ABSTRACT

IRON ABSORPTION FROM A NATURAL MINERAL WATER (SPATONE IRON+)

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The value of Spatone Iron+ as an effective iron supplement needs at be assessed in the context of iron requirements.

In addition it should be noted that iron absorption from food is in the range of 5 – 20% depending on diet. Meat and fish-containing diets give the highest absorption.

Men lose about 1mg iron per day, regardless of the amount of iron in the body, and this loss is generally replaced by dietary iron alone.

Children's iron requirements are high relative to body weight because of the demand for iron caused by rapid growth. Menstruation and childbirth increase iron losses in women to the order of 2mg a day or more. This extra iron requirement must generally be obtained from a total dietary intake, which is, on average, less than the male intake.

It is not, therefore, surprising that iron insufficiency is relatively common in children and women of childbearing age.

Our studies of the absorption of Spatone Iron+ show that people who are short of iron (having serum ferritin concentrations of less that 15ug/1 and therefore low iron stores) will absorb up to 40% of a 20ml sachet of Spatone Iron+ taken on an empty stomach.

This means that a sachet of Spatone Iron+ will provide approximately 2mg Iron/day, which will provide sufficient additional iron to cover the average extra needs of pregnancy and to prevent the development of iron insufficiency in women and children. Young female athletes have relatively high iron requirements which will also be met at this level of supplementation.

Our study indicates that people, with high iron stores will absorb little iron from Spatone. Spatone Iron+ is thus a safe, effective iron supplement, which provides iron for those requiring it, but should not cause iron overload in those with adequate iron stores.

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Iron absorption from a natural mineral water (Spatone Iron-Plus)

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Summary Spatone Iron-Plus is a naturally occurring mineral water from Trefriw Wells Spa in Gwynedd, Wales, UK which contains approximately 0.3 mg of iron per ml as ferrous sulphate. The water has been taken as a tonic since Victorian times. Iron absorption from Spatone Iron-Plus was measured in a whole body counter after labelling the water with [59Fe] ferrous sulphate. Absorption studies were carried out in 13 subjects. Mean absorption for 10 ml of Spatone Iron-Plus taken on an empty stomach was 23%. Absorption was related to body iron stores as assessed by serum ferritin. In subjects with a serum ferritin concentration of <10 μg/l, absorption was approximately 40% but was <10% in subjects with ferritin concentrations of approximately 200 μg/l. This study indicates that Spatone Iron-Plus provides iron in a highly bio-available form.

Keywords Iron absorption, mineral water, iron deficiency

Introduction

The human body contains about 4 g iron, which is found in many proteins but mostly in haemoglobin in the red blood cell. A variable amount is found in the tissues as ferritin and haemosiderin. This is 'storage iron' which is available for haem synthesis if required (Worwood 1994a). In adult males iron losses are limited to about 1 mg Fe per day but menstruation and childbirth increase iron losses in younger women, and in children iron requirements are high relative to body weight because of the demand for iron caused by rapid growth. The iron content of the body is largely regulated by variation in the amount of iron absorbed from the diet. Western diets usually contain about 10–15 mg Fe per day. This level of intake is adequate for men but women and children must generally obtain more iron from an intake which is less than that of men (British Nutrition Foundation 1995).

It is not surprising therefore that iron deficiency, defined as an absence of storage iron, is more common in children and women of childbearing age than in men. The level of storage iron may be estimated by measuring the serum ferritin concentration. Concentrations of less than 12–15 μg/l indicate the absence of storage iron and a value of 300 μg/l is equivalent to iron stores of about 2 g (the maximum normally found). Hallberg *et al.* (1993) consider that the best indicator of iron deficiency is a serum ferritin concentration of less than 16 μg/l, and have concluded that approximately 30% of young women in Europe and North America are iron deficient. The application of multiple measures (i.e. a low serum ferritin and a low transferrin saturation) enhances specificity but at the expense of sensitivity. Such an analysis indicates that approx 12% of young women in North America may be iron deficient (Cook *et al.* 1986).

There is concern that iron deficiency, even in the absence of anaemia, may cause both physical and behavioural abnormalities in children (Baynes 1994). If iron losses in excess of iron intake continue, iron deficiency anaemia will develop. In North America and Europe the incidence of iron deficiency anaemia is in the range 10–15% and in parts of the developing world the incidence of iron deficiency anaemia in women is over 50% (De Maeyer & Adiels-Tegman 1985).

The frequency of iron deficiency and its possible deleterious effects have given treatment and prevention high priority. Groups at high risk may be targeted. For example, in many hospitals prophylactic iron is given to all women during pregnancy but agreement about the desirability of this practice is not universal (Hibbard 1988; Horn 1988). General fortification of food has been practised for many years. In the UK white flour is supplemented with iron to increase the iron content to that of wholemeal flour.

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However this iron is poorly available and there is little evidence about its efficacy in reducing the incidence of iron deficiency in the population (Department of Health and Social Security 1981).

Universal fortification of food may have undesirable consequences. Haemochromatosis (Bothwell *et al.* 1989) is the most common autosomal recessive, inherited disorder in populations of European origin, with an incidence varying between 1 and 3 per 1000 people (Worwood 1994b). This is a disorder in which too much iron is absorbed, causing iron accumulation in the tissues, particularly the liver, and cirrhosis, diabetes and other pathological changes can occur. About 10% of the population carry the gene which is located on the short arm of chromosome 6. There is no evidence to suggest that these heterozygotes are at risk of developing major iron overload through fortification or supplementation of the diet, but subjects who are homozygous for the gene (two copies) almost certainly are.

It has also been suggested that those with iron stores at the top of the normal range (as indicated by high levels of serum ferritin) may have enhanced susceptibility to infection, cancer and coronary heart disease, but there is little evidence that high levels of tissue iron, as opposed to high levels of serum ferritin, are implicated (British Nutrition Foundation 1995).

Tonics and food supplements containing iron are widely available and have been used for centuries. Such supplements should ideally contain iron which is highly available, should not cause side-effects and should not be absorbed by those with high levels of storage iron who do not need additional iron. Spatone Iron-Plus is a natural, iron-containing mineral water from Trefriw Wells in Gwynedd, Wales, UK. Analysis by Laboratorium für Wasseruntersuchungen, Hannover gave the following constituents: cations, 28.5 mmol/l, of which 11.3 mmol/l was Fe^{2+}, 11.0 mmol/l was Ca^{2+}, 5.3 mmol/l Mg^{2+} and 0.4 mmol/l Fe^{3+}; anions 29.8 mol/l, of which 29.3 mmol/l was sulphate. Undissolved solid amounted to 21 mg out of 2063 mg/l.

The water has been taken as a tonic for many years. The aim of this study was to measure the availability of the iron from Spatone Iron-Plus and to study its absorption in normal subjects with varying levels of iron stores.

Methods

Permission to perform the trial was obtained from the ethics committee of the South Glamorgan Health Authority and the Department of Health Administration of Radioactive Substances Advisory Committee. Volunteers were sought by placing advertisements in the teaching hospital and the College of Medicine. Each volunteer gave informed consent after having the study explained in detail. Subjects in the age range 18–65 years were accepted, although women who were pregnant or thought that they may be pregnant were excluded, as were those taking iron-containing medications or tonics. In this way, 15 volunteers were recruited. Iron status was determined in each subject, although only 13 (6 males) were available to take part in the iron absorption study. Details of age and sex are given in Table 1.

A blood sample of approximately 10 ml was taken for a full blood count (Technicon H1 counter) and for preparation of serum. Serum ferritin concentrations were determined by enzyme linked immunoassay (Dawson *et al.* 1991).

The iron content of the Spatone Iron-Plus solution was determined by colorimetric analysis with ferrozine as the chromogen (Worwood & Darke 1993) in four sachets of 10 ml from the box provided by Trefriw Wells Spa Ltd and used for all studies. Spatone Iron-Plus contains iron in the form of ferrous sulphate at pH 2.9. The sachets are filled under nitrogen to prevent oxidation. Therefore it was feasible to label the mineral water with ^{59}Fe ferrous sulphate (Product code NEZ049 from Dupont UK Ltd, Stevenage, Herts, containing 37 μg Fe/ml in 0.05 M H_2SO_4 solution, specific activity 1 GBq/mg Fe at the reference date). An appropriate volume of this stock solution was diluted in Spatone Iron-Plus to give an activity concentration of 185 kBq/ml; this was done once a week during the duration of the trial.

For each iron absorption measurement, 100 μl of the diluted solution (18.5 kBq) was added to the contents of one sachet of Spatone Iron-Plus (10 ml) in a beaker; this was further diluted with 10 ml deionized water and adjusted to pH 2.9 with 0.05 M sulphuric acid in order to increase the volume as suggested by the manufacturer. Thus each drink contained approximately 3 mg of iron, of which less than 0.3 μg was in the form of ^{59}Fe.

Iron absorption studies

The iron absorption measurements were carried out using the Cardiff whole body counter (Davies *et al.* 1974; Thomson 1981) which has recently been refurbished and modernized. The counter is in the form of a low background shielded room with six uncollimated NaI(Tl) scintillation detectors (thickness 10.2 cm, diameter 15.2 cm) mounted radially on a large steel annulus. The subject lies on a couch which is co-axial with the gantry and a stepper motor is used to drive the detectors along its length at a speed of 0.5 cm/s (total scan distance = 144 cm). For each

Table 1. Haematological values and % iron absorption in volunteers

Volunteer no.	Sex	Age (y)	Hb g/dl	MCH (pg)	Serum ferritin μg/l	% ^{59}Fe absorption
1	M	51	15.2	32.4	102	27.1
2	F	30	14.1	30.0	40	23.1
3	M	38	15.1	29.7	19	12.1
4	F	60	13.1	30.0	41	24.0
5	F	35	12.3	29.7	3	39.3
6	M	64	15.3	30.5	194	6.8
7	M	59	15.9	30.3	52	10.8
8	M	21	15.6	30.5	162	7.3
9	M	55	15.5	31.8	49	23.3
10	F	55	13.0	31.8	14	17.7
11	F	48	14.1	31.0	43	21.1
12	F	48	13.7	33.2	9	43.3
13	F	35	12.6	30.6	10	37.4
Normal range	M	–	>13.0	>27	15–300	–
	F	–	>11.5	>27	15–200	–

traverse the counts from all the detectors are recorded as a pulse height spectrum using commercial gamma spectroscopy software (Genie PC from Canberra Packard, Pangbourne, UK), which is also used to control the scanning motion.

In order to minimize sensitivity changes due to redistribution of ^{59}Fe within the body, an optimized counting technique was used; this has two distinct components (Walters *et al.* 1975; Thomson 1981). Firstly, count rates were measured within a wide energy band (0.10–0.95 MeV) lying below the main photopeaks (at 1.10 and 1.29 MeV). Secondly, count rates in this band were measured during two traverses along the subject and also over 40 s periods, with the gantry static at each end of the subject; subsequently, a fraction (0.28) of the combined static count rates was added to the traverse count rate.

Volunteers were measured in the whole body counter on three occasions on two separate days: before the test drink to estimate background radioactivity, approximately 20 min after the drink to establish a baseline count rate and, finally, 14 days later to measure absorption (retention). Each measurement took approximately 15 min. The drink of ^{59}Fe labelled Spatone Iron-Plus was administered after an overnight fast and subjects were asked to continue fasting for at least a further hour after completion of the baseline count. (Measurements on a scintillation counter indicated that less than 0.5% of the activity was retained on the plastic beaker.)

On each measurement day, room background and a reference standard of ^{59}Fe were counted. The ratio of the subject to standard count rate was calculated at baseline and 14 days, with iron absorption being equal to the 14-day ratio expressed as a percentage of the baseline ratio. This procedure automatically corrects for radioactivity decay and temporal variations in whole body counter sensitivity. The total radiation dose to each subject was approximately 50 μSv.

Results

Table 1 gives information about iron status and iron absorption for the volunteers. All had haemoglobin concentrations and MCH values within the normal range for the laboratory. Serum ferritin concentrations varied from <15 μg/l to nearly 200 μg/l. The iron concentration in the batch of sachets used for the study was found to be 0.24 mg/ml—somewhat lower than the official analysis (see earlier). The mean iron absorption from the test dose was 23%. However, when iron absorption was plotted against log serum ferritin there was a highly significant negative correlation between the two (Figure 1).

After the absorption study had been commenced, it was learned that one subject (No. 10) had previously been anaemic, had not responded to oral iron and had been given intramuscular iron to correct the anaemia. It seemed advisable to omit this subject from the absorption study as there was a possibility of intestinal malabsorption. If the relationship between iron absorption and serum ferritin is examined in the 12 remaining subjects the value of r is −0.785, $P = 0.002$.

Discussion

Spatone Iron-Plus contains ferrous sulphate, with the only other cations present in significant concentrations being

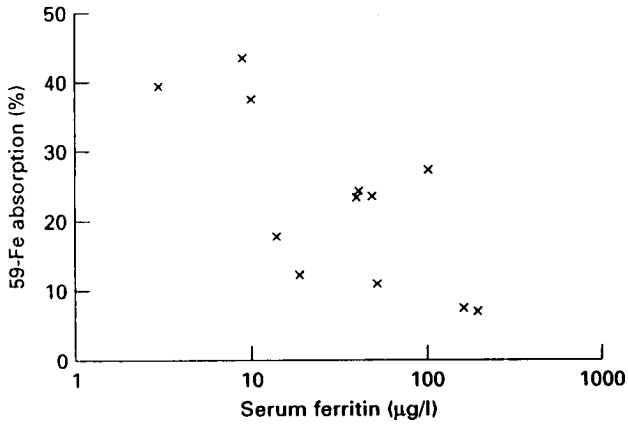

Figure 1. Per cent absorption of ^{59}Fe is inversely related to $\log_{10}$ serum ferritin concentration: % absorption = 50.6–18.0 ($\log_{10}$ serum ferritin), $n = 12$, $r = -0.785$, $P = 0.002$.

calcium and magnesium. Calcium reduces iron absorption from food but there is no agreement on the longer-term effects of high calcium intake on iron status (British Nutritional Foundation 1995). Studies in rats showed that inhibition of ferrous iron absorption by calcium required a 10-fold excess of calcium, so it is unlikely that iron absorption will be inhibited by the concentration of calcium present in Spatone (Barton *et al.* 1983).

The iron from Spatone Iron-Plus is an effective supplement to dietary iron as it is readily available. Normally iron absorption from diets varies from 5–15% and similar % absorption is found for therapeutic iron preparations (Bothwell *et al.* 1979). The mean absorption was 23% for Spatone Iron-Plus, but % absorption is related to the level of body iron stores as indicated by serum ferritin concentration. Thus our study suggests that subjects with absent iron stores (serum ferritin 10 μg/l or less) will absorb approximately 40% of a dose of about 3 mg Fe, whereas those with high levels of storage iron (serum ferritin approximately 200 μg/l) will absorb less than 10% of the dose. This relationship between % absorption of a standard 3 mg dose of ferrous sulphate and iron stores is well known (Cook *et al.* 1974; Magnusson *et al.* 1981).

The value of Spatone Iron-Plus as a tonic should be assessed in the context of iron requirements. Total iron requirements (absorbed iron), as the median (95 percentile) mg/d, are 1.05 (1.37) in adult males, 1.68 (3.27) in girls aged 11–14 y and 1.46 (2.94) in women (18–45 y). These translate into dietary iron requirements (95 percentile) from 9 mg/d for adult males to over 20 mg for girls and young women (Commission of the European Communities 1993). Pregnancy imposes a cost of an additional 2 mg Fe/d (averaged throughout the pregnancy) over male requirements.

Our studies of the absorption of Spatone Iron-Plus suggest that iron deficient subjects (having serum ferritin concentrations of less than 10 μg/l) will absorb up to 40% of the nominal 3 mg Fe in a 10 ml sachet taken on an empty stomach. There is no reason to suppose that absorption will decrease significantly in % terms if the dose is increased to 20 ml/d; 20 ml of Spatone Iron-Plus will thus provide an additional iron uptake of 2.4 mg which is sufficient to provide the median extra iron needed by young women or the extra needs of pregnancy. However, if the mineral water is taken with a meal then absorption may be reduced or even increased depending on the composition of the meal.

Our study suggests that people with high iron stores will absorb less iron from Spatone Iron-Plus. This is in accordance with the behaviour of 'reference' doses of ferrous sulphate (3 mg of iron). Thus subjects with high iron stores should not readily develop iron overload at this level of supplementation. Continuous use may, of course, accelerate the development or iron overload in subjects who have genetic haemochromatosis, but this is a risk which is common to all supplements and fortified foods.

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References

Barton J.C., Conrad M.E. & Parmley R.T. (1983) Calcium inhibition of inorganic iron absorption in rats. *Gastroenterology* **84**, 90–101.

Baynes R.D. (1994) Iron deficiency. In *Iron Metabolism in Health & Disease* (eds J.H. Brock, J.W. Halliday, M.J. Pippard & L.W. Powell), W.B. Saunders, London, pp. 189–225.

Bothwell T.H., Charlton R.W., Cook J.D. & Finch C.A. (1979) *Iron Metabolism in Man.* Blackwell Science, Oxford.

Bothwell T.H., Charlton R.W. & Motulsky A.G. (1989) Haemochromatosis. In *the Metabolic Basis of Inherited Disease* (eds C.R. Scriver, A.L. Beaudet, W.S. Sly & D. Valle). McGraw-Hill, New York, pp. 1433–1462.

British Nutrition Foundation (1995) *Report of the Task Force. Iron—Nutritional and Physiological Significance.* Chapman and Hall, London.

Commission of the European Communities (EC) (1993) *Nutrient and Energy Intakes for the European Food, 31st Series.* Office for Official Publications of the European Communities, Luxembourg.

Cook J.D., Lipschitz D.A., Miles L.E.M. & Finch C.A. (1974) Serum ferritin as a measure of iron stores in normal subjects. *American Journal of Clinical Nutrition* **27**, 681–687.

Cook J.D., Skikne B.S., Lynch S.R. & Reusser M.E. (1986) Estimates of iron sufficiency in the US population. *Blood* **68**, 726–731.

Davies I.H., Jacobs A. & Owen G.M. (1974) The use of a whole body counter for measuring bone marrow erythroid activity by profile scanning. *Journal of Nuclear Medicine and Biology* **1**, 145–147.

Dawson D.W., Hoffbrand A.V. & Worwood M. (1991) Investigation of megaloblastic and iron-deficiency anaemias. In *Practical Haematology* 7th edn (eds Dacie J.V. & Lewis S.M.), Churchill Livingstone, Edinburgh, pp. 387–420.

De Maeyer E. & Adiels-Tegman M. (1985) The prevalence of anemia in the world. *World Health Statistics Quarterly* **38**, 302–316.

Department of Health and Social Security (1981) *Nutritional Aspects of Bread and Flour. Report on Health and Social Subjects No. 23*. HMSO, London.

Hallberg L., Bengtsson C., Lapidus L., Lindstedt G., Lundberg P-A. & Hulten, L. (1993) Screening for iron deficiency: an analysis based on bone-marrow examinations and serum ferritin determinations in a population sample of women. *British Journal of Haematology* **85**, 787–798.

Hibbard B.M. (1988) Iron and folate supplements during pregnancy: supplementing everyone treats those at risk and is cost effective. *British Medical Journal* **297**, 1324–1326.

Horn E. (1988) Iron and folate supplements during pregnancy: supplementing everyone treats those at risk and is cost effective. *British Medical Journal* **297**, 1325–1327.

Magnusson B., Bjorn-Rasmussen E., Hallberg L. & Rossander L. (1981) Iron absorption in relation to iron status. Model proposed to express results to food iron absorption measurements. *Scandinavian Journal of Haematology* **27**, 201–208.

Thomson W.H. (1981) *Calibration and Clinical Use of a Scanning Whole Body Counter*. Unpublished PhD thesis, University of Wales College of Medicine, Cardiff.

Walters G.O., Jacobs A., Worwood M., Trevett D. & Thomson W. (1975) Iron absorption in normal subjects and patients with idiopathic haemochromatosis: relationship with serum ferritin concentration. *Gut* **16**, 188–192.

Worwood M. (1994a) Disorders of iron metabolism. *In Scientific Foundations of Biochemistry in Clinical Practice*. 2nd edn (eds D. Williams & V. Marks), Butterworth-Heinemann, Oxford, pp. 446–452.

Worwood M. (1994b) Genetics of haemochromatosis. *Balliere's Clinical Haematology* **7**, 903–918.

Worwood M. & Darke C. (1993) Serum ferritin, blood donation, iron stores and haemochromatosis. *Transfusion Medicine* **3**, 21–28.