

1-1-2023

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10.1111/jhn.13180

Johnston, M., O'Sullivan, T., Devine, A., Wallace, R., Costello, L., & Sambell, R. (2023). Toddlers may be getting enough iron in long day-care services after all. *Journal of Human Nutrition and Dietetics*. Advance online publication. <https://doi.org/10.1111/jhn.13180>

This Journal Article is posted at Research Online.
<https://ro.ecu.edu.au/ecuworks2022-2026/2435>

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Toddlers may be getting enough iron in long day-care services after all

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Abstract

Background: Previous research has suggested that toddlers are not provided with adequate dietary iron in long-day care (LDC) services. However, the iron bioavailability provided is unknown. The present study aimed to investigate the amount and bioavailability of iron provided to toddlers aged 2–3 years at LDC services.

Methods: A cross-sectional audit was conducted using a 2-day weighed food record of 30 LDC services. Iron provision (not child intake) in LDC services across Perth, Australia was compared with the estimated average requirements (EAR) and LDC services provision guidelines (50% of EAR = 2 mg/day based on a 14% bioavailability factor). Bioavailability was estimated per mealtime using haem and non-haem iron, ascorbic acid, animal protein, calcium, soy, eggs and phytates using two pre-existing algorithms (by A. P. Rickard and colleagues and H. Hallberg and H. Hulthen).

Results: Median iron supplied (2.52 mg/day, interquartile range [IQR] = 2.43–3.17) was above the 50% of EAR of 2.0 mg/day ($p < 0.001$). Median bioavailable iron was 0.6 mg/day (IQR = 0.54–0.8) using the method of Rickard et al. and 0.51 mg/day (IQR = 0.43, 0.76 using that of Hallberg and Hulthen). The top three foods contributing to iron provision were bread, breakfast cereals and beef.

Conclusions: Our results suggest that LDC services in Perth are meeting the minimum recommendation of provision of 50% of the iron EAR, and also that toddlers are provided with sufficient bioavailable iron. Future strategies should focus on promoting food combinations to maintain the iron bioavailability in meals currently served at LDC services.

KEYWORDS

bioavailability, dietary iron, Early Childhood Education and Care, iron, Long-day-care services, toddlers

Key points

- Food provision of dietary iron at mealtimes was highly bioavailable to toddlers attending Australian metropolitan long day care (LDC) services.
- Utilising two existing algorithms, the iron provided was found to be highly bioavailable.
- Future education strategies for LDC services to maintain iron bioavailability should focus on mealtime food combinations, reflecting a variety of foods.

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INTRODUCTION

Iron deficiency is the most common childhood micronutrient deficiency in the western world.¹ In the first 3 years of life, iron requirements are significantly higher (per mg/kg) than other life stages.^{2,3} This is because of iron's role in blood expansion, growth and development.³ Low iron stores have been associated with poor cognition, motor and social emotions in pre-schoolers aged 4 years.^{4,5} It is suggested that iron deficiency occurring before the age of 3 years could lead to permanent cognitive and neurological impairments,^{4,5} thus affecting a child's long-term developmental outcomes.

Cross-sectional Australian and international dietary surveys show that toddlers and babies (aged 10–20 months) under consume iron.^{6–9} For the Australian studies, it was reported that between 10%–18% of children are not consuming enough iron, putting them at risk of possible iron deficiency (aged 12–20 months).^{6,8} These studies found children consumed most of their iron from fortified breakfast cereals, yet red meat and dark leafy vegetables were not substantial sources, despite being iron-rich foods.^{6,8,10}

Haem and non-haem are the two types of dietary iron.² Haem iron is found in animal tissue and blood including lamb, beef, chicken, fish and pork, with an average bioavailability of 25%.² Although non-haem iron is found in cereals, dark leafy greens and legumes where approximately 10% of non-haem iron is absorbed, but is dependent on other dietary factors consumed at the same time of the meal.² Because iron bioavailability is an indicator of iron status, it is important to recognise foods and nutrients which enhance and inhibit its absorption.¹¹ This is especially important for toddlers as a result of low levels of intake^{6–9} of highly bioavailable iron and the potential impact on developmental outcomes.⁴

Dietary factors that enhance iron absorption include ascorbic acid and animal protein.¹¹ Overall, ascorbic acid is a well-researched enhancer of non-haem iron.¹² Numerous studies have shown a progressive increase in iron absorption through vitamin C supplementation and decreased risk of iron deficiency when vitamin C rich food such as citrus fruits, mangoes, sweet potato and capsicum were consumed concurrently with iron-rich foods.^{12,13} Its effect has also been seen to overcome most inhibitors including calcium and phytates.^{14,15} Animal protein, including meat, poultry, fish and seafood, has also been shown to enhance non-haem iron absorption.¹¹ Various older studies have observed this enhancement with an increase of 100%–150% in non-haem iron absorption when meat was concurrently consumed.¹⁶

Inhibitors of iron absorption include calcium, phytates, polyphenols soy and egg.¹¹ Multiple international studies show a positive correlation between the high consumption of calcium-rich foods, low dietary iron intake and iron deficiency in toddlers.^{17,18} In addition to

the inhibitory effects of calcium, high consumption of milk has shown to displace the consumption of iron-rich foods in infants and toddlers.¹⁹ However, when the inhibitor effect of calcium is applied to a multiple-meal approach, with a wide variety of foods and enhancers, the effect of calcium is limited.²⁰ The predominant inhibitor of iron is phytates. Phytates occur in a relatively high amount in whole-grain cereals and legumes. The adverse effect of phytates on iron bioavailability is dose-dependent and occurs in amounts of approximately 2–10 mg per meal.^{21,22} The inhibitory effects of phytates on iron are prominent in plant-based diets, where its effect on iron may become more apparent because of the societal shift to the inclusion of more plant-based protein.²³

Long day care (LDC) services provide opportunities to promote healthy eating considering approximately 1.3 million children attend childcare services in Australia, where LDC services are the most common type of service attended.^{24,25} International and national studies analysing the nutritional intake of children attending LDC services, from a range of age groups, found an inadequate provision of iron-rich meat and vitamin C rich fruits and vegetables compared to the country-specific guidelines.^{7,26–29} Moreover, to our knowledge, there is no national or international research specifically investigating the bioavailability of iron provided at mealtimes by LDC services.

Thus, the present study aimed to analyse the amount, source and bioavailability of iron provided to toddlers (2–3 years) in Western Australia LDC services and compare the findings with the respective estimated average requirements (EAR) for toddlers aged 1–3 years. We hypothesised that toddlers were not being provided with adequate bioavailable iron when compared to the EAR for iron.³⁰ This research will contribute to the current evidence of food provision in LDC by providing a deeper understanding of food provision from a mealtime perspective to support interpretation of future nutrition guidelines and guiding resource and training narratives for this sector.

METHODS

Study design

The present study was a part of broader research into the food and drink provision at LDC services in Western Australia. The original protocol has been described previously.³¹ This study utilised a cross-sectional weighed ingredient audit of food and drink served to toddlers aged 2–3 years attending LDC services in Perth, Western Australia, over 3 years: 2015, 2016 and 2017. Socio-demographics of the services were analysed via the Socio-Economic Indexes of Areas (SEIFA).³² The Index of Relative Socio-economic Disadvantage (IRSD) ranks

areas within Australia from one to ten in order of most to least disadvantaged.³²

Study population

A minimum of 27 LDC services was required to detect statistical outcomes as determined through the Wilcoxon signed-rank test calculation (confidence 5%, power 80% with medium effect size [$d=0.5$]). The services were selected through the Australian Children's Education and Care Quality Authority register. Every 10th service was randomly selected and asked to participate via a telephone call (maximum of three calls before removal). Inclusion criteria for selection included operating for 8 h or more in a day, 5 days a week, and both preparing and serving food onsite. Likewise, the participants were included if they resided in the Perth Metropolitan Area Western Australia, with a postcode between 6000 and 6199. If the services wished to participate, when meeting the inclusion criteria, they were forwarded an information letter in addition to a telephone call, 2 days post agreement to participate, to arrange appropriate days for data collection.

Data collection

A cross-sectional audit was conducted using a 2-day weighed food record to determine provision, this research did not capture child food intake. This prospective method of data collection was utilised as it is considered the 'gold standard' of dietary measures.^{33,34} Six trained research assistants, who were undergoing an undergraduate or postgraduate nutrition or dietetic degree at the time, collected the data. Preceding collection, the assistants were trained, which comprised of a 4-h standardised workshop, and included the use of calibrated scales for food measurement, demonstration of daily tasks and recording of weights. The assistants also completed an "I'M ALERT" safety course³⁵ which detailed basic food safety and handling practices in a commercial setting. Before the visit, the assistants also attended an orientation and completed any necessary Occupational Health and Safety inductions, completed a risk assessment and shared a certificate of currency for insurance.

The weights, before and after cooking, of all food served, were collected for morning tea (MT), lunch (L), afternoon tea (AT) over two consecutive days using calibrated scales (SJ-5001HS; A&D Australasia). Foods offered to toddlers with dietary restrictions, including vegetarian, food allergies and intolerances, as well as additional servings meals, were captured within the data collection. A spare set of calibrated scales (same model) were on hand if needed. On the first day, the assistants weighed ingredients (raw and cooked) and prepared MT, where weights were added to a prepopulated Excel spreadsheet (Microsoft Corp.).³⁶ This was recorded, along

with attendance numbers of all children, staff who would consume a 'child size' meal and the average final weight of one serve of the meal (weighed three times and the average taken). This was repeated for L and AT over two consecutive days. Post data collection, raw weights and ingredients were analysed via the recipe and meal plan function using Xyris Foodworks, version 10.³⁷ These data were then compared against 50% of the EAR for iron (2.0 mg/day) for a reference person aged 2–3 years.³⁰ Male and female iron requirements are the same.³⁰ The EAR was chosen as a comparison for this project because it is suitable for population-based nutrient requirements. LDC services are recommended to provide at least 50% of a child's nutrient intake across a main meal and two snacks.^{38–40,31} Bioavailable iron was analysed against a benchmark of 0.28 mg/day, which was calculated by using a 14% absorption factor of 50% of the EAR for iron, because this is the absorption factor used in the EAR calculations.³⁰ Our project did not capture foods and drinks offered at home.

Iron bioavailability algorithms

To increase rigor in our findings, two established algorithmic calculations were utilised to assess iron bioavailability at each meal: the algorithms of Rickard et al.⁴¹ and Hallberg and Hulthen,⁴² respectively, as detailed below.

Algorithm of Rickard et al.⁴¹

The algorithm created by Rickard et al.⁴¹ uses ascorbic acid, calcium, phytate, non-haem iron and animal protein to estimate iron bioavailability. The calculation was chosen for its application in other similar population-based dietary surveys.⁴¹ It utilises dietary data from known enhancers and inhibitors to estimate iron bioavailability.⁴¹ The algorithm is:

$$\text{Available Fe(mg)} = (\text{percentage of available non} \\ \text{-haem Fe} \times \text{NH}) + (0.25 \times \text{HI})$$

Percentage of available non – haem Fe

$$\begin{aligned} & (1 + 1n(1 + 0.00056 \times AA)) \\ = 22.42 \times & \frac{\times (1 + 1n(1 + 0.0008 \times AT))}{(1 + 1n(1 + 0.0008 \times C))} \\ & \times (1 + 1n(1 + 0.0033 \times P)) \\ & \times (1 + 1n(1 + 0.0424 \times NH)) \end{aligned}$$

where HI is haem iron (mg), AA is ascorbic acid (mg), AT is red meat, poultry⁴¹ and fish (g), C is calcium (mg), P is phytates (mg), and NH is non-haem iron (mg).

Algorithm of Hallberg and Hulthen⁴²

The algorithm by Hallberg and Hulthen⁴² was chosen as internal validation has shown good prediction of iron bioavailability. This algorithm was developed using the absorption of iron from a basal meal.⁴² Hallberg and Hulthen⁴² estimated the mean absorption of iron from this meal, without the presence of known enhancers or inhibitors, was 22.1% + 0.18%. By contrast to the algorithm of Rickard et al.,⁴¹ the algorithm by Hallberg and Hulthen⁴² considered iron status, which is a non-dietary factor that influences iron bioavailability. Thus, this algorithm adjusts to a reference dose absorption of 40%, which equates to a 23 µg/l serum ferritin concentration.⁴² This algorithm utilises enhancers including ascorbic acid, animal protein (meat, fish and seafood). Additionally, it also accounted for soy protein, eggs, phytates and calcium to estimate the non-haem iron bioavailability.⁴² There were also special considerations for interactions between individual factors including animal protein with phytates and ascorbic acid with phytates, which was not included in the algorithm by Rickard et al.⁴¹ A calculation was used for each enhancer and inhibitor to obtain a factor that is detailed by Hallberg and Hulthen.⁴² Then, the basal absorption of 22.1% was multiplied by each individual factor.⁴² Thus, the value produced is the percentage of non-haem iron absorbed in a meal. Furthermore, the algorithm estimate haem bioavailability includes:

$$\text{Log haem iron absorption (\%)} = 1.9897 - 0.3092 \times \log \text{ serum ferritin}$$

This was then corrected to the calcium content in the meal by multiplying the value obtained by the calcium factor used for non-haem iron bioavailability.⁴² The estimated bioavailability for haem and non-haem iron was then totalled to obtain the estimated total iron bioavailability for each meal.

Dietary analysis

A nutritionist categorised iron-rich foods into haem and non-haem iron utilising current literature, which was subsequently checked by an experienced Dietetic researcher (Table 1). Because some foods contain both haem and non-haem iron, foods were allocated to either the haem or non-haem category based on the most prevalent form in the food.

Phytate containing foods (mg/100 g) were derived from a meta-analysis where the average amount was used per food type.²³ Heavily processed grains were not included in the list, as a result of the removal of the majority of phytates from processing, soaking, heating and fermentation, thus being impractical.²³ Therefore, only unprocessed grains (wholewheat, corn, brown rice) or grains that had

TABLE 1 Classification of iron containing foods into either predominantly haem or non-haem iron.⁴³

Haem iron	Non-haem iron
Organ meat/offal	Vegetables (e.g., kale, spinach, potato)
Poultry	Grains and cereals (bread, cereals)
Beef	Beans
Fish and shellfish	Legumes
Game meat	Nuts and seeds
Lamb	Soy products (tofu, soymilk)
Pork	Fortified or enriched products (bread, pasta and cereals)
	Peas
	Chocolate and cocoa powder
	Fruit/dried fruit
	Eggs
	Dairy products (milk, cheese, yoghurt)
	Herbs, spices and seasonings
	Yeast and yeast products

minimal processing, such as nuts, seeds, legumes (taking into account soaking) and soy were included.²³ Levels of calcium (mg), vitamin C (mg), total iron (mg), soy protein (mg), egg (g) and animal meat including fish, poultry and red meat (g) were obtained via analysis using Xyris Foodworks, version 10.³⁷ This was then applied to each meal (morning tea, lunch and afternoon tea) to calculate haem iron (mg/serve), non-haem iron (mg/serve) and bioavailable iron for both algorithms (% and mg/serve) in Access and Excel (Microsoft Corp.).³⁶ The sum of total iron, haem iron, non-haem iron and bioavailable iron for each day was calculated, followed by an average over the two consecutive days. The iron bioavailability (% and mg/day) for both algorithms was then averaged. The top 10 foods, with the highest contribution of iron (mean mg/serve), were ranked.

Statistical analysis

Data was analysed via SPSS, version 26 (IBM Corp.) using $p < 0.05$ for significance and 95% confidence intervals.⁴⁴ First, descriptive statistics were used to describe total iron (mg/serve) and bioavailable iron (mg/serve), amount of iron bioavailable (percentage), haem iron (mg/serve and percentage) and non-haem iron (mg/serve and percentage), averaged over two consecutive days at the 30 services. If normally distributed, the mean ± SD values were used, whereas median and interquartile ranges (IQR) were used for non-normal data. The data were then compared against recommendations or reference ranges.³⁰ This

included total iron (mg/day vs. EAR of 2 mg/day [50%]), bioavailable iron (mg/serve vs. 0.28 mg/serve [50%]) and percentage of iron bioavailable (compared with the value of 14% used in the EAR).³⁰ This was calculated using a one-sample Wilcoxon signed rank because the data were not normally distributed (normality assessed using Shapiro–Wilk, normal Q–Q plot and histograms). To determine whether the iron bioavailability estimated by the two algorithms differed from one another, root mean square error (RMSE), bias estimates, a paired sample *t*-test and a Bland–Altman plot were performed.

RESULTS

Study population

The average provision of total iron (mg/serve) per mealtime is shown in Table 2. The median provision of iron at the participating LDC services is shown in Table 3. Median total iron amount offered was above 50% of EAR recommendations for total iron, and 26 (86.7%) of the 30 services met or exceeded the EAR ($p < 0.001$, $z = 3.861$, $r = 0.7$ large effect), across the 2 days.

TABLE 2 Average total dietary iron per mealtime, served to children aged 2–3 years attending long day care services in the Perth Metropolitan Area over a 2-day period.

	Average total iron provision (mg/serve)		
	Day 1	Day 2	Average
Morning tea	0.67	0.80	0.74
Lunch	1.68	1.34	1.51
Afternoon tea	0.68	0.53	0.61
Total	3.04	2.67	2.86

TABLE 3 Total iron, haem iron, non-haem iron and bioavailable iron (based on Rickard et al.⁴¹ and Hallberg and Hulthen⁴²) compared to age-specific recommendations/reference values, served to children ages 2–3 years attending long day care services in the Perth Metropolitan Area.

	<i>N</i>	Median (mg/day IQR)	EAR (mg/day) recommendation for LDC services	<i>p</i> value ^a	LDC services meeting recommendation (%)
Total iron	30	2.52 (2.43, 3.17)	2.00 ^b	$p < 0.001$	87%
Haem-iron	30	0.40 (0.17, 0.56)	NA	NA	NA
Non-haem iron	30	2.17 (1.91, 2.79)	NA	NA	NA
Bioavailable iron (Rickard et al. ⁴¹)	30	0.60 (0.54, 0.80) ^c	0.28 ^c	$p < 0.001$	100%
Bioavailable iron (Hallberg and Hulthen ⁴²)	30	0.51 (0.43, 0.76) ^d	0.28 ^c	$p < 0.001$	100%

Abbreviations: EAR, Estimated Average Recommendation; IQR, interquartile range (25th percentile and 75th percentile); LDC, long day care; NA, not available.

^aSignificant difference was calculated via Wilcoxon one sample signed-rank test (95% confidence interval, $p < 0.005$).

^b50% of the estimated average recommendation of iron. Reference child: 1–3 years old.³⁰

^cIron bioavailability was measured using pre-existing algorithms by Rickard et al.⁴¹

^dIron bioavailability was measured using pre-existing algorithms by Hallberg and Hulthen.⁴²

^e50% of the Estimated Average Recommendation of iron. Reference child: 1–3 years old, taking into account 14% absorbability.³⁰

Iron bioavailability using algorithm of Rickard et al.⁴¹ compared to recommendations

Using the algorithm by Rickard et al.,⁴¹ the median bioavailable iron (0.6 mg/day) was statistically higher than what is accounted for by the EAR (14% of 2 mg/day = 0.28 mg/day, $p < 0.001$, $z = 4.782$, $r = 0.8$ large effect). On average, 24.3% of iron provided at LDC services was bioavailable, which was significantly higher than the 14% used for the EAR calculations ($p < 0.001$).

Iron bioavailability using the algorithm of Hallberg and Hulthen⁴² compared to recommendations

Similarly, to the Rickard et al. algorithm, the median bioavailable iron (0.51 mg/day) was statistically higher than what is accounted for by the EAR (14% of 2 mg/day = 0.28 mg/day, $p < 0.001$, $z = 4.792$, $r = 0.8$ large effect) using the algorithm by Hallberg and Hulthen.⁴² On average, 23.7% of iron provided at LDC services was bioavailable. This was significantly higher than the 14% recommended for the EAR ($p > 0.001$).

Comparison of the algorithms by Rickard et al.⁴¹ and Hallberg and Hulthen⁴² with respect to estimating iron bioavailability

A Bland–Altman plot describing the relationship between the two algorithms is shown in Figure 1. The plot demonstrates relatively low systemic bias; however, a cluster of values below zero can be seen which spread out as the values increase. The 95% limits of agreement (two standard deviations) of the Bland–Altman plot were

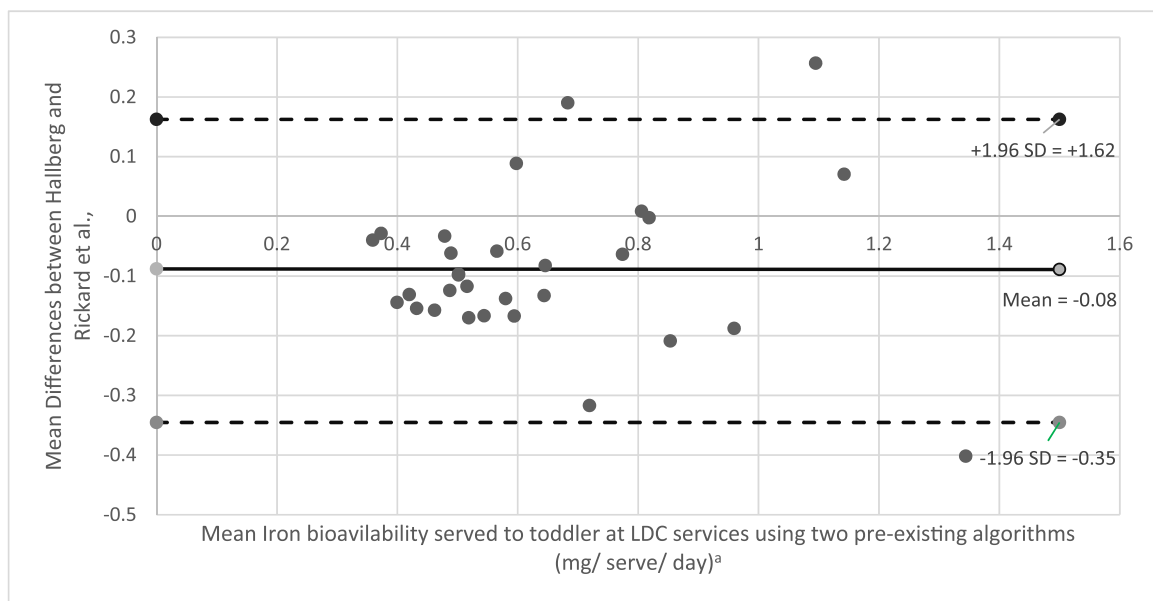


FIGURE 1 Bland–Altman plot for agreement in estimating iron bioavailability between two iron bioavailability algorithms. The solid line represents the mean difference of iron bioavailability and the dashed lines represent the 95% limits of agreement between the two methods. LDC, long-day care. ^aIron bioavailability was measured using pre-existing algorithms by Rickard et al.⁴¹ and Hallberg and Hulthen.⁴²

TABLE 4 Top 10 contributors of total dietary iron by percentage provided in this sample of long day-care services.

Food	Contribution to total iron (%)	Iron type (haem or non-haem)
Bread ^a	12.2	Non-haem
Breakfast cereals ^b	11.7	Non-haem
Beef mince	8.4	Haem
White flour	4.1	Non-haem
Pasta	3.9	Non-haem
Frozen mixed vegetables	3.9	Non-haem
Processed meat ^c	3.2	Haem
Baked beans	2.4	Non-haem
Egg	2.3	Non-haem
Peas, frozen	2.2	Non-haem

^aBread included white, wholemeal and multigrain sliced bread and white and wholemeal English muffins.

^bBreakfast cereals included Weetabix, rice puffs, cornflakes, oats, bran flakes and cheerio's.

^cProcessed meats included bacon, ham, polony, salami and sausages.

+1.62 and −0.35. On average, iron bioavailability calculated using the algorithm by Hallberg and Hulthen⁴² was 0.08 mg/serve/day below that of the algorithm by Rickard et al.,⁴¹ with a RMSE of 0.17 mg/serve/day. Furthermore, there was no statistical difference between the estimated bioavailability produced by the two algorithms from the paired sample *t*-test ($t = -1.63$, $p < 0.114$, d.f. = 29).

Iron-rich foods

Individual food items ranked by contribution to total iron provision in participating LDC services are shown in Table 4. The biggest contributor to iron provision was bread (12.2%), followed by breakfast cereals (11.7%) and beef mince (8.4%). Within the top 10 contributing foods, 80% of the foods were classified as predominantly non-haem sources, whereas 20% were haem iron sources.

DISCUSSION

The results of the present study illustrate similarity in estimating iron bioavailability between the two algorithms with a mean difference of 0.08 mg/serve/day. The average iron bioavailability calculated by both algorithms (24.3% and 23.8%) was significantly higher than the 14% accounted for in the EAR. Our results did not compare the accuracy of both algorithms because this is beyond the scope of our research. To date, there has not been a comprehensive validation study for adults or children, although a study⁴⁵ showed the algorithm of Hallberg and Hulthen⁴² to be the best predictor of serum ferritin concentrations in women compared to other iron bioavailability algorithms.

Participating LDC services provided a median of 2.52 mg/day, which is 26% above the recommended levels of total iron for toddlers aged 2–3 years (vs. EAR of 2.0 mg/day), across M, L and AT. It is important to emphasise that the present study only analysed provision not actual intake. We observed that 26 of the 30 services (87%) met or exceeded the recommended total iron

provision. This is inconsistent with other international studies, which reported an under provision of iron in LDC settings for children (3–5-year-olds) and babies/toddlers (aged 10–48 months), respectively.^{7,26} However, these studies compared dietary provision of iron with recommended dietary intake (RDI), which had a higher requirement than EAR (2.5 mg/day vs. 2.0 mg/day). These studies reported that LDC services provided 6.0–6.3 mg/day versus country-specific RDI values of 10 mg/day (USA) and 8 mg/day (the Netherlands).^{7,26} Furthermore, both studies had an average provision recommendation that was higher than our findings because of the inclusion of breakfast in their data.^{7,26} In Australia, it is estimated that 8% of pre-school children have anaemia and this prevalence increases in other vulnerable groups.^{46,47} Because iron deficiency is a leading cause of anaemia, this suggests that toddlers are likely provided with suboptimal iron within home settings.⁴⁷ Additionally, one study assessing children's intake in the home setting (full day including all mains meals and snacks) showed the inadequate total iron intake of children aged 1–5 years.⁸ LDC services provide opportunities for observational learning from peers, which has been associated with an increased interest and acceptance of new foods.⁴⁸ These habits learned during childhood have been shown to continue into adulthood and further illustrates the importance of adequate iron provision by LDC services for toddlers.

Our results illustrate LDC services provide significantly higher iron bioavailability (mean of 24.1% from both algorithms) compared to the EAR recommendation (14%) and other studies.⁴⁹ This suggests the iron provided to toddlers attending LDC services is being served with known enhancers such as ascorbic acid at the same time as limiting inhibitors. The highest contributor of ascorbic acid in this study included oranges, capsicum and white potato. The highest contributor of inhibitors to iron bioavailability included calcium from dairy (shredded cheese, full cream milk and yoghurt) and phytates including frozen peas, corn and brown rice. In our study, 86% ($n = 30$) of services provided toddlers with adequate bioavailable iron, which reflects a 14% improvement in compliance from total iron provision to bioavailable iron provision. This demonstrates that recommendations for ingredient combination at mealtimes may provide a “strengths based” approach to support LDC services meeting EAR recommendations through bioavailability.

In the present study, bread and cereals contributed most to the iron provision at LDC services with a combined provision of 23.8%. Our findings are consistent with other American, Australian, and Flemish studies that found the highest contribution of dietary iron in toddlers included iron-fortified bread or breakfast cereals, where meat including beef was also in the top three of iron providing foods.^{6,8,26,49} It is noted that breakfast cereal consumption was a high contributor of total iron provision even though breakfast provision was not analysed at LDC services.

Breakfast cereals were either used as an ingredient in recipes or given traditionally with milk at other meal opportunities. These findings further illustrate toddlers' dependency on non-haem iron sources, specifically breakfast cereals, and the importance of considering their value in this setting, when public health messaging suggests a reduction in processed foods which would include breakfast cereals.^{6,8,29} Furthermore, this is a particularly important message in light of the societal shift to plant-based protein sources which typically have higher amounts of phytates.²³ This should not be a deterrent to include plant-based protein sources in the diet because dietary modelling suggests that nutritional requirements can be met, when a proportion of haem sources of iron are exchanged with non-haem iron sources, in a well-planned diet.^{50–52}

The important contribution to iron provision of both refined breakfast cereals (ranked second highest contributor) and processed meat (ranked seventh) poses a challenge to LDC services because these are classified as discretionary foods which are not recommended in the childcare setting.²⁹ Research indicates overprovision of discretionary foods by toddlers at LDC services and at home can displace other core food groups such as vegetables.^{8,28,53} It is critical that training, guidelines and policy advice consider this and recommend alternatives to LDC services that align with the emerging considerations to limit discretionary food provision in LDC services that do not compromise iron bioavailability and provision. Such recommendations might include using plant-based sources of protein such as beans and legumes that contain non-haem iron at the same time as being classified as a vegetable.⁵⁴

Aligning with the reduction of refined grains and discretionary food provision, simple recipe modification that maintains iron provision and bioavailability is recommended (Table 5). Simple modifications to recipes commonly provided at LDC services could improve iron bioavailability, offering practical and realistic solutions to LDC services. The main aim for LDC services should be to serve iron-rich foods at every meal and to maximise iron absorption through providing accompanying vitamin C rich fruits and/or vegetables. Cost estimation of morning tea, lunch and afternoon tea meals and modified meals (Table 5), based on a large chain supermarkets online pricing at the time of this research, found that there was a negligible difference in price per child/day (\$2.51/\$2.53 AUD). This suggests modifications to meals are not necessarily more expensive and cost should not be considered as a deterrent.

Additionally, limiting inhibitors of iron will also increase iron bioavailability. Regarding calcium, this is a key nutrient in toddlers' growth and development, and thus it is not feasible to reduce dairy and dairy alternatives on the menu. However, research indicates that when ascorbic acid is served with non-haem iron, this prevents the inhibitor effects of calcium, polyphenols and phytates.^{14,15} This further emphasises the focus on

TABLE 5 Common meals served at LDC services and example ingredient exchanges to increase bioavailability of iron.

Example meal by LDC service	Bioavailable iron ^a (0.63 mg/day) ^b	Modified meal ^c	Bioavailable iron (0.63 mg/day) ^b
Morning tea: Multigrain toast with margarine, honey, vegemite and jam ^d	0.15 mg/serve	MT: Multigrain toast with baked beans ^e	0.44 mg/serve
Lunch: Bacon carbonara ^f	0.37 mg/serve	L: Tuna pasta with spinach/kale ^g	0.76 mg/serve
Afternoon tea: Mixed fresh fruit ^h	0.00 mg/serve	AT: Hummus and carrot sticks with fresh fruit ⁱ	0.17 mg/serve
Total	0.52 mg/serve/day		1.37 mg/serve/day

Abbreviations: AT, afternoon tea; L, lunch; LDC, long-day care; MT, morning tea; RDI, recommended dietary intake.

^aBioavailability was calculated via existing algorithm using type of iron (haem and non-haem iron) while factors in enhancers (vitamin C and animal protein) and inhibitors (calcium, phytates and non-haem iron).⁴¹

^b50% of the Recommended Dietary Intake of iron. Reference child: 1–3 years old male, taking into account 14% absorbability.³⁰

^cRecommendation amounts were based on Australian Dietary Guidelines serving size.⁵⁵

^dRecipe included 125 g/serve = multigrain bread (41.2 g/serve), margarine (8.1 g/serve), honey (1.8 g/serve), vegemite (1.6 g/serve) and jam (4.4 g/serve).

^eRecipe included 102 g/serve = multigrain bread (41.2 g/serve), baked beans (60 g).

^fRecipe included 220 g/serve = white pasta (59.3 g), bacon (42.6 g), garlic paste (0.9 g), stock (2.8 g), cream (33.4 g), mixed frozen vegetables (28 g) and onion (13 g).

^gRecipe included 250 g/serve = white pasta (60 g), tuna (100 g/serve), kale/spinach (75 g), garlic (0.9 g), stock (2.8 g), cream (33.4 g) and onion (13 g).

^hRecipe included 65 g/serve = rockmelon (19 g), banana (22 g), apple (15 g) and pear (19 g).

ⁱRecipe included 110 g/serve = carrot sticks (75 g), hummus (2 Tbsp) and rockmelon (10 g).

continuing to serve iron with ascorbic acid rich foods because of its dual role in enhancing absorption and limiting inhibitors. Second, methods for removing phytates in foods such as beans and legumes via soaking/pre-soaking could also improve the iron bioavailability.⁵⁶ Utilising legumes as a key source of iron does not compromise protein content, but increases vegetable protein content while also increasing other phytochemicals and resistant starch, which have proven health benefits.⁵⁴

Strengths and limitations

A strength of the present study was the use of a 2-day-weighted food record, particularly because a weighed food record is considered gold standard in dietary measurement for individual food intake, and an adapted version of this method has potential to more accurately measure food provision at the service level because it minimises reliance on memory and estimations,^{33,34} ultimately improving the accuracy of the results³³; however this requires further research to validate. A 3-day record could have been employed but was omitted as a result of feasibility and participant burden.³¹ Another strength of the present study is the employment of systemic sampling of services where every 10th service was contacted from the Australian Children's Education and Care Quality Authority database.⁵⁷ This helped the recruitment of a cohort representative of the population and minimised response bias.

The present study was limited to analysing provision of dietary iron and not actual child intake, on the premise that toddlers should be provided with the opportunity to consume adequate dietary iron. We are

therefore unable to make assumptions regarding the dietary iron consumption of toddlers attending LDC services. Because food refusal is common among toddlers, a closer examination of the consumption of dietary iron in the LDC setting would be an interesting area for future research.⁵⁸

The use and comparison of two iron bioavailability algorithms strengthened our study. The calculation by Rickard et al.⁴¹ has been employed in other diet record studies but is not yet fully validated for use in determining the iron bioavailability.⁵⁹ This algorithm was based on post-prandial serum ferritin, not isotopic absorption, which has shown to alter the usual ratios of enhancers and inhibitors.^{41,59} The second algorithm has shown the highest predictive value (98.7%) for iron status compared to other iron algorithms that have been developed.⁴⁵ In addition, it adjusts the bioavailability to iron status and is the only algorithm to our knowledge that considers the interaction between inhibitors and enhancers.

Both algorithms have limitations. They both did not account for additional antinutrients of iron such as oxalic acid or the effect of heating of ascorbic acid. This introduced error as ascorbic acid is not heat stable and is often lost in cooking.⁶⁰ Thus, food and cooking recommendations, such as steaming, may be needed to retain ascorbic acid for its enhancer effect for iron bioavailability.⁶⁰ It should be noted that polyphenols were not included because LDC services do not provide tea, red wine and coffee to toddlers, which are a predominant sources of polyphenols.⁶¹ However, polyphenols can also be found in smaller amounts in fruits, vegetables, cereals, dry legumes, chocolate, and some beverages, oils and spices.⁶¹ Therefore, the exclusion of polyphenols as an inhibitor is a limitation. In addition, iron status for the algorithm of Hallberg and Hulthen⁴² was assumed to be

normal. This created bias because it is recommended to alter the algorithm to the individual's iron status, aiming to improve accuracy of the results. This was not within the scope of the project and thus is a limitation. Additionally, our study was epidemiological and did not analyse the effect that dietary iron provision at LDC services had on toddler's iron status. Therefore, it can only be inferred that the provision of bioavailable iron at LDC services was sufficient to achieve optimal blood iron status because child dietary intake and absorption was not measured.

Recommendations

The findings from the present study indicate the need for education strategies and training of staff, specifically around maintaining iron provision and maximising iron bioavailability when menu planning for LDC services. This translation should include information such as providing iron-rich food at every mealtime and serving with enhancers through the use of standardised recipes. In addition, formal vocational Early Childhood Education and Care training certificates could extend current information relating to the dietary requirements of toddlers to include the value of food combinations to enhance iron bioavailability. This research could also be used to guide broader national and state policy and legislative action to support food provision quality, inclusive of iron provision and bioavailability recommendations for LDC services. This would ultimately provide toddlers the opportunity to consume adequate bioavailable iron that meets their needs to optimise health and development. It is recommended that focusing on perceived barriers to food and nutrition at LDC services be addressed. This can include providing appropriate nutrition information and standardised recipes to improve consistency of nutrition provided at LDC services, which facilitate ingredient combinations that increase iron bioavailability. Further research into the subsequent adoption of recommendations including changes in training, education and policies is also warranted. In addition, validity of iron bioavailability algorithms is recommended to further to update existing research and evaluate the accuracy of iron algorithm estimates.

CONCLUSIONS

The provision of total dietary iron for toddlers attending LDC services was adequate when compared to a minimum target of 50% of the EAR. In total, 86% of services met the recommendation for iron provision, and this increased when bioavailability was considered. Along with other foods, we found that some less healthy dietary choices including refined grains and processed meats were important contributors to the provision of iron. Future education for menu planners should

therefore include strategies to limit these foods without compromising total iron provision.

AUTHOR CONTRIBUTIONS

Michaela Johnston was the lead data analyst for this project. Ros Sambell and Therese O'Sullivan helped formulate the research question, design the study. Ros Sambell coordinated the collection and input of data. Therese O'Sullivan supported analysis. Therese O'Sullivan, Ruth Wallace, Amanda Devine, Leesa Costello and Ros Sambell provided ongoing support and guidance on the manuscript.

ACKNOWLEDGEMENTS

This research received no specific grant from any funding agency, commercial or not-for-profit sectors. We thank the research assistants: Andrea Gracie, Melissa Dunham, Jacqueline Van Zyl, Clare Richards, Julia Main, Michaela Johnston and Krystal Kotkis. We also thank the 30 services who participated in the research. Open access publishing facilitated by Edith Cowan University, as part of the Wiley - Edith Cowan University agreement via the Council of Australian University Librarians.

CONFLICTS OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are subject to third party restrictions. Restrictions apply to the availability of these data, which were used under license for this study. Data subject to third party restrictions. Data are currently embargoed as it is part of a larger study.

ETHICAL APPROVAL

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects/patients were approved by the Human Research Ethics Committee at Edith Cowan University (#18486). Written informed consent was obtained from all subjects/patients.

TRANSPARENCY DECLARATION

The lead author affirms that this manuscript is an honest, accurate and transparent account of the study being reported. The reporting of this work is compliant with STROBE guidelines. The lead author affirms that no important aspects of the study have been omitted and that any discrepancies from the study as planned have been explained.

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PEER REVIEW

The peer review history for this article is available at <https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/jhn.13180>.

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How to cite this article: Johnston M, O'Sullivan T, Devine A, Wallace R, Costello L, Sambell R. Toddlers may be getting enough iron in long day-care services after all. *J Hum Nutr Diet*. 2023;1–11. <https://doi.org/10.1111/jhn.13180>