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Effect of replacing bread, egg, milk, and yogurt with equivalent ω-3 enriched foods on ω-3 LCPUFA intake of Australian children

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Abstract

Objective In countries with traditionally low fish consumption such as Australia, foods enriched with ω-3 long-chain polyunsaturated fatty acids (ω-3 LCPUFA) may play a role in meeting ω-3 LCPUFA intakes for optimal health. The aim of this study was to assess the effect of replacing bread, egg, milk, and yogurt with ω-3 LCPUFA enrichment of these foods on total ω-3 LCPUFA intake in Australian children's diets. Methods Dietary modeling was undertaken using survey data from a nationally representative sample of 4487 children (2249 boys, 2238 girls) ages 2 to 16 y in whom the Multiple Source Method was used to estimate usual ω-3 LPUFA intakes distributions from two 24-h dietary recalls, corrected for within-person variation; 15 models were constructed. Results The adjusted mean ± SD and median and interquartile range (IQR) of usual dietary intakes of ω-3 LCPUFA gradually increased from 2.5 ± 0.8 to 7.1 ± 4.9 mg/d and 2.3 (1.9–2.9) to 5.4 (3.6–9.2), respectively, after the modeling (P = 0.001 for each model). Median (IQR) intake of total ω-3 LCPUFAs in non-fish eaters and fish eaters was 1.4 (0.8–2.3) and 2.3 (1.0–6.1) mg/d, respectively, which increased threefold to 4.3 (2.6–7.8) and 7.5 (3.9–13) mg/d, respectively, after replacement of all four ω-3 enriched foods. Conclusion Replacement of four core foods with ω-3 enriched alternatives resulted in improved simulated ω-3 LCPUFA intakes in Australian children but not to optimal levels of intake. Increased fish consumption is still the most effective strategy for increasing ω-3 LCPUFA intake.

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Title page

Effect of replacement of bread, egg, milk and yogurt with equivalent n-3 enriched foods on n-3 LCPUFA intake of Australian children

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Running head: Dietary modelling of n-3 LCPUFA enriched foods

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Keywords: *dietary modelling, n-3 enriched food, n-3 LCPUFA intakes, children, Australia*

Abstract

In countries with traditionally low fish consumption such as Australia, foods enriched with omega-3 long chain polyunsaturated fatty acids (n-3 LCPUFA) may play a role in meeting n-3 LCPUFA intakes for optimal health. The aim of the present study was to assess the effect of replacement of bread, egg, milk and yogurt with n-3 LCPUFA enrichment of these foods on total n-3 LCPUFA intake in Australian children's diets. Dietary modelling was undertaken using survey data from a nationally representative sample of 4487 children (2249 boys, 2238 girls) aged 2-16 years in whom the Multiple Source Method (MSM) was used to estimate usual n-3 LPUFA intakes distributions from 2 x 24-h dietary recalls, corrected for within-person variation and fifteen models were constructed. The adjusted mean ± SD and median and inter quartile range (IQR) of usual dietary intakes of n-3 LCPUFA gradually increased from 2.5 ± 0.8 to 7.1 ± 4.9 mg/d and 2.3 (1.9-2.9) to 5.4 (3.6-9.2), respectively, after the modelling ($p = 0.001$ for each model). Median (IQR) intake of total n-3 LCPUFA's in non-fish eaters and fish eaters was 1.4 (0.8-2.3) and 2.3 (1.0-6.1) mg/d, respectively which increased by 3 fold to 4.3 (2.6-7.8) and 7.5 (3.9-13) mg/d, respectively after replacement of all four n-3 enriched foods. Replacement of four core foods with n-3 enriched alternatives resulted in improved simulated n-3 LCPUFA intakes in Australian children but not to optimal levels of intake. Increased fish consumption is still the most effective strategy to increase n-3 LCPUFA intake.

Introduction

Cardiovascular disease (CVD) is the leading cause of death in Australia[1]. Ninety percent of Australian adults have at least one modifiable CVD risk factor and 25% have three or more modifiable risk factors[2]. The progression of CVD can be delayed by lifestyle choices[3], and early intake of n-3 long chain polyunsaturated fatty acid (n-3 LCPUFA) has been shown to have beneficial effects on the prevention of CVD in later life[4,5].

Improved clinical outcomes for cardiovascular risk have been demonstrated in randomized double-blind controlled studies in children aged between 2-18 years following n-3 LCPUFA supplementation. These include reductions in biomarkers of CVD risk in healthy children[6], modulation of vascular function and inflammatory markers in obese adolescents[7], and improvement in vascular health in hyperlipidemic children at risk for early heart disease[8].

De novo synthesis of n-3 LCPUFA, including eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA) is inefficient in humans, especially for DHA[9], and these n-3LCPUFAs therefore need to be obtained from dietary sources, mainly from fish with smaller amounts supplied by meat and egg products[10]. Moderate amounts of n-3 LCPUFA are also found in commercially available foods that have been enriched with n-3 LCPUFA, including selected brands of bread, cereal, milk, yogurt and eggs[11]. For cardiovascular health, the National Heart Foundation of Australia (NHFA) recommends that children should follow the adult guideline of about 500 mg/d for EPA plus DHA, or 2 to 3 serves per week of 150 g fish, preferably oily fish, combined with food and drinks enriched with marine n-3 fatty acids[12,13]. In Australia, the National Health and Medical Research Council (NHMRC) has established the nutrient reference value for n-3 LCPUFA in children less than 14 years based on the adequate intake (AI) or observed median intake from the National Dietary Survey[14]. For children aged 14-16 years, a suggested dietary target (SDT) recommended for prevention of chronic diseases, has been set at 610 and 430 mg/d for boys and girls, respectively based on the observed 90th percentile of the population intake[14]. Meyer and Kolanu (2011) have extrapolated SDT for children younger than 14 years by adjusting for energy intakes, by sex and age group[15].

The number of foods with added n-3 LCPUFA is increasing, particularly in western countries such as Australia[11] where milk, cereal and bread enriched with n-3 LCPUFA are potential contributors to n-3 LCPUFA intakes[16]. However less than 7 % of Australian children are reported to consume foods enriched with n-3 LCPUFA[15], which has been proposed as a strategy to increase n-3 LCPUFA intake at a population level[16,17]. The effectiveness of substituting foods with n-3 LCPUFA enriched alternatives has not yet been investigated in the population of Australian children. Previous prospective studies have demonstrated that consumption of foods enriched with n-3 LCPUFA improves n-3 LCPUFA intake [17,18] and CVD risks[18] but that this strategy requires

individual behavior changes which may not be maintained over the long term. The current study aimed to assess whether replacement of actual consumption levels of bread, milk, egg and yogurt with n-3 LCPUFA enriched brands would in simulated intakes enable Australian children to meet the recommendation for n-3 LCPUFA for optimal health, without the need to change individual food behaviours.

Material and methods

Subjects

Dietary data were available from children participating in the Australian National Children's Nutrition and Physical Activity Survey (Children's Survey) which was conducted between 22 February and 30 August 2007. The survey was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the National Health and Medical Research Council (NHMRC) of Australia registered ethics committees of the Commonwealth Scientific and Industrial Research Organization (CSIRO) and University of South Australia[19]. The data used in this study were obtained with permission from the Australian Social Science Data Archive[20]. The protocol of the survey has been reported elsewhere[21]. Participants in the survey were children aged between 2 and 16 years (n = 4487) randomly selected using random digit dialing from all Australian states and territories in metropolitan, rural and remote areas.

Dietary data

Two 24-hour dietary recalls were available for each subject, collected using a standardized 24-hour dietary recall methodology during a computer assisted personal interview (CAPI) and a computer assisted telephone interview (CATI)[21]. Analyses of the 24-hour dietary recall data into daily nutrient intake were conducted using the Australian nutrient composition database (AUSNUT) 2007 developed specifically for the 2007 Children's Survey[19]. The intake of total n-3 LCPUFA was determined by summing individual intakes of EPA, DPA and DHA. The usual daily dietary n-3 LCPUFA intake, corrected for within-person variation, was estimated using data from the two 24-hour dietary recalls and applying the Multiple Source Method (MSM)[22]. With this method, the total variance was adjusted for the intra-individual variances due to day-to-day variability[22]. Special handling information appeared in the result section during the analysis by the MSM, where only positive and negative values for skewness of variable were encountered during Box-Cox transformation. "When only positive values for skewness are countered during Box-Cox transformation of residual, MSM will use the best parameter estimates that lead to the residual distribution skewness closest to zero. When only negative values for skewness are encountered

during Box-Cox transformation of residuals, MSM will use the parameters that lead to the best skewness value for the residual distribution (which is defined as being is closest to zero) to determining Lambda, W and skewness for the Box-Cox transformation" [22].

Dietary modelling

The dietary modelling was conducted by incrementally replacing all types of bread, egg, milk and yogurt items in the two 24-hour food recalls for each child with the corresponding n-3 LCPUFA enriched products. The dietary modelling was run in four stages: (1) replacement with one type n-3 LCPUFA enriched food (bread, eggs, milk or yogurt); (2) replacement with two types n-3 LCPUFA enriched foods (bread + egg, bread + milk, bread + yogurt or bread + egg); (3) replacement with three types n-3 LCPUFA enriched foods (bread + milk + yogurt, bread + yogurt + egg, milk + yogurt + egg or bread + egg + milk) and (4) replacement with four types n-3 LCPUFA enriched foods (bread + milk + yogurt + egg). This procedure generated a total of 15 models of dietary n-3 LCPUFA intake. The content of EPA and DHA in enriched items was obtained from a survey of nutrient content on food labels conducted at three major supermarket chains (Woolworths, Coles and Aldi) in Wollongong and Sydney in December 2009 (Table 1A). DPA was not included, as this was not used by manufactures to enrich food items. The modeled average intakes of total n-3 LCPUFA were compared to the AI and the SDT, where the NHMRC of Australia has defined AI as "The average daily nutrient intake level based on observed or experimentally-determined approximations or estimates of nutrient intake by a group (or groups) of apparently healthy people that are assumed to be adequate" and the SDT as "A daily average intake from food and beverages for certain nutrients that may help in prevention of chronic disease"[(14)]. Hence, the AI reflects the median intakes of the population and is not a recommended intake per se, while SDTs are target intakes for optimal health, rather than prevention of deficiency states.

The cost of the meal was calculated with the assumption that the amount (g) of bread, egg, milks and yogurt consumed by each child was enriched with n-3 LCPUFA. The differences in price between bread, egg, milk and yogurt with and without n-3 LCPUFA enrichment were used in calculating the cost of meals. The price of these foods was obtained from the supermarkets survey as described above.

The Microsoft Excel VLOOKUP function was used to run the replacement of the n-3 LCPUFA enriched foods in each model as well as the cost of meals. Briefly, the master database of foods in the AUSNUT 2007 specifically used to calculate EPA, DPA, DHA and n-3 LCPUFA and the data of foods consumed by each child from two 24-hour dietary recalls were provided in an Excel spread. The nutrient content of EPA, DPA and DHA in bread, egg, milk and yogurt items in the Children's Survey was replaced with the corresponding n-3 LCPUFA enriched items in each

model. The food identity (Food ID) in the AUSNUT 2007 was used as a key to merge the EPA, DHA and n-3 LCPUFA value to the data consumed by the total group of children in each model.

Statistical analysis

The statistical analysis was carried out using Statistical Package for the Social Sciences (SPSS) software version 17.0, Chicago IL, USA. Intakes of EPA, DHA and total n-3 LCPUFA (mg/d) are presented as mean ± SD and median (IQR). Intakes of n-3 LCPUFA were determined by fish eater status, based on consumption of at least one serve of fish (yes/no) from the two-24 hour dietary recalls. The data were tested for normality using the Kolmogorov-Smirnov test and as the data were skewed, the differences in n-3 LCPUFA intake before and after replacement were assessed using non-parametric tests. The Friedman test was used to compare the difference between the mean ranks of the related models. Post-hoc analysis with Wilcoxon Signed-Rank tests were conducted to locate significant differences; a significance level using the Bonferroni adjustment for multiple comparison correction was set at $p<0.003$.

Results

Dietary n-3 LCPUFA intake and cost of meals before and after modelling

The average amount of bread, egg, milk and yogurt consumed by the children varied between age groups and ranged from 52 to 84 g/d for bread, 22 to 35 g/d for egg, 114 to173 mL/d for milk, and 56 to 86 g/d for yogurt (Table 1B). These portion sizes resulted in a range of intakes for n-3 LCPUFA: bread: 22.9 to 37.0 mg/d; egg: 44.0 to 70 mg/d; milk: 13.7 to 20.8 mg/d; and yogurt: 37.5 to 57.6 for children aged 2-3, 4-8, 9-13 and 14-16 years, respectively. The mean cost of meals in children who consumed bread, egg, milk and yogurt increased by AUD 45 cents (€ 0.36 or US$ 0.47) with replacement of all of the potential corresponding foods (model 15).

Median (IQR) of total n-3 LCPUFAs for the whole group adjusted for the intra-individual variances was 2.3 (1.9-2.9) mg/d and this increased by 3.1 mg (115 %) to 5.4 (3.6-9.2) mg/d after replacement of all four items (model 15). Corresponding intakes of individual fatty acids also increased. With EPA, the greatest increase occurred with replacement of milk (model 3) and further small increases occurred with replacement of bread, yogurt and egg (model 5, 12). With DHA, the greatest increases occurred with replacement of yogurt (model 4) and milk (model 3) or combination of both (model 8) and further with additional replacement of bread and egg (model 11 and 15). Statistically significant differences ($p = 0.001$) were observed between intakes of EPA, DPA, DHA and total n-3 LCPUFA between actual diet (before replacement) and each level of replacement, except for EPA intake in model 4 (Table 2).

Twenty percent of all children consumed fish on at least one of the days of the survey. Median (IQR) baseline intake of total LCPUFA's in non-fish eaters was 1.4 (0.8-2.3) mg/d and this increased by 2.9 mg (207 %) to 4.3 (2.6-7.8) mg/d after replacement of all four items (Table 3A). Median (IQR) baseline intake of total n-3 LCPUFA's in fish eaters was 2.3 (1.0-6.1) mg/d and this increased by 5.2 mg (226 %) to 7.5 (3.9-13) mg/d after replacement of all four items (Table 3B).

DHA intake in non-fish eating children increased 23-fold, as compared to the 7-fold increase in the fish eater group, due to 6-fold higher starting levels in the latter. In non-fish eaters, DHA increased from 0.1 to 2.3mg/d, whilst in the fish eaters group increased from 0.6 to 4.4mg/d. This suggests that children who reported consuming fish were also consuming higher quantities of bread, milk, eggs and yogurt, therefore these enriched foods contributed more DHA (i.e. 3.8 mg increase) than in non-fish eating children (2.2 mg).

Distribution of n-3 LCPUFA intake and comparison between modelled diet and nutrient reference values for n-3 LCPUFA

As a result of the modelling, the distribution of intakes adjusted for intra-individual variances shifted towards higher intakes of n-3 LCPUFA (Figure 1). Substitution of n-3 enriched milk for un-enriched milk (model 3) virtually doubled the n-3 LCPUFA intakes and shifted the curve to the right (Figure 1). The addition of the other n-3 enriched foods (bread, eggs and yoghurt) to the enriched milk (model 15) shifted the curve further to the right (Figure 1).

Based on average intake of n-3 LCPUFA (non-adjusted intra-individual variances), there was a greater proportion of children reach the AI for total n-3 LCPUFA intake (28.1 % to 43.3 %) and minimal change in the proportion of children who reached the SDT (3.8 % to 4.2 %) (Online Supplement Figure 1). The percentage of children that reach the AI for n-3 LCPUFA before and after modeling increased from 13 % to 31 % in the non-fish and 85 % to 91 % in the fish eater groups, while for SDT a minimal increase from 0.05 % to 0.1 % in the non-fish eater and 18 % to 20 % in the fish eater groups were demonstrated. Overall, the percentage of total children that reach the AI for n-3 LCPUFA increased with the incremental inclusion of the four enriched items, while the percentage of children that met the SDT showed minimal change (Online Supplement Figure 2).

Discussion

This study is the first to assess the effectiveness of a dietary strategy designed to increase n-3 LCPUFA intake in Australian children by consuming enriched foods. Food fortification can lead to relatively rapid changes in the specific nutritional status of a community, and is a cost-effective public health intervention, however the items need to be consumed in adequate amounts by a large proportion of target individuals in the population and the levels of fortification must be high enough

to substantially increase the intakes[23]. Our modelling was based on children's diets actual dietary practices with regard to serving size and frequency of intake of bread, egg, milk and yogurt, hence requiring no dietary behavior change, apart from brand substitution.

The large inter-individual variation in the amount of bread, egg, milk and yogurt consumed by children in this study resulted in wide changes to total n-3 LCPUFA intake as a result of the dietary modelling. Our data showed a gradual increase in the mean and median intakes of total n-3 LCPUFA (sum EPA+DPA+DHA) adjusted for intra-individual variances of 2.8 and 2.4 fold increase, respectively, by replacing bread, egg, milk and yogurt with n-3 LCPUFA enriched choices for these foods in the actual diet of each child. However, this improvement did not result in children reaching the SDT for n-3 LCPUFA intake. Notably was the magnitude of increase in median DHA in the non-fish eater consumers which was 3-fold higher compared to the increase observed in the fish consumers. This suggests that consumption of n-3 LCPUFA enriched foods benefits non-fish eaters more so than fish eaters, however, the improvement after modelling was still below the SDT for both groups. Recommending increased fish consumption is still the most effective option to increase n-3 LCPUFA intake.

One limitation to recommending an increase in foods enriched with n-3 LCPUFA is the need for the food items to be consumed in large enough quantities to meet the recommendation of 500 mg/d[24]. According to manufacturers' data for the marine fish oil LCPUFA content of bread and milk, meeting this target would require consumption of 10 slices of bread or 1 L of milk per day. This is impractical and does not comply with current dietary guidelines that recommend smaller portions from a variety of nutritious foods[25]. It has been reported that regular consumption of a variety of n-3 LCPUFA enriched foods, consumed over eight servings per day, providing between 50 and 150 mg EPA plus DHA per serving, increased the daily n-3 LCPUFA intake of Australian adults from 200 mg/d to 960 mg/d[18], nearly two-fold higher than the recommendation for n-3 LCPUFA of 500 mg/d[13]. Consumption of 600 mL of milk per day providing 120 and 60 mg DHA and EPA, respectively, increased the daily intake of n-3 LCPUFA (EPA + DHA) of healthy children by 180 mg/d[6].

The present study was a conceptual model and did not assess biochemical markers of n-3 LCPUFA. However, other studies have reported that increases in plasma, platelet and mononuclear cell phospholipid content of n-3 LCPUFA can be achieved by consumption n-3 LCPUFA enriched foods without the simultaneous ingestion of supplements or a change in dietary habits[26]. Regular consumption of a variety of n-3 LCPUFA enriched foods providing between 50 and 150 mg EPA plus DHA per serving increased in EPA, DHA and n-3 LCPUFA concentration in erythrocytes by 82, 111 and 35 % and 53, 76 and 53 % at 3 and 6 months respectively, after supplementation[18]. Additionally, this improvement was associated with reduction in CVD risks, including a positive

association with arterial compliance and a negative association with serum C reactive protein and urinary 11-dehydro-Thromboxane B2 excretion[18]. The concentration of EPA and DHA expressed as the percentage of total fatty acid (omega-3 index) increased from 4 to 7 % over 6 months study[18], placing these subjects in lower risk for cardiac death[27].

In our study, we assumed that the replacement with n-3 LCPUFA enriched foods would not negatively impact on other nutrients, particularly lipid profiles as have been shown in healthy volunteers[28-30] as well as in hypercholesterolemic subjects[31]. A number of intervention studies that have focused on change in dietary behaviour in order to achieve a daily intake of 1 to 1.8 g/d n-3 LCPUFA have resulted in weight gain in the participants[17,18]. Since our study used actual food consumption data from a nationally representative sample of children to model scenarios of replacing foods with similar energy content, no impact on weight gain would be expected.

Uptake of foods enriched with n-3 LCPUFA by Australian consumers is low[32], with barriers including excessive price[33], undesirable sensory qualities (fishy after taste)[16] and a possibility of overdosing[33,34]. Consumers want realistic advice[35] in terms of how to adopt a dietary recommendation on a daily basis. Our dietary modelling provides a realistic example of how changes to n-3 LCPUFA intakes can be achieved by consuming different combinations of n-3 LCPUFA enriched foods. This study demonstrates that in children who do not consume fish, promotion of foods enriched with n-3 LCPUFA may be a strategy to increase intakes, however the dietary recommendations of 500 mg/d for cardiovascular health[13] or SDT range of 300 to 610 mg/d[15] will still not be reached. A wider range of appropriate n-3 LCPUFA enriched foods would help children to meet the recommendation. Increasing the levels of n-3 LCPUFA added to enriched products is unlikely, due to cost, as well as the existence of Australian food regulations that allow permitted maximum levels within food categories. In our dietary modelling, replacement of bread, egg, milk and yogurt with the corresponding n-3 LCPUFA enriched products increased the average cost of consuming these foods by AUD 45 cent per day, which is considered to be an insignificant budgetary increase. However, it remains to be demonstrated whether enrichment as a public health strategy is an acceptable option for Australian families.

A potential limitation of this study is that the use of two 24-hour food recalls to obtain the n-3 LCPUFA intake may introduce errors in estimating habitual consumption of n-3 LCPUFA food sources, such as enriched foods and especially fish, which was only occasionally consumed. This may lead to potential under or over-reporting bias. However given the large dataset used this study the individual noise is limited. Furthermore, previous work on the National Nutrition Survey that looked at FFQ as well as 24 hr recalls and there were no significant differences between n-3 LC PUFA intakes calculated from FFQ and 24 hr recall data[36]. Compared to previous national dietary surveys in 1985 and 1995, as well as in Western Australia, the trend of low fish consumption which

results in the low n-3 LCPUFA intakes appears to have remained unchanged in the population of Australian children. Similar findings have also been reported in a recent study of overweight and obese children[37] as well as in our previous survey in Australian families with young children[35].

Our estimation using adjusted data found lower intakes of EPA, DPA, DHA and total n-3 LCPUFA than average n-3 LCPUFA intake estimations using unadjusted data (Online Supplementary Table 1, 2A and 2B). This could be explained by "extreme" values of n-3 LCPUFA which may result in non-normal data which could be responsible for wide distributions of intakes and enlarged variances. Our analysis using the MSM showed an indication that the Box-Cox transformation algorithm did not find an optimal lambda. The MSM selected the "best" not optimal transformation parameter lambda and searched for lambda over a grid to minimize the skewness to account for intra- and inter-individual variations in usual intake distribution. Therefore, the distribution intake of this study should be carefully interpreted[22].

In conclusion, this dietary modelling scenario provides a realistic example of practical ways that Australian children can increase their n-3 LCPUFA intakes without changing their dietary behavior by consuming products enriched with n-3LCPUFA. Consumption of bread, egg, milk and yogurt enriched with n-3 LCPUFA in this scenario doubled the median population intake of n-3 LCPUFA and importantly increased median DHA intakes 7-fold. The increased DHA intakes were more pronounced in the non-fish eating group, as median DHA increased 23-fold. However, consumption of n-3 LCPUFA enriched foods does not increase intake sufficiently to achieve the SDT for prevention of chronic disease . The best way to meet n-3 LCPUFA intakes for optimal health is consumption of fish, or fish oil supplements (or micro-algal supplements) for non-fish consumers.

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Conflicts of interest

No competing interests were identified.

Table 1A. Omega-3 LCPUFA content in n-3 LCPUFA enriched bread, egg, milk and yogurt

	EPA (mg/100g)	DHA (mg/100g)	Total n-3 LCPUFA* (mg/100g)
Bread (*n* = 4)	8.0[a]	36.0	44.0
Egg (*n* = 3)	60.0	140.0	200.0
Milk (*n* = 1)	0.3	11.7	12.0
Yogurt (*n* = 4)	0.0	67.0	67.0

From nutrient content listed on item labels, obtained from a supermarket survey conducted by N Kolanu and S Rahmawaty in Wollongong and Sydney, December 2009 (unpublished data)

[a]Average amount

[*]Sum EPA+DPA+DHA, but DPA content is zero

Table 1B. Intake of bread, egg, milk and yogurt in consumers

	Mean ± SD intake (in consumers)				
	2-3 y (*n*, %)	4-8 y (*n*, %)	9-13 y (*n*, %)	14-16 y (*n*, %)	All ages
Bread (g/d)	52 ± 42[a] (1035, 24)[b]	58 ± 47 (1188, 28)	72 ± 63 (1052, 25)	84 ± 74 (1018, 24)	63 ± 59 (4293)
Egg (g/d)	22 ± 19 (254, 25)	28 ± 21 (271, 27)	31 ± 26 (242, 24)	35 ± 29 (250, 25)	7 ± 17 (1017)
Milk (g/d)	114 ± 67 (1051, 25)	139 ± 89 (1169, 28)	156 ± 103 (1041, 24)	173 ± 130 (982, 23)	137 ± 104 (4243)
Yogurt (g/d)	56 ± 31 (541, 37)	57 ± 29 (459, 31)	72 ± 44 (255, 17)	86 ± 60 (210, 14)	21 ± 37 (1465)

[a]Mean ± SD intake (in consumer during the two days of survey)

[b]Number of consumers, percentage of consumers

Table 2. Modelling of usual daily intake of EPA, DHA and total n-3 LCPUFA before and after replacement with n-3 enriched foods for all children (n = 4487)[a]

	n-3 enriched foods	EPA		DHA		Total n-3 LCPUFA[b]	
		Mean ± SD	Median (IQR)	Mean ± SD	Median (IQR)	Mean ± SD	Median (IQR)
Baseline (actual diet)		0.9 ± 1.9	0.4 (0.2-0.9)	1.0 ± 2.5	0.4 (0.3-0.7)	2.5 ± 0.8	2.3 (1.9-2.9)
1 item							
Model 1	(bread)	1.0 ± 2.5	0.5* (0.2-1.1)	1.3 ± 2.4	0.7* (0.5-1.1)	2.9 ± 0.8	2.7* (2.3-3.2)
Model 2	(egg)	1.0 ± 2.1	0.5* (0.2-1.1)	1.1 ± 3.1	0.5* (0.3-0.8)	2.6 ± 1.1	2.4* (1.9-3.1)
Model 3	(milk)	0.8 ± 0.8	0.6* (0.3-1.0)	3.3 ± 4.4	1.5* (1.1-2.7)	5.3 ± 3.9	4.0* (2.6-6.7)
Model 4	(yogurt)	0.9 ± 1.9	0.4 (0.2-1.0)	3.1 ± 4.3	1.8* (1.5-2.2)	4.2 ± 8.2	1.9* (0.8-4.5)
2 items							
Model 5	(bread, egg)	1.0 ± 0.4	0.9* (0.8-1.0)	1.5 ± 2.8	0.7* (0.5-1.2)	3.0 ± 1.1	2.8* (2.3-3.6)
Model 6	(bread, milk)	0.9 ± 0.9	0.6* (0.4-1.0)	3.6 ± 3.9	1.8* (1.4-3.1)	5.6 ± 3.9	4.3* (2.9-7.3)
Model 7	(bread, yogurt)	1.0 ± 2.5	0.5* (0.2-1.1)	2.9 ± 4.2	1.6* (1.1-2.6)	4.7 ± 1.3	4.5* (3.7-5.4)
Model 8	(milk, yogurt)	0.8 ± 0.8	0.6* (0.3-1.0)	4.6 ± 5.1	2.4* (1.7-4.6)	6.6 ± 4.6	5.0* (3.4-8.6)
Model 9	(milk, egg)	0.9 ± 0.9	0.6* (0.3-1.0)	3.4 ± 4.6	1.6* (1.1-2.9)	5.5 ± 4.2	4.1* (2.7-7.0)
Model 10	(yogurt, egg)	1.0 ± 2.1	0.5* (0.2-1.1)	3.3 ± 4.8	1.9* (1.5-2.6)	4.4 ± 0.3	4.4* (4.2-4.6)
3 items							
Model 11	(bread, milk, yogurt)	1.0 ± 0.6	0.8* (0.6-1.2)	4.8 ± 4.8	2.7* (1.9-5.3)	6.9 ± 4.7	5.3* (3.6-9.0)
Model 12	(bread, yogurt, egg)	1.0 ± 0.4	0.9* (0.8-1.0)	3.1 ± 4.6	1.7* (1.1-2.8)	4.9 ± 1.7	4.5* (3.6-5.8)
Model 13	(milk, yogurt, egg)	0.9 ± 0.9	0.6* (0.3-1.0)	4.7 ± 5.4	2.5* (1.7-4.7)	6.8 ± 4.9	5.2* (3.4-5.2)
Model 14	(bread, egg, milk)	0.9 ± 1.0	0.7* (0.4-1.1)	3.7 ± 4.1	1.9* (1.4-3.4)	5.8 ± 4.1	4.4* (2.9-7.5)
4 items							
Model 15	(bread, egg, milk, yogurt)[c]	1.0 ± 1.0	0.7* (0.4-1.1)	4.9 ± 4.9	2.8* (2.0-5.4)	7.1 ± 4.9	5.4* (3.6-9.2)

[a]Estimated from two 24-hour dietary recalls using the Multiple Source Method (MSM) software to account for day today variation[(22)]

EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; n-3 LCPUFA, omega-3 long chain polyunsaturated fatty acid; total n-3 LCPUFA, sum EPA+DPA+DHA; SD, standard deviation; IQR, Interquartile range

[c]The average cost of meal in children who ate bread, egg, milk and yogurt increased AUD 45 cent per day

*Significant difference between the baseline (actual intake) and intake from each model ($p = 0.001$) using Post-hoc analysis with Wilcoxon Signed-Rank Tests with a Bonferroni correction applied

Table 3A. Modelling of usual daily intake of EPA, DHA and total n-3 LCPUFA before and after replacement with n-3 enriched foods[a] (n = 3554)[b] in non-fish eater[c]

		Non-fish eater (n = 3554)					
		EPA		DHA		Total n-3 LCPUFA	
		Mean ± SD	Median (IQR)	Mean ± SD	Median (IQR)	Mean ± SD	Median (IQR)
Baseline (actual diet)		0.7 ± 0.5	0.5 (0.4-0.9)	0.4 ± 1.6	0.1 (0.1-0.3)	1.9 ± 1.8	1.4 (0.8-2.3)
1 item							
Model 1	(bread)	0.7 ± 0.6	0.5 (0.4-0.8)	0.7 ± 1.5	0.3* (0.2-0.6)	2.2 ± 1.5	1.8* (1.2-2.8)
Model 2	(egg)	0.8 ± 0.6	0.6* (0.4-1.0)	0.5 ± 2.4	0.1 (0.1-0.4)	2.0 ± 2.1	1.4 (0.8-2.4)
Model 3	(milk)	0.7 ± 0.9	0.4 (0.2-0.7)	2.7 ± 3.6	1.3* (0.8-2.3)	4.4 ± 4.0	3.1* (1.9-5.5)
Model 4	(yogurt)	0.7 ± 0.5	0.5 (0.4-0.9)	1.9 ± 3.7	0.9* (0.5-1.9)	3.4 ± 3.0	2.5* (1.5-4.3)
2 items							
Model 5	(bread, egg)	0.8 ± 0.7	0.6* (0.4-0.9)	0.8 ± 2.0	0.3* (0.2-0.7)	2.4 ± 1.9	1.9* (1.2-3.0)
Model 6	(bread, milk)	0.7 ± 0.9	0.5 (0.2-0.8)	3.0 ± 3.5	1.5* (1.0-2.7)	4.7 ± 3.9	3.4* (2.1-6.0)
Model 7	(bread, yogurt)	0.7 ± 0.6	0.5 (0.4-0.8)	1.8 ± 3.4	0.9* (0.4-1.8)	3.6 ± 3.1	2.8* (1.7-4.5)
Model 8	(milk, yogurt)	0.7 ± 0.9	0.4 (0.2-0.7)	3.9 ± 4.6	2.0* (1.3-3.7)	5.5 ± 4.8	4.0* (2.4-7.0)
Model 9	(milk, egg)	0.7 ± 1.1	0.4 (0.2-0.8)	2.8 ± 4.0	1.3* (0.8-2.4)	4.6 ± 4.3	3.2* (1.9-5.8)
Model 10	(yogurt, egg)	0.8 ± 0.6	0.6* (0.4-1.0)	2.1 ± 4.2	0.9* (0.5-2.1)	3.6 ± 3.5	2.5* (1.5-4.5)
3 items							
Model 11	(bread, milk, yogurt)	0.7 ± 0.9	0.5 (0.2-0.8)	4.1 ± 4.6	2.3* (1.5-4.2)	5.8 ± 4.9	4.2* (2.6-7.6)
Model 12	(bread, yogurt, egg)	0.8 ± 0.7	0.6* (0.4-0.9)	2.0 ± 3.8	0.9* (0.4-1.9)	3.9 ± 3.5	2.8* (1.7-4.8)
Model 13	(milk, yogurt, egg)	0.7 ± 1.1	0.4 (0.2-0.8)	4.0 ± 4.9	2.1* (1.3-3.9)	5.7 ± 5.2	4.1* (2.4-7.4)
Model 14	(bread, egg, milk)	0.8 ± 1.1	0.5 (0.2-0.9)	3.1 ± 3.8	1.6* (1.0-2.8)	4.8 ± 4.3	3.5* (2.1-6.2)
4 items							
Model 15	(bread, egg, milk, yogurt)	0.8 ± 1.1	0.5 (0.2-0.9)	4.2 ± 4.8	2.3* (1.5-4.4)	6.0 ± 5.3	4.3* (2.6-7.8)

[a]Modelled diet by replacing bread, egg, milk and yogurt with n-3 enriched for these foods in the actual diet of each child for all children (as note in Table 1)

[b]Estimated from two 24-hour dietary recalls using the Multiple Source Method (MSM) software to account for day today variation[(22)]

[c]Fish eater, ate fish from the two 24-hour recalls; Non-fish eater, did not eat fish from the two 24-hour recalls

EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; n-3 LCPUFA, omega-3 long chain polyunsaturated fatty acid; total n-3 LCPUFA, sum EPA+DPA+DHA; SD, standard deviation; IQR, Interquartile range

*Significant difference between the baseline (actual intake) and intake from each model ($p = 0.001$) using Post-hoc analysis with Wilcoxon Signed-Rank Tests with a Bonferroni correction applied

Table 3B. Modelling of usual daily intake of EPA, DHA and total n-3 LCPUFA before and after replacement with n-3 enriched foods[a] (n = 933)[b] in fish eater[c]

		Fish eater (n = 933)					
		EPA		DHA		Total n-3 LCPUFA	
		Mean ± SD	Median (IQR)	Mean ± SD	Median (IQR)	Mean ± SD	Median (IQR)
Baseline (actual diet)		2.7 ± 9.9	0.7 (0.2-2.1)	7.4 ± 40.0	0.6 (0.2-2.8)	7.1 ± 20.0	2.3 (1.0-6.1)
1 item							
Model 1	(bread)	2.6 ± 7.7	0.8* (0.3-2.3)	6.0 ± 28.0	0.9* (0.3-3.4)	7.6 ± 20.0	2.5* (1.1-6.8)
Model 2	(egg)	2.7 ± 9.9	0.7 (0.2-2.1)	7.4 ± 40.0	0.6 (0.2-2.8)	7.1 ± 20.0	2.3 (1.0-6.1)
Model 3	(milk)	1.8 ± 3.7	0.8* (0.4-1.7)	5.9 ± 12.0	2.5* (1.2-5.6)	8.9 ± 12.0	5.9* (2.8-10.7)
Model 4	(yogurt)	2.7 ± 9.9	0.7 (0.2-2.1)	8.2 ± 31.0	1.4* (0.4-5.2)	10.0 ± 28.0	3.2* (1.3-9.3)
2 items							
Model 5	(bread, egg)	2.6 ± 7.7	0.8* (0.3-2.3)	6.0 ± 28.0	0.9* (0.3-3.4)	7.6 ± 20.0	2.5* (1.1-6.8)
Model 6	(bread, milk)	1.8 ± 3.5	0.9* (0.4-1.9)	6.0 ± 10.0	3.0* (1.5-6.5)	9.1 ± 12.0	6.2* (3.2-11.4)
Model 7	(bread, yogurt)	2.6 ± 7.7	0.8* (0.3-2.3)	7.2 ± 24.0	1.9* (0.6-5.7)	10.0 ± 27.0	3.5* (1.5-9.7)
Model 8	(milk, yogurt)	1.8 ± 3.7	0.8* (0.4-1.7)	7.3 ± 12.0	3.7* (1.8-8.2)	10.0 ± 13.0	6.9* (3.5-12.8)
Model 9	(milk, egg)	1.8 ± 3.7	0.8* (0.4-1.7)	5.9 ± 12.0	2.5* (1.2-5.6)	8.9 ± 12.0	5.9* (2.8-10.7)
Model 10	(yogurt, egg)	2.7 ± 9.9	0.7 (0.2-2.1)	8.2 ± 31.0	1.4* (0.4-5.2)	10.0 ± 28.0	3.2* (1.3-9.3)
3 items							
Model 11	(bread, milk, yogurt)	1.8 ± 3.5	0.9* (0.4-1.9)	7.4 ± 11.0	4.4* (2.2-8.7)	10.0 ± 12.0	7.5* (3.9-13)
Model 12	(bread, yogurt, egg)	2.6 ± 7.7	0.8* (0.3-2.3)	7.2 ± 24.0	1.9* (0.6-5.7)	10.0 ± 27.0	3.5* (1.5-9.7)
Model 13	(milk, yogurt, egg)	1.8 ± 3.7	0.8* (0.4-1.7)	7.3 ± 12.0	3.7* (1.8-8.2)	10.0 ± 13.0	6.9* (3.5-12.8)
Model 14	(bread, egg, milk)	1.8 ± 3.5	0.9* (0.4-1.9)	6.0 ± 10.0	3.0* (1.5-6.5)	9.1 ± 12.0	6.2* (3.2-11.4)
4 items							
Model 15	(bread, egg, milk, yogurt)	1.8 ± 3.5	0.9* (0.4-1.9)	7.4 ± 11.0	4.4* (2.2-8.7)	10.0 ± 12.0	7.5* (3.9-13)

[a]Modelled diet by replacing bread, egg, milk and yogurt with n-3 enriched for these foods in the actual diet of each child for all children (as note in Table 1)

[b]Estimated from two 24-hour dietary recalls using the Multiple Source Method (MSM) software to account for day today variation[(22)]

[c]Fish eater, ate fish from the two 24-hour recalls

EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; n-3 LCPUFA, omega-3 long chain polyunsaturated fatty acid; total n-3 LCPUFA, sum EPA+DPA+DHA; SD, standard deviation; IQR, Interquartile range

*Significant difference between the baseline (actual intake) and intake from each model ($p = 0.001$) using Post-hoc analysis with Wilcoxon Signed-Rank Tests with a Bonferroni correction applied

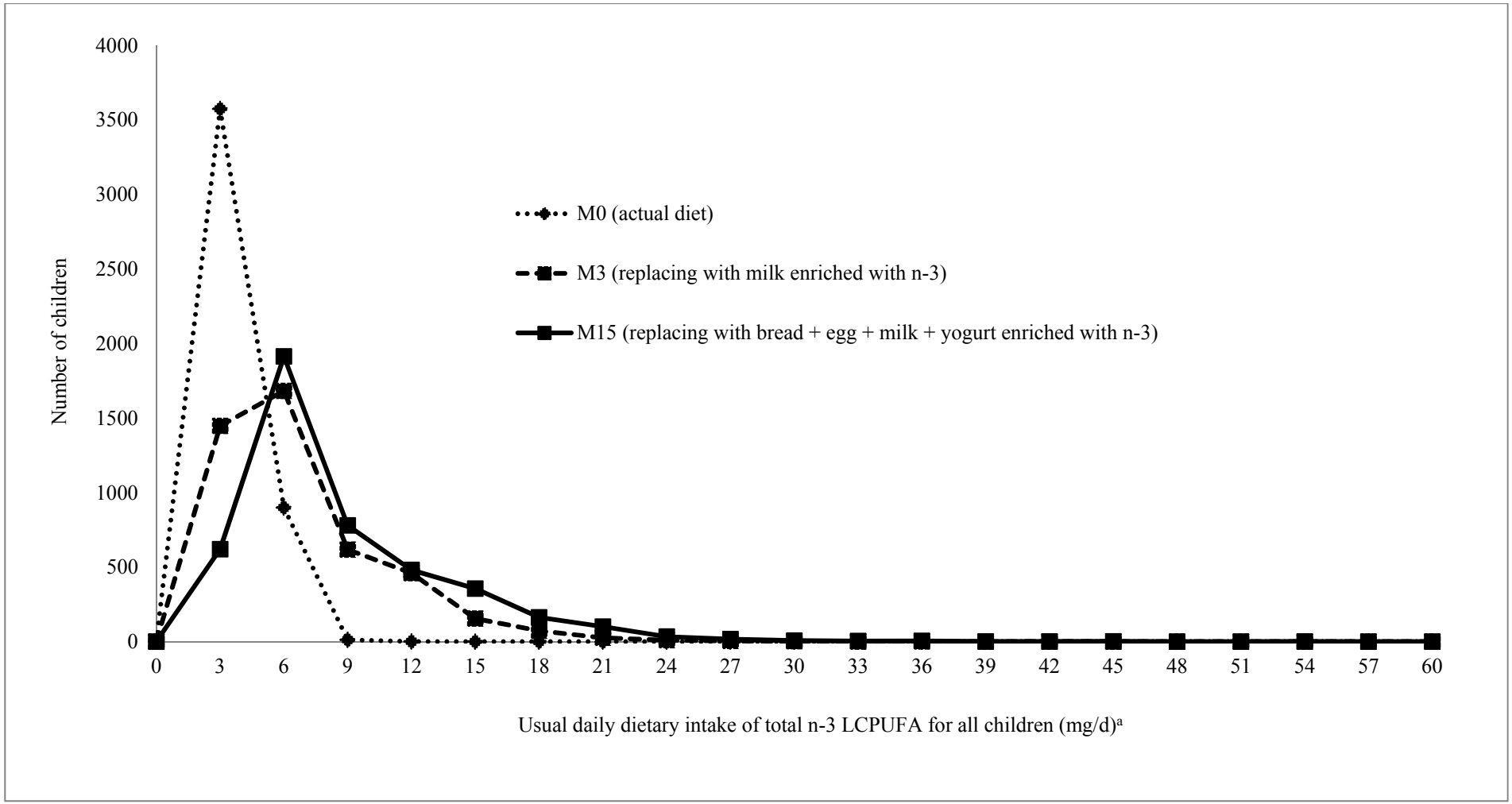
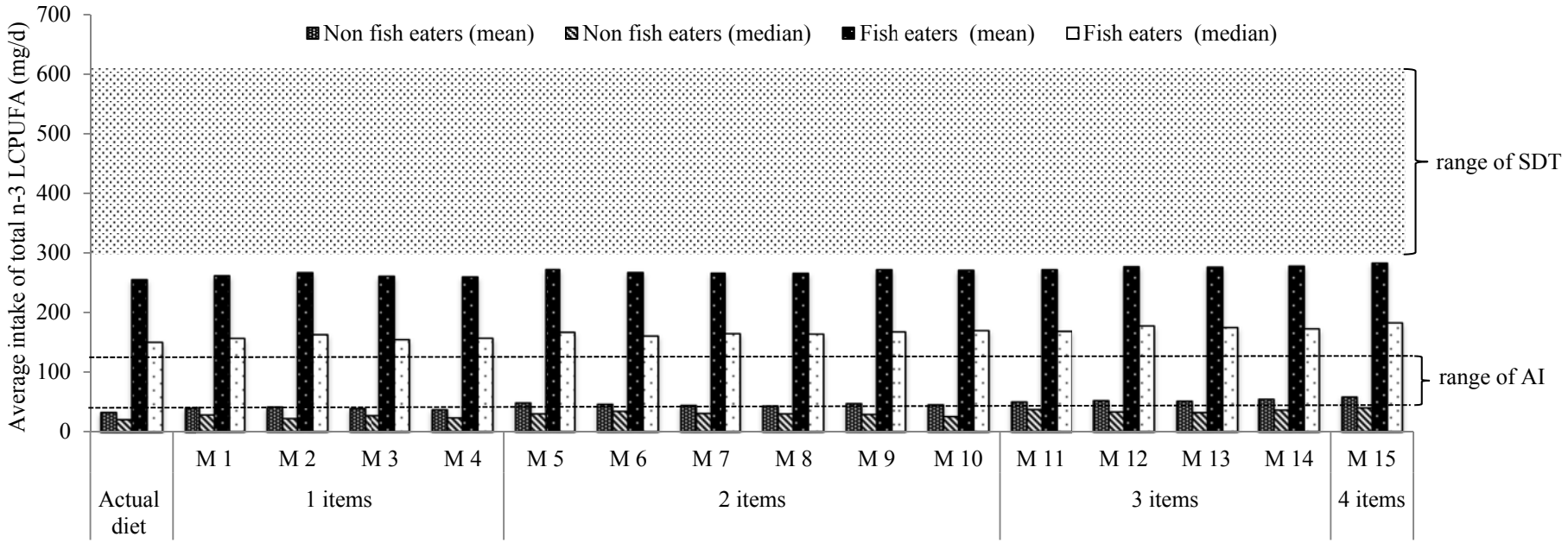


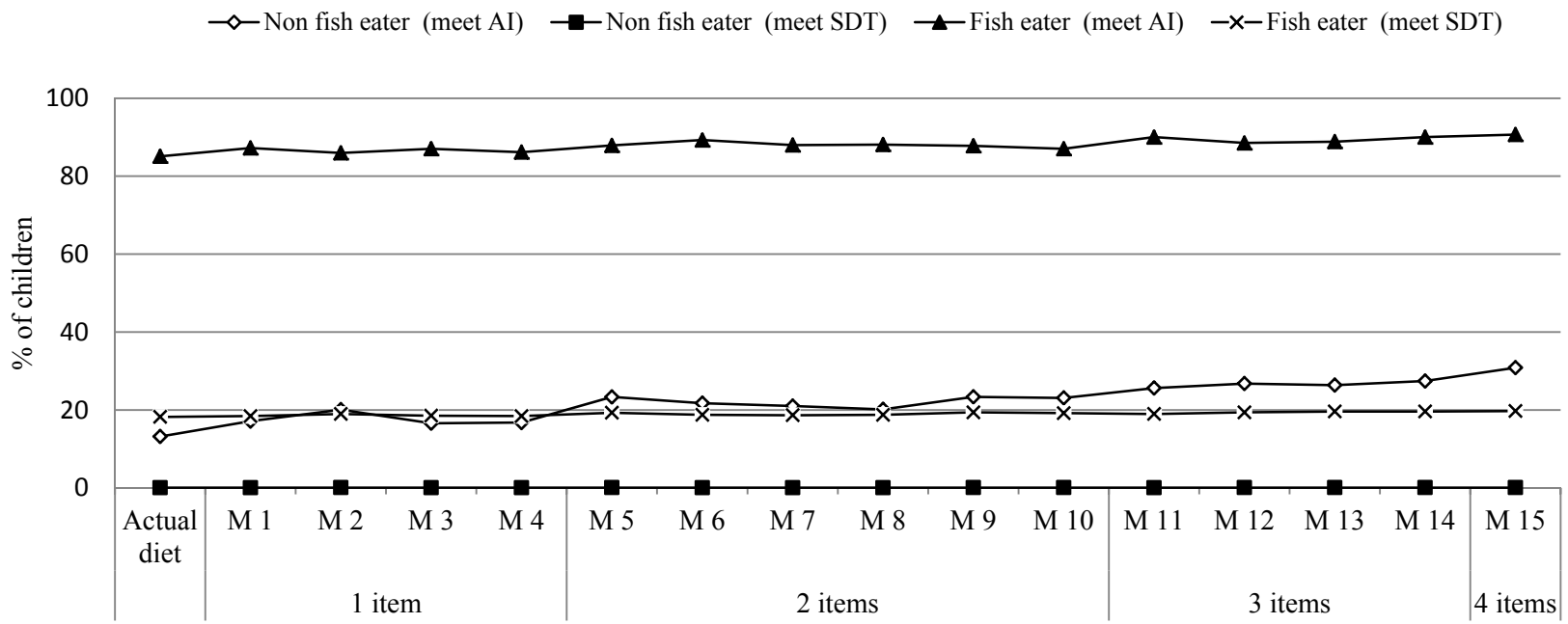
Fig 1. The changes distribution of usual daily dietary intake of total n-3 LCPUFA of the Australian children's diet (n=4487) before and after replacing bread, milk, yogurt and egg with enriched n-3 for these foods.

[a]Estimated from two 24-hour dietary recalls using the Multiple Source Method (MSM) software to account for day today variation[22]



Supplement Fig 1. Modelling intake of total n-3 LCPUFA (non-adjusted intra individual variances) before and after replacement with n-3 enriched foods from (n = 4487) in relation to the nutrient reference values[15]

SDT, suggested dietary target; AI, adequate intake; M1 to M15 refers to Models 1 to 15 for replacement with n-3 enriched foods (see details in Table 2)



Supplement Fig 2. Percentage of children (n = 4487) that meet AI and SDT by fish eater status based on average intake of total n-3 LCPUFA (non-adjusted intra individual variances)

SDT, suggested dietary target; AI, adequate intake; M1 to M15 refers to Models 1 to 15 for replacement with n-3 enriched foods (see details in Table 2)

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