

## Beneficial Effect of Goat Milk on Nutritive Utilization of Iron and Copper in Malabsorption Syndrome

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### ABSTRACT

The search for diets that improve the digestive and metabolic use of iron and copper in malabsorption syndrome led us to study goat milk for particular nutritional characteristics and compare it with cow milk, which is usually supplied. We studied the metabolism of iron and copper in transected rats (control) and in resected rats (resection of 50% of the distal small intestine). The diets used were the standard diet recommended by the American Institute of Nutrition and diets based on goat or cow milk. Intestinal resection reduced the apparent digestibility coefficient (ADC) of iron in the three diets tested. In the transected and resected rats, the ADC of iron was highest with the goat milk diet, followed by the standard diet and lowest with the cow milk diet. The ADC of copper was not affected by intestinal resection in the animals fed the goat milk diet, and was higher than that in the two groups of animals fed the other diets. Intestinal resection reduced the ADC of copper with the standard diet and the cow milk diet. When both groups of animals were fed the goat milk diet, the deposit of iron in the organs was greater than with those fed the cow milk diet and similar to that in those animals given the standard diet. The copper content in the kidneys was lower in the resected than in the transected animals, except in the case of those fed the goat milk diet, in which it was similar to that of the control (transected) rats. This study shows the beneficial effect of goat milk, with respect to cow milk, on the metabolism of iron and copper in control rats, especially those with malabsorption syndrome.

(**Key words:** goat milk, cow milk, iron malabsorption, copper malabsorption)

**Abbreviation key:** ADC = apparent digestibility coefficient, DSI = distal small intestine, MCT = medium-chain triglycerides.

### INTRODUCTION

Iron is an element of fundamental biological importance that participates in the transport (hemoglobin) and storage (myoglobin) of oxygen and in oxidative metabolism (cytochromes) (Linder, 1996).

Copper, on the other hand, is essential to mobilize the iron in the synthesis of hemoglobin. Thus, ceruloplasmin, a plasma enzyme that contains copper, catalyzes the oxidation of the ferrous ion into the ferric ion, thus ensuring that the iron is taken up by the transferrin and transported to the tissues for the synthesis of iron-containing compounds, chief among which is hemoglobin (Anderson et al., 1985).

The lack of either or both iron and copper leads to the appearance of anemia (Anderson et al., 1985; Czajka-Narins, 1988). One of the causes of the deficiency of these two elements is the resection of 50% of the distal small intestine (**DSI**), that is, in cases of malabsorption syndrome, which produces a significant decrease in the digestive and metabolic use of iron (Hartiti et al., 1995a) and copper (Hartiti et al., 1995b).

In recent studies we have found that when lyophilized goat milk is incorporated into the diet of rats for 37 d, it produces a greater nutritive use of iron in relation to animals fed with cow milk (López Aliaga et al., 2000).

Therefore, our objective was to determine the effects of the particular nutritional characteristics of goat milk on digestive and metabolic use and on the distribution of iron and copper in various organs of transected and

Received March 28, 2001.

Accepted October 5, 2001.

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resected (50% of DSI) rats. These effects were compared with those of a cow milk-based diet and the standard diet recommended by the American Institute of Nutrition (1977), to improve the nutritive use of these minerals in malabsorption syndrome.

## MATERIALS AND METHODS

### Animals

We studied a total of 69 male albino Wistar rats with an initial body weight of  $177 \pm 3$  g (6 to 7 wk old), obtained from the University of Granada Laboratory Animal Service. All experiments and surgical procedures using rats conformed to the guidelines and legal requirements established in the United States for the proper care and use of laboratory animals. After surgery both the transected and resected animals were housed in individual ventilated thermoregulated cages ( $22 \pm 2^\circ\text{C}$ ) with a 12 h:12 h light/dark period. Food and distilled water were available ad libitum to all rats. At the start of the experimental period, the rats were 10 to 11 wk old.

### Diets

The diets and mineral and vitamin supplements were prepared according to the recommendations of the AIN (1977) except that the level of fat in the diets was 10% rather than 5%. The standard diet (Diet S; Table 1) was prepared by using olive oil as the source of fat (10%) and casein as the protein source (20%). The milk-based diets were created with lyophilized cow or goat milk (Diet C and Diet G, respectively; Table 1). These were analyzed to determine the fat content (cow milk, 35.23%; goat milk, 43.63%), protein content (cow milk, 23.92%; goat milk, 25.27%), lactose content (cow milk, 37.55%; goat milk, 31.10%), and mineral composition (mg/100 g of lyophylate) (cow milk: Ca, 1031.5; P, 731.3; Mg, 76.3; Fe, 0.61; Cu, 0.11; and Zn, 3.72; goat milk: Ca, 1215.2; P, 843.3; Mg, 82.5; Fe, 1.13; Cu, 0.42; and Zn, 4.15). The quantities of lyophilized goat or cow milk used were those that were necessary to obtain a diet with a 10% fat content. To obtain the 20% protein content (as recommended by the AIN, 1977), the diet was supplemented with casein (12.53 g of casein/100 g diet of cow milk and 14.05 g of casein/100 g diet of goat milk), because the protein provided by the lyophylate used for the milk-based diets was insufficient.

The mineral corrector was prepared according to recommendations by AIN (1977) for the standard diet and to our own specifications for the milk-based diets. These following specific correctors were formulated by considering the mineral content of the lyophilized milks supplied to the rats, to meet the mineral-content recom-

**Table 1.** Composition of the experimental diets.

Component	g/kg diet (dry weight)
Diet S <sup>1</sup>	
Protein (casein)	209
DL-Methionine	3
Fat (olive oil)	112
Fiber (micronized cellulose)	50
Mineral supplement <sup>2</sup>	36
Vitamin supplement <sup>2</sup>	10
Choline chloride	2
Wheat starch	156
Sucrose	450
Diet C <sup>1</sup>	
Protein (casein + protein cow milk)	190
DL-Methionine	3
Fat (cow milk) (MCT <sup>3</sup> , 20.6 g)	98
Fiber (micronized cellulose)	40
Mineral supplement <sup>2</sup>	35
Vitamin supplement <sup>2</sup>	10
Choline chloride	2
Wheat starch	121
Sucrose	387
Lactose (cow milk)	131
Diet G <sup>1</sup>	
Protein (casein + protein goat milk)	194
DL-Methionine	3
Fat (goat milk) (MCT <sup>3</sup> , 33.2 g)	92
Fiber (micronized cellulose)	46
Mineral supplement <sup>2</sup>	36
Vitamin supplement <sup>2</sup>	10
Choline chloride	2
Wheat starch	148
Sucrose	405
Lactose (goat milk)	85

<sup>1</sup>Standard diet (Diet S), lyophilized cow and goat milk-based diets (Diet C and Diet G, respectively).

<sup>2</sup>Mineral and vitamin supplements were prepared according to the recommendations of the American Institute of Nutrition (1977).

<sup>3</sup>Medium-chain triglycerides.

mendations of the AIN (1977): 35 mg of Fe/kg of diet; and 5 mg of Cu/kg of diet. The iron and copper contents in the diets (mg/kg diet) after analysis were as follows: standard diet: Fe, 31.15; Cu, 5.19; cow milk diet: Fe, 30.02; Cu, 5.19; and goat milk diet: Fe, 30.03; Cu, 5.33. The iron was added as ferric citrate and the copper as cupric carbonate.

The lactose content of the milk diets was subtracted from the total carbohydrate content of the standard diet, and wheat starch and sucrose were added corresponding to the difference (Table 1).

### Resection and Transection Procedures

The method described by Hartiti et al. (1994) was used to perform the resection of 50% of the DSI and the transection groups.

### Experimental Design

The following six experimental groups were formed: 1) group T-S: transected rats, standard diet (n = 11); 2)

group R-S: resected rats, standard diet (n = 13); 3) group T-C: transected rats, cow milk diet (n = 10); 4) group R-C: resected rats, cow milk diet (n = 11); 5) group T-G: transected rats, goat milk diet (n = 14); and 6) group R-G: resected rats, goat milk diet (n = 10). All animals were fed up to the time of surgery and were given access to water containing 50 g of glucose/L for 24 h after surgery. Thereafter, a period of 30 d was allowed for adaptation to the diet, during which feed and double-distilled water were available ad libitum to all animals. Beginning 30 d after surgery, feed intake (the amount of food consumed daily by each rat, determined by weighing the amounts of diet given, refused, and spilled) was measured and urine and feces were collected for a period of 7 d (Thomas and Mitchell, 1927). Body weight was recorded at the beginning and end of the experimental period. Throughout the experimental period all rats had access to double-distilled water. At the end of this period the rats were fasted for 24 h and killed after intraperitoneal anesthesia with sodium pentobarbital (5 mg/100 g of body weight) and totally bled by cannulation of the abdominal aorta. Kidney, liver, heart, sternum, both femurs, and spleen were removed and frozen at  $-80^{\circ}\text{C}$  in liquid nitrogen, then stored at  $-40^{\circ}\text{C}$  for iron and copper analysis.

### Biological Indices

The apparent digestibility coefficient (ADC) and balance (R retention) were calculated as follows:  $\text{ADC} = (I - F) \times 100/I$ , and  $\text{Balance (R retention)} = I - (F + U)$ , with  $R/I = \text{retention/intake} \times 100$ ; where  $I = \text{intake}$ ,  $F = \text{fecal excretion}$ , and  $U = \text{urinary excretion}$ .

### Analytical Methods

Water contents of diet, feces, kidney, liver, heart, sternum, femur, and spleen were determined by drying the materials at  $105 \pm 2^{\circ}\text{C}$  until the weight remained constant. An appropriate amount of the resulting material was ashed at  $450^{\circ}\text{C}$ , and the residue was extracted with 5 M HCl and brought up to an appropriate volume with double-distilled water. Atomic absorption spectrophotometry (Perkin-Elmer 1100B, Shelton, CT) was used for iron and copper determination.

### Quality Control

Given the importance of accurate determination of the various parameters studied, the measurement of these was subjected to a quality control procedure. This consisted of analyzing a lyophilized bovine liver (certified reference material BCR 185; Community Bureau of References, Brussels, Belgium), which yielded an iron

value of  $209 \pm 7 \mu\text{g/g}$  and a copper value of  $181 \pm 5 \mu\text{g/g}$  (mean  $\pm$  SEM values of five determinations) (certified value: Fe,  $214 \pm 5 \mu\text{g/g}$ ; and Cu,  $189 \pm 4 \mu\text{g/g}$ ).

### Statistical Analysis

We calculated the mean value and the standard error of the mean value for each parameter studied. Variance analysis (the one-way method of the SPSSPC) (SPSS, 2001) and the Bonferroni post hoc test were used to compare the different diets supplied to the two groups of animals (transected and resected rats). To compare the two groups given the same diet, we used a Student's *t*-test for independent samples (the SPSSPC *t*-test procedure). Values of  $P < 0.05$  were considered significant.

## RESULTS

### ADC and Balance of Iron

The digestive use of iron was greater ( $P < 0.001$ ) for the control rats (transected) than for those subjected to intestinal resection, irrespective of the type of diet. Comparison of the effects of the three diets revealed that both among the transected and the resected rats, the ADC of iron is greater ( $P < 0.05$ ) when the animals are given the standard diet, rather than the cow milk diet. The animals fed with the goat milk diet presented a higher ( $P < 0.025$ ) level of iron digestibility than those given the other two diets (standard diet and cow milk diet), among both the control (transected) animals and the resected rats (ADC of iron:  $G > S > C$ ) (Table 2).

With respect to the iron balance, we found that higher ( $P < 0.001$ ) levels of retention were obtained for transected animals than resected ones, for all the diets studied. Both in the control (transected) rats and in the resected rats, iron retention was lower among the animals fed with the cow milk diet than among those receiving the other two diets (standard diet:  $P < 0.001$ , transected rats, and  $P < 0.025$ , resected rats; goat milk diet:  $P < 0.025$ , transected and resected rats). These latter two did not differ significantly (balance of iron:  $S = G > C$ ). (Table 2).

### ADC and Balance of Copper

The ADC of copper was affected by intestinal resection for the standard diet and the cow milk-based diet, and was higher ( $P < 0.001$ ) for the control (transected) animals.

Nevertheless, the animals with intestinal resection given the goat milk diet presented levels of digestive use of copper of an order similar to those of the control (transected) animals. Comparison of the different diets shows that for the transected animals given the goat

**Table 2.** Digestive and metabolic use of iron in transected and resected rats.<sup>1</sup>

Group	n	Fe intake ( $\mu\text{g}/\text{rat}/\text{day}$ )	Fecal Fe ( $\mu\text{g}/\text{rat}/\text{day}$ )	Absorbed Fe ( $\mu\text{g}/\text{rat}/\text{day}$ )	ADC, <sup>2</sup> (%)	Urinary Fe ( $\mu\text{g}/\text{rat}/\text{day}$ )	Fe balance ( $\mu\text{g}/\text{rat}/\text{day}$ )	R/I <sup>3</sup> (%)
T - S <sup>4</sup>	11	579.9 $\pm$ 18.5 <sup>a,b</sup>	451.2 $\pm$ 18.8 <sup>b,g</sup>	128.1 $\pm$ 5.6 <sup>g</sup>	22.1 $\pm$ 1.1 <sup>a,b,g</sup>	2.8 $\pm$ 0.2 <sup>a,b,g</sup>	125.8 $\pm$ 5.6 <sup>a,g</sup>	21.6 $\pm$ 1.1 <sup>a,b,g</sup>
R - S <sup>4</sup>	13	597.5 $\pm$ 20.1 <sup>c,d</sup>	522.8 $\pm$ 17.4 <sup>c,d</sup>	74.7 $\pm$ 7.1	12.5 $\pm$ 1.1 <sup>c,d</sup>	4.1 $\pm$ 0.6 <sup>c,d</sup>	70.6 $\pm$ 7.2 <sup>c</sup>	11.8 $\pm$ 1.1 <sup>c</sup>
T - C <sup>4</sup>	10	621.6 $\pm$ 15.0 <sup>e,h</sup>	509.1 $\pm$ 17.7 <sup>e,h</sup>	112.5 $\pm$ 8.0 <sup>h</sup>	18.1 $\pm$ 1.1 <sup>e,h</sup>	7.8 $\pm$ 0.6 <sup>e,h</sup>	104.7 $\pm$ 7.8 <sup>e,h</sup>	16.8 $\pm$ 1.1 <sup>e,h</sup>
R - C <sup>4</sup>	11	665.1 $\pm$ 17.4	611.9 $\pm$ 18.4 <sup>f</sup>	61.2 $\pm$ 3.7 <sup>f</sup>	9.2 $\pm$ 0.7 <sup>f</sup>	13.8 $\pm$ 1.0 <sup>d</sup>	47.4 $\pm$ 3.5 <sup>f</sup>	7.1 $\pm$ 0.6 <sup>f</sup>
T - G <sup>4</sup>	14	487.7 $\pm$ 10.1	357.1 $\pm$ 9.2 <sup>i</sup>	130.6 $\pm$ 9.3 <sup>i</sup>	26.6 $\pm$ 1.7 <sup>i</sup>	3.5 $\pm$ 0.4 <sup>i</sup>	127.1 $\pm$ 9.4 <sup>i</sup>	26.1 $\pm$ 1.7 <sup>i</sup>
R - G <sup>4</sup>	10	505.9 $\pm$ 18.5	425.3 $\pm$ 18.5	80.6 $\pm$ 5.7	16.1 $\pm$ 1.2	7.0 $\pm$ 0.7	73.6 $\pm$ 5.5	14.6 $\pm$ 1.2

<sup>1</sup>All data are expressed as mean  $\pm$  SEM values. a = Significant difference between T - S and T - C; b = significant difference between T - S and T - G; c = significant difference between R - S and R - C; d = significant difference between R - S and R - G; e = significant difference between T - C and T - G; f = significant difference between R - C and R - G; g = significant difference between T - S and R - S; h = significant difference between T - C and R - C; i = significant difference between T - G and R - G.

<sup>2</sup>Apparent digestibility coefficient.

<sup>3</sup>Retention/intake.

<sup>4</sup>T = Transected, R = resected, S = Diet S, C = Diet C, G = Diet G.

milk diet, the ADC of copper is similar to that obtained with the standard diet and higher ( $P < 0.001$ ) than that of the animals fed with cow milk (ADC of copper: G = S > C). Among the resected animals, the digestive use of copper was highest ( $P < 0.001$ , G-C and S-C; and  $P < 0.01$ , S-G) with the goat milk diet, followed by the standard diet, and lowest with the cow milk diet (ADC of copper: G > S > C) (Table 3).

With respect to the copper balance, no differences were found between the transected and resected animals with the standard and goat milk diets, but the levels of retained copper were lower ( $P < 0.005$ ) among the resected animals that were fed cow milk than among the equivalent transected animals. Moreover, comparison of the different diets revealed that the copper balance is similar for the control (transected) rats and the resected animals fed with the standard diet and the goat milk diet, and greater than that of the rats consuming cow milk; and was significant for the resected rats ( $P < 0.001$ ), whereas in the transected (control) rats the copper balance was higher among the

rats given the standard diet ( $P < 0.025$ ) than among those given cow milk (balance of Cu : S = G > C) (Table 3).

### Iron and Copper Content in Organs

The two groups of animals fed with the standard diet (transected and resected) presented no differences with respect to the iron content of the different organs studied. Concerning the rats given milk-based diets, no differences were found in the organs that are representative of the metabolic use of the mineral (the liver, spleen, and sternum) between the control and the resected animals (Table 4).

Comparison of the three diets shows that among both the transected and the resected animals given cow milk, in general, the iron content is lower in the organs studied, particularly the liver ( $P < 0.001$ ) and the spleen ( $P < 0.001$ ), with respect to the standard and the goat milk diets (Table 4).

**Table 3.** Digestive and metabolic use of copper in transected and resected rats.<sup>1</sup>

Group	n	Cu intake ( $\mu\text{g}/\text{rat}/\text{day}$ )	Fecal Cu ( $\mu\text{g}/\text{rat}/\text{day}$ )	Absorbed Cu ( $\mu\text{g}/\text{rat}/\text{day}$ )	ADC <sup>2</sup> (%)	Urinary Cu ( $\mu\text{g}/\text{rat}/\text{day}$ )	Cu balance ( $\mu\text{g}/\text{rat}/\text{day}$ )	R/I <sup>3</sup> (%)
T - S <sup>4</sup>	11	96.6 $\pm$ 3.1 <sup>a,b</sup>	54.9 $\pm$ 2.8 <sup>a,b,g</sup>	41.7 $\pm$ 0.8 <sup>a</sup>	43.4 $\pm$ 1.2 <sup>a,g</sup>	6.0 $\pm$ 0.3	35.7 $\pm$ 0.9 <sup>a</sup>	37.2 $\pm$ 1.2 <sup>a,g</sup>
R - S <sup>4</sup>	13	99.5 $\pm$ 3.3 <sup>c,d</sup>	63.3 $\pm$ 1.5 <sup>c,d</sup>	36.2 $\pm$ 2.7 <sup>c</sup>	36.4 $\pm$ 1.6 <sup>c,d</sup>	5.8 $\pm$ 0.2 <sup>c,d</sup>	30.6 $\pm$ 2.7 <sup>c</sup>	30.8 $\pm$ 1.7 <sup>c,d</sup>
T - C <sup>4</sup>	10	105.4 $\pm$ 2.5 <sup>e</sup>	68.5 $\pm$ 2.7 <sup>e,h</sup>	36.9 $\pm$ 1.9 <sup>h</sup>	35.0 $\pm$ 1.8 <sup>e,h</sup>	5.9 $\pm$ 0.7 <sup>h</sup>	31.0 $\pm$ 1.8 <sup>h</sup>	29.4 $\pm$ 1.7 <sup>e,h</sup>
R - C <sup>4</sup>	11	112.8 $\pm$ 2.9 <sup>f</sup>	87.7 $\pm$ 1.4 <sup>f</sup>	25.1 $\pm$ 2.5 <sup>f</sup>	21.9 $\pm$ 1.6 <sup>f</sup>	4.2 $\pm$ 0.2 <sup>f</sup>	20.8 $\pm$ 2.4 <sup>f</sup>	18.2 $\pm$ 1.6 <sup>f</sup>
T - G <sup>4</sup>	14	86.6 $\pm$ 1.8	48.3 $\pm$ 1.1	38.2 $\pm$ 1.4	44.1 $\pm$ 1.0	5.8 $\pm$ 0.3	32.5 $\pm$ 1.3	37.4 $\pm$ 1.1
R - G <sup>4</sup>	10	89.8 $\pm$ 3.3	50.7 $\pm$ 2.6	39.0 $\pm$ 2.1	43.6 $\pm$ 2.0	5.7 $\pm$ 0.5	33.3 $\pm$ 2.0	37.1 $\pm$ 1.8

<sup>1</sup>All data are expressed as mean  $\pm$  SEM values. a = Significant difference between T - S and T - C; b = significant difference between T - S and T - G; c = significant difference between R - S and R - C; d = significant difference between R - S and R - G; e = significant difference between T - C and T - G; f = significant difference between R - C and R - G; g = significant difference between T - S and R - S; h = significant difference between T - C and R - C.

<sup>2</sup>Apparent digestibility coefficient.

<sup>3</sup>Retention/intake.

<sup>4</sup>T = Transected, R = resected, S = Diet S, C = Diet C, G = Diet G.

**Table 4.** Iron concentrations ( $\mu\text{g/g}$  of dry weight) in several organs in transected and resected rats.<sup>1</sup>

Group	n	Liver	Spleen	Sternum	Heart	Femur
T - S <sup>2</sup>	11	240.3 $\pm$ 3.4 <sup>a</sup>	1299.1 $\pm$ 48.8 <sup>a</sup>	73.06 $\pm$ 2.4	258.5 $\pm$ 17.9	50.87 $\pm$ 1.4 <sup>a,g</sup>
R - S <sup>2</sup>	13	245.8 $\pm$ 3.8 <sup>b</sup>	1252.6 $\pm$ 32.4 <sup>b</sup>	69.8 $\pm$ 2.2 <sup>e</sup>	283.9 $\pm$ 12.2	53.55 $\pm$ 2.6 <sup>b</sup>
T - C <sup>2</sup>	10	212.3 $\pm$ 2.4 <sup>c</sup>	1102.8 $\pm$ 26.7 <sup>c</sup>	71.1 $\pm$ 0.9 <sup>c</sup>	244.6 $\pm$ 3.2 <sup>c</sup>	43.81 $\pm$ 0.6
R - C <sup>2</sup>	11	220.8 $\pm$ 3.7 <sup>d</sup>	1047.8 $\pm$ 74.9 <sup>d</sup>	66.4 $\pm$ 1.3 <sup>d</sup>	278.6 $\pm$ 3.1	45.41 $\pm$ 1.5
T - G <sup>2</sup>	14	230.3 $\pm$ 6.9	1284.9 $\pm$ 28.2	74.0 $\pm$ 0.8	255.1 $\pm$ 2.1 <sup>f</sup>	45.61 $\pm$ 0.7 <sup>f</sup>
R - G <sup>2</sup>	10	242.1 $\pm$ 3.4	1306.7 $\pm$ 30.9	82.2 $\pm$ 4.8	287.2 $\pm$ 3.5	49.39 $\pm$ 0.7

<sup>1</sup>All data are expressed as mean  $\pm$  SEM values. a = Significant difference between T - S and T - C; b = significant difference between R - S and R - C, c = significant difference between T - C and T - G; d = significant difference between R - C and R - G; e = significant difference between R - S and R - G; f = significant difference between T - G and R - G; g = significant difference between T - S and T - G.

<sup>2</sup>T = Transected, R = resected, S = Diet S, C = Diet C, G = Diet G.

When we compared the goat milk diet with the standard diet, we found that among the animals given the former, the iron content in the sternum was greater ( $P < 0.01$ ) among the resected rats, whereas no differences were found among the control animals. In the femur, however, the iron content in the transected rats was higher ( $P < 0.001$ ) for the rats given the standard diet than with those that consumed goat milk (Table 4).

The deposits of copper in the organs presented no differences between the transected and the resected animals given the standard diet, except in the kidneys (T > R) ( $P < 0.05$ ). Of the rats given cow milk, the copper content was higher among the transected rats than among the resected animals ( $P < 0.001$ , kidney and sternum; and  $P < 0.01$ , liver, spleen, and femur), except in the heart (T = R). With respect to the goat milk diet, there were no differences in the copper content in any of the organs studied (Table 5).

When we compared the different diets given to the control (transected) animals, we found that in general the standard diet produced a higher copper content in all the organs than the milk-based diets (whether goat or cow milk) (standard diet/cow milk diet:  $P < 0.001$ , liver and femur;  $P < 0.005$ , sternum and heart; standard diet/goat milk diet:  $P < 0.001$ , femur;  $P < 0.01$ , sternum;  $P < 0.05$ , liver, spleen, and heart), except in the kidneys,

spleen (S-C), and kidneys (S-G). The copper content in the kidneys ( $P < 0.025$ ) and in the femur ( $P < 0.005$ ) was greater when the transected animals consumed a goat milk diet than a cow milk diet; this tendency was also evident for the other organs studied, but it was not significant (Table 5).

With respect to the resected animals, we found that the copper content was greater when the animals were given the standard diet than when they consumed the cow milk diet, for all the organs studied ( $P < 0.001$ ) except the kidneys and the heart, although here, too, the same tendency was observed (but it was not significant). Comparison of the standard diet with that made up of goat milk shows that the levels of copper deposits in resected rats are of the same order, except in the kidneys, where they are greater ( $P < 0.001$ ) in the animals fed a goat milk diet. When considering the milk-based diets, we found higher levels ( $P < 0.001$ , kidneys, liver, sternum, and femur; and  $P < 0.05$ , spleen) of copper deposits in the different organs studied when the resected animals consumed a goat milk-based diet than when they were given cow milk, except in the heart, where these differences were not observed, although the same tendency was noted (but it was not significant) (Table 5).

**Table 5.** Copper concentrations ( $\mu\text{g/g}$  of dry weight) in several organs in transected and resected rats.<sup>1</sup>

Group	n	Kidney	Liver	Heart	Sternum	Femur	Spleen
T - S <sup>2</sup>	11	30.3 $\pm$ 1.6 <sup>a</sup>	17.5 $\pm$ 0.4 <sup>f,g</sup>	19.1 $\pm$ 0.5 <sup>f,g</sup>	9.5 $\pm$ 0.4 <sup>f,g</sup>	7.0 $\pm$ 0.2 <sup>f,g</sup>	9.0 $\pm$ 0.4 <sup>f,g</sup>
R - S <sup>2</sup>	13	25.2 $\pm$ 1.9 <sup>b</sup>	17.0 $\pm$ 0.4 <sup>h</sup>	18.1 $\pm$ 0.7	8.7 $\pm$ 0.3 <sup>h</sup>	6.6 $\pm$ 0.3 <sup>h</sup>	8.9 $\pm$ 0.5 <sup>h</sup>
T - C <sup>2</sup>	10	27.1 $\pm$ 0.8 <sup>c,d</sup>	15.0 $\pm$ 0.5 <sup>c</sup>	16.2 $\pm$ 0.4	7.2 $\pm$ 0.2 <sup>c</sup>	5.1 $\pm$ 0.2 <sup>c,d</sup>	8.0 $\pm$ 0.2 <sup>c</sup>
R - C <sup>2</sup>	11	22.1 $\pm$ 0.5 <sup>e</sup>	12.7 $\pm$ 0.6 <sup>e</sup>	16.9 $\pm$ 0.4	4.8 $\pm$ 0.3 <sup>e</sup>	4.2 $\pm$ 0.2 <sup>e</sup>	6.6 $\pm$ 0.5 <sup>e</sup>
T - G <sup>2</sup>	14	31.6 $\pm$ 1.3	16.2 $\pm$ 0.4	17.3 $\pm$ 0.7	7.8 $\pm$ 0.6	6.1 $\pm$ 0.2	8.0 $\pm$ 0.4
R - G <sup>2</sup>	10	32.7 $\pm$ 1.8	16.7 $\pm$ 0.9	18.1 $\pm$ 0.8	9.2 $\pm$ 0.2	6.6 $\pm$ 0.3	8.2 $\pm$ 0.4

<sup>1</sup>All data are expressed as mean  $\pm$  SEM values. a = Significant difference between T - S and R - S; b = significant difference between R - S and R - G; c = significant difference between T - C and R - C; d = significant difference between T - C and T - G; e = significant difference between R - C and R - G; f = significant difference between T - S and T - C; g = significant difference between T - S and T - G; h = significant difference between R - S and R - C.

<sup>2</sup>T = Transected, R = resected, S = Diet S, C = Diet C, G = Diet G.

## DISCUSSION

The digestive use (ADC) of iron in rats with a resection of 50% of the DSI, when fed one of the three test diets (S, C, or G), was lower than in the respective control groups. The lower absorption of iron in animals with intestinal resection may be caused by the reduction of the absorptive area, which leads to an increase in iron levels, from digestion in the intestinal lumen; therefore, there is an increase in the competition for binding sites to the carrier protein and thus a decrease in the absorption of iron (Hartiti et al., 1994), despite that the intestinal resection is distal and the duodenum, the preferential segment for iron absorption, is retained (Conrad and Umbreit, 1993).

Furthermore, according to Gómez-Ayala et al. (1998), it has been shown by an *in vivo* intestinal perfusion technique that iron is also absorbed in the jejunum-ileum segment, both by active transport and by diffusion, and so when all the ileum and a large part of the jejunum are removed, it is only logical that there should be a pronounced decline in iron absorption among animals with a resection of 50% of DSI.

The type of diet supplied influences the digestive use of iron; the diet based on goat milk presents a greater coefficient of apparent digestibility of iron than do the standard and cow milk diets. All the diets include the same iron content, but the fat quality is different, and so the high medium-chain triglycerides (MCT) content of the goat milk (36%), in comparison with the 21% of the cow milk and the 0% of the standard diet, could be influential in the improved absorption of iron, both in the resected animals and in the transected (control) animals. The MCT in the diet are rapidly absorbed and metabolized to obtain energy (García Unciti, 1996) and so could increase the synthesis of carrier proteins and thus the absorption of iron. Moreover, according to Tappenden et al. (1997), short-chain fatty acids favor intestinal adaptation after resection, probably because of the increased quantity of the other nutrients transported through the basolateral membrane. It is possible that medium-chain fatty acids, which are absorbed within the intestinal cells without reesterification, directly entering portal circulation, have the same effect on intestinal adaptation. Thus, not only is energy retention favored directly by the presence of MCT in the diet, but it could also increase as a consequence of the greater absorption of the other nutrients in the diet.

The more beneficial effects of the goat milk diet on the digestive use of iron, with respect to the cow milk diet, could be because of various nutritional factors that are present in greater quantity in goat milk; these include cysteine (Souci et al., 1989; Van Campen, 1973), an amino acid that is influential in improving iron ab-

sorption, and lysine, which by the solubilization of ferric or ferrous iron by the formation of tridentate chelates is the main factor associated with an enhanced uptake of the metal by the gut, in the presence of amino acid, because the stereoisomer of lysine has been shown to be effective in enhancing iron absorption (Van Campen, 1973). Moreover, the higher ascorbic acid (vitamin C) content in goat milk (Jandal, 1996; Souci et al., 1989) could contribute to increasing the absorption of iron in rats given the goat milk diet, because vitamin C is known to form a chelate with this mineral, which remains soluble at a higher pH within the small intestine (Czajka-Narins, 1988). Moreover, although the three diets tested contained casein, which as animal protein favors iron absorption (Layrisse and Martínez-Torres, 1972), from a qualitative viewpoint the casein derived from goat milk is more soluble and contains a higher proportion of other soluble proteins ( $\beta$ -lactoglobulin,  $\alpha$ -lactoalbumin, and serum albumin) (Boza and Sanz Sampelayo, 1997). This greater protein solubility offered by a goat milk diet could favor the absorption of iron.

According to a study by Sakai et al. (1992), large quantities (800 to 1000 ml/d) of cow milk consumed by infants could produce an iron deficiency. The present study reveals the beneficial effect of goat milk, compared with cow milk, on the digestive and metabolic use of iron, both among the control animals and among those with malabsorption syndrome. This implies that the consumption of goat milk, rather than cow milk, might prevent iron deficiency and thus the incidence of nutritional ferropenic anemia, a highly prevalent pathology (Caballo et al., 1993).

In transected (controls) and resected rats the deposit of iron in the liver (the preferential reserve organ for iron in the form of ferritin), the spleen (an organ that contains a lower proportion of iron, in the form of hemosiderin), and the sternum (a notably erythropoietic organ) follows the pattern observed for the absorption and retention (in absolute values) of iron, for the three diets. Thus, the iron content in the liver and spleen is higher with the standard and goat milk diets than with the cow milk diet, whereas in the sternum, despite that the same tendency is observed, the differences are very small. The lower iron content in the liver and spleen in rats fed with the cow milk diet may be explained by the observations of Hallberg et al. (1991), who showed that the calcium derived from cow milk interferes with the absorption of iron in the diet, which could explain the lower level of iron deposited in the organs. This effect does not occur when the rats consume the goat milk diet, which may be because this type of milk decreases the calcium-iron interference; this is in agreement with the conclusions of Park et al. (1986),

who suggest that goat milk increases the bioavailability of iron.

The digestive use of copper is reduced by the resection of 50% of the DSI among the animals consuming the standard diet, a consequence of the smaller absorptive area available; this conclusion is in accordance with the result obtained by Urban and Campbell (1984) and by Hartiti et al. (1995b).

Previous studies in rats have shown that the resection of the DSI modifies biliary physiology (Gómez-Ayala et al., 1994; Lisbona et al., 1991), because bile is the principal means of excreting copper, via the feces (Linder and Roboz, 1989), although this is not the only means, because in both humans and rats a small proportion of the copper is excreted in the urine; moreover, significant quantities of this mineral are found in the gastrointestinal tract with the saliva and the gastric, pancreatic, and intestinal juices (Linder and Roboz, 1989). Practically 50% of the copper excreted is via the bile, and because this excretion is affected by the distal resection of the intestine, the digestive use of this mineral is affected (Hartiti et al., 1995b).

After resection, there is a 16% reduction in the digestive use of copper in the rats given the standard diet, a value that is lower than that found for iron, which was around 40%. This less pronounced effect of intestinal resection on the digestive use of copper may be because the stomach and the proximal duodenum are the sites where copper absorption is greatest and fastest (Harris, 1991); in the type of resection performed, these sites have been preserved, and only the DSI has been removed.

This negative effect of intestinal resection on the digestive use of copper is more evident when the rats consume the cow milk-based diet, whereas copper absorption among the rats given the goat milk diet was not affected. Considering the type of diet supplied, we see that the high MCT content (36%) of goat milk must have a positive effect on copper absorption, among both the transected animals and the resected ones, with respect to cow milk, which has a lower MCT content (21%). These findings agree with those of Hartiti et al. (1995b), in that a high MCT content in the diet improves the digestive use of copper. This effect may be caused by the modification of the biliary function by these kinds of fatty acids (Lisbona et al., 1991) because, according to Johnson et al. (1992), the homeostasis of copper is evidently regulated by biliary excretion.

The beneficial effect of dietary goat milk on copper absorption may also be attributed to the higher cysteine content in goat milk (83 mg/100 g) than in cow milk (28 mg/100 g) (Souci et al., 1989), because this amino acid is the higher component in the small protein metallothionein (Linder, 1996); moreover in metallothionein,

cysteine is the amino acid responsible for the extremely tight covalent bond with the copper (Hallman et al., 1971) and this protein mediates copper absorption before passing into the plasma.

The distribution of copper throughout the organism is performed in two phases, first by means of a transfer from the intestine to the liver and the kidneys, and a subsequent phase from the liver to the rest of the organism (Linder, 1996). The kidney is rich in copper (Anderson et al., 1985) and, as this study shows, is the organ that best reflects events at the digestive and metabolic levels. Thus, the copper content of the kidney was lower among the resected animals, except when they consumed the goat milk-based diet, the beneficial effect of which on the absorption and retention of copper means that it is present in quantities similar to those found in the control (transected) rats. The same tendency is evident in the other organs studied (liver, heart, sternum, femur, and spleen) for the goat milk diet. This is not the case for the other two diets, in particular, that based on cow milk.

## CONCLUSIONS

The present study demonstrates the beneficial effect of goat milk, compared with cow milk, on the metabolism of iron and copper in control rats and those with resection of the DSI. This study suggests that goat milk should be studied for use in human malabsorption syndrome.

## ACKNOWLEDGMENTS

This study was supported by the Interministerial Commission of Science and Technology, Madrid, Spain (Project ALI96-1024-C02-02). We thank Ms. Elisa Alcover for her efficient administrative support and Ms. Francisca Gil Extremera and Ms. Rosa Jiménez for their competent technical assistance.

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