THE HAEMATINIC EFFECT OF 
IRON IN FLOUR

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(Received 18 July 1970)

SUMMARY

1. Two large-scale community based studies of the haematinic effect of iron added to flour used to bake bread are reported. These were designed to simulate conditions in the community as closely as possible.

2. Neither trial gave conclusive evidence of benefit in terms of an effect on circulating haemoglobin level, but in one trial the results suggested a small haematinic effect.

3. The availability of an iron salt from bread eaten in a normal varied diet is clearly much lower than has been suggested from highly controlled radioactive isotope studies.

White flour is an important source of dietary iron in most Western communities and in many countries iron is added to 'restore' its content to that of a high extraction or a wholemeal flour (Elwood, Newton, Eakins & Brown, 1968). In Great Britain the Bread and Flour Regulations (1963) require that either reduced iron (of a specification laid down in the regulations) or ferric ammonium citrate (B.P. or B.P.C.) shall be added, if necessary, to restore the iron content of flours other than wholemeal (e.g. low extraction white flour) to at least 1.65 mg/100 g.

In a recent report by the Ministry of Health in Great Britain (1968) it was shown by means of radioactive labelled iron (a) that the form of iron (referred to here as 'powdered iron') which is currently added to flour to comply with the Bread and Flour Regulations (1963) is unavailable to volunteers when fed in a 'standard breakfast', (b) that both ferric ammonium citrate (which could legally be added to flour under the same regulations) and ferrous sulphate are well absorbed from bread eaten as part of a 'standard breakfast', and (c) that the incorporation of an egg in the 'standard breakfast', very markedly reduces the proportion of iron absorbed.

As there may be many substances in a normal diet which interfere with iron absorption, it was felt that a more realistic test of the value, if any, of ferric ammonium citrate as an additive to flour would be to incorporate this into the entire bread supply of a number of anaemic women and to maintain this supply for a considerable period. During this time the effect of the added iron on circulating haemoglobin level would be studied.

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We have conducted two such studies. In the first of these the effect of ferric ammonium citrate in bread on the haemoglobin levels of anaemic women selected from a community sample was studied. As a rise in haemoglobin level was looked for in this study, it is for convenience referred to as the 'therapeutic' trial. In the second trial a more realistic prophylactic design was used. All the anaemic women in a large community sample were identified and given sufficient conventional treatment with oral iron to raise their haemoglobin levels. A random sample of these treated women were then given bread containing ferric ammonium citrate. The effect of the iron in the bread was assessed by the degree to which it prevented a subsequent fall in circulating haemoglobin level. For convenience this is therefore referred to as the 'prophylactic' trial.

Women were selected for inclusion in these trials simply on the basis of an estimate of their circulating haemoglobin level. Selection was sequential, that is the women with the lowest levels were visited first and the level for selection was raised until a sufficient number had been obtained for the trial. The total number that could be admitted was limited by the facilities for daily deliveries of bread. All women who were pregnant, were already receiving iron treatment, were found to be seriously anaemic or were suffering from any contraindicative disease were excluded. So also were all women who were on special diets or who ate no white bread. At the close of the study all who were still anaemic received appropriate treatment with medicinal iron. In view of the results of other studies in which the morbidity of anaemia is assessed (Elwood, 1968; Elwood & Hughes, 1970) it was considered justifiable to delay the conventional treatment of these women until the trials were completed.

The analysis of the data is based on a comparison between means in the subgroups given various 'treatments'. It is unfortunate that it was not possible in these trials to have a true 'control' group to which bread with no added iron was given. It is illegal to offer such bread for sale in Great Britain. However, in the preliminary radioactive studies (Ministry of Health, 1968) the mean absorption of powdered iron by anaemic subjects under ideal conditions was found to be under 1%. Bread containing powdered iron was therefore given to the control groups in the trials which follow.

**MATERIALS AND METHODS**

**The 'therapeutic' trial**

Just over 3000 adult women were seen at a preliminary haematological screening survey (Table 1). Women found to have haemoglobin levels below 8.0 g/100 ml were given conventional iron therapy and do not feature in this report. The 391 women with the next lowest haemoglobin levels were all asked to cooperate in a trial and 304 were suitable and agreed. For 9 months the homes of half of these women, selected at random, were supplied with ordinary bread. This contained powdered iron at a level sufficient to comply with the Bread and Flour Regulations (1963). The homes of the other women were supplied with bread made from flour containing ferric ammonium citrate. This had been added at a level 10% higher than the powdered iron to allow for flour eaten as cakes, etc. The ferric ammonium citrate supplied on average about 1 mg Fe per subject per day. Both breads were produced by a normal commercial process.

**The 'prophylactic' trial**

This trial was larger than the first and a higher level of ferric ammonium citrate was added to the flour. This achieved a total level similar to that in wholemeal flour (Fe 3.5 mg/100 g flour,
or about 2.7 mg added Fe) which is the level defined in the U.S.A. for ‘enriched’ bread. In this trial 450 women, selected in a similar way to the first trial but drawn from a wider geographical area which included a further population of 5245 women, agreed to cooperate (Table 1). These 450 women were all given a short course of oral iron which led to a mean rise of 0.8 g Hb/100 ml and 1.9% PCV. Following this they were admitted to the trial and allocated at random to one of three groups. One group received bread containing the Fe salt and this supplied on average about 2.7 mg Fe per woman per day. During the trial it was estimated that about 85% of the bread eaten by these women during the trial came from this supply. The other women in the trial were allotted at random to two groups: one third were given a daily tablet which supplied the same amount of iron as the special bread, and two thirds received a placebo tablet which contained no iron. The women in both the groups given tablets obtained ordinary bread which supplied about 0.8 mg powdered iron per person per day.

**RESULTS**

The changes during the ‘therapeutic’ trial in mean haemoglobin level and mean packed cell volume are shown in Table 2. The difference in the mean changes in haemoglobin level, but not in PCV, in the total groups is statistically significant ($P<0.05$) and the direction of the difference suggests that the powdered iron in the control bread had a more favourable effect than the ferric ammonium citrate. However the changes in the groups with the lowest haemoglobin levels are inconsistent with this hypothesis.

Tables 3 and 4 show the mean changes in haemoglobin level and PCV during the ‘prophylactic’ trial. The mean Hb level and PCV, having been raised by the iron therapy before admission, fell gradually during the trial. This fall is not quite consistent, as the data for the placebo group (0 mg Fe tablets) showed a mean fall at 6 months, which increased for PCV but not**
haemoglobin at 12 months. It is possible that this discrepancy was caused by a slight change in the standards used for haemoglobinometry but whether or not this happened comparisons between groups are unbiased. Although the trends shown by the data are not entirely consistent

TABLE 2. The ‘therapeutic’ trial. Mean changes (±SE) in circulating haemoglobin level (g/100 ml) and haematocrit (%) in groups of women given bread containing powdered iron, or ferric ammonium citrate for 9 months

<table>
<thead>
<tr>
<th>Hb level (g/100 ml)</th>
<th>Changes in Hb (g/100 ml) and PCV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bread with powdered Fe</td>
</tr>
<tr>
<td>8—8.9</td>
<td>0.12±0.13 g/l00 ml (10)</td>
</tr>
<tr>
<td>9—9.9</td>
<td>0.25±0.25 g/100 ml (25)</td>
</tr>
<tr>
<td>10—10.9</td>
<td>0.15±0.18 g/100 ml (49)</td>
</tr>
<tr>
<td>11+</td>
<td>0.37±0.19 g/100 ml (40)</td>
</tr>
<tr>
<td>All</td>
<td>0.24±0.11 g/100 ml (124)</td>
</tr>
</tbody>
</table>

Numbers in subgroups shown in parentheses.

TABLE 3. The ‘prophylactic’ trial. Mean changes (±SE) in women given bread containing added ferric ammonium citrate or tablets

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean changes during trial (g/100 ml Hb and % PCV)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>After 6 months</td>
</tr>
<tr>
<td>Bread (2.7 mg Fe)</td>
<td>-0.14±0.06 g/100 ml (192)</td>
</tr>
<tr>
<td>Tablets (0 mg Fe)</td>
<td>-0.44±0.09 g/100 ml (103)</td>
</tr>
<tr>
<td>Tablets (2.7 mg Fe)</td>
<td>-0.08±0.12 g/100 ml (54)</td>
</tr>
</tbody>
</table>

(Numbers in subgroups shown in parentheses)

Note: Hb and PCV changes show significant ($P<0.01$) heterogeneity after 6 months but not after 12 months.

they suggest that the iron supplement had some effect, albeit small, on haemoglobin level, whether it was given in bread or in tablets. At the end of 6 months this benefit is statistically significant (Table 4) but after 12 months the evidence of benefit is not convincing and the changes
within the groups do not differ significantly. This same analysis was repeated in the women aged 50 years and over, as these can be presumed to have ceased to menstruate, and are therefore free of an important factor affecting iron balance. The changes they show are very similar in size and in direction to those shown by the total groups.

Table 4. The ‘prophylactic’ trial. The effect of a 2.7 mg Fe daily supplement given in bread or as a tablet. Data represent the differences (± SE) between the bread and the Fe tablet groups, and the group given 0 mg Fe in Table 3

<table>
<thead>
<tr>
<th>Supplement</th>
<th>After 6 months</th>
<th>After 12 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bread (2.7 mg Fe)</td>
<td>+0.31 ± 0.11 g/100 ml*</td>
<td>+0.18 ± 0.12 g/100 ml</td>
</tr>
<tr>
<td>Tablet (2.7 mg Fe)</td>
<td>+0.37 ± 0.15 g/100 ml*</td>
<td>+0.03 ± 0.16 g/100 ml</td>
</tr>
</tbody>
</table>

* *P* < 0.05.

DISCUSSION

Two fundamental aspects of these trials which require consideration are first, the realism of their designs, and second, their sensitivity.

Every attempt was made to simulate normal conditions within the milling and baking industry, and in order as far as possible to avoid disturbance to the usual diets of the subjects, the bread was distributed and sold in a normal commercial way. Although the design of the first trial was not entirely realistic in that the ‘therapeutic’ effect of a prophylactic measure was tested, this limitation was entirely removed in the second trial.

The sensitivity of the trials can be examined from the standard errors of the mean changes shown in the Tables. If one makes certain physiological assumptions these statistics can be translated into estimates of proportionate absorptions of the added iron. If one further assumes that the absorption of powdered iron by the control group was under 1%, then it can be shown that in the first trial, in order to obtain a statistically significant (*P* < 0.05) and clinically important effect (a rise of, say, 0.25 g Hb/100 ml), the absorption of iron from the ferric ammonium citrate would have to be 16% or more. A proportion absorption of at least this size, in a population sample of anaemic women, had been predicted on the basis of the preliminary radioactive isotope studies (Ministry of Health, 1968). The second trial was considerably more sensitive than this, as absorption of the iron in the ferric ammonium citrate of about 6% in the first 6 months or about 4% in the 12 months would probably have led to a statistically significant result. The trials were therefore reasonably realistic and sensitive. In fact dietary surveys in the area in which the trials were conducted showed that the mean total dietary iron intake was about 11.5 mg per day so the ferric ammonium citrate gave an ‘effective’ increase of about 10% in the first and about 25% in the second trial.
However there are certain inevitable uncertainties in these trials. The bread was supplied to homes and not to individual subjects, so it could be that the women themselves did not eat the bread. We think this unlikely as the women were visited frequently and most seemed to be very cooperative, and the amount of bread which was sold during the trials was close to what we had predicted on the basis of preliminary dietary studies in the area. On the other hand there may have been a falling off in those taking iron tablets, particularly during the second 6 months. This is quite likely, as following the tests at the end of 6 months, the subjects were told that their results were satisfactory (except for a few who had to be withdrawn because of a marked fall in haemoglobin level). Further bias may have occurred because of selection of subjects. In the first trial 22% and in the second 13% of the women eligible for inclusion on the basis of their haemoglobin level were not admitted because they were unsuitable or refused to participate. A further 22% in the first trial and 28% in the second dropped out for various reasons, but there is no indication that any of these omissions predominated in any one subgroup.

Despite these limitations we feel that the results of these trials genuinely reflect what happens when flour is enriched with iron, and there are several possible explanations of our findings. The first is that powdered iron is more readily available to man than the iron in ferric ammonium citrate (thus accounting for some of the results in the first trial). We cannot accept this explanation for several reasons. Detailed studies of the absorption of several radioactive labelled iron preparations gave consistent evidence that powdered iron is almost completely unavailable from bread (Elwood, 1968). Furthermore, in the first trial reported here the changes in the most anaemic subjects conflict with this hypothesis (Table 2). A second possible explanation is that although the ferric ammonium citrate had a beneficial effect, this was too small to be detected as statistically significant. This hypothesis seems to us to be reasonable, but if it is true, it is clear that iron added to flour used to bake bread is very poorly available to man from a normal diet.

Unfortunately the low availability of iron from bread cannot be compensated for by adding larger amounts of iron. Iron salts induce rancidity in flour on storage, and have a harmful effect on its baking qualities. Even the level used in the prophylactic trial described above caused slight, but noticeable changes in the bread and extreme care had to be taken during preparation and baking to ensure an acceptable loaf.

However the decision whether or not iron should be added to flour depends on an epidemiological assessment of the importance of iron deficiency in the community. There is remarkably little valid evidence on which to base such an assessment (Elwood, 1968, 1970).

ACKNOWLEDGMENTS

We are grateful to the very large number of people who helped with this work. The Panel on Iron in Flour of the Ministry of Health, and in particular its secretary, Dr W. T. C. Berry, gave constant encouragement and advice. Members at every level of the flour milling and baking industry cooperated in every way possible. Local doctors, physicians and the Medical Officer of Health and his staff gave considerable assistance. Our director, Professor A. L. Cochrane and the staff of the Epidemiology Unit gave unfailing help and support.

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