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The effect of prolonged dietary supplementation with guar gum on subsequent iron absorption and retention in rats

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1. The effect of prolonged consumption of guar gum on iron absorption and Fe status was investigated in rats. Experiments with closed loops of duodenum, isolated *in situ*, were designed to reveal changes in the short-term regulation of duodenal Fe uptake, induced by challenge with low- and high-Fe meals. In separate experiments, the effect of guar gum on the capacity of intact rats to maintain Fe status and to absorb Fe from a test-meal was investigated.

2. Male Wistar rats were given either a control, semi-synthetic diet (C) for 21 d or a similar diet containing 100 g guar gum (G)/kg for 27 d. Both diets contained 36 mg Fe/kg. Two subgroups were then challenged with meals containing low-Fe (8 mg/kg) or high-Fe (566 mg/kg), while a third subgroup received a meal of the control diet (36 mg Fe/kg). At intervals of 12, 36, 60 and 84 h after the dietary challenge, the uptake of [^{59}Fe] ferric citrate was measured using closed duodenal loops *in situ*. All G-supplemented animals absorbed less Fe than their C-fed counterparts. Within group C, animals given the high-Fe challenge had lower absorptions 12, 36 and 60 h later, compared with those given the maintenance diet, whilst those given the low-Fe meal showed much increased uptake 12 and 36 h later. The latter effect was virtually abolished by guar gum.

3. In the second experiment, male Wistar rats were fed on the C or G diets containing 8, 15, 20, 26 or 36 mg Fe/kg for approximately 10 weeks *ad lib*. Fe retention from a starch–sucrose test meal extrinsically labelled with ^{59}Fe was then measured, together with packed cell volume and Fe content of the liver as indices of Fe status. No effect of feeding guar gum on Fe retention nor Fe status at any level of Fe intake was observed.

4. It was concluded that feeding guar gum leads to a small reduction in the rate of uptake of Fe in the rat duodenum and to an impairment of the regulation of uptake in response to brief episodes of dietary Fe depletion. However, these changes do not apparently lead to any nutritionally significant reduction in the capacity of the intact animals to absorb Fe and maintain their Fe status.

Guar gum is a soluble galactomannan which falls within the definition of dietary fibre, and which increases the viscosity of the gut contents. The simultaneous ingestion of guar gum with food leads to a reduction in the rate of nutrient absorption which may be exploited clinically in the management of diabetes (Blackburn *et al.* 1984; Johnson, 1984). In addition to this effect, prolonged consumption of guar gum is known to alter mucosal cell proliferation and, under some circumstances, to reduce enzyme and carrier activity in the proximal small intestinal mucosa of the rat (Johnson *et al.* 1984). The purpose of the present study was to discover whether such adaptive changes lead to an alteration in Fe absorption or to impairment of Fe status. In particular, the experimental approach was designed to reveal any possibly effect of prolonged guar gum consumption on the short-term regulation of Fe absorption which occurs in response to a challenge with a single high- or low-Fe test meal, and which appears to be mediated by the intestinal mucosal cells, independently of any change in body Fe status (Fairweather-Tait & Wright, 1984).

MATERIALS AND METHODS

Animals

Male Wistar rats were used in all the experiments and were randomly allocated into the experimental groups. They were caged in pairs in Expt 1, and individually in Expt 2, using stainless-steel and plastic cages with wire bottoms, and were provided with distilled water *ad lib*. Body-weights were measured twice weekly and food intakes were recorded daily.

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Table 1. *Expt 1. Composition of the control and guar-gum-supplemented diets (g/kg diet)*

Diet... Ingredients	Control	Guar-gum-supplemented
Starch*	326	226
Sucrose	326	326
Casein†	168	168
Maize oil	80	80
Cellulose‡	40	40
Guar gum§	—	100
Mineral mix‖	40	40
Vitamin mix¶	20	20

* 'Snoflake' maize flour; Corn Products Ltd, Manchester.
† Edible casein; Glaxo Farley Foods, Plymouth.
‡ Solka floc; Johnson Jorgensen Wettre Ltd, Woking, Berks.
§ Sigma Chemical Co., St Louis, MO, USA.
‖ Levels of minerals in the diet (g/kg): $CaHPO_4$ 13·0, $CaCO_3$ 8·2, KCl 7·03, $NaHPO_4$ 7·4, $MgSO_4.H_2O$ 4·0, $MnSO_4.H_2O$ 0·18, $ZnCl_3$ 0·10, $FeSO_4.7H_2O$ 0·144, $CuSO_4$ 0·015, KIO_3 0·001.
¶ Levels of vitamins in the diet (mg/kg): nicotinic acid 60, cyanocobalamin in mannitol 50, calcium-D-pantothenate 40, thiamin hydrochloride 10, riboflavin 10, pyridoxine 10, pteroylmonoglutamic acid 5, D-biotin 1, menadione 1, Rovimix E50 (Roche Products Ltd, Dunstable, Beds) 150, Rovimix A-500 (Roche) 25, Rovimix $D_3$500 (Roche) 15, choline bitartrate 1800.

Diets

The compositions of the semi-synthetic control (C) and guar-gum-supplemented (G) diets are given in Table 1. Both diets had a total Fe content of 36 mg/kg. In Expt 1 the appropriate high- and low-Fe challenge meals were prepared by adding extra ferrous sulphate to the diets or by omitting it from the mineral mix. In Expt 2, the $FeSO_4$ content of the diets was varied to give a range of final Fe concentrations as shown in Table 3 (p. 250).

Whole-body gamma counting

The use of the NE8112 (Nuclear Enterprises; Edinburgh) small animal whole-body gamma counter has been described by Fairweather-Tait & Wright (1984).

Atomic absorption spectroscopy (AAS)

Diets, livers and biological standards (National Bureau of Standards, Office of Standard Reference Materials, Washington, USA) were analysed for total Fe. Samples were ashed in silica crucibles at 480° for 48 h, the ash was taken up in warm, concentrated hydrochloric acid, diluted to an appropriate volume with distilled water and subsequently filtered. The solutions were analysed for Fe using a PU 9000 AAS (Pye Unicam, Cambridge) with background correction.

Packed cell volume

Packed cell volumes (PCV) were analysed in whole heparinized blood samples by the microhaematocrit method.

In situ *loop preparation*

The incubation medium for the preparation contained 16 mM-Hepes (Sigma Chemical Co., Poole, Dorset) together with 125 mM-sodium chloride, 3·5 mM-potassium chloride, 1 mM-calcium chloride, 10 mM-magnesium sulphate and 10 mM-D-glucose. The pH was adjusted before use, using 0·1 M-sodium hydroxide. Ferric citrate was prepared using 0·1 M-ferric chloride in 0·1 M-HCl, equilibrated with $^{59}FeCl_3$ (Amersham International plc, Amersham, Bucks) to which was added citrate ions as 1·0 M-trisodium citrate so that the molar ratio,

Fe:citrate ions, was 1:10. The ferric citrate was diluted with the incubation medium, the resulting ferric citrate concentration being 0·5 mM and 1μCi ^{59}Fe/ml (37 kBq ^{59}Fe/ml).

The rats were anaesthetized with sodium pentobarbital (Sagatal; May and Baker Ltd, Dagenham), administered by intra-peritoneal injection at 60 mg/kg body-weight. The abdomen was opened, and a loop of duodenum was exposed, care being taken to ensure the maintenance of an intact blood supply. Loops were prepared by ligating the intestine 20 mm from the pylorus and approximately 70 mm distally. Before the second ligature was tightened, the loops were filled with approximately 0·5 ml ferric citrate solution by injection from a syringe, the exact volume being determined gravimetrically. The loop was then replaced in the abdominal cavity, the incision covered with moist cotton wool and the ambient temperature maintained at 37° using a heated operating table. At 30 min after injection of the test solution the loops were excised, the intestinal fluid collected and the loops rinsed with 20 ml ice-cold incubation medium. Before killing by cervical dislocation, cardiac punctures were performed to obtain blood samples for PCV determinations. Livers were removed, rinsed in saline (9 g NaCl/l), blotted and freeze-dried. The excised loops were dried overnight at 85°, weighed and counted in the gamma counter for 20 min. The intestinal fluid contents were counted separately. Carcass ^{59}Fe content was measured by counting in the whole-body gamma counter, using appropriate standards to calibrate and correlate the two counters. The Fe uptake is defined as the sum of gut and carcass contents.

Expt 1

Male rats (180, mean weight 120 g) were randomly allocated into two equal groups and given either the C diet for 14 d *ad lib.* or the G diet for 20 d *ad lib.*, to ensure the attainment of similar body-weights in both groups. They were then trained to meal feed over 7 d. Animals from each group were then divided into three subgroups. The medium-Fe subgroups received further single meals of the C or G diets containing 36 mg Fe/kg, whilst the other subgroups were challenged with single meals of C or G diet having a low (8 mg Fe/kg) or high (566 mg/kg) Fe content. Absorption studies were carried out at 12, 36, 60 and 84 h after the low- or high-Fe meals, and at 12 and 84 h in the case of the medium-Fe subgroup. Further meals of the C or G diets containing 36 mg Fe/kg were given at 24 h intervals after the challenge meals. The final numbers of animals used at each time point are indicated in Fig. 1 (p. 249).

Expt 2

Male rats (eighty, mean weight 140 g) were randomly allocated into ten groups of eight animals and given diets C or G, with Fe contents as shown in Table 3 (p. 250). The diets were given *ad lib.* for 67 d in the case of groups C1–C5 and for 73 d in the case of groups G1–G5 (Table 3). Samples of tail blood were taken on day 0 and on the last day of the feeding period for PCV determinations.

On day 60 in the case of groups C1–C5, and on day 66 in the case of G1–G5, the rats were given a ^{59}Fe-labelled meal after an overnight fast and whole body Fe retention was measured. The rats were given a meal consisting of 5 g starch-sucrose (50:50, w/w) paste and 120 μg Fe (as $FeSO_4.7H_2O$ solution) extrinsically labelled with 0·5 μCi ^{59}Fe (18·5 kBq ^{59}Fe, $FeCl_3$; Amersham International plc). After consuming the meal, their whole-body radioactivity was measured in the whole body gamma counter and again 7 d later. At 3 h after the test meal they all received their appropriate diets *ad lib.* Whole body retention was calculated by measuring the difference in the ^{59}Fe content of the animal immediately post-dosing and 7 d later, adjusting for decay, when all the unretained ^{59}Fe from the test meal had been excreted. At the end of the experiment the animals were killed by cervical dislocation and the livers removed, rinsed in saline, blotted and freeze-dried for subsequent determination of the whole liver Fe content.

Statistical analysis

In both experiments the equality of the means was assessed by a one-way analysis of variance, followed by comparison with the least significant difference (LSD) for the data (Cochran & Cox, 1964). The significances of trends in Fe status and Fe retention within the C- and G-fed groups of Expt 2 were tested using linear regression analysis.

RESULTS

Expt 1

The mean food intakes of the two groups of animals over the two feeding periods are shown in Table 2. G-fed animals ate 18% less food than those fed on the C diet over the first 10 d, but there was no significant difference in total food consumption of the groups over the period during which they were meal-fed, nor in the final body-weights of the groups (Table 2). There was no difference in Fe status as determined by the total liver Fe content, in the two groups (Table 2).

The percentage uptake of [^{59}Fe]ferric citrate by both G-fed and C-fed rats after the low-, medium- or high-Fe meals is illustrated in Fig. 1. Within the groups C and G, Fe-uptake by those animals consistently given medium-Fe meals, and hence not receiving a dietary Fe challenge, was virtually identical at 12 and 84 h. In the C-fed group, the animals challenged with the high-Fe meals showed reduced Fe-uptake at 12 and 60 h, in comparison with those given the medium-Fe meal, but there was no difference at 84 h. In the C-fed group, animals given a low-Fe meal showed a considerable enhancement of Fe uptake at 12 and 36 h, but not at 60 or 84 h.

G-fed rats given the high-Fe challenge also showed lower percentage Fe uptake than the medium-Fe subgroup at 12, 36 and 60 h. However, the G-fed rats given a low-Fe meal showed a much smaller enhancement of uptake than that seen in the C-fed group; the enhancement was significant only at 12 h after the meal. Uptake of Fe by the G-fed rats was always lower than that of their C-fed counterparts. The differences between equivalent G- and C-fed subgroups were statistically significant ($P < 0{\cdot}05$) in every case other than at 12 h for the medium-Fe subgroups and at 12 and 60 h for the subgroups given the high-Fe challenge.

Fig. 2 shows the percentage of the total Fe uptake transferred into the carcass in each of the subgroups, at each time-point, following the challenge with dietary Fe. The percentage transferred into the carcass was increased initially by the low-Fe meal and decreased by high-Fe challenge, but in all the subgroups the percentage was close to 50 after 84 h.

Expt 2

The mean body-weights and food intakes for each group are shown in Table 3. Both the food intakes and to a lesser degree the final body-weights, fell as the Fe level of the diet, whether G or C based, was reduced. The G diets of similar Fe contents were again less readily accepted over the first 10 d in comparison with the C diets. The mean initial PCV values were similar in all the groups (Table 4).

The final PCV values and liver Fe contents of the C- and G-fed rats are shown in Table 4. The analysis of variance for these data indicated significant inequality among the means, but this was almost entirely due to the expected reduction in Fe status in those subgroups receiving Fe-depleted diets. In one instance only, the difference between the means for the PCV values of equivalent subgroups exceeded the calculated LSD ($P < 0{\cdot}05$). There was therefore no consistent evidence for any effect of guar gum on Fe status at any level of Fe intake. In a further analysis, the total Fe intake was determined for each animal individually,

Table 2. *Expt 1. Food intakes, final body-weights and total liver iron content in the guar-gum fed and control-fed animals*

(Mean values with their standard errors)

Diet	*n*	Total food intake over *ad lib.* period (g/rat)		Total food intake over meal-feeding period (g/rat)		Final body-wt (g)		Total liver Fe content (mg)	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Control	77	320·9	3·6	115·9	1·2	242·5	1·8	3·51	0·16
Guar gum	87	365·0	3·5	116·3	1·8	238·9	1·4	3·38	0·13

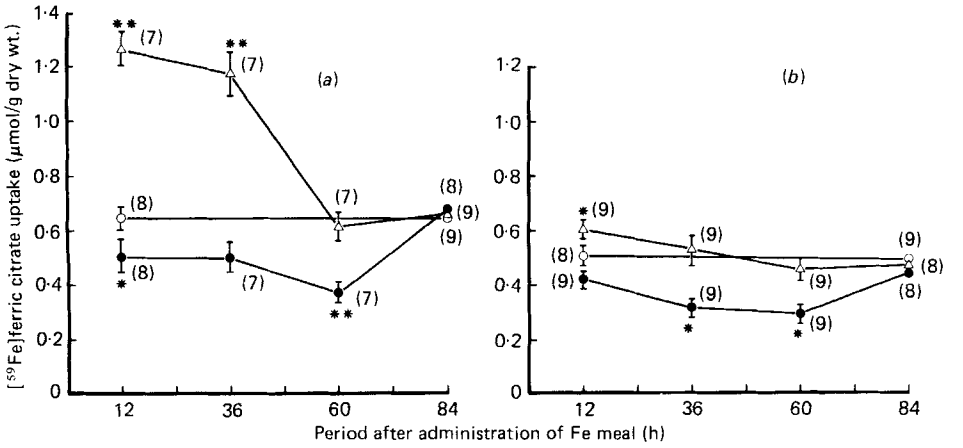

Fig. 1. Expt 1. Uptake of [^{59}Fe]ferric citrate measured in (a) guar-gum-fed or (b) control-fed animals at various time-intervals after the administration of a low- (△), medium- (○), or high- (●) Fe meal. Points are mean values with their standard errors represented by vertical bars obtained using the numbers of animals shown in parentheses. Mean values were significantly different from those for medium-Fe fed rats (○) at 12 h and 84 h: $*P < 0{\cdot}05$, $**P < 0{\cdot}01$.

and the regression of PCV and liver-Fe *v.* $\log_{10}$ Fe intake was calculated. In each instance positive linear relation with gradients significantly greater than zero ($P < 0{\cdot}001$) were obtained, but there were no significant differences between C- and G-fed groups.

The whole body Fe retention for each of the groups is also shown in Table 4. In both the C- and G-fed rats the percentage Fe retention increased as dietary intake fell. However, as with the Fe status, there was no evidence of any effect of guar gum on Fe retention. A significant inverse linear relation between retention and $\log_{10}$ Fe intake for individual animals was obtained, but there were no significant differences in gradient between C- and G-fed groups.

DISCUSSION

In the absence of any major excretory pathway in mammals, Fe balance is maintained by regulation of intestinal absorption. Thus in man, and in the rat, Fe absorption is increased in anaemia and suppressed in response to Fe overloading (Bothwell *et al.* 1979). A short-term regulatory mechanism which is independent of changes in body Fe status has also been shown to exist, whereby absorption responds to previous dietary Fe intake

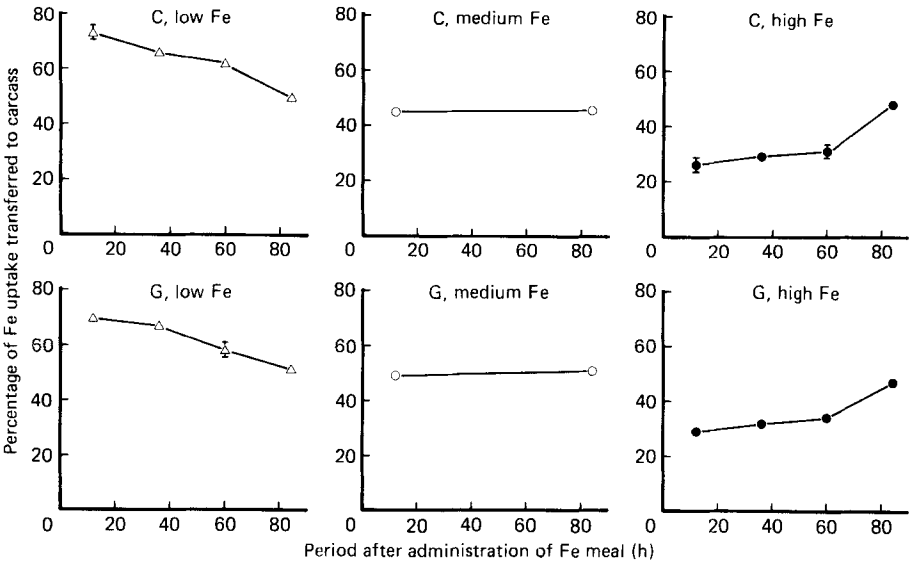

Fig. 2. Expt 1. Percentage of total iron uptake transferred to the carcass in guar-gum- (G) fed or control- (C) fed rats at various time intervals following low-, medium- or high-Fe meals. Points are mean values with their standard error represented by vertical bars where the size of the error exceeded that of the symbol.

Table 3. *Expt 2. Final body-weights (g), total food intakes (g/rat) and the iron contents of control (C) and guar-gum-supplemented (G) diets given to each group*

(Mean values with their standard errors)

Group	Dietary Fe concentration (mg/g diet)	Final body-wt		Total food intake (g)	
		Mean	SEM	Mean	SEM
G1	8	365·6	12·0	1182	31
G2	15	373·2	10·3	1259	17
G3	20	398·5	9·7	1293	26
G4	26	396·3	10·6	1382	31
G5*	36	410·3	8·8	1411	20
C1	8	384·5	10·5	1230	17
C2	15	420·4	13·8	1303	11
C3	20	434·5	4·7	1307	22
C4	26	431·1	10·8	1348	13
C5*	36	433·8	5·6	1381	17

* G5 and C5 are identical to the guar-gum-supplemented and control diets respectively of Table 1.

(Hegenauer *et al.* 1977; Fairweather-Tait & Wright, 1984; Fairweather-Tait *et al.* 1985). The first experiment in the present study was designed to examine the impact of prolonged guar gum consumption on the rate of Fe uptake in the duodenum of the rat. The procedure was designed to minimize any acute effect of guar gum in the gut lumen and to reveal changes in the short-term regulation of uptake.

As expected on the basis of previous studies (Fairweather-Tait & Wright, 1984;

Table 4. *Expt 2. Iron retention, initial and final packed cell volumes (PCV), and total liver Fe contents in groups of rats fed on either control (C) or guar-gum-supplemented (G) diets of varying Fe concentrations*

(Mean values with their standard errors. All values represent the mean for eight animals, except where indicated)

Group	Dietary Fe concentration (mg/g diet)	Whole body Fe retention (% of dose)		PCV				Total liver Fe content (mg)	
				Initial		Final			
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
G1	8	73·0	0·87	45·5	1·6	27·9	3·1	1·86	0·15
G2	15	69·4	0·77	45·7	1·3	34·8*	2·1	2·32	0·24
G3	20	65·9	1·03	44·0	0·9	42·0	1·7	2·56	0·43
G4	26	66·4	1·21	44·7	1·1	41·2	3·5	3·12	0·23
G5	36	64·4	1·0	44·2	1·6	44·8	1·2	3·18	0·30
C1	8	73·3	1·60	45·2	1·1	28·4	2·4	1·55	0·11
C2	15	69·8	0·67	44·8	0·9	31·8*	2·8	2·52	0·07
C3	20	67·8	0·84	44·6	0·9	40·9	1·9	2·68	0·21
C4	26	66·7	1·45	45·1	1·1	42·3	3·6	3·55	0·16
C5	36	65·8†	1·16	45·4	1·3	45·6†	0·5	3·37†	0·21

Equivalent C- or G-fed subgroups did not differ significantly, except where indicated: $*P < 0·05$

Fairweather-Tait *et al.* 1985), in both the G- and C- fed groups there was a suppression of Fe uptake in animals given a high-Fe meal before investigation and an increase in those given a low-Fe meal. However, Fe uptake by all the G-fed subgroups was lower than their equivalent control groups at every time point (Fig. 1).

An important problem with experiments of this type is imposed by the need to normalize the uptake values to an anatomical index which may differ between groups. Most of the differences between the G- and C-fed subgroups are relatively small, but they seem unlikely to be due simply to a reduction in the contribution of absorptive tissue to the total weight of duodenum because, as we have previously shown, the weight of mucosa as a proportion of whole gut is increased in the jejunum of rats fed on guar gum at this level (Johnson *et al.* 1984).

A second possibility is that a residual layer of guar gum remained within the duodenum after the challenge meal, and that this reduced the rate of uptake of Fe by increasing the resistance to diffusion of the mucosal unstirred fluid layer, as has been shown to occur in the case of actively absorbed sugars under experimental conditions (Johnson & Gee, 1981; Blackburn & Johnson, 1983). This possibility cannot be wholly excluded, although it seems unlikely, given the 12 h delay between the last meal and the experiment. Further evidence against this interpretation lies in the observation that the effect of guar gum depended on the previous Fe loading, suggesting a specific adaptive change in these animals, operating at the level of mucosal cell function. In none of the subgroups was there any effect of guar gum on the percentage of the absorbed Fe which was transferred from the mucosa into the carcass. This suggests that the adaptive response to guar gum consumption operates on the mechanism by which Fe is taken up from the lumen rather than on the subsequent process of transfer into the circulation.

Evidence from previous studies regarding the effects of guar gum on Fe absorption is both limited and conflicting. Gillooly *et al.* (1984) reported a direct inhibitory effect on Fe

absorption in human volunteers given a liquid test-meal containing guar gum. On the other hand, Hallberg (1983) has reported increased Fe absorption in humans given guar gum incorporated into bread. Of more relevance to the present study are the results of Wölbling *et al.* (1980) who reported that Fe-replete rats fed on guar gum for 3 d before an ^{59}Fe-labelled test-meal showed reduced Fe retention compared with controls, although there was no such difference between groups of Fe-deficient rats. The authors also reported a direct inhibition of Fe uptake when guar gum was present in closed jejunal loops of both Fe-replete and Fe-deficient rats.

In the present study, dietary supplementation with guar gum for approximately 10 weeks had no significant effect on Fe status of the rats. In groups receiving diets with a less than adequate Fe content, the final Fe status was reduced to similar levels in both the C- and G-fed groups. As expected, the decrease in Fe status of the rats fed on Fe-deficient diets led to an increase in the retention of labelled ^{59}Fe from a test meal. However, no significant differences in Fe status or Fe retention between the C- and G-fed groups were seen at any level of Fe intake. These results suggest that prolonged consumption of guar gum, even at the relatively high level used in this study, has no adverse effect on the overall capacity of the rat to absorb Fe, nor upon its capacity to increase absorption in response to reduced Fe stores. Evidently the rate of uptake of Fe in the duodenum is not a limiting factor for whole-body absorption in this particular situation, presumably because the dietary Fe remains in the gut long enough for the potentially available fraction to be fully absorbed. These observations are consistent with those of Harmuth-Hoene & Schelenz (1980), who found no reduction in carcass Fe content in rats fed on guar gum (100 g/kg) for 21 weeks and no change in Fe balance in weanling rats fed on guar gum for 8 d.

We conclude that the prolonged consumption of guar gum, at a level of 100 g/kg dry weight, is associated with a small reduction in the rate of Fe uptake in the duodenum. The effect is most marked in animals previously challenged with a low-Fe meal, indicating that the adaptive response of the mucosa to brief dietary Fe depletion is reduced. However, these effects do not appear to reduce the capacity of the animals to regulate their body Fe status during prolonged marginal intake, nor is there any apparent impairment of their ability to retain Fe from a test-meal. The present study does not therefore provide evidence of any mechanism whereby the long-term consumption of guar gum by humans might lead to an impairment of Fe nutriture.

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