acid[b]	MRP addition[c]	Interaction[d]
	C	GL	GM	L	M			
Bodyweight(g)	141.8 ± 7.2	132.4 ± 7.2	131.2 ± 6.4	132.6 ± 2.1	120.9 ± 3.2*	NS	NS	NS
Liver								
weight (g)	6.57 ± 0.36	6.02 ± 0.51	6.69 ± 0.57$^{\#}$	4.82 ± 0.77*	5.30 ± 0.17*	NS	$p = 0.007$	NS
Mg (mg)	1.44 ± 0.09	1.45 ± 0.11$^{\#}$	1.72 ± 0.19$^{\#}$	1.04 ± 0.02*	1.16 ± 0.07*	NS	$p = 0.001$	NS
Mg (mg/g)	0.22 ± 0.02	0.24 ± 0.01$^{*\#}$	0.24 ± 0.01*	0.22 ± 0.01	0.22 ± 0.01	NS	$p = 0.025$	NS
Kidney								
weight (g)	0.73 ± 0.03	0.70 ± 0.03	0.74 ± 0.07	0.63 ± 0.03*	0.65 ± 0.04	NS	NS	NS
Mg (mg)	0.16 ± 0.01	0.19 ± 0.01$^{\#}$	0.18 ± 0.01$^{\#}$	0.12 ± 0.01*	0.13 ± 0.01*	NS	$p = 0.000$	NS
Mg (mg/g)	0.22 ± 0.01	0.27 ± 0.01$^{*\#}$	0.24 ± 0.01$^{\#}$	0.20 ± 0.01	0.20 ± 0.01	NS	$p = 0.000$	NS
Femur								
weight (g)	0.24 ± 0.01	0.22 ± 0.01	0.22 ± 0.21$^{\#}$	0.20 ± 0.01*	0.17 ± 0.01*	NS	$p = 0.007$	NS
Mg (mg)	0.86 ± 0.03	0.76 ± 0.03*	0.74 ± 0.06*	0.81 ± 0.02	0.68 ± 0.03*	NS	NS	NS
Mg (mg/g)	3.6 ± 0.1	3.4 ± 0.1$^{\#}$	3.4 ± 0.1$^{\#}$	4.0 ± 0.1*	3.9 ± 0.1*	NS	$p = 0.000$	NS
Ca (mg/g)	198 ± 8	181 ± 3$^{*\#}$	181 ± 2$^{*\#}$	214 ± 4*	195 ± 4	$p = 0.047$	$p = 0.006$	NS
Cecum content								
weight (g)	1.36 ± 0.09	1.76 ± 0.13$^{*\#}$	1.70 ± 0.18	0.99 ± 0.12*	1.13 ± 0.22	NS	$p = 0.001$	NS
Mg (mg/g)	0.98 ± 0.09	0.62 ± 0.05*	0.67 ± 0.09*	0.84 ± 0.13	0.67 ± 0.08*	NS	NS	NS
Serum (mg/dl)	2.3 ± 0.1	2.0 ± 0.1	2.3 ± 0.2	2.3 ± 0.1	2.4 ± 0.1	NS	NS	NS

[a] Data are means ± S.E. of five animals. Superscript * within the same row indicates significant difference from the C group (control) ($P < 0.05$). Superscript$^{\#}$ within the same row indicates significant difference from the corresponding pair-fed group ($p < 0.05$).

[b] Amino acid: lysine or methionine. NS: no significant effect.

[c] MRP addition: presence or not of MRP (GL or GM samples) in the diet. NS: no significant differences.

[d] Interaction amino acid × MRP addition. NS: no significant differences.

a diet containing casein, a lysine-rich protein, have improved bone quality compared with those fed other protein sources (Budek et al., 2006). On the other hand, lysine deficiency compromises skeletal growth and trabecular development in rats (Soon, Ammann, Williamson, Ginty, & Offord, 2005). Results of the present assay suggest that MRP mask the amino acid-enhancing effect on magnesium bone. The effect could be of interest, since 50-60% of the total body magnesium is present in the bone (Cashman & Flynn, 1999), and decreased bone concentration has been described in some bone disorders, e.g. osteoporosis (Cohen, 1988; Cohen & Laor, 1990). In this context, some authors have used peroral magnesium administration as the sole treatment in postmenopausal osteoporosis (Stending-Lindberg, Koeller, Bauer, & Rob, 2004).

The effects on bone magnesium are of major importance when we take into account the statistically significant decrease in bone calcium among animals fed MRP-diets, as previously observed by our research group (Delgado-Andrade, Seiquer, & Navarro, 2005).The data for calcium concentration in the femur (Table 3) showed a significant decrease in MRP-groups, with respect to the control group and their pair-fed groups. The L group also presented a significant increase compared with the control group, which is evidence for the positive role of lysine in improving bone mineral status. Moreover, the magnesium and calcium contents of the femurs of animals fed MRP diets were highly correlated ($r = 0.9855$ and $r = 0.9783$ for the GL and GM groups, respectively, $P < 0.01$ in both cases), a relationship which did not appear in the control or the free amino acid-supplemented groups.

It has been proposed that the effects of dietary MRP may be related to the action of their endogenously formed compounds, termed advanced glycation end-products (AGEs). Bone collagen is a protein that is very sensitive to *in vitro* glycation (Sajithlal, Chithra, & Chandrakasan, 1999) and the formation of AGEs, observed in collagen aging and osteoporosis, affects bone matrix functionality and bone formation (Yamamoto et al., 2001).

Liver weights decreased among animals fed the L and M diets, compared with the control group and with respect to the MRP groups ($p = 0.007$) (Table 3). As a consequence, both groups also exhibited lower total magnesium contents in the liver, with no variations of the mineral concentration. In this respect, the literature only reports increases in the liver weights of animals fed diets that are highly supplemented with methionine (Benevenga & Steele, 1984), and there are no data concerning the intake of small quantities. The absence of variations in the hepatic total magnesium contents of the GL and GM groups with respect to the control animals, and the increase compared with their pair-fed groups, indicate that MRP present in these diets could have counteracted the decrease caused by the free amino acid still present in them. In fact, liver magnesium concentrations among GL and GM animals were higher than those in control rats.

The tendency for increased magnesium content in the kidneys of animals fed MRP diets compared with the control group is emphasized when the data are expressed as a concentration value, reaching statistical significance in the GL group (Table 3). Moreover, magnesium deposits in the kidneys were lower in the L and M groups than in the controls and also than in those animals fed MRP ($p = 0.000$), which suggests that MRP consumption could induce an increase in magnesium deposition in this organ.

On the basis of the results observed for the liver and the kidney, and taking into account that these organs have been described as specific targets for MRP accumulation in the body (Faist & Erbersdobler, 2001), it can be suggested that some MRP-Mg complexes might be absorbed and once inside the organism they could act as carriers of magnesium toward the liver and the kidney, to the detriment of the natural reserve, the bone. The passage of MRP by the kidney is supported by the appearance of the brown colour observed in the urine of the groups fed MRP diets, especially in those consuming diets with the added glucose-lysine mixture (Delgado-Andrade, 2002).

The weights of the cecum contents were higher in animals fed MRP diets than in animals fed amino acid diets ($p = 0.001$); in the case of the GL group, the value was significantly higher than in the control or the L groups. Some modifications of magnesium concentration in the cecal mass were observed. The tendency for a decrease in this respect among the animals consuming browned diets could be related to the higher water content of the faeces (10, 16 and 21% for the control, GL and GM groups, respectively), although diarrhoea, a symptom described by some authors as a consequence of browning products consumption (Andrieux & Saquet, 1984), was not observed.

In summary, the different effects observed, after the consumption of MRP from a glucose-methionine or glucose-lysine heated mixture, on magnesium intake and fractional absorption, confirm the importance of reactants in the final effects of the compounds formed. The decrease in food intake in the glucose-methionine group is deduced not to be related to the browning products but to the residual methionine present. Moreover, although these MRP do not modify the magnesium balance, the browned derivates from both mixtures seem to increase renal and hepatic magnesium and mask the positive effect of free amino acids on bone magnesium. All of this, together with the more important effect described concerning bone calcium, suggests that the consumption of the MRP assayed could diminish bone mineralization. Further work, including the evaluation of some other bone health indices, will be required to test this hypothesis and to study possible implications of MRP consumption on bone health.

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