Nutritional Assessment of Magnesium from Raw and Processed Chickpea (Cicer arietinum L.) in Growing Rats

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We studied the effect of different processing methods (dry heating, soaking in distilled water, acidic solution, or basic solution, and soaking + cooking) on the nutritive utilization of magnesium from chickpeas (Cicer arietinum L.) in growing rats. We also investigated the effects of processing on several seed components that affect magnesium utilization. Chemical and biological methods were used for nutritional determinations. Although a large amount of the magnesium from raw chickpeas was absorbed (3.92 ± 0.36 mg/rat/day), the digestive utilization of magnesium (apparent digestibility coefficient) from unprocessed chickpeas was low. Processing led to an overall decrease in magnesium absorption and digestive utilization, because of modifications in certain components (e.g., fiber) of the legume. Under our experimental conditions feeding with raw or processed chickpeas led to elevated urinary excretion of magnesium (4.22 ± 0.37 mg/rat/day) in comparison with that found in growing rats fed a standard diet (1.11 ± 0.09 mg/rat/day). This increase resulted in a negative metabolic balance of this element. However, muscle and bone concentrations of magnesium were not affected by any of the experimental diets.

Keywords: Chickpea; fecal excretion; fiber; magnesium; nutritive utilization; processing techniques

INTRODUCTION

The fundamental role of magnesium in various physiological processes in humans and animals has been described in detail in several reviews of this cation. Nevertheless, this information has apparently not been sufficiently disseminated; therefore dietary intake in the general public is often deficient (Hazell, 1985; Wester, 1987; Moreiras et al., 1990). Many studies have noted the relationship between dietary magnesium deficiency and the incidence of certain diseases (including atherosclerosis); the links between intake and disease are especially worrisome in children (Aikawa, 1978; Hazell, 1985; Wester, 1987).

Recent investigations (Moreu et al., 1995; Fernández et al., 1997; Nestares et al., 1997) have shown that legumes can be a good source not only of protein but also of minerals. After soybean, chickpea (Cicer arietinum L.) is the legume that contains the greatest amounts of magnesium; this species constitutes a major source of magnesium in the Mediterranean diet (Mataix et al., 1995). The mean amounts of this mineral do not differ significantly between known varieties of chickpea ( desi and kabuli). This is in contrast to the findings for fiber (Jambunathan and Singh, 1981; Rossi et al., 1984) or in crops grown in different locations, a factor known to influence protein content (Singh et al., 1983).

Other components of chickpeas such as protein, fiber, vitamin D, phytic acid, and other minerals affect the nutritive utilization of magnesium directly or indirectly, however (Wester, 1987; Brink et al., 1991; Torre et al., 1993). These factors, like the amount of magnesium in chickpeas, are modified by processing (Meiners et al., 1976; Khan et al., 1988; Vidal and Frías, 1991; Attia et al., 1994; Nestares et al., 1996), and notable differences in how these nutrients change have been reported (Singh, 1985; Vidal and Frías, 1991). Because chickpeas must be processed for human consumption, information on the relationships between these changes may help in determining optimum methods for preparing this legume.

This study investigated the effects of commonly used processing methods on the composition and nutritive utilization of magnesium from chickpeas and sought to identify the main factors that modify these parameters. Chickpeas are the most commonly consumed legume in Spain (Varela et al., 1995), and the processing methods tested here were chosen to reproduce, as closely as possible, different cooking methods used in the home. Dry heating was tested because chickpea flour is used in a number of dishes and in dietary foods. We also tested the effects of simmering with or without prior soaking in water alone, water with bicarbonate (baking soda), or water with citric acid (lemon juice).

MATERIALS AND METHODS

Samples and Processing Techniques. Raw, dried chickpeas (R) (Cicer arietinum L.) were grown in Andalusia (southern Spain). The seeds were subjected to seven different treatments: H = dry heating, S = soaking in distilled water, SA = soaking in acidic medium, SB = soaking in basic medium, SC = S + cooking, SAC = SA + cooking, and SBC = SB + cooking.

Raw chickpeas were dry heated under pressure at 120 °C, 1 atm, for 15 min. In processes S, SA, and SB, raw seeds were soaked at room temperature for 9 h in distilled water (pH = 5.3), citric acid solution (0.1%, pH = 2.6), or sodium bicarbonate solution (0.07%, pH = 8.4), respectively. The seed-to-solution ratio was 1:3 (wt/vol). The soaking liquid was drained off, and the seeds were blended and lyophilized. Soaked chickpeas were cooked (SC, SAC, SBC) by boiling in distilled water for 35 min, at a seed-to-water ratio of 1:6.67 (wt/vol). The cooking water was drained off, and the seeds were blended and lyophilized.

Analytical Techniques. Water content was determined by oven-drying at 105 ± 1 °C until a constant weight was obtained. Ash was measured by calcination at 500 °C to a
constant weight. Magnesium content was measured by atomic absorption spectrometry (Perkin Elmer 1100-B apparatus). Aliquots of raw and processed chickpeas diets, feces, femur, and longissimus dorsi muscle of rats were reduced to ash in a muffle furnace at 450 °C and then dissolved in 6 N HCl for analysis. Urine samples were measured as such. Lanthanum chloride (1–0.1%) was added to avoid interferences during the analysis.

The method of Van Soest and Wine (1968) as modified by McQueen and Nicholson (1979) was used to determine neutral detergent fiber (NDF), cellulose (CL), hemicellulose (HMC), and lignin (LN). To remove starch, the samples were incubated overnight with a solution of 0.5% bacterial α-amylase (Vidal-Valverde et al., 1992).

**Biological Methods.** Experimental Design and Diet. We used a biological balance technique. Food intake and changes in body weight were recorded, and magnesium intake and fecal and urinary magnesium excretion were calculated.

Eight experiments were done in which raw or processed chickpeas were the only source of food: group R, raw chickpeas; group H, chickpeas dry heated under pressure; group S, chickpeas soaked in distilled water; group SB, chickpeas soaked in basic medium; SA, chickpeas soaked in acidic medium; group SC, chickpeas soaked in double-distilled water and cooked; group SAC, chickpeas soaked in basic medium and cooked; SAC, chickpeas soaked in acidic medium and cooked.

Each experiment lasted 10 days. During the first 3 days the rats were allowed to adapt to the diet and experimental conditions. The main experimental period comprised the next 7 days, during which body weight and food intake were recorded and feces and urine were collected for subsequent analysis. The diet and double-distilled water were available ad libitum throughout the experimental period.

**Animals.** In each experiment we used 10 young albino Wistar rats (5 male, 5 female) reared in the University of Granada Laboratory Animal Services. The growing animals (recently weaned), with an initial body weight of 58.8 g, were housed in individual metabolic cages kept in a thermostatically controlled 12 h light: dark period (lights on at 9:00). The rats were handled at all times in accordance with current European regulations regarding laboratory animals.

**Biological Indices.** The following indices and parameters were determined for each group, according to the formulas given below: apparent digestibility coefficient (ADC) (1) for magnesium and magnesium retention (balance) (2).

\[ \text{ADC} = \frac{1 - \frac{F}{I}}{1} \times 100 \]

\[ \text{balance} = 1 - (F + U) \]

In accordance with the formulas recommended by the FAO/WHO (1966), the factors used were I (magnesium intake), F (fecal magnesium), and U (urinary magnesium). Magnesium intake is expressed as mg/at.

**Safety Precautions.** All reasonable precautions were taken to avoid mishaps in the use of volatile or caustic reagents such as hydrochloric acid.

**Statistical Methods.** The results from all experiments and analyses were tested statistically by analysis of variance using Statgraphic Statistical Graphics 2.1 System software (Statistical Graphics Corp., Rockville, MD) with an IBM Personal System/2 Model 20 computer (International Business Machines Corp., North Harbour Portsmouth, U.K.).

**RESULTS**

**Chemical Analysis.** Table 1 gives the values for magnesium and ash content in raw and processed chickpea diets. Raw chickpeas contained 134.26 mg of magnesium/100 g of sample. Soaking slightly decreased Mg content (by 4–12%), and cooking led to larger reductions (22–27%). As expected, dry heating under pressure (process H) did not affect Mg content.

| Table 1. Composition of Ash and Magnesium in Raw and Processed Chickpeas in Dry Matter* |
|---|---|---|
| diats | ash content (%) | magnesium content (mg/100 g of diet) |
| R | 2.88 | 134.26 |
| H | 2.91 | 138.93 |
| S | 2.77 | 126.24 |
| SA | 2.63 | 119.36 |
| SB | 2.69 | 129.34 |
| SC | 2.97 | 105.16 |
| SAC | 1.91 | 98.40 |
| SBC | 1.96 | 101.15 |

* R = raw chickpeas; H = heated chickpeas; S = soaked chickpeas; SA = chickpeas soaked in acidic medium; SB = chickpeas soaked in basic medium; SAC = chickpeas soaked in acidic medium and cooked; SBC = chickpeas soaked in basic medium and cooked.

**Table 2. Composition of Fiber in Raw and Processed Chickpeas in Dry Matter (g/100 g of diet)**

<table>
<thead>
<tr>
<th>group</th>
<th>neutral fiber (NDF)</th>
<th>cellulose (CL)</th>
<th>hemicellulose (HMC)</th>
<th>lignin (LN)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td>14.36</td>
<td>0.70</td>
<td>2.81</td>
<td>0.09</td>
</tr>
<tr>
<td>H</td>
<td>13.20</td>
<td>0.21</td>
<td>3.33</td>
<td>0.14</td>
</tr>
<tr>
<td>S</td>
<td>7.00</td>
<td>0.49</td>
<td>0.15</td>
<td>3.26</td>
</tr>
<tr>
<td>SA</td>
<td>10.43</td>
<td>0.06</td>
<td>2.45</td>
<td>0.44</td>
</tr>
<tr>
<td>SB</td>
<td>6.13</td>
<td>0.25</td>
<td>2.56</td>
<td>0.15</td>
</tr>
<tr>
<td>SC</td>
<td>11.52</td>
<td>0.26</td>
<td>7.03</td>
<td>0.19</td>
</tr>
<tr>
<td>SAC</td>
<td>11.31</td>
<td>0.40</td>
<td>5.42</td>
<td>0.10</td>
</tr>
<tr>
<td>SBC</td>
<td>7.27</td>
<td>0.47</td>
<td>4.81</td>
<td>0.49</td>
</tr>
</tbody>
</table>

* R = raw chickpeas; H = heated chickpeas; S = soaked chickpeas; SA = chickpeas soaked in acidic medium; SB = chickpeas soaked in basic medium; SC = soaked and cooked chickpeas; SAC = chickpeas soaked in acidic medium and cooked; SBC = chickpeas soaked in basic medium and cooked.

The fiber content (NDF, CL, HMC, and LN) in raw and processed chickpea diets is shown in Table 2. Raw chickpeas contained 14.36% NDF; most of this amount (9.71%) was HMC. Heating (H) and soaking followed by cooking (SC, SAC, and SBC) increased the relative CL content. Soaking with or without cooking decreased percent HMC. The decrease after soaking in acidic medium was smaller than after soaking in basic solution or distilled water. No significant change in HMC was seen in samples subjected to treatment H. Lignin was not affected significantly by any of the treatments.

**Biological Analysis.** Magnesium intake was significantly higher in rats fed with diets H, S, and SB (P < 0.05) than in the other groups (Table 3). Feces weight expressed as dry matter (Table 3) was significantly higher in groups H, S, and SA than in animals fed raw chickpeas (P < 0.05). Soaking + cooking and soaking in basic medium without cooking significantly increased feces weight in comparison with the rest of the processing methods (P < 0.05).

We found no direct correlation between food intake and feces weight, although feces weight in dry matter did correlate with fecal water content. Fecal magnesium excretion (Table 3) was lowest in rats fed raw chickpeas or chickpeas that had been soaked before cooking. In comparison, fecal magnesium content was significantly higher in groups S and SA (P < 0.05) and was greatest in groups H and SB (P < 0.05).

Digestive utilization of magnesium, calculated as the ADC, was 49.2% in raw chickpeas. Magnesium absorption in absolute values (Table 3) was significantly reduced in all experimental groups except group S in comparison with the group fed raw chickpeas. Magnesium retention (Table 4) was negative in most experimental groups.
as reported in other analyses of cooked chickpeas
content was significantly reduced by soaking in acidic
Singh, 1981; Chavan et al., 1989; Attia et al., 1994). Ash

DISCUSSION

Chemical Analysis of Diets. The ash and magne-
ium contents of the chickpea variety assayed in this
study were within the range reported by others (Meiners
et al., 1976; Tiwari et al., 1977; Jambunathan and
Singh, 1981; Chavan et al., 1989; Attia et al., 1994). Ash
content was significantly reduced by soaking in acidic
(33.7%) or basic (31.9%) medium followed by cooking,
reported in other analyses of cooked chickpeas
(Meiners et al., 1976; Attia et al., 1994), although the
decreases we found were slightly smaller.

The reduction in Mg caused by cooking under our
experimental conditions was smaller than the 50%
decrease found by Meiners et al. (1976). The smaller
decreases we obtained may have been due in part to
prior soaking. The differences in mineral loss due to
processing between our figures and those reported by
others may be related to differences in experimental or
growth conditions used.

Soaking, regardless of whether it was followed by
cooking, decreased HMC as a result of solubilization.
That the decrease was smaller when chickpeas were
soaked in acidic solution is strongly supported by the
reduction in Mg caused by cooking in these experiments.

The increase in fecal dry weight in rats fed processed
chickpeas (Table 3) and animals given a control diet containing 12%
casein-methionine (Nestares et al., 1993). The differ-

Fecal Excretion. Interestingly, dry weight of feces
in rats fed raw chickpeas (Table 3) was significantly
lower than in animals fed processed chickpeas (Table
3) and animals given a control diet containing 12%
casein-methionine (Nestares et al., 1993). The differ-
ingen food intake (Nestares et al., 1996) and fecal
dry weight were lowest in rats given raw chickpeas.

The increase in fecal dry weight in rats fed processed
chickpeas (Table 2) probably resulted from the increase
in cellulose, as found by Saito and Sato (1988) in their
study of a control diet containing casein. Processing led
to fecal dry weights that approached the values we
found with a casein-methionine diet that contained 8%
fiber (1365 ± 69 mg of feces/100 g diet) (Nestares et al.,
1993).

Total fiber content was also indirectly increased by
soaking, as a result of the loss of nutrients through
solubilization (Vidal and Frías, 1991). This loss may
account for part of the lower fecal dry weight in rats
fed with dry-heated chickpeas.

Although our analytical methods detected “resistant
starch” in fiber, soaking in basic medium (SB) may have
transformed the starch into a more readily utilizable
form (Nestares et al., 1996). This would account for the
increase in total fecal excretion in group SB despite the
lack of a significant increase in cellulose content.

Biological Analysis of Magnesium. In all the diets
we tested, magnesium content remained above the
required level for growing rats (40 mg/100 g of diet)

| Table 3. Digestive Utilization of Magnesium |
|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| group | food intake (g/100 g rat/day) | magnesium intake (mg/rat/day) | feces weight (mg/rat/day) | fecal magnesium (mg/rat/day) | absorbed magnesium (mg/rat/day) | ADC |
| R | 9.73 ± 0.37 | 8.06 ± 0.30 | 672.7 ± 91.4 | 4.14 ± 0.46 | 3.92 ± 0.36 | 49.17 ± 4.71 |
| H | 10.79 ± 0.22 | 10.12 ± 0.33 | 1160 ± 57.7 | 7.24 ± 0.74 | 2.88 ± 0.16 | 28.05 ± 6.83 |
| S | 10.88 ± 0.17 | 9.58 ± 0.33 | 1216.9 ± 138.2 | 5.94 ± 0.50 | 3.64 ± 0.29 | 38.78 ± 3.95 |
| SA | 10.56 ± 0.18 | 7.52 ± 0.13 | 1084.4 ± 50.0 | 5.14 ± 0.16 | 2.38 ± 0.21 | 31.47 ± 2.43 |
| SB | 12.19 ± 0.25 | 11.59 ± 0.23 | 1569.4 ± 100.6 | 8.85 ± 0.66 | 2.74 ± 0.54 | 24.02 ± 4.76 |
| SC | 12.32 ± 0.40 | 7.86 ± 0.37 | 1422.7 ± 51.8 | 5.03 ± 0.26 | 2.83 ± 0.48 | 34.64 ± 4.35 |
| SAC | 12.00 ± 0.00 | 6.92 ± 0.12 | 1452.7 ± 31.3 | 4.45 ± 0.12 | 2.47 ± 0.15 | 35.56 ± 1.84 |
| SBC | 11.36 ± 0.26 | 7.26 ± 0.23 | 1396.7 ± 72.4 | 4.96 ± 0.25 | 2.30 ± 0.28 | 31.33 ± 3.54 |

| Table 4. Metabolic Utilization of Magnesium |
|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| group | total urinary magnesium (mg/rat/day) | balance |
| R | 4.22 ± 0.37a | |
| H | 4.29 ± 0.28b | |
| SA | 4.93 ± 0.32 | |
| SB | 3.59 ± 0.21 | |
| SC | 4.02 ± 0.11 | |
| SAC | 2.61 ± 0.17 | |
| SBC | 2.34 ± 0.14 | |

| Table 5. Magnesium Content in Femur and Longissimus Dorsi Muscle |
|--------------------------|--------------------------|--------------------------|--------------------------|
| group | magnesium content (mg/g of ash) |
| femur | muscle |
| R | 9.59 ± 0.26a | 22.39 ± 0.31a |
| H | 10.28 ± 0.18b | 20.23 ± 0.41a |
| SA | 9.39 ± 0.69abc | 20.86 ± 0.22a |
| SB | 10.21 ± 0.24abc | 20.26 ± 0.37a |
| SC | 12.57 ± 0.24c | 19.99 ± 0.50a |
| SAC | 11.82 ± 0.94def | 18.90 ± 0.14a |
| SBC | 12.49 ± 0.23ef | 22.38 ± 0.45a |

M magnesium content in muscle and bone (expressed per gram of ash) differed significantly between group R rats and animals fed processed chickpeas (Table 5). However, this difference was without biological signifi-
cance.

DISCUSSION

Chemical Analysis of Diets. The ash and magne-
sium contents of the chickpea variety assayed in this
study were within the range reported by others (Meiners
et al., 1976; Tiwari et al., 1977; Jambunathan and
Singh, 1981; Chavan et al., 1989; Attia et al., 1994). Ash
content was significantly reduced by soaking in acidic
(33.7%) or basic (31.9%) medium followed by cooking,
reported in other analyses of cooked chickpeas
(NCR, 1990) despite the losses in mineral content caused by processing.

The digestive utilization of magnesium, expressed as the ADC, was approximately 50% (Table 3), a figure lower than the 88% found by Hardwick et al. (1990) in growing rats fed an adjusted diet that contained appropriate amounts of calcium (0.5%) and magnesium (0.05%) (AIN, 1977). The lower ADC for magnesium in our experiments was expected, in view of the high magnesium intakes from the chickpea diets (Table 3). The lower quality of protein in the diets we tested may have decreased the digestive utilization of magnesium (Aikawa, 1978). This effect is supported by the work of Moreu et al. (1995), who obtained and ADC of 50% in growing rats fed exclusively with raw fava beans (Vicia faba), a legume similar in protein quality to chickpea. Moreover, the vitamin D deficiency in the chickpea (Mataix et al., 1995) would be expected to reduce the active transport of magnesium (Ebel and Gunther, 1980).

Despite the low ADC for magnesium in growing rats fed raw chickpeas, net magnesium absorption was high. In fact, absorption was greater than the value (1.91 ± 0.22 mg of Mg/rat/day) found in rats fed an adjusted diet containing 20% casein-methionine (Moreu et al., 1995). This may have been the result of the high magnesium intake in our chickpea-fed animals. Moreover, because of the calcium deficit in chickpea diets (Nestares et al., 1997), the level of parathyroid hormone would be expected to increase, and this, together with the absence of calcium–magnesium interactions, would favor magnesium absorption.

The effect of protein quality on the digestive utilization of magnesium was most evident in group H (Table 3). Because Mg intake was so high in this group, the effect of diminished protein quality (Nestares et al., 1996) was also more obvious. However, the low ADC for magnesium in group H was accompanied by a lower net Mg absorption in comparison with the raw chickpea diet (Table 3), despite the greater magnesium intake in group H. This finding confirms that other factors, possibly including dietary fiber content, affect magnesium absorption.

The increase in fecal excretion, favored by the increase in fiber (mainly cellulose) intake as a result of processing, led to greater fecal losses of magnesium (Torre et al., 1991) by decreasing the absorption of this mineral as a result of solvent drag. In quantitative terms, magnesium is especially susceptible to this effect mechanism (Chutkow, 1964). In fact, under our experimental conditions increased fecal excretion was associated with increased fecal water content.

The relation between fecal losses of magnesium and increased fecal excretion was evident in rats fed processed chickpeas; these changes resulted in a lower net absorption of magnesium in comparison with the raw chickpea diet (Table 3). Loss of magnesium through the feces was greatest in group SB (soaking in basic solution), in which total fecal excretion and fecal loss of magnesium were 2 times the values in the group fed raw chickpeas. As a result, net magnesium absorption was lower in the former group (Table 3).

Loss of soluble magnesium as a result of processing and especially processing + cooking (Table 1) resulted in lower magnesium intake and lower net absorption (Table 3). This effect accounts for the higher net absorption in group S (soaking in distilled water) than in any other experimental group, despite the high rate of magnesium excretion (Table 3). This reflects the lower loss of soluble cation in distilled water (Table 1).

Despite the high net absorption of magnesium in diets consisting of raw or processed chickpeas, the high rate of urinary excretion led to negative magnesium balances (Table 4). In rats fed the chickpea diets, urinary magnesium excretion was significantly higher than 1.11 ± 0.09 mg of Mg/rat/day, the value found in rats fed an adjusted diet with 20% casein-methionine (Moreu et al., 1995). The lower quality of chickpea protein in comparison with casein may have been responsible for the increase in urinary magnesium excretion in animals fed with this legume, as increased proteinuria caused by low-quality dietary protein inhibits the tubular reabsorption of this cation and increases urinary loss (Brink et al., 1991). Moreover, high magnesium intakes both decrease absorption and increase urinary excretion of this cation (Aikawa, 1978; Brink et al., 1991; Sutton and Domrongkitchaiporn, 1993).

The magnesium contents in femur and muscle in the different experimental groups showed that the mineral was not mobilized from these tissues (Table 5). This finding was not surprising in view of the high dietary content and net absorption of magnesium, which probably obviated the need for parathyroid hormone to mobilize magnesium stores, as occurs when intestinal absorption is impaired (MDntyre et al., 1961). The experimental period we used (10 days) may have been too short to detect the effects of excess dietary magnesium and low-quality protein (Nestares et al., 1996) on the metabolic utilization of magnesium (Lerma et al., 1993).

In conclusion, although processing of legumes decreases the ADC of magnesium, the content of this mineral in chickpeas is, in principle, high enough to ensure that the amount of this nutrient actually absorbed by persons who consume this food in typical diets is sufficient to negate any Mg losses caused by processing and to partially satisfy nutritional requirements. The negative metabolic balance found under our experimental conditions was probably the result of rapid fecal and urinary losses. Nevertheless, when chickpeas are consumed together with other foods such as meat, potatoes, and other vegetables (as would normally occur in human diets), interactions with dietary protein probably offset these losses.

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