Ability of a Cocoa Product To Prevent Chronic Mg Deficiency in Rats

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The ability of a cocoa-derived product, used in some European countries as a dietary supplement added to milk, to prevent chronic Mg deficiency was investigated. Wistar rats were fed an Mg-deficient diet supplemented with 3% (w:w) cocoa product (271 mg of Mg^{2+}/kg of food). The concentration of Mg^{2+}, Ca^{2+}, and PO_4^{3-} in plasma, whole blood, skeletal muscle, heart, kidney, and femur was measured after the rats had been fed the diet for 35, 42, 49, and 56 days. These values were compared with the results from rats fed a diet that was deficient in Mg (225 mg/kg of food) and in rats fed a control diet (557 mg of Mg^{2+}/kg of food). On day 56, dietary supplementation with the cocoa product had significantly reduced hypomagnesemia and hypercalcemia and had prevented the appearance of hypophosphatemia caused by Mg deficiency. The cocoa-supplemented diet also substantially reduced the decreases in Mg in muscle, heart, and bone caused by Mg deficiency. Ca deposits in skeletal muscle and kidney were reduced, as were P deposits in kidney. Mobilization of P from bone was also prevented. These findings show that the regular use of the cocoa product as a dietary supplement effectively prevented the negative nutritional effects of the long-term intake of a diet moderately deficient in Mg.

Keywords: *Cocoa; Mg deficiency; prevention*

INTRODUCTION

Magnesium deficiency is known to be associated with cardiovascular alterations and many renal, gastrointestinal, neurological, and muscular disorders (Seelig, 1986; Shils, 1988). The symptoms and signs of Mg deficiency have been traced to complex electrolytic alterations secondary to deficiency.

Epidemiological studies (Wester, 1987) have reported that the amount of Mg consumed by many persons is below the recommended intake. Most individuals obtain from 20−30% less than the recommended dietary allowances (RDA) during prolonged periods (Wester, 1987).

Low intakes of Mg for long periods may be responsible for the appearance of symptoms that have thus far remained unexplained. Examples include (1) the relationship between low erythrocyte concentrations of Mg and sleep alterations (Depoortere et al., 1993), (2) chronic fatigue syndrome (Cox et al., 1991), (3) dementia of the Alzheimer type (Lemke, 1995), (4) depression (Widmer et al., 1995), and (5) increased levels of free radicals (Günther, 1991).

Mg deficiency is also known to alter phosphorus and calcium metabolism (Shils, 1988; Lemay and Gasçon-Barre, 1992). Earlier work in our laboratory showed that a low dietary supply of Mg increased Ca absorption and balance, a change that was accompanied by tissue calcification (particularly in the kidney) and increased phosphaturia, which led to negative phosphorus balances and decreased concentrations of this ion in bone and heart (Planells et al., 1993, 1995).

Because of these findings we investigated a product derived from cocoa, an Mg-rich food, to determine whether it was able to prevent Mg deficiency. Cocoa products are currently widely consumed as dietary supplements in Western countries. The product tested here is used in Spain and other European countries mixed with milk.

We designed experiments in the rat to determine the ability of this product to protect the organism from possible chronic Mg deficiency. The cocoa product was added to a diet moderately deficient in Mg (satisfying 50% of this species' Mg requirements) at a proportion similar to that normally used for human consumption (3%). The changes in the distribution of Mg in different tissues were analyzed, as were the Ca and P distributions, since these ions are also affected by Mg deficiency. A long (7-week) time-frame was used so that our experimental model would more nearly approximate the situation described in Western populations.

MATERIALS AND METHODS

Animals and Diets. Male Wistar rats used in this study weighed an average of approximately 180 g (7 weeks old approximately) at the start of the experiment. They were given distilled water and a semisynthetic diet during 8 weeks. The control diet contained (g/kg) protein (casein) (Musal & Chemical, Granada, Spain), 200; DL-methionine (Roche SA, Madrid), 5; sucrose (Musal & Chemical), 310; wheat starch (Musal & Chemical), 310; fiber (cellulose) (Musal & Chemical), 80; olive oil, 5; AIN-76 mineral mix (National Research Council, 1979), 35; AIN-76 vitamin mix (National Research Council, 1979) and 10; choline bitartrate (Merck), 2. In all, these components provide 557 mg of Mg^{2+}, 5274 mg of Ca^{2+}, and 4410 mg of PO_4^{3-} per kilogram of feed.

The animals were divided into three groups, each of which received a different amount of dietary Mg. Rats in the Mg-

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deficient (D) group received the same diet except that the amount of Mg was 225 mg/kg of food. In the cocoa-supplement group (D+CC), the diet was the same as in group D, except that it was supplemented with 3% (w:w) cocoa product. This diet contained 271 mg of Mg^{2+}, 5274 mg of Ca^{2+}, and 4670 mg of PO_4^{3-} per kilogram feed. The analytical composition of the cocoa product (per 100 g of food) was as follows: protein, 6.5 g; carbohydrates, 78 g; fat, 2.4 g; and ash, 2.2 g. The Mg, Ca, and P contents were 1268 mg of Mg^{2+}, 5718 mg of Ca^{2+}, and 4871 mg of PO_4^{3-} per kilogram feed.

Throughout the experimental period all animals were housed in individual metabolic cages in a well-ventilated, thermoregulated room (21 ± 2 °C, with a 12-h light−dark period.

Ten rats from each group were killed on days 35, 43, 49, and 56 of the experiment, and samples of blood, plasma, Longissimus dorsi muscle, heart, kidney, and femur were taken for analysis.

Analytical Techniques. Mg and Ca in the diet, whole blood, muscle, heart, kidney, and femur were determined by atomic absorption spectrophotometry (AAS) (Perkin-Elmer 1100 B apparatus) of samples previously ashed at 450 °C and extracted with a 6 N solution of HCl and lanthanum chloride (1.0−0.1%) (Merck). Plasma Mg and Ca were also determined by AAS in samples that were not previously ashed.

Phosphorus was measured according to the colorimetric method of Fiske and Subbarow (1925) in ashed samples dissolved in 6 N HCl (Merck), except for plasma, which was tested without prior treatment.

The quality control measures used were as follows: Precinorm U (ref 180 509) (Boehringer Mannheim, Spain) for Mg and Ca and Seniscann N (ref 994148) (Química Clínica Aplicada S.A., Spain) for P.

Statistical Analysis. The data were subjected to analysis of variance with the one-way procedure of the SPSS/PC software package. Means were compared with Duncan's test for all three means. Differences between the means were considered significant at the 1% level.

RESULTS

Plasma (Figure 1). At all four time points when data were obtained, plasma levels of Mg were significantly higher in control animals than in rats fed the deficient (D) or supplemented diet (D+CC). However, plasma Mg levels decreased by only 16.9% in group D+CC, whereas in the deficient group (D) the decrease was 29.4% of the control value (day 56).

The cocoa-supplemented diet also prevented the large increase in calcemia caused by the Mg-deficient diet. However, calcemia in rats fed the supplemented diet was significantly higher than that found in control animals on days 42 and 49.

Rats given diet D+CC had plasma phosphate levels similar to those in control animals throughout the experimental period. In both of these groups, phosphatemia was significantly higher than in the Mg-deficient group on days 49 and 56.

Whole Blood (Figure 2). In whole blood we found changes similar to those in plasma. Rats fed with the cocoa-supplemented diet (D+CC) had significantly higher levels of Mg in whole blood than did Mg-deficient animals (D) at all time points studied.

Although Ca in whole blood was significantly lower in control rats than in group D+CC or group D at all four time points, the 12% increase on day 56 in group D+CC was much smaller than the corresponding 84.7% increase in group D. Phosphorus levels in whole blood decreased by only 11% in group D+CC, whereas in group D the decrease was 30% of the control value (day 56).

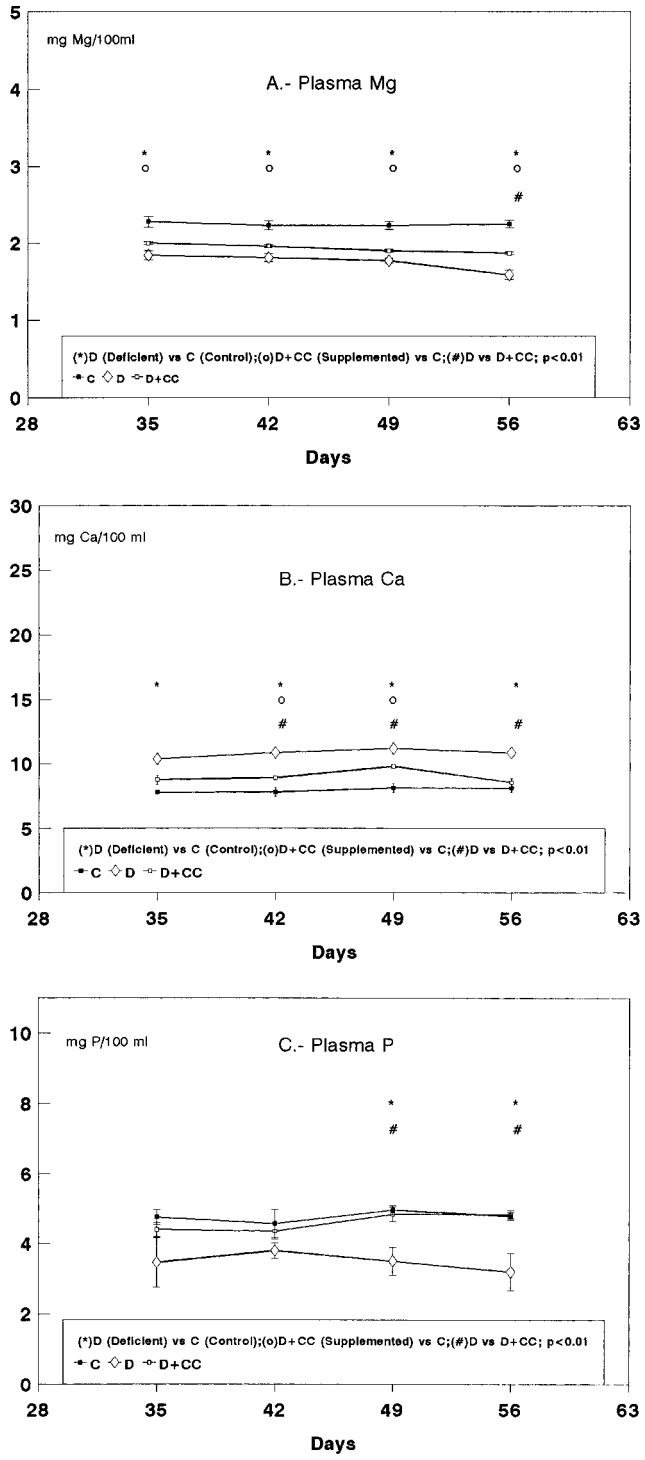

Figure 1. Mg (A), Ca (B), and P (C) content in plasma.

Muscle (Longissimus dorsi) (Figure 3). The level of Mg in skeletal muscle of rats given the cocoa-supplemented (D+CC) diet was similar to that in control animals; the only finding of note was an 8% decrease on day 56. However, in rats fed the Mg-deficient diet, Mg levels in skeletal muscle were consistently much lower: 27% below the control value on day 35 and 37% below the control value on day 56.

The findings for Ca concentrations were similar: in group D+CC, the differences in comparison with controls were not significant, whereas in group D the difference became significant on day 35 and increased steadily with time (Figure 3). The levels of P in skeletal

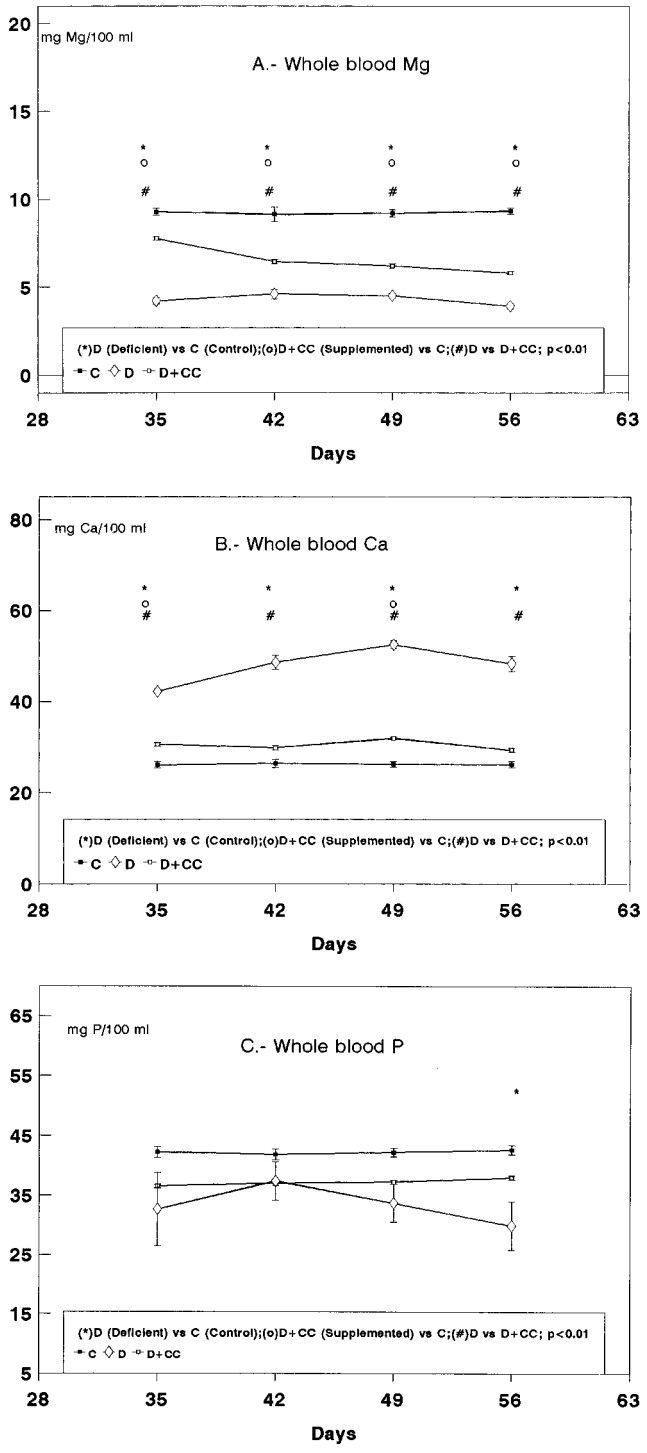

Figure 2. Mg (A), Ca (B), and P (C) content in total blood.

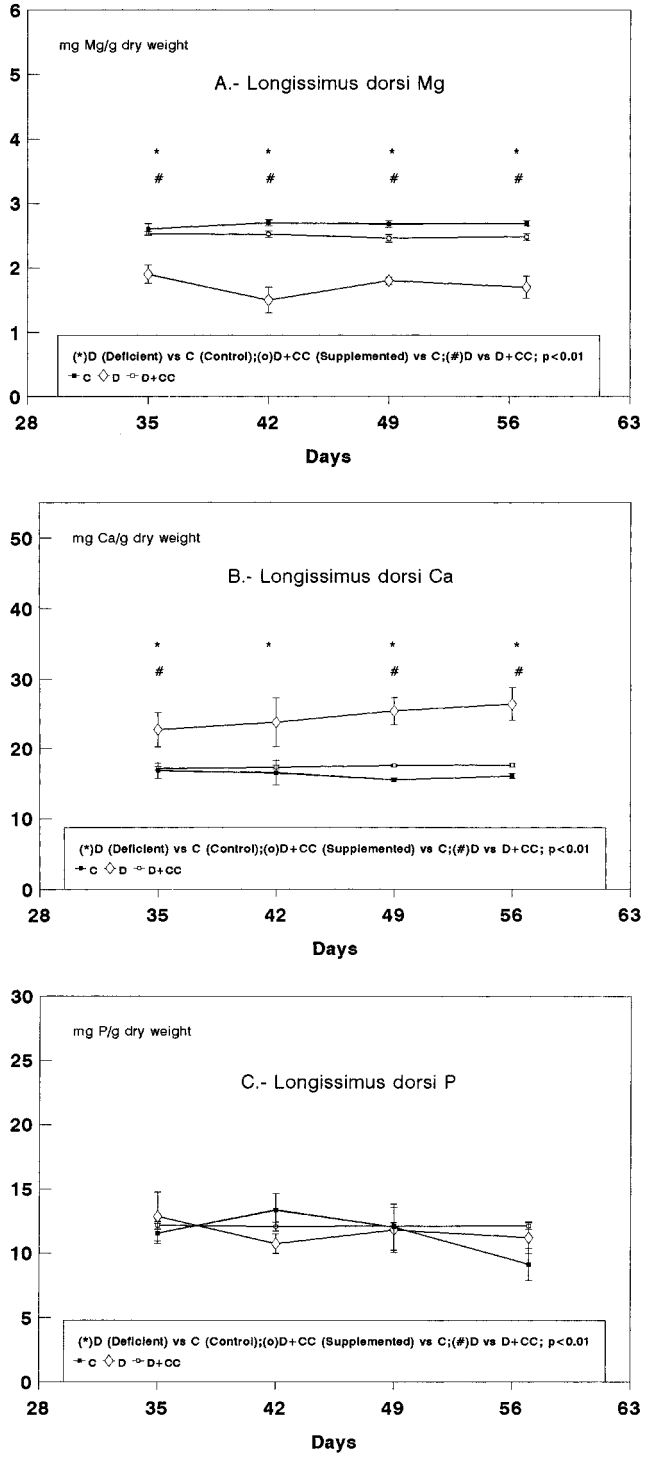

Figure 3. Mg (A), Ca (B), and P (C) content in muscle Longissimus dorsi.

muscle in group D+CC and in Mg-deficient animals (group D) were not significantly different from control values.

Heart (Figure 4). Rats fed the Mg-deficient diet showed a significant decrease in Mg levels in heart on day 56. Dietary supplementation with the cocoa product prevented the significant decline in Mg levels in this organ, in comparison with control values.

Neither Mg deficiency nor dietary supplementation with the cocoa product significantly affected heart levels of Ca during the experiment. In contrast, rats fed with the Mg-deficent diet showed a significant decrease in heart phosphorus levels from day 49 until the end of the experiment. Dietary supplementation with cocoa product also prevented the significant decline in phosphorus concentration in cardiac muscle seen in control rats.

Kidney (Figure 5). In kidney, slight but significant differences in Mg levels were found between groups D and D+CC. In Mg-deficient rats, Mg content in kidney decreased during the experimental period, especially from day 42 onward. Rats fed the Mg-deficient diet had levels similar to those in control animals on days 49 and 56, whereas in animals given the cocoa-supplemented diet, Mg levels were slightly but significantly higher than in the control and deficient groups.

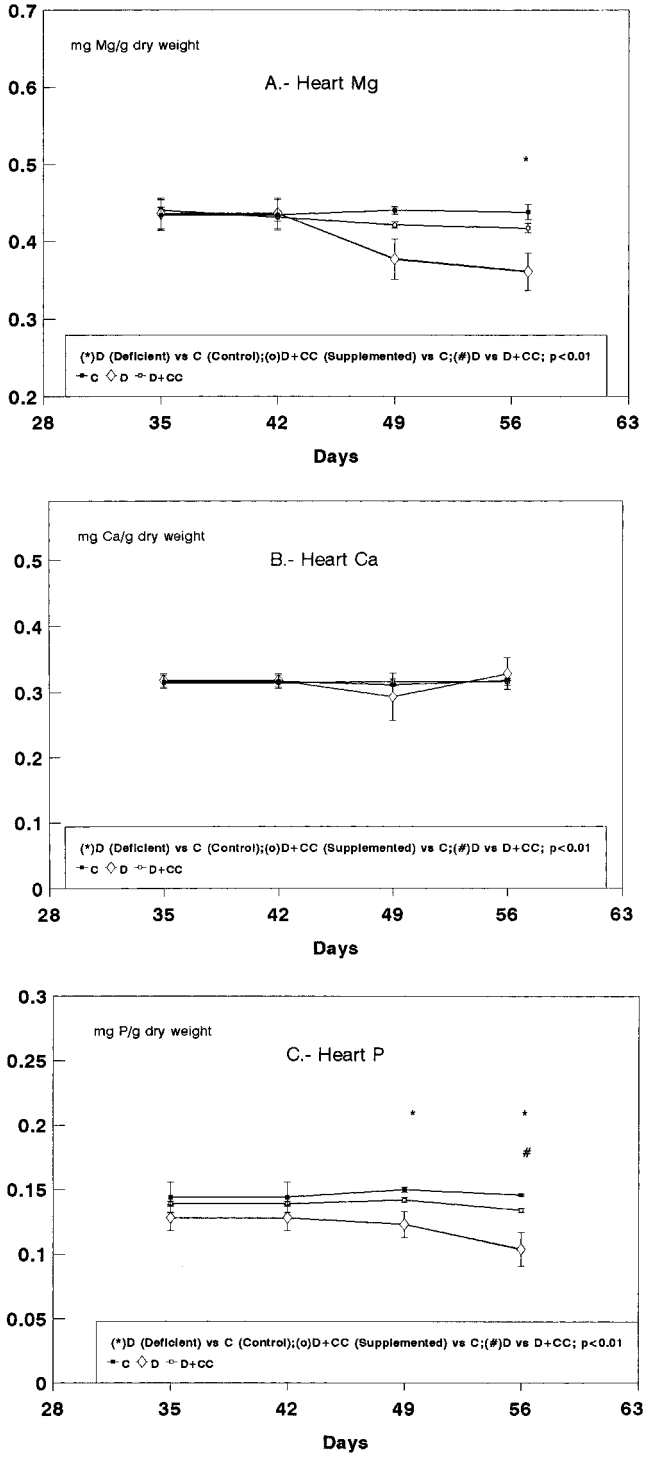

Figure 4. Mg (A), Ca (B), and P (C) content in heart.

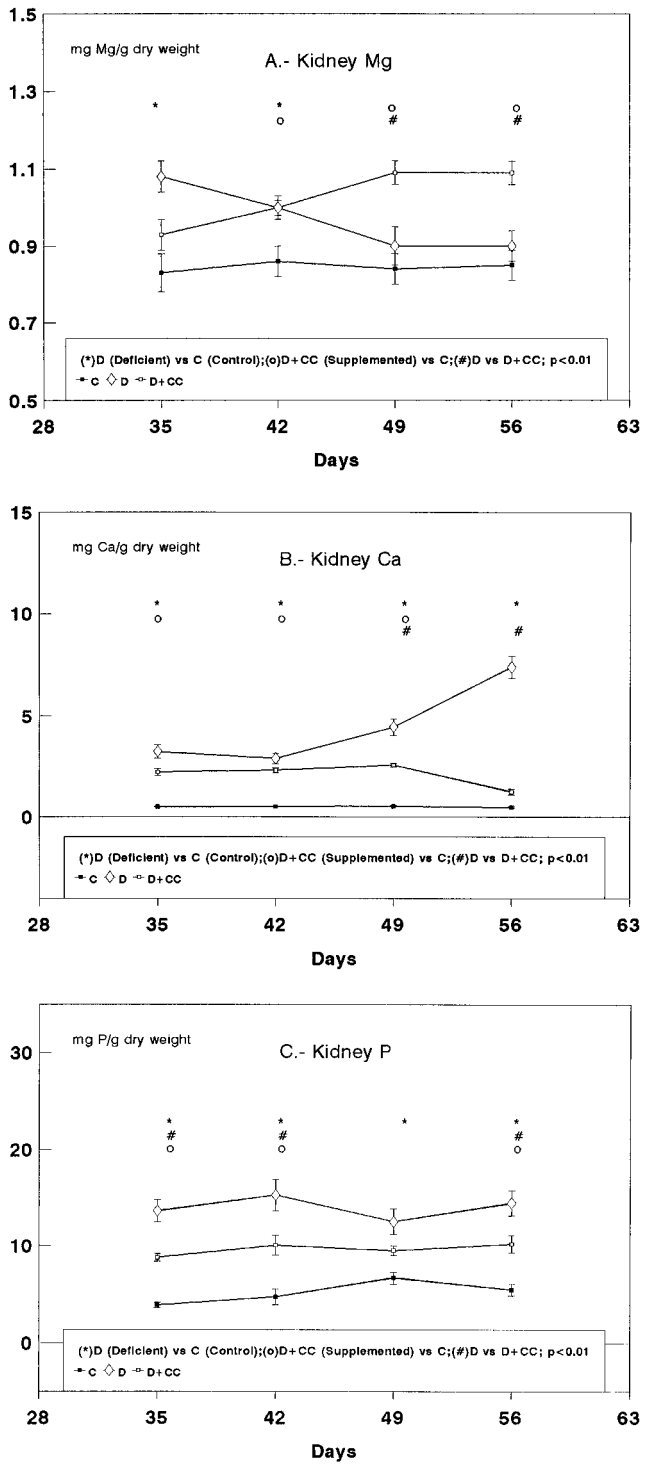

Figure 5. Mg, (A) Ca (B), and P (C) content in kidney.

In group D+CC, Ca levels also increased significantly. However, in this group we did not find the large increase in kidney Ca levels that was seen in Mg-deficient rats on days 49 and 56. In contrast with the findings in deficient rats (D), we found that in rats fed the cocoa-supplemented diet, the Ca deposits accumulated in response to the Mg deficiency tended to decrease. By day 56, renal Ca concentration had increased only 2.5-fold (not significant) in group D+CC animals, whereas Ca concentration in group D was 15-fold higher than the control value. The changes in kidney P levels were similar to those for Ca, although the difference between group D+CC and group D was evident as early as day 35. In group D, renal phosphorus concentration increased 2.6-fold, whereas in group D+CC this increase was only 1.8-fold at the end of the experiment (day 56) (Figure 5).

Bone (Femur) (Figure 6). The Mg-deficient diet significantly decreased Mg concentration in the femur as early as day 42, whereas in rats fed the cocoa-supplemented diet, although bone Mg levels tended to decrease, the differences, in comparison with the control group, did not reach significance. The cocoa product prevented the slight calcification of the femur due to Mg deficiency, but bone Ca levels were not significantly different from control values from day 49 onward. However, feeding the cocoa-supplemented diet pre-

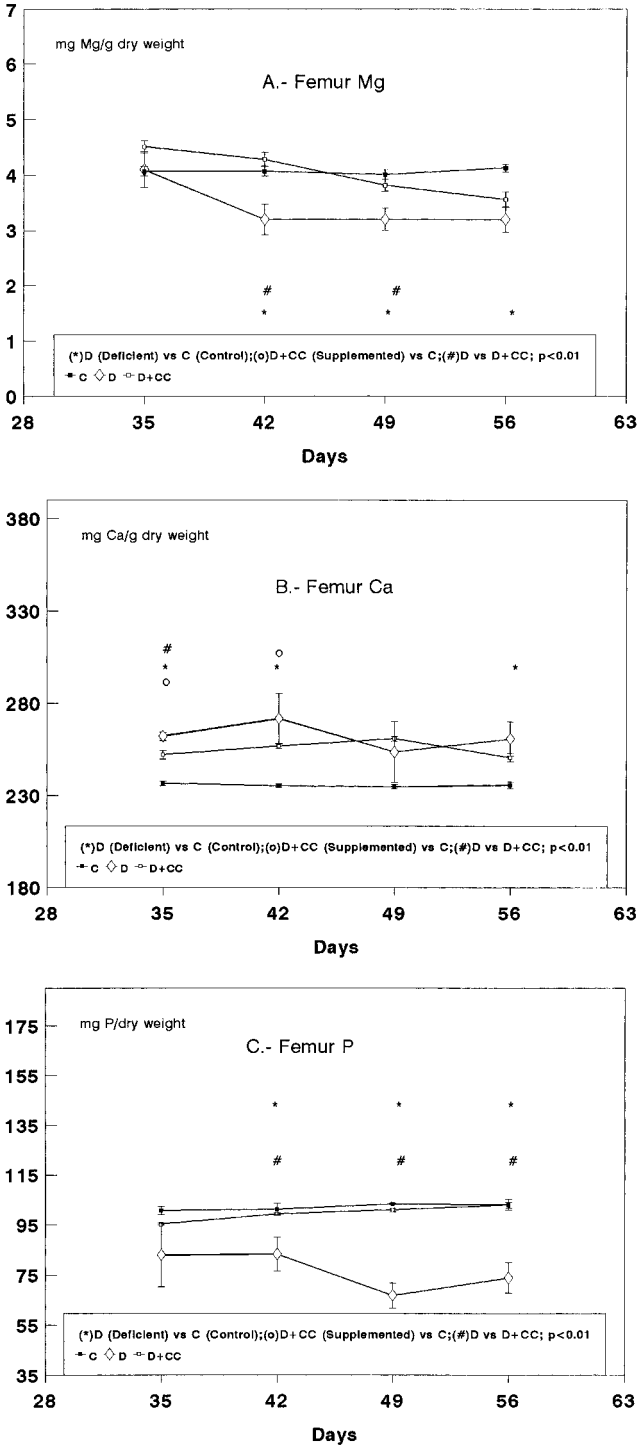

Figure 6. Mg (A), Ca (B), and P (C) content in femur.

vented the significant P losses seen in Mg-deficient animals (Figure 6).

DISCUSSION

The decline in Mg concentrations in plasma, blood, muscle, heart, kidney, and bone in rats fed the Mg-deficient diet are similar to the decreases found in earlier studies (Lerma et al., 1993; Planells et al., 1995). These decreases in tissue Mg levels were accompanied by an increase in Ca levels in muscle, kidney, femur, plasma, and erythrocytes (the changes in plasma levels of the ion do not completely account for the differences found in whole blood). Another effect of Mg deficiency was a decrease in P concentrations in plasma, heart, and femur, and an increase in kidney P.

The changes in plasma (Figure 1) and muscle (Figure 3) Ca may result from increased absorption and retention in response to Mg deficiency (Planells et al., 1993), whereas the changes in erythrocyte levels (Figure 2) may be related to alterations in membrane erythrocyte transport systems (Agus and Morad, 1991) and changes in the lipid composition of the erythrocyte membrane (Lerma et al., 1993; Tongyai et al., 1989).

The decrease in plasma P (Figure 1) resulted from hyperphosphaturia caused by Mg deficiency (Planells et al., 1993), together with the formation of insoluble phosphates in the kidney (Figure 5) (Planells et al., 1995).

Long-term Mg deficiency leads to losses of P and to calcification of the myocardium (Planells et al., 1995), increasing the vulnerability of myocytes to subsequent acute myocardial infarction (Lockards and Blooms, 1991). In the present experiment we found no signs of calcification in heart muscle (Figure 4), perhaps because the experimental period was too short for this to develop. The different results in skeletal and cardiac muscle may have been due to different sensitivities to Mg deficiency (Altura et al., 1993).

In the kidney (Figure 5), Mg deficiency leads to the formation of Ca phosphate and Ca oxalate deposits (Bruce and King, 1989). The changes in femur (Figure 6) Ca and P content probably reflect alterations in the exchange of Ca and P together with modifications in osteoblast, ostocyte, and osteoclast function (Weawer and Welsh, 1993), rather than mobilizations due to hypercalcemia or hypophosphatemia.

The results reported here indicate that the addition of 3% (w:w) cocoa product to the diet was able to palliate the effects of prolonged intake of an Mg-deficient diet. The regular use of this or similar products may therefore help lower the nutritional risk associated with inadequate Mg intake and the high incidence of hypomagnesemia in Western populations (Schimatschek and Classen, 1992; Zirm et al., 1995; Laserre et al., 1995).

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