multiple birth. Familial occurrences of dizygotic twinning found in this and other epidemiological studies are at least in part due to genetic factors.

The risk of twin births to monozygotic mothers was not significantly increased, but monozygotic mothers had significantly more same sex twin births. Comparison of estimated and expected numbers of monozygotic twin births suggests that this increase is a result of monozygotic twinning. Although other studies have suggested a familial component in monozygotic twinning, samples have been small and the methods susceptible to recall bias.^{4,6}

This population based study shows that women who are twins have an increased risk of giving birth to twins. Contrary to results based on self reports,⁴ this registry based study, free from recall bias, does not support the existence of a common aetiology for monozygotic and dizygotic twinning.

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Haem iron intake in 12-36 month old children depleted in iron: case-control study

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Abstract

Objective—To compare the intakes of haem and non-haem iron in iron depleted and iron replete children.

Design—Case-control study.

Setting—Early Childhood Centres and a long day care centre in Sydney, Australia.

Subjects—Children aged 12-36 months depleted in iron and controls matched for age and sex.

Mean outcome measures—Iron status by using plasma ferritin concentration. A three day weighed dietary intake record completed by the parents. Risk factors for iron deficiency assessed by questionnaire.

Results—Fifty six iron depleted and 68 iron replete children participated. The average daily intake of haem iron was significantly lower in the iron depleted group ($t=2.392$, $P=0.018$); there was a tendency towards a lower average daily intake of non-haem iron ($t=1.724$, $P=0.086$) and vitamin C ($t=1.921$, $P=0.057$) for iron depleted children. Low intake of haem iron (<0.71 mg/day) was significantly associated with iron depletion with an odds ratio of 3.0 ($P=0.005$). The proportion of iron depleted children who were given whole cows' milk before 12 months of age was almost double that of iron replete children; multivariate analysis showed that both haem iron intake and age of introduction of cows' milk were independently associated with iron depletion.

Conclusions—The results of this study show that, in young children in developed countries, a lower haem iron intake is a major risk factor for iron

depletion; the introduction of whole cows' milk before 12 months is further confirmed as a risk factor. Parental education on nutrition should now focus on these two aspects of nutrition for infants and young children.

Introduction

Young children, whether in developing or developed countries, are at risk of iron deficiency because rapid growth imposes large iron needs and the bioavailability of iron in the diet of infants is low.^{1,2} Recent studies have shown that iron deficiency may have profound non-haematological consequences, including lower developmental scores and impaired behaviour.^{3,4} Prospective studies in Costa Rica and Chile have demonstrated that the effects of iron deficiency anaemia on intelligence quotient (IQ) may be irreversible.^{5,5}

Adult studies have shown that iron from meats (haem iron) is important in iron nutrition because it may be seven times more readily absorbed than iron from vegetable sources (non-haem iron).⁶ Several factors have been reported to be associated with poor iron status in infants and young children, including low birth weight,⁷ low socioeconomic status,⁸ early introduction of cows' milk (before 12 months),⁹ excessive intake of cows' milk after 12 months,¹⁰ late introduction of solids,^{11,12} and low total iron intake.¹³

We have not been able to find any published studies which have directly measured the intake of haem iron in children in the 12-36 month age group. We tested

the hypotheses that iron depleted children aged 12-36 months have a lower intake of haem and non-haem iron than iron replete children of the same age.

Subjects and methods

Informed consent for the measurement of plasma ferritin concentration was obtained from parents whose children were being screened for increased blood lead concentrations. Most of these children, in Sydney, Australia, were attending early childhood centres and a long day care centre, a small number being participants in a community prevalence survey of increased blood lead concentrations. Children aged 12-36 months were recruited between February and September 1994.

Iron depletion was defined as a plasma ferritin concentration of $\leq 10 \mu\text{g/l}$.² Of the 479 children screened for iron status, 76 were found to have a concentration of $\leq 10 \mu\text{g/l}$. The parents of these children were approached and asked to complete a three day weighed record of their child's dietary intake. A control group was formed by identifying the iron replete child who was nearest in the sampling order to each identified iron depleted child, was of the same sex, and was within 6 months of age. Parents of the children selected for the control group were subsequently contacted and invited to undertake a three day weighed dietary record for their child. Parents were aware of their child's lead and iron status but were requested not to make any changes to their child's diet until after the dietary record had been completed.

Parents of children in the two study groups completed a questionnaire which inquired about demographic details and known risk factors for the development of iron deficiency in infants and preschool children. A dietitian instructed parents of participating children on the method of keeping a three day weighed and measured food record; electronic scales and a record book were provided. The dietitian also weighed the children when possible—for example, when the child was cooperative.

Data from the records were entered into a dietary analysis computer program¹⁴ which utilises the Australian standard food composition tables.¹⁵ When

necessary, recipes were entered or dietary analysis was obtained from manufacturers of processed foods. Iron intake from meat, poultry, fish, and processed meats was calculated manually from the records. From earlier laboratory studies it is generally assumed that 40% of the iron in all animal tissues, including meat, liver, poultry, and fish, is haem iron.¹⁶ Outside of the laboratory, however, it is impossible to separate the effects of the haem and non-haem iron content of meat, fish, and poultry because of the absorption enhancing qualities of haem iron as well as other factors. For the purposes of this study all the iron in meat has been considered as haem iron. Data were entered on a computerised database¹⁷ and were analysed with SPIDA.¹⁸ Categorical data were analysed by using χ^2 tests or the conditional binomial exact test (when cell sizes were lower than five). Dietary intake and other continuous data were analysed with two sample t tests. A square root transformation of haem and non-haem iron intakes was used to fulfil the assumptions of normality; haem iron intakes were compared with a t test with unpooled standard deviations as the variance differed significantly between the two groups. Family incomes were compared by using the Wilcoxon rank sum test.

To report the effects of low nutrient intakes in a way which is useful for clinicians, data were dichotomised. Haem and non-haem iron intakes were dichotomised around the median of the daily intake for the control group (0.71 mg/day and 5.07 mg/day, respectively). This was done because there is no information available on recommended daily intakes for haem and non-haem iron. The only recommendation available is for total iron intake (6-8 mg/day for children aged 1-11 years).¹⁹ Vitamin C was similarly dichotomised around the median of the daily intake for the control group (85.9 mg/day) and also around the Australian recommended dietary intake of 30 mg/day.¹⁹ The effects of low nutrient intakes were analysed by using logistic regression analysis with potential confounders controlled for.

Ethical approval for all components of this study was obtained from the Central Sydney Area Health Service ethics committee before the study started.

Results

A total of 124 children participated in the dietary intake study: 56 iron depleted children and 68 iron replete control children. The two groups did not differ in age or weight (table 1).

There were no significant differences between the groups in socioeconomic status as measured by family income (median \$A32 001-40 000 for the iron depleted group and greater than \$A50 001 for the iron replete group, $z=1.35$, $P=0.178$). There were no significant differences between the groups in the proportions of children who were born prematurely (<36 weeks gestation) (odds ratio (95% confidence interval) 1.34 (0.37 to 4.86), $P=0.655$); breast fed (3.47 (0.85 to 14.18), $P=0.083$); or given a vitamin supplement (1.13 (0.18 to 7.0) $P=0.900$) or iron supplement (1.26 (0.29 to 5.55), $P=0.757$). Only one child (who was iron depleted) was reported to be vegetarian. A significantly higher proportion of iron depleted children than iron replete children had cows' milk introduced before the age of 1 year (2.72 (1.24 to 5.93), $P=0.012$).

Table 2 shows the average daily intakes of carbohydrate, fat, protein, energy, vitamin C, calcium, and haem and non-haem iron for the two groups. In the iron depleted group only the average intake of haem iron was significantly lower.

When the intakes of haem and non-haem iron were dichotomised around the median of the daily intakes for the control group, only low intake of haem iron was

Table 1—Descriptive details of children aged 12-36 months in central Sydney. Figures are means (SD)

Variable	Iron depleted children (n=56)	Iron replete children (n=68)	Significance
Age (months)	20.7 (4.3)	20.1 (4.5)	$t=0.805$; $P=0.423$
Weight (kg)	12.9 (1.8)	12.7 (1.8)	$t=0.728$; $P=0.469$
Ferritin ($\mu\text{g/l}$)	7.1 (2.7) (n=56)	22.2 (9.6) (n=68)	

Table 2—Average daily nutrient intake of iron replete and iron depleted children aged 12-36 months. Figures are means (SD)

Nutrient	Iron depleted children (n=56)	Iron replete children (n=68)	Significance
Carbohydrate (g)	117 (31)	125 (36)	$t=1.378$; $P=0.171$
Fat (g)	42.1 (9.0)	42.5 (13.1)	$t=0.175$; $P=0.861$
Protein (g)	42.9 (10.1)	45.2 (14.3)	$t=1.026$; $P=0.114$
Total energy (MJ)	4.157 (1.011)	4.451 (1.101)	$t=1.537$; $P=0.127$
Vitamin C (mg)	98 (55)	111 (68)	$t=0.921$; $P=0.057$
Calcium (mg)	858 (252)	797 (269)	$t=1.311$; $P=0.192$
Haem iron (mg)	0.28 (0.24)	0.42 (0.35)	$t=2.329$; $P=0.018$
Non-haem iron (mg)	4.7 (2.17)	5.29 (2.03)	$t=1.724$; $P=0.086$

significantly associated with iron depletion with an odds ratio of 3.0 (1.4 to 6.5; $P=0.005$). A multivariate analysis determined that the effects of the introduction of cows' milk before 12 months of age and intake of haem iron were independent. The odds ratio for low intake of haem iron in this model was 3.0 (1.3 to 6.8; $P=0.009$) and for cows' milk introduced before 12 months of age was 2.44 (1.09 to 5.44; $P=0.03$). There was no association in these data between low intake of vitamin C and iron depletion.

Discussion

Low iron stores represent a state of vulnerability for progression to iron deficiency and iron deficiency anaemia. The body's regulation of iron metabolism is very effective in working against the development of iron deficiency if the diet supplies sufficient absorbable iron.²⁰ We were unable to find published reports of other studies in which the intake of haem and non-haem iron of iron depleted and iron replete preschool children has been directly measured. The results of our study show that iron depleted children have a significantly lower intake of haem iron than iron replete children. These data indicate that in the context of a developed country haem iron intake is an important factor in the development of iron depletion. There was no significant difference between the two groups in the intake of non-haem iron, suggesting that non-haem iron intake per se is less important than haem iron intake. While there was no difference in vitamin C intake between the two groups, the average intake of vitamin C for both groups exceeded the Australian recommended daily intake of 30 mg/day.¹⁹

Children with low haem iron intake (defined as <0.71 mg/day, the median of the control group daily intake) were three times as likely to be iron depleted than those with a haem iron intake ≥ 0.71 mg/day. This strong association was independent of the effect of introduction of cows' milk before 12 months of age. There was no significant association between low non-haem iron intake (<5.07 mg/day) or low vitamin C intake (<30 mg/day or <85.9 mg/day (median of the control group daily intake)) and iron depletion.

Australia, like Great Britain, most Western European countries, the United States of America, and other developed Western nations, has a high standard of living. Australia also has a well developed social security system and a range of free services which provide advice and support (including nutrition counselling) to parents of infants and young children. The results of this study indicate that in developed countries public health campaigns which aim to reduce iron depletion and iron deficiency in preschool children should encourage adequate meat intake. This is in addition to the current recommendations—that is, no cows' milk before 12 months; restrict volume of cows' milk to less than 600 ml a day after 12 months of age; introduce solids at 4–6 months; introduce lean meat at 6–9 months.

The median of the control group's daily intake of haem iron of 0.71 mg/day can be obtained from one heaped tablespoon of finely chopped grilled lean beef or lamb, about $3\frac{1}{2}$ –7 heaped tablespoons of skinless, chopped, baked chicken, or about eight heaped tablespoons of steamed, mashed fish fillet.¹⁵

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Key messages

- Iron deficiency is the most common nutritional deficiency worldwide
- Iron deficiency may cause lowering of developmental scores and may impair behaviour
- Iron depleted 1 and 2 year old children have lower haem iron intake
- Introduction of cows' milk as the main milk drink should be delayed until after 12 months of age
- Public health campaigns to reduce iron depletion and iron deficiency in preschool children should encourage adequate meat intake

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