Effects of intestinal resection, cholecalciferol and ascorbic acid on iron metabolism in rats

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The effect of dietary supplementation with ascorbic acid or cholecalciferol on Fe utilization was studied using the metabolic balance technique, in rats in which 50% of the distal small intestine was removed, or in which the mid small intestine was transected and reanastomosed (controls). Three different diets were used. The first (basal diet) contained (g/kg dry wt): protein (casein + 50 mg D,L-methionine/g) 120 and fat (medium-chain triacylglycerols, olive oil and sunflower oil, in equal parts) 40. The other diets were obtained by adding ascorbic acid (150 mg/kg diet) or cholecalciferol (0·4 mg/kg diet) to the basal diet. Apparent digestibility coefficient (ADC) and Fe retention were significantly lower in resected animals than in their respective control groups (transected rats). However, the addition of ascorbic acid or cholecalciferol to the basal diet increased the ADC and Fe retention in both transected and resected rats. Five weeks after surgery, resection also resulted in a reduced concentration of Fe in the sternum, but did not reduce the concentration of haemoglobin or serum Fe total Fe-binding capacity or the concentration of Fe in liver, testes, femur or muscle (*longissimus dorsi*). Supplementation with ascorbic acid increased serum Fe concentration, while the concentration of Fe in muscle was reduced by supplementation with both ascorbic acid and cholecalciferol. Neither supplementation had any effect on the Fe concentration in other tissues, on haemoglobin concentration or plasma total Fe-binding capacity. Thus, supplementation with ascorbic acid or with cholecalciferol increased Fe absorption and reduced the concentration of Fe in muscle.

Resected rats: Iron: Ascorbic acid: Cholecalciferol

Intestinal resection is frequently used to treat diseases of the small intestine. Nevertheless, the consequences of this surgical intervention may influence its therapeutic value. A reduction in the absorptive mucosal surface and reduced intestinal transit time could reduce nutrient absorption. Many investigators have confirmed the development of anatomical and functional adaptations (Grey & Morin, 1985; Hazel *et al.* 1986; Wilson *et al.* 1986; Kwan *et al.* 1987) in the remaining segment. Experiments in laboratory animals have tried to enhance this response by varying the amount of bowel removed (Hanson *et al.* 1977*a*), the site of resection (Antonson & Vanderhoof, 1982; Wittmann *et al.* 1985), the post-operative time interval (Hanson *et al.* 1977*b*) or the diet (Biasco *et al.* 1984; Ford *et al.* 1985). In humans the management of these factors is limited and depends on the patient's state (Devine & Kelly, 1989). From a nutritional standpoint the critical factor is composition of the diet (Barrionuevo *et al.* 1989; Campos *et al.* 1989; Coves *et al.* 1991*a*, *b*; López-Aliaga *et al.* 1991). However, very few studies have examined the possible effects of diet in reducing the negative consequences of intestinal resection on Fe utilization. Fe depletion directly affects the activity of critical cellular enzymes such as ribonucleotide reductase (*EC* 1.17.4.1), cytochromes or aconitase (*EC* 4.2.1.3) and is known to impair

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cell proliferation (reviewed by Kühn *et al.* 1990), the key factor in the development of adaptive responses in the remaining intestine (Grey & Morin, 1985; Hazel *et al.* 1986). We therefore designed the present study to investigate the effects of intestinal resection on Fe utilization in rats, and to determine the influence of ascorbic acid and cholecalciferol in reversing these. We used a basal diet in which the fat component consisted of equal amounts of medium-chain triacylglycerols (MCT), sunflower oil and olive oil, as this composition has already been shown to enhance lipid metabolism in resected rats (Lisbona *et al.* 1991; Gómez-Ayala *et al.* 1994). To this basal diet we added ascorbic acid, which is known to increase Fe absorption (Hallberg *et al.* 1987, 1989). Mineral interactions, especially between Ca and Fe, might be expected in intestinal surfaces that have been surgically reduced (Bothwell *et al.* 1989; Kochanowsky & McMahan, 1990; Cook *et al.* 1991). Ca is an inhibitor of Fe absorption (Bothwell *et al.* 1989); moreover, cholecalciferol is well known as a potent promoter of Ca metabolism (Campos *et al.* 1989). We therefore tested the effects on Fe metabolism of the addition of cholecalciferol to the basal diet.

MATERIALS AND METHODS

Animals

Adult male albino Wistar rats (*Rattus norvegicus*) weighing an average of 190 (SE 7) g were used in all experiments. The animals were obtained from the University of Granada Laboratory Animal Service, and were housed throughout the experiment in individual metabolism cages designed for the separate collection of faeces and urine. The cages were kept in a well-ventilated, thermostatically controlled room ($22 \pm 2°$) with a 12 h light–12 h dark cycle.

Assurance of compliance

All experiments and surgical procedures with rats conformed to guidelines established by legal requirements in the UK for the proper care and use of laboratory animals.

Diets

The semi-purified basal diet was prepared according to the recommendations of the American Institute of Nutrition (1977) for a standard rat diet. The standard diet contains (g/kg dry wt): protein (casein + 50 mg D,L-methionine/g) 120, fat (olive oil) 40, fibre (micronized cellulose) 80, mineral supplement 35, vitamin supplement 10, and choline chloride 2. Olive oil in the standard diet was replaced in our basal diet by 40 g/kg medium-chain triacylglycerols, sunflower oil and olive oil in equal parts. Equal amounts (356·5 g) of maize starch and sucrose were added to all diets to make up to 1 kg dry matter. The mineral supplement provided 35 mg Fe/kg diet. The remaining diets were obtained by adding ascorbic acid (150 mg/kg diet) or cholecalciferol (0·4 mg/kg diet) to the basal diet.

The amount of ascorbic acid added to our basal diet was calculated for rats (/kg body weight) from that normally used in human clinical practice. The amount of cholecalciferol added was that which most effectively enhanced the nutritive utilization of Ca (Campos *et al.* 1989), P (Barrionuevo *et al.* 1989) and Mg (López-Aliaga *et al.* 1991).

Resection and transection procedures

Before surgery each rat was weighed. After general anaesthesia was induced with sodium pentobarbitone (50 mg/kg body weight) the animals were tested by tail-pinch reflex until the reflex had completely disappeared. The abdomen was shaved with clippers and cleaned with betadine. A standard abdominal approach was used, with care to ensure that the rat remained under adequate anaesthesia at all times. Using sterile instruments and aseptic

technique, 50% of the distal small intestine (DSI) was removed. The rat was placed on a warm heating pad and an electrical thermometer was inserted into the rectum. The body cavity was exposed with a midline abdominal incision. Surfaces were kept moist at all times with sterile saline at 37°, and fingers and tools were also kept wet. The intestine was exteriorized onto wet gauze sponges. For 50% resection leaving the ileocaecal valve intact (30 mm from the distal end of the ileum), total intestinal length was measured with sterile string knotted every 100 mm. Using a haemostat, several vascular arcades were picked up, no. 3 silk suture was pulled through and tied off. This procedure was repeated until all arcades supplying the 500 mm to be excised were ligated. The blood vessels were transected and the intestinal segment was excised on sterile gauze sponges so that any blood lost did not enter the abdominal cavity. The remaining intestinal segment was reanastomosed using 6-0 silk suture. The peritoneum and muscle were closed with 4-0 silk suture, and the skin was secured with wound clips. Rectal temperature was measured every 15 min. Animals in which the intestine was transected were treated identically except that the small intestine was divided and reanastomosed at the mid-small intestine, and the blood supply was not tied off in this procedure.

Experimental design

Six experimental groups were formed: (1) group T-B, transected rats, basal diet (*n* 10); (2) group R-B, resected rats, basal diet (*n* 13); (3) group T-B + ascorbic acid, transected rats, basal diet with ascorbic acid (*n* 11); (4) group R-B + ascorbic acid, resected rats, basal diet with ascorbic acid (*n* 14); (5) group T-B + cholecalciferol, transected rats, basal diet with cholecalciferol (*n* 11); (6) group R-B + cholecalciferol, resected rats, basal diet with cholecalciferol (*n* 11).

All animals were fed up to the time of surgery, and were given access to water containing 50 g 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 lib.* to all animals. Beginning 30 d after surgery, feed intake was measured and urine and faeces were collected for a period of 7 d (Thomas & Mitchell, 1923). At the end of this period rats were exsanguinated by cannulation of the abdominal aorta. The liver, sternum and both femurs, testes and *longissimus dorsi* (LD) muscle were removed and frozen at −80° in liquid N_2. All samples were stored at −40° before the analysis of Fe.

The following indices and variables were determined in all experiments: Fe intake, faecal Fe excretion, absolute Fe absorption, apparent digestibility coefficient (ADC), urinary Fe excretion, and absolute Fe retention (balance). Fe levels were also measured in the liver, both femurs, sternum, both testes and LD muscles. Serum Fe, haematological indices and total Fe-binding capacity (TIBC) were also determined.

Analytical techniques

The Fe content of the diet, faeces, and different organs were determined by ashing a 1 g sample at 450°. The resulting residues were dissolved in 5 M-HCl and diluted to an appropriate volume with double-distilled water. To determine Fe content, atomic absorption spectrophotometry (Perkin-Elmer 1100 B; Bodenseewerk, Germany) was used.

Packed cell volume (PCV), erythrocyte and leucocyte counts, and haemoglobin concentration were obtained with a Symex CC-130 automatic cell counter (Symex, Kobe, Japan). The leucocyte index was determined by microscopic observation of stained slides. Serum concentrations of Fe were obtained by colorimetry (Trinder, 1956). A Ferrimat-Kit (Ramsay, 1957) was used to determine TIBC.

Biological indices

The apparent digestibility coefficient (ADC) and Fe retention (R) over the 7 d experimental period were calculated as follows:

$$ADC = (I - F) \times 100/I,$$

and

$$R = I - (F + U),$$

where I is intake of Fe, F is faecal Fe and U is urinary Fe.

Statistical treatment

For each variable investigated the mean value and standard error of the mean (SEM) were found. The differences between groups (transected and resected rats) and diets (basal diet, basal diet with ascorbic acid, and basal diet with cholecalciferol) were compared by one-way ANOVA. All data were analysed with two-way ANOVA to evaluate the effect of resection and type of diet. A P value of less than 0·05 was considered significant.

RESULTS

ADC and iron balance

One month after resection, there was a significant decrease in ADC and retention of Fe in all three groups of resected rats compared with the corresponding transected rats ($P < 0·001$). The addition of ascorbic acid to the basal diet at a dose of 150 mg/kg diet increased both Fe absorption and retention in transected and resected rats ($P < 0·001$), leading to a greater digestive and metabolic utilization of this element (Table 1).

Cholecalciferol at a dose of 0·4 mg/kg diet significantly enhanced apparent Fe absorption in transected ($P < 0·01$) and resected rats ($P < 0·001$) in comparison with their respective controls fed on the basal diet alone. Similarly, Fe balance was significantly higher in transected ($P < 0·001$) and resected rats ($P < 0·01$) that consumed this vitamin.

Haematological indices

No significant differences in haemoglobin concentration or TIBC were found between any of the experimental groups (Table 2). These values remained within normal physiological limits for rats in all groups (Charles River Laboratories, 1982). The mean values of the haematological indices were close to normal values in all groups: erythrocytes 8·4 (SE 0·4) $\times 10^{12}$/l, packed cell volume 50 (SE 1)%, haemoglobin 170 (SE 5) g/l, mean corpuscular volume 57 (SE 2) fl, mean corpuscular haemoglobin 19 (SE 0·7) μg, mean corpuscular haemoglobin concentration 34 (SE 1)%, leucocytes 7 (SE 0·6) $\times 10^6$/l, neutrophils 23 (SE 2)%, band neutrophils 0%, lymphocytes 66 (SE 1)%, monocytes 3 (SE 0·4)%, eosinophils 2 (SE 0·3)%, basophils 0%.

However, serum Fe concentration was significantly higher in transected and resected rats ($P < 0·001$) that consumed the basal diet with ascorbic acid at a dose of 150 mg/kg diet (Table 2).

Iron concentrations in different organs

No differences in Fe concentrations were found in the liver or testes between transected and resected rats fed on different diets (Table 3). However, Fe content was significantly lower in the femurs of resected animals given the basal diet supplemented with cholecalciferol ($P < 0·001$) in comparison with resected rats fed on the basal diet alone. Intestinal resection slightly decreased Fe concentrations in the sternum regardless of the type of diet ($P < 0·05$) (Table 3). Intestinal resection itself did not affect Fe concentration in the LD muscle: no

Table 1. *Apparent digestibility coefficient (ADC) and iron retention in transected and resected rats fed on basal or vitamin-supplemented diets*‡

(Mean values with their standard errors)

Treatment group	*n*	Fe intake (mg/rat per d) Mean	SE	Faecal Fe (mg/rat per d) Mean	SE	Absorbed Fe (mg/rat per d) Mean	SE	ADC (%) Mean	SE	Urinary Fe (mg/rat per d) Mean	SE	Fe retention (mg/rat per d) Mean	SE
T-B	10	0·667	0·022	0·545	0·022	0·122	0·007	18·42	1·07	0·011	0·001	0·111	0·007
R-B	13	0·671	0·039	0·599	0·034	0·072	0·006	10·64***	1·37	0·009	0·001	0·064***	0·006
T-B+ascorbic acid	11	0·807	0·023	0·618	0·017	0·189	0·007	23·42†††	0·34	0·012	0·001	0·177†††	0·006
R-B+ascorbic acid	14	0·842	0·027	0·705	0·023	0·137	0·005	16·28***†††	0·37	0·011	0·001	0·126***†††	0·005
T-B+cholecalciferol	11	0·826	0·036	0·634	0·029	0·192	0·012	23·24††	1·02	0·007	0·001	0·185†††	0·012
R-B+cholecalciferol	11	0·696	0·039	0·591	0·033	0·105	0·007	15·04***†††	0·38	0·007	0·001	0·098***††	0·007

T-B, transected rats fed on the basal diet; R-B, resected rats fed on the basal diet.

Mean values were significantly different from those of the corresponding transected group: ** $P < 0·01$, *** $P < 0·001$.

Mean values were significantly different from those of the corresponding basal group: †† $P < 0·01$, ††† $P < 0·001$.

‡ For details of diets and procedures, see pp. 872–874.

Table 2. *Plasma values of iron and total iron-binding capacity (TIBC) in transected and resected rats fed on basal or vitamin-supplemented diets*‡

(Mean values with their standard errors)

Treatment group	*n*	Plasma iron (μg/l) Mean	SE	TIBC (μg/l) Mean	SE
T-B	10	1328	19	4680	242
R-B	13	1301	36	5065	268
T-B+ascorbic acid	11	1647***	86	5210	265
R-B+ascorbic acid	14	1768†††	45	4758	319
T-B+cholecalciferol	11	1336	17	5236	321
R-B+cholecalciferol	11	1297	74	5480	285

T-B, transected rats fed on the basal diet; R-B, resected rats fed on the basal diet.

*** Mean value was significantly different from that for the T-B group, $P < 0·001$.

††† Mean value was significantly different from that for the R-B group, $P < 0·001$.

‡ For details of diets and procedures, see pp. 872–874.

significant differences were found between transected and resected rats fed on the same diet. In contrast, ascorbic acid significantly decreased muscular Fe content in both transected and resected rats ($P < 0·001$) in comparison with their respective control groups. Similarly, cholecalciferol decreased muscular Fe content in transected ($P < 0·01$) and resected rats ($P < 0·001$) in comparison with their respective control groups (Table 3).

Table 3. *Iron concentrations in organs in transected and resected rats fed on basal or vitamin-supplemented diets*‡

(Mean values with their standard errors)

Treatment group	*n*	Liver (μg/g dry wt) Mean	SE	Femur (μg/g) Mean	SE	Sternum (μg/g) Mean	SE	Muscle (μg/g dry wt) Mean	SE	Testes (μg/g dry wt) Mean	SE
T-B	10	246·5	12·2	48·3	2·7	79·7	1·6	76·6	5·7	118·9	10·9
R-B	13	250·5	12·3	48·2	1·4	72·9*	2·0	80·7	4·3	120·2	06·5
T-B + ascorbic acid	11	248·2	19·1	45·4	1·4	81·7	3·2	57·7†††	3·2	134·6	03·3
R-B + ascorbic acid	14	247·4	15·8	45·2	1·3	73·6*	2·2	62·1†††	1·7	131·7	03·0
T-B + cholecalciferol	11	242·4	14·2	45·6	1·9	79·5	2·6	53·6††	3·1	128·4	06·1
R-B + cholecalciferol	11	262·6	09·5	36·5**†††	1·5	68·0*	3·5	50·9†††	2·6	132·9	03·8

T-B, transected rats fed on the basal diet; R-B, resected rats fed on the basal diet.

Mean values were significantly different from those of the corresponding transected group: * $P < 0·05$, ** $P < 0·01$.

Mean values were significantly different from those of the corresponding basal group: †† $P < 0·01$, ††† $P < 0·001$.

‡ For details of diets and procedures, see pp. 872–874.

DISCUSSION

One month after resection of 50% of the DSI there was a significant decrease in digestive and metabolic utilization of Fe in all resected animals regardless of the diet they were fed on. These findings were probably a consequence of higher mineral concentrations resulting from digestion in a smaller absorptive surface. Competition for binding sites was greater, and Fe absorption was consequently lower. The occurrence of Fe absorption in the distal intestinal segment, although lower than in the proximal segment (A. E. Gómez-Ayala, unpublished results) might have contributed to this reduction, although the surgical technique left the duodenum (the site of maximum Fe absorption) intact, and spared the proximal part of the jejunum, which is also able to absorb Fe (Muir & Hopfer, 1985; Conrad *et al.* 1987; Mugitani, 1989; Schümann *et al.* 1990).

In contrast, the addition of ascorbic acid to the basal diet at a dose of 150 mg/kg diet significantly increased both Fe absorption and retention in transected and resected rats. The key role of ascorbic acid in the absorption of dietary non-haem Fe is well known (Conrad & Barton, 1981; Hallberg *et al.* 1987, 1989; Bothwell *et al.* 1989). The reasons for its action are twofold: (1) the prevention of the formation of insoluble and unabsorbable Fe compounds, and (2) the reduction of ferric to ferrous Fe, which seems to be a requirement for Fe uptake into mucosal cells (Hallberg *et al.* 1989).

When the basal diet was supplemented with cholecalciferol at a dose of 0·4 mg/kg diet the digestive and metabolic efficiency of Fe increased in comparison with the values obtained with the basal diet alone. The improvement caused by this vitamin might have been due to both direct and indirect effects on Fe absorption. On one hand, this vitamin increases the concentration of binding protein in the remnant small intestine of DSI-resected rats, with a parallel increase in the active component of Ca (Campos *et al.* 1989) and Mg absorption (López-Aliaga *et al.* 1991). This leads to a lower concentration of divalent cations in the intestinal lumen, and thus curtails ionic interaction between these minerals. This condition improves Fe absorption by simple diffusion (Zhang *et al.* 1989). On the other hand, cholecalciferol may directly favour Fe absorption, increasing the

concentration of Fe-binding protein. This protein, called mobilferrin, was first identified by Conrad & Umbreit (1993) in rat duodenal homogenates and in the intestinal mucosa of rats and humans (Conrad *et al.* 1990, 1992). The inhibition of the active component of Fe absorption in rats by 2,4-dinitrophenol (A. E. Gómez-Ayala, unpublished results) supports this hypothesis. The positive role of cholecalciferol as a promoter of the active component in the absorptive process of several minerals has been reported previously (Barrionuevo *et al.* 1989; Campos *et al.* 1989; López-Aliaga *et al.* 1991).

The addition of ascorbic acid or cholecalciferol to the diet failed to eliminate completely the negative consequences of resection. However, dietary supplementation did attenuate these consequences to some extent, since both vitamins, through different mechanisms of action, enhanced Fe absorption in the remaining small intestine.

Although the ADC and Fe balance were lower in resected rats in comparison with their respective controls regardless of the type of diet consumed, the values obtained were within the normal range described for this species (Pallarés *et al.* 1993).

Despite our knowledge of the distribution of Fe throughout the body in normal physiological states, and of factors that promote or predispose to depletion, the effects of intestinal resection on the nutritive utilization of Fe remain for the most part unknown. We evaluated haemoglobin concentration, TIBC, several haematological indices and Fe distribution in rats that had undergone this operation. All values remained within normal limits for this species under all experimental conditions tested (Charles River Laboratories, 1982). Our findings suggest that erythropoiesis remained optimal (Huebers *et al.* 1990) and confirm that the phagocytic mononuclear system was not altered by intestinal resection, since Refsum & Schreiner (1984) attributed a major role to macrophages in Fe homeostasis.

Fe concentrations in the liver of transected and resected rats showed no significant changes, suggesting that the main Fe reserve was not modified by this operation, despite the significant decrease in the digestive and metabolic utilization of Fe. Surgery likewise had no effect on Fe concentration in the testes, in which proliferation is the predominant function. These findings confirm once again that the values obtained for Fe ADC and balance remained within physiological limits for the rat (Zhang *et al.* 1989; Pallarés *et al.* 1993).

Fe concentration in bone (femur) was significantly reduced only in resected rats fed on the basal diet supplemented with cholecalciferol (0·4 mg/kg diet). This vitamin is known to promote the nutritive utilization of other minerals such as Ca and Mg (Campos *et al.* 1989; López-Aliaga *et al.* 1991). The significant increase in Ca and Mg deposition in the femur of resected rats given a cholecalciferol supplement might explain this result: this increase probably occurred at the expense of Fe reserves, which in turn might have decreased as a result of diminished expression of transferrin receptors. In this connection, Tanaka & Teitelbaum (1990) found a 30% decline in the expression of transferrin receptors as a consequence of exposure to this vitamin.

However, in the sternum, where erythropoiesis takes place, intestinal resection led to a small decrease in Fe content under all experimental conditions. The decline was probably due to a slight increase in erythropoiesis required to maintain haemoglobin concentrations, the mechanism of homeostatic Fe regulation described by several investigators (Shull & Theil, 1982; Aziz & Munro, 1986; May *et al.* 1990; Dandekar *et al.* 1991; Kühn, 1991). In an erythropoietic organ such as the sternum, there is a tendency toward haem biosynthesis to maintain adequate haemoglobin concentrations, limiting Fe storage in ferritin.

In muscle tissue, intestinal resection itself did not affect Fe concentration, whereas dietary vitamin supplementation decreased this value. Molecular studies have reported that ascorbic acid increases Fe bioavailability in cells by directly mobilizing Fe from ferritin (Beinfait & Van Den Briel, 1981); in addition, ascorbic acid greatly retards the autophagic

uptake of ferritin clusters into lysosomes (haemosiderin) (Hoffman *et al.* 1991). This effect of ascorbic acid is probably more intense in muscle cells than in any other type of cell. The intensity of Fe mobilization is probably dependent on the importance of the organ in maintaining Fe homeostasis.

Supplementation of the basal diet with cholecalciferol also decreased muscle Fe concentration. A new hypothesis suggests that this vitamin may play a role in regulating Fe homeostasis, as this vitamin might influence the rate of transferrin receptor expression (Tanaka & Teitelbaum, 1990), which in turn affects cellular iron intake (Huebers & Finch, 1987; Huebers *et al.* 1990; Kühn *et al.* 1990). Moreover, muscle is not considered as important in Fe homeostasis as the other organs studied here. The influence of cholecalciferol on the expression of transferrin receptors, and hence on Fe uptake, probably depends on Fe status of the body, and on the importance of a given organ or tissue in Fe homeostasis.

Despite recent progress in our understanding of Fe metabolism, numerous aspects of the mechanism by which cholecalciferol or ascorbic acid improve the nutritive utilization of this element require further investigation.

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