Iron deficiency in children with coeliac disease on treatment with gluten-free diet

Role of intestinal blood loss

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SUMMARY 24 children in whom coeliac disease had been diagnosed between one and 10 years earlier were re-examined for intestinal loss of iron and activity of the disease. Mild iron deficiency by laboratory criteria and by response to iron medication was common. The children with biopsy evidence of flat mucosa or intraepithelial lymphocytosis had a greater iron loss in their stools than those patients with normal, or almost normal, histology. The data suggest that loss of iron was mainly due to intestinal blood loss rather than to shedding of mucosal cells. We conclude that treatment with iron is indicated in many children with coeliac disease as the increased losses of iron persist for long periods although absorption of iron seems to improve or be negligibly affected.

Mild iron deficiency is often found in patients with coeliac disease being treated with gluten-free diets (Visakorpi *et al.*, 1970; Shmerling and Zimmerli-Häring, 1971). The potential aetiological factors are decreased absorption of iron, increased loss of iron with the rapidly turning over epithelium cells, and pathological bleeding through intestinal tract (Sutton *et al.*, 1970; Anand *et al.*, 1977; Harms *et al.*, 1977). However, impaired absorption is less likely to be the reason for iron deficiency as ferrous iron generally seems to be better absorbed after starting treatment (Badenoch and Callender, 1960; Anand *et al.*, 1977) and the absorption of haemoglobin iron, in particular, does not depend on the integrity of the mucosa of the proximal small bowel and is not reduced even in untreated patients (Webb *et al.*, 1967; Anand *et al.*, 1977).

We studied the role of intestinal loss of iron in a group of children with coeliac disease on long-term treatment with gluten-free diet, with or without evidence of active disease. This was done to obtain information on the pathogenic mechanism of iron deficiency and on the indications for treatment with iron.

Subjects

A group of 24 children (13 boys and 11 girls) with coeliac disease were re-evaluated. The initial diagnosis had been based on the presence of a flat proximal jejunal mucosa between 9 months and 10.7 years before the study (Interlaken agreement; Visakorpi, 1970). The patients had been treated by excluding gluten from their diet and were being followed up at least annually at this hospital. During this time, most of the patients had been given courses of iron when they were found to be anaemic. At the time of the study the children were aged between 3 and 17.2 years.

Methods

Hb and RBC indices were measured by a Model S Coulter Counter from venous blood drawn into EDTA. Reticulocytes were counted microscopically. A blood sample was also drawn in the morning for serum analyses. Serum iron and total iron-binding capacity were determined spectrophotometrically in duplicate (Bothwell *et al.*, 1972; Rice and Fenner, 1974) and transferrin iron saturation was calculated. Storage iron was estimated by serum ferritin assay in triplicate (Siimes *et al.*, 1974).

The patients with transferrin saturation <16%

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and/or serum ferritin $<1 \mu\text{g}/100 \text{ ml}$ were started on treatment with iron; 2 mg ferrous iron/kg daily, given 1 to 2 hours before the meal. After a period of about 3 months the blood tests were repeated.

In 19 patients intestinal loss of iron was estimated by means of a 5-day stool collection 2 days after IV administration of 100 nCi $^{59}\text{Fe Cl}_3$. The stools were then counted in a whole body counter with four detectors at 90° angles. The accuracy of the counting was $\pm 20\%$ in the activities of $<0.5 \text{ nCi}$, $\pm 10\%$ and $\pm 7\%$ in the activities of 1 and 2 nCi respectively. There are no control values from healthy children to indicate the physiological loss of iron by this technique. However, we have studied several children with severe iron deficiency anaemia and with suspicion of intestinal blood loss and found that presumed healthy children usually have $<0.2 \text{ nCi}$ (% of the injected label/5 days) in their stools. An arbitrary value of 0.5 nCi was used in this study as an upper limit of normal, although this value may be slightly too high.

In 5 additional patients daily stools were collected for between 7 and 10 days and analysed separately after giving the same dose of ^{59}Fe . The results of these counts were more accurate as there was only one stool container each day per measurement. The lowest detectable level of radioactivity was about 0.04 nCi .

In all 24 patients biopsy specimens were obtained from the first loop of jejunum using a Crosby-Kugler capsule of paediatric size; these were fixed in formalin, then routinely embedded, sectioned at $7 \mu\text{m}$, and stained with haematoxylin and eosin. The histological findings were graded as total villous atrophy, partial villous atrophy, slight villous changes, and normal.

In order to obtain an additional and more sensitive quantitative criterion of the activity of the disease, the intraepithelial lymphocytes were counted in an area of the slide where the tissue was orientated, so that the plane of the section passed vertically through

the epithelium and the basement membrane was easily visible. For each specimen at least 500 cell nuclei were counted. The results were expressed as the number of lymphocytes per 100 villous cells (Ferguson *et al.*, 1976).

The patients participated in this investigation voluntarily. The dose of radioactivity used was in accordance with the Scandinavian regulations on paediatric research (Radiation Protection Institutes in Denmark, Finland, Iceland, Norway, and Sweden, 1976)—i.e. a dose of less than the annual endogenous exposure.

Results

There was evidence of mild iron deficiency in the group of 24 children with coeliac disease. Hb, transferrin saturation, MCV of red blood cells, and serum ferritin were below the lower limits of normal in 6, 7, 6, and 8 patients respectively. However, anaemia was mild since the lowest Hb was 11.5 g/dl . The mean reticulocyte count was slightly raised. In 7 patients the values were above 1.5% , ranging from 1.6 to 2.3% .

A total of 10 children were considered to have iron deficiency by meeting at least two criteria denoting an abnormality. These patients were started on iron treatment, and later the laboratory values were all normal except in 2 of the 10 patients in whom the concentration of serum ferritin remained $<1 \mu\text{g}/100 \text{ ml}$. The mean levels of Hb, transferrin saturation, and serum ferritin rose from 12.6 to 13.4 g/dl , from 18.8 to 40.5% ($P < 0.01$), and from 1.2 to $1.5 \mu\text{g}/100 \text{ ml}$ respectively.

In 19 patients the mean amount of radioactivity in the 5-day stool specimens was 1.0 nCi (1.0% of injected dose) ranging from 0.2 to 3.0 nCi . In 17 of them the value was considered abnormal: 0.5 nCi or more. The daily excretion of radioactivity was measured in 5 other patients. The results are shown in the Figure.

Figure Radioactivity in stools of 5 patients collected daily after an intravenous injection of 100 nCi of $^{59}\text{Fe Cl}_3$. Pattern of the secretion remained similar for 10 days, although there was a pronounced variation in individual stools from day to day. Each stool specimen contained some radioactivity. No specimen was obtained from 2 patients on day 6 and from some after 7 days.

Abnormal proximal jejunal mucosa was found in 9 patients of whom 6 had total villous atrophy and 3 had only slight mucosal changes. The patients with flat mucosa had more radioactivity in their stools (from 1.2 to 3.0 nCi) than those with slight changes or normal mucosa (from 0.2 to 2.1 nCi).

In the patients with histological changes in mucosa the intraepithelial lymphocyte counts were high. The mean counts were 67, 55, and 32 and the total ranges from 51 to 87, from 44 to 64, and from 23 to 61 per 100 cells in patients with total villous atrophy, slight mucosal changes, and normal mucosa, respectively. The last range was also somewhat raised as in healthy individuals it is considered to be <34 (Kosnai and Kuitunen, 1977) or <38 lymphocytes/100 cells (Ferguson *et al.*, 1976). The intraepithelial lymphocyte count and the quantity of radioactivity in the stools showed a positive correlation in the 24 patients ($P < 0.01$).

A negative correlation was found between the concentration of serum ferritin and the radioactivity in the stools ($P < 0.001$).

Discussion

The results show a pronounced loss of radioactivity in stools after intravenous injection of labelled iron in a group of children with coeliac disease treated with gluten-free diet for at least one year. The loss could be attributed either to intestinal bleeding or to accelerated turnover of epithelial cells in which iron is lost in stools (Sutton *et al.*, 1970). Our results show that both mechanisms could be involved. However, the findings suggest that intestinal bleeding would account for a major proportion. The iron lost through epithelial cells should derive from serum and appear in stools during the first days after the injection, as the normally short turnover of these cells of about 3 days should be even more rapid in these patients. On the other hand, the incorporation of iron into haemoglobin starts immediately, and it is practically completed within 5 days. Accordingly, the radioactivity derived from red cells should gradually increase in the stools and then reach a certain plateau. It was evident that neither of these patterns was found. In addition, there seemed to be a pronounced variation in the loss of label from day to day in all patients.

The intestinal blood loss and signs of mild iron deficiency were greater in patients with total villous atrophy of the jejunum and in those with intraepithelial lymphocytosis. These findings suggest that iron treatment should be considered, for instance annually or semiannually, in these patients who are clearly unable to follow the dietary instructions. On the other hand, the patients who presumably kept

to the diet more rigidly and who had a normal or almost normal mucosal histology also bled, although to a lesser extent. Some of these patients must therefore also have an increased need for iron, perhaps because some breaks in diet are inevitable even with strongly motivated families. Alternatively it is possible that subtle abnormalities in mucosal integrity remain, even with strict dietary management.

Several studies show that iron absorption is not a limiting factor in patients being treated for coeliac disease (Anand *et al.*, 1977). Our patients were able to compensate for most of the continuous loss of iron by increased absorption since any anaemia was mild. Patients with signs of iron deficiency responded well to iron, although they were unable to fill their iron stores as shown by the small change in concentration of serum ferritin (Siimes *et al.*, 1974). We feel that most children with coeliac disease require some iron. Reticulocytosis or reduced level of serum ferritin may be practical and sensitive criteria to detect these individuals.

References

- Anand, B. S., Callender, S. T., and Warner, G. T. (1977). Absorption of inorganic and haemoglobin iron in coeliac disease. *British Journal of Haematology*, **37**, 409–414.
- Badenoch, J., and Callender, S. T. (1960). Effect of corticosteroids and gluten-free diet on absorption of iron in idiopathic steatorrhea and coeliac disease. *Lancet*, **1**, 192–194.
- Bothwell, T. H., Conrad, M. E., Cook, J. D., Crosby, W. H., Fielding, J., Hallberg, L., Izak, G., Layrisse, M., and Ramsay, W. N. M. (1972). Studies on the standardization of serum iron and iron binding capacity assays. In *Modern Concepts in Hematology*, p. 153. Edited by G. Izak and S. M. Lewis. Academic Press: New York.
- Ferguson, A., McClure, J. P., and Townley, R. R. W. (1976). Intraepithelial lymphocyte counts in small intestinal biopsies from children with diarrhoea. *Acta paediatrica Scandinavica*, **65**, 541–546.
- Harms, H. K., Böhne, W., Kracke, W., Sansoni, B., Mader, T., and Linderkamp, O. (1977). Iron metabolism in untreated coeliac disease; haematological indices, serum iron concentrations, iron absorption, and excretion. *Acta paediatrica Belgica*, **30**, 247.
- Kosnai, I., and Kuitunen, P. (1977). Intraepithelial lymphocytes in various malabsorption states. In *Proceedings of XIX Congressus Paediatricus cum Participatione Internationali*, Prague.
- Radiation Protection Institutes in Denmark, Finland, Iceland, Norway, and Sweden. (1976). *Exposé of Persons for Research Purpose. Report on the Applicability on International Radiation Protection Recommendation in the Nordic Countries*, p. 73. Edited by B. Lindell. Liber Tryck: Stockholm.
- Rice, E. W., and Fenner, H. E. (1974). Study of the ICSH proposed reference method for serum iron assay: obtaining optically clear filtrates and substitution of ferrozine. *Clinica chimica acta*, **53**, 391–393.
- Shmerling, D. H., and Zimmerli-Häring, S. M. (1971). Die floride Zöliake. Untersuchungsergebnisse bei 88 Patienten zwischen 1963 und 1969. *Helvetica paediatrica acta*, **26**, 565–584.

- Siimes, M. A., Addiego, J. E., and Dallman, P. R. (1974). Ferritin in serum: diagnosis of iron deficiency and iron overload in infants and children. *Blood*, **43**, 581–589.
- Sutton, D. R., McLean Baird, I., Stewart, J. S., and Coghill, N. F. (1970). 'Free' iron loss in atrophic gastritis, post-gastrectomy states, and adult coeliac disease. *Lancet*, **2**, 387–390.
- Visakorpi, J. K. (1970). An international enquiry concerning the diagnostic criteria of coeliac disease. *Acta paediatrica Scandinavica*, **59**, 463–464.
- Visakorpi, J. K., Kuitunen, P., and Savilahti, E. (1970). Frequency and nature in children suffering from the malabsorption syndrome with gluten intolerance. *Acta paediatrica Scandinavica*, **59**, 481–486.
- Webb, M. G. T., Taylor, M. R. H., and Gatenby, P. B. B. (1977). Iron absorption in coeliac disease of childhood and adolescence. *British Medical Journal*, **2**, 151–152.

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