

Nutritional Adequacy, Diet Quality and Calcium Intake on the Royal Prince Alfred Hospital Elimination Diet

Erica Bessell (SID 450269747)



Allergy Unit

Department of Clinical Immunology

Royal Prince Alfred Hospital

9-11 Layton Street, Camperdown NSW 2006, Australia

Supervisors:

Dr Robert H Loblay, MB, BS, FRACP, PhD – Director, RPAH Allergy Unit

Dr Velencia Soutter, MB, BS, FRACP (Paed) – Paediatrician, RPAH Allergy Unit

Dr Anne R Swain, Dip Nutr Diet, PhD – Head Dietitian, RPAH Allergy Unit

Carling Chan, BSc (Hons) MND – Research Dietitian, RPAH Allergy Unit

Brooke McKinnon, BSc Nutr (Hons) – Dietitian, RPAH Allergy Unit

Neelam Pun, BSc Nutr (Hons) – Research Dietitian, RPAH Allergy Unit

Amy Wu, BSc Nutr (Hons) – Research Dietitian, RPAH Allergy Unit

Wendy Stuart-Smith, MND – Lecturer, USYD

28th October 2016

Manuscript formatted for the Journal of Nutrition and Dietetics

Declaration

The candidate, *Erica Bessell*, hereby declares that none of the work presented in this essay has been submitted to any other University or Institution for higher degree and that to the best of her knowledge contains no material written or published by another person, except where due reference is made in the text.

Signature

Erica Bessell

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Authors:

Erica Bessell¹, BBiotechMedRes (Hons) MND Research Student

Carling Chan², MND, APD

Brooke Scott², BSc Nutr (Hons), APD

Neelam Pun², BSc Nutr (Hons), APD

Amy Wu², BSc Nutr (Hons), APD

Wendy Stuart-Smith^{1,2}, MND, APD

Anne R Swain², Dip Nutr Diet, PhD, APD

Author Affiliations:

¹University of Sydney, Sydney NSW 2006

²Royal Prince Alfred Hospital Allergy Unit, Sydney NSW 2050

Authorship: EB drafted the manuscript and analysed and interpreted data. CC, BS, NP, AW and AS contributed to the conceptualisation of the study and critical revisions of the manuscript. WS contributed to the conceptualisation of the study.

Address: Royal Prince Alfred Hospital Allergy Unit, 9-11 Layton Street, Camperdown NSW 2006, Australia

Email: Erica Bessell – ebes5887@uni.sydney.edu.au

Funding and Conflicts of Interest: There were no financial grants or funding provided for this study. There were no conflicts of interest.

Running title: Diet quality and calcium on an elimination diet.

Word Count: 3507 words (excluding title page, abstract, references, figures and tables)

Abstract

Aim: This study aimed to assess and compare nutritional adequacy, diet quality and calcium intake of patients before and on the Royal Prince Alfred Hospital (RPAH) Elimination Diet (ED).

Methods: This study used data from a five-year prospective observational study at the RPAH Allergy Unit. Participants completed a four-day weighed food record (WFR) prior to their first appointment (baseline) and one month after commencing the ED (on ED). WFRs were entered into Xyris FoodWorks8 (2012) and nutritional adequacy was determined using the Nutrient Reference Values. Diet quality was assessed using the Healthy Eating Index for Australian Adults (HEIFA).

Results: A total of 121 baseline WFRs and 81 on ED WFRs were collected. Mean HEIFA score improved on the ED compared to baseline (52.7 ± 8.1 to 58.0 ± 8.6). Thirty-eight participants at baseline (31%) and 11 participants on the ED (14%) met calcium requirements. Calcium supplementation on the ED improved the proportion meeting requirements, regardless of inclusion or exclusion of dairy. Seventeen participants on the ED (21%) were drinking calcium-fortified soy milk and 20 participants (25%) were drinking other calcium-fortified alternatives (e.g. rice milk).

Conclusion: Adequate intake of dairy and alternatives should be promoted on the ED, with supplementation recommended when calcium intake is likely to be inadequate.

Key Words: calcium, dairy, diet quality, elimination diet, food intolerance

Introduction

Food intolerances are non-immune reactions to naturally-occurring chemicals (e.g. salicylates, amines, glutamate) or additives (e.g. colours, flavours, preservatives)¹.

Symptoms of food intolerance can be many and varied, and can affect multiple systems including the gastrointestinal system (e.g. bloating), skin (e.g. urticaria), central nervous system (e.g. headaches) and respiratory system (e.g. sinus problems). Reactions are dose-dependent and can be delayed, making it difficult to identify the trigger(s)¹.

The best diagnostic test for identifying food intolerance is the Royal Prince Alfred Hospital (RPAH) Elimination Diet (ED) and challenge protocol. The ED involves eliminating naturally-occurring food chemicals and additives from the diet. If the patient is experiencing significant gastrointestinal symptoms, eliminating gluten, dairy and soy may also be warranted. Patients follow the ED until symptoms have ceased or significantly eased (usually 2-6 weeks). Challenges are then conducted to identify which chemicals cause reactions. After challenges, a personalised diet is prescribed, and through liberalisation, the threshold of tolerance is identified¹.

Previous research at the RPAH Allergy Unit found the ED improved the alignment of patients' diets with the Acceptable Macronutrient Distribution Range (AMDR) for carbohydrate, fat and saturated fat intake, when compared with their diets before commencing the ED (Tripodi E, 2016; unpublished data). Overall diet quality, as measured by the Healthy Eating Index for Australian Adults (HEIFA)², also improved on the ED from 51 to 58. Mean intake of dairy and alternatives, however, slightly decreased on the ED (1.2 serves/day vs 1.8 serves/day prior to commencing the ED). At both stages, intake of dairy and alternatives was below the recommended 2.5 serves/day³. This is reflected in mean calcium intake, which also decreased on the ED,

with patients meeting on average 65% of their Estimated Average Requirements (EAR) compared to 91% of their EAR prior to commencing the ED. Although the ED is a short-term diagnostic tool, highly sensitive patients may be unable to liberalise their diet much and therefore remain on a limited diet longer term. Calcium intake is thus of concern, as it is a vital micronutrient to ensure adequate bone mineralisation. Inadequate calcium intake is associated with low bone mineral density and osteoporosis in the elderly, resulting in an increased risk of fractures and morbidity⁴.

Inadequate calcium intake is not limited to patients on the ED. The most recent Australian Health Survey (AHS; 2011-12) found 73% of females and 51% of males aged ≥ 2 years did not meet their EARs⁵. Major sources of calcium included milk products and dishes (providing 42% of calcium intake), cereals and cereal products (13%), and cereal-based products and dishes (13%)⁶. Milk alone provided 21% of calcium intake, followed by cheese (10%) and yoghurt (5%). Amongst the patients presenting to the Allergy Unit for education on the ED, half were already restricting dairy in their diet (Tripodi E, 2016; unpublished data). On the ED, many major sources of calcium are excluded, including flavoured milks and yoghurts, and hard and tasty cheeses. Patients additionally excluding dairy would also have to remove plain milk and yoghurt, and fresh cheeses, from their diet¹. Rich sources of calcium may be limited to calcium-fortified soy products (which may also be excluded), or calcium-fortified milk substitutes, such as rice milk.

This study aimed to assess nutritional adequacy, diet quality and calcium intake of patients on the ED, and compare this with their diets before commencing the ED. This study is the first to include a comprehensive analysis of calcium intake on the ED, and thus will contribute to improvements in clinical practice.

Methods

This study utilised data from the Nutritional Adequacy Study, a five-year prospective observational study, commenced in 2013 at the RPAH Allergy Unit. It was designed to assess the nutritional adequacy and diet quality of the ED, with comparison to dietary habits prior to commencing the ED. The study also aimed to investigate factors contributing to nutritional adequacy. Approval was received from the Ethics Review Committee (RPAH Zone) of the Sydney Local Health District (protocol no. X13-0208), and the study conforms to the standards of the Declaration of Helsinki (2008 revisions).

Patients were contacted by research dietitians one week prior to their first appointment at the Allergy Unit. Eligible patients were 18 years or older, had suspected food intolerance based on described symptoms (e.g. urticaria, angioedema, eczema, irritable bowel, migraine), and had no previous dietetic education on food intolerance or the ED. Those who were eligible and consented to participate were sent an information pack and completed a four-day weighed food record (WFR) over three weekdays and one weekend day, including details of physical activity and nutritional supplementation. This was the 'baseline WFR'.

While at the Allergy Unit for their appointment, participants completed a consent form and Patient Information Form (PIF) to gather data regarding dietary habits, had their height and weight measured, and returned their completed baseline WFR. An additional WFR was completed one month after commencing the ED, and collected at follow-up appointments, or returned via post or email. This was the 'on ED WFR'.

Analysis of dietary data was conducted using Xyris FoodWorks8 (2012) and collated with data from the PIF using Microsoft Excel (2007). Age and gender-specific Nutrient

Reference Values were used to assess nutritional adequacy. Dietary data was coded using an amplified version of the HEIFA², based on the 2013 Australian Dietary Guidelines (ADGs)³. Mixed dishes were broken down into ingredients using patient-provided recipes or standardised recipes, as per the AUSNUT2013 Food Recipe file⁷. Microsoft Excel (2007) was used to convert this coding to the HEIFA score, representing diet quality as a number between 0 and 100 with higher scores indicating better adherence to the ADGs. Statistical analysis was performed using Microsoft Excel (2007) and GraphPad Prism 7.02 (2016). Continuous variables were tested for normality using the D'Agostino-Pearson test. Parametric variables were analysed using t-tests, and presented as means with standard deviation (SD). Non-parametric variables were analysed using Mann-Whitney tests, and presented as medians with inter-quartile range (IQR). Nutrient intake and HEIFA sub-scores are presented as means with SD. Discrete variables were analysed using the Chi-squared test or Fisher's exact test. $P < 0.05$ was considered statistically significant.

Results

The study sample consisted of 121 participants, including 95 females (79%) and 26 males (21%). All participants completed a baseline WFR, and 81 participants completed a WFR on the ED. Participants with only a baseline WFR ($n=40$) were included as they were determined to be demographically equivalent to participants who had completed both WFRs (see Appendix 1). At baseline, the median age of the participants was 41 years (IQR 31-54 years) and the median body mass index (BMI) was 23.5kg/m^2 (IQR $21.0\text{-}26.3\text{kg/m}^2$). Participants who completed an on ED WFR included 65 females (80%) and 16 males (20%). The median age of these participants was 41 years (IQR 33-54.5 years) and the median BMI was 23.1kg/m^2 (IQR $20.4\text{-}26.7\text{kg/m}^2$).

The majority of participants (n=108) reported symptoms in more than one system at baseline; the most commonly affected system was the gastrointestinal system (105 participants reported symptoms). Forty-eight participants identified dairy as a trigger for their symptoms, and 51 participants reported to be limiting dairy in their diet. Thirteen baseline WFRs were excluding dairy entirely. In the 81 on ED WFRs, 55 participants were excluding dairy, with 34 participants excluding both dairy and soy.

Diet quality, as assessed by the HEIFA, improved on the ED compared to baseline (52.7 ± 8.1 to 58.0 ± 8.6 , $P < 0.05$), due to significant improvements in the sub-scores for serves of unsaturated fats and non-core foods, sodium intake, and saturated fat intake as a percent of energy (see Table 1). The proportion of participants meeting recommendations for most of these categories also significantly improved. There were significant decreases in the sub-scores for serves and variety of fruit, and serves of dairy and alternatives. The proportion of participants meeting the recommendations for serves of fruit also decreased ($P < 0.05$). At baseline, 20 participants (17%) were meeting the recommendations for serves of dairy and alternatives, compared to 10 participants (12%) on the ED ($P > 0.05$).

The proportion of participants consuming carbohydrate and fat within the AMDRs significantly increased on the ED compared to baseline (see Table 2). Mean carbohydrate intake (as a percent of energy) increased to be within the AMDR on the ED compared to baseline. On the ED, mean intake was below the EAR for vitamin A and calcium. Mean vitamin A intake at baseline was adequate, however mean calcium intake was inadequate. The proportion of participants meeting the EAR on the ED compared to baseline significantly decreased for thiamin, riboflavin, folate, vitamin A, and calcium. The proportion of participants consuming sodium below the upper limit

significantly increased on the ED compared to baseline. At baseline, 38 participants (31%) were meeting the EAR for calcium compared to 11 participants (14%) on the ED.

Calcium intake at baseline from food alone did not change regardless of the exclusion (n=13) or inclusion (n=108) of dairy (see Figure 1a). On the ED, dairy exclusion was associated with a significantly lower calcium intake from food compared to those including dairy (see Figure 1a), and a lower proportion meeting requirements (4 of 55 participants excluding dairy [7%] vs 7 of 26 participants including dairy [27%]; $P<0.05$). At baseline and on ED, participants who took calcium supplements (n=14 at baseline, n=34 on ED) had a higher calcium intake than those who did not ($P<0.05$ for both; see Figure 1b), and a higher proportion met requirements ($P<0.05$ for both).

Considering both supplementation and dairy exclusion on the ED, those who were not supplementing and excluding dairy (n=26) had the lowest calcium intake (see Figure 2). Only two participants in this group met the EAR, predominantly through the use of soy milk. Those who were supplementing, with (n=5) and without (n=29) dairy inclusion, had the highest calcium intake, with 4 participants and 22 participants meeting requirements, respectively. The eight participants taking supplements with inadequate calcium intake were close to requirements (62% to 96% of the EAR).

At baseline, 29 participants (24%) included calcium-fortified soy milk in their diet (mean intake 0.9 serves/day). Similarly, this was included by 17 participants (21%) on the ED, but with a higher mean intake (1.4 serves/day). More participants were using other dairy alternatives (e.g. calcium-fortified rice milk) on the ED compared to baseline (20 participants [25%] vs 6 participants [5%], respectively). Mean intake on the ED was 0.9 serves/day compared to 0.5 serves/day at baseline. Another 20 participants

on the ED were including unfortified rice milk, which contains 73.5mg/100mL compared to 126mg/100mL in calcium-fortified brands. Thirty-four participants on the ED were excluding both dairy and soy, with 14 of these participants taking a calcium supplement and 25 of these participants consuming rice milk (including fortified and unfortified varieties). Mean intake of rice milk in these participants was 1.1 serves/day, however the majority (n=15) were having unfortified varieties.

Discussion

Results from this study indicated that diet quality improved to better align with the ADGs on the ED compared to baseline, and a larger proportion of participants were adherent to the AMDRs. The ED was, however, associated with a higher proportion of inadequate intake for multiple key nutrients compared to baseline. Calcium intake was found to be largely inadequate at baseline and on the ED. The exclusion of dairy from the ED, warranted in patients with gastrointestinal symptoms, resulted in significantly lower calcium intakes compared to those including dairy. Mean calcium intake was still below the EAR for both groups. Supplementation significantly improved the proportion of participants meeting calcium recommendations on the ED, regardless of dairy inclusion or exclusion. Some participants excluding both dairy and soy on the ED were including calcium-fortified rice milk, but more were using unfortified varieties.

The increased HEIFA scores on the ED compared to baseline were due to significant increases in serves of unsaturated fats, and decreases in serves of non-core foods, sodium intake, and saturated fat intake as a proportion of energy. On the ED, patients avoid certain food additives often found in processed and packaged foods. These are commonly non-core foods as they contain higher amounts of sodium and saturated fat, explaining the observed changes in HEIFA sub-scores. This is supported by a report on

the CSIRO Healthy Diet Score, which found that people who avoided certain foods or dietary components had a higher score than non-avoiders, due to a decreased intake of discretionary foods⁸. On the ED, patients are also encouraged to use products such as rice bran oil and Nuttelex, which contain unsaturated fats, instead of vegetable oil or butter. This could contribute to a higher intake of unsaturated fats.

The ED did result in some decreases in diet quality, including decreases in serves and variety of fruit, and serves of dairy and alternatives. Pears are the only fruit allowed on the strictest form of the ED, as all others contain natural chemicals which must be avoided, explaining these results. Additionally, the majority of participants were excluding dairy on the ED (55 of 81 participants) compared to only roughly one-tenth at baseline, contributing to the observed decrease in intake. Patients are encouraged to boost intake with dairy alternatives such as calcium-fortified rice milk, however this was only employed by 20 participants on the ED.

The proportion of participants consuming carbohydrate and fat within the AMDR increased on the ED compared to baseline, and mean energy from carbohydrate increased to be within the AMDR. Mean energy from fat and protein was within the AMDR at both stages. Carbohydrate intake below the AMDR is characteristic of the Australian population⁵, and increased carbohydrate intake on the ED may result from a higher intake of starchy vegetables compared to baseline, as indicated by previous research at the Allergy Unit (Nearchou M, 2015; unpublished data). The increase in the proportion of people consuming fat within the AMDR may be related to the decreased intake of non-core foods, which can contain large amounts of saturated fats.

The proportion of participants meeting the EAR on the ED significantly decreased compared to baseline for thiamin, riboflavin, folate, vitamin A and calcium. Decreases

for thiamin and folate may be due to the higher proportion of participants excluding gluten on the ED compared to baseline (45 of 81 participants vs 22 of 121 participants, respectively). In Australia, fortification of wheat flour with thiamin and folate is mandatory⁹, but fortification of flour used in gluten-free products is voluntary, thus contributing to a possible decreased intake in people following a gluten-free diet. The major source of riboflavin in the Australian diet is milk and milk products⁵, thus explaining the observed result as intake of dairy and alternatives decreased and more participants were excluding dairy on the ED compared to baseline. Mean intake on the ED was below the EAR for vitamin A and calcium. At baseline, mean intake of vitamin A was above the EAR, and 86 participants (71%) met requirements compared to only 23 participants (28%) on the ED. Previous research at the Allergy Unit attributed this decrease to the small variety of vegetables allowed on the ED (Tripodi E, 2016; unpublished data). Patients on a stricter ED must exclude carrot, sweet potato and pumpkin, which are all rich sources of vitamin A. Calcium intake was inadequate at baseline and on ED, with 31% (n=38) and 14% (n=11) meeting the EAR, respectively. These results are unsurprising, as participants in this study were predominantly female and a large proportion presented to the Allergy Unit limiting dairy in their diet, with roughly one-tenth excluding dairy entirely. In the 2011-12 AHS, only 27% of females and 49% of males aged two years and over met their EAR for calcium⁵.

At baseline, calcium intake in those excluding dairy (n=13) was similar to those including dairy (n=108) due to the use of dairy alternatives, such as calcium-fortified soy milk, used by 29 participants at baseline. On the ED, calcium intake was lower in those excluding dairy (n=55) compared to those including dairy (n=26). Patients who present to the Allergy Unit with gastrointestinal symptoms are often required to exclude

dairy on the ED. This was seen in this study as 105 of 121 participants (87%) presented with gastrointestinal symptoms and 55 of 81 participants (68%) were excluding dairy on the ED. Thirty-four of these participants were additionally excluding soy. The exclusion of dairy and soy from the diets of these participants could explain the decreased calcium intake observed here. Those with adequate calcium intake in this group were drinking ≥ 1.4 serves/day of calcium-fortified soy milk or rice milk.

Patients on the ED are encouraged to have three serves/day of dairy or calcium-fortified dairy alternatives. Only 10 of the 34 participants excluding both dairy and soy on the ED included calcium-fortified rice milk, with an additional 15 participants including unfortified varieties. Additionally, the mean intake was only 1.1 serves/day. Unfortified rice milk is listed as containing 73.5mg/100mL on FoodWorks8, while fortified rice milk is listed as containing 126mg/100mL. Unfortified rice milk is, however, a better option than other unfortified dairy alternatives (e.g. unfortified soy milk listed as containing 13mg/100mL). This highlights the importance of recommending particular brands of rice milk to patients on the ED, especially those excluding dairy and soy, to ensure calcium-fortified varieties are selected.

As the Australian population has inadequate intake of calcium and dairy and alternatives^{5,10}, it may be difficult to encourage all patients on the ED to adequately include dairy alternatives in their diet. Thus, it is important to be aware of other sources of calcium on the ED, including tofu (if allowed soy), cabbage, and eggs¹. These foods provide substantially less calcium per serve than dairy and alternatives, however results from the 2011-12 AHS indicate up to 40% of dietary calcium can come from sources other than dairy and alternatives⁴. Most importantly, supplementation should be considered in patients who are unlikely to meet calcium requirements with food alone.

In this study, supplementation was found to increase the proportion of participants meeting calcium requirements on the ED, regardless of dairy inclusion or exclusion.

Adequate calcium intake is encouraged to maintain bone health, however it is important to be aware of other major influencing factors, including vitamin D status and exercise⁴.

All three factors are required for bone maintenance and reducing risk of osteoporosis.

Additionally, other dietary characteristics can influence calcium metabolism and risk of osteoporosis. In the EPIC-Oxford study, vegans and vegetarians with a calcium intake of ≥ 525 mg/day had similar fracture risks to meat-eaters¹¹. This may result from some of the typical characteristics of these diets which decrease calcium resorption from bone and loss in the kidneys, including high intakes of fruit and vegetables, and low sodium intake¹². A lower sodium intake already results on the ED, however encouraging fruit and vegetable intake may aid bone health even if calcium requirements are not met.

An advantage of this study was the wider variety of participants at baseline, adding to the statistical power of the study and reducing the selection bias that may be introduced by limiting the study to only participants who complete two WFRs. This approach, however, excluded the use of paired analyses, which may be an area of interest for future studies as more participants are recruited. The participants were predominantly female, however this was not viewed as a limitation as it is representative of the food intolerance population. Use of the HEIFA² was both a strength and limitation of this study. It allowed diet quality based on the ADGs to be compared using a single score, and also allowed more in depth analysis using sub-scores for various criteria. The HEIFA, however, is validated in 19 to 30 year olds, when these participants were 19 to 80 years old. Discrepancies were also identified in the coding process over time, and though these were rectified where possible, remaining discrepancies could introduce

error to the results. Particularly, it was not always possible to identify dairy alternatives as calcium-fortified or unfortified if no brand was provided. In these cases, generic options were selected which may or may not have met the calcium cut-off used in this study. Future studies may wish to stratify the participants by gender, which was not conducted in this study due to the small number of males. Results from the 2011-12 AHS show that nutrient intakes can vary greatly between genders, especially for calcium⁵, thus stratifying the participants may reveal differences between genders which could influence clinical practice.

Overall, the ED improved the diet quality of participants due to decreases in intake of discretionary foods and deleterious nutrients. Intake of some key micronutrients decreased on the ED due to its restrictive nature, thus appropriate dietary education is important. Despite similarly inadequate calcium intakes at baseline and in the Australian population⁵, promoting adequate intake on the ED is important for maintaining bone health. As a nutrient at risk, it is important to include education of allowed sources of calcium on the ED, as well as encouraging the use of supplementation if required and integrating rich sources of calcium into recipes available to patients. It is recommended that future studies consider stratifying the participants by gender, and include investigations into diet quality and nutritional adequacy after liberalisation.

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Appendix 1**Comparison of baseline demographic characteristics for participants with baseline WFR only (n=40) and participants with both baseline and on ED WFRs (n=81).**

	Baseline WFR Only	Baseline & On ED WFR	P-value
Age, yrs: mean (SD)	41.4 (14.7)	44.0 (13.8)	0.359
Gender: n (%)			0.509
Female	30 (75)	65 (80)	
Male	10 (25)	16 (20)	
BMI, kg/m ² : median (IQR)	23.2 (21.5-25.0)	23.5 (20.8-26.9)	0.753

BMI – Body Mass Index, ED – Elimination Diet, IQR – Inter-Quartile Range, SD – Standard Deviation, WFR – Weighed Food Record

Figure Legends

Figure 1. Effect of dairy exclusion and calcium supplementation on calcium intake, at baseline and on Elimination Diet (ED). Figure 1a compares calcium intake from food only (i.e. not including calcium from supplements) in those excluding dairy at baseline (n=13) and on ED (n=55), and those including dairy at baseline (n=108) and on ED (n=26). Figure 1b compares calcium intake in non-supplementers at baseline (n=107) and on ED (n=47), and supplementers at baseline (n=14) and on ED (n=34). Dotted line indicates Estimated Average Requirement (EAR). Calcium intake presented as percent of EAR (%EAR), as Box and Whiskers plots with outliers determined by the Tukey method. Mean intake represented as a cross (+). Data compared using Mann-Whitney tests. $P < 0.05$ considered statistically significant. *** indicates $P < 0.001$, and ns indicates not significant.

Figure 2. Comparison of calcium intake between four groups on the Elimination Diet: those excluding dairy and not supplementing (n=26), those excluding dairy and supplementing (n=29), those including dairy and not supplementing (n=21), and those including dairy and supplementing (n=5). Dotted line indicates Estimated Average Requirement (EAR). Calcium intake presented as percent of EAR (%EAR). Data for the first three groups represented as Box and Whiskers plots with outliers determined by the Tukey method and mean intake represented as a cross (+). Data for the last group represented as dots for each point due to small sample size and mean intake represented as a solid line.

Table 1. Comparison of diet quality in baseline (n=121) and on ED WFRs (n=81), using total HEIFA score and composite sub-scores.

HEIFA Categories	Maximum Score	HEIFA Score: mean (SD)			Proportion Meeting Recommendations: n (%)		
		Baseline	On ED	P-value [^]	Baseline	On ED	P-value
Vegetables (serves)	5	3.1 (1.5)	3.2 (1.6)	0.374	29 (24)	25 (31)	0.278
Vegetables (variety)	5	1.5 (1.1)	1.8 (1.0)	0.069	-	-	-
Fruits (serves)	5	2.3 (1.8)	1.4 (1.5)	0.001	25 (21)	5 (6)	0.005 ↓
Fruits (variety)	5	0.4 (1.4)	0.0 (0.0)	0.006	-	-	-
Total Grains (serves)	5	2.9 (1.4)	2.9 (1.4)	0.715	21 (17)	14 (17)	0.990
Whole Grains (serves)	5	1.4 (1.6)	1.3 (1.7)	0.747	6 (5)	8 (10)	0.177
Meat and Alternatives (serves)	10	8.4 (2.5)	8.3 (2.7)	0.894	74 (61)	51 (63)	0.796
Dairy and Alternatives (serves)	10	4.6 (3.4)	3.2 (3.6)	0.003	20 (17)	10 (12)	0.413
Unsaturated Fats (serves)	5	2.1 (1.8)	3.0 (2.0)	0.003	24 (20)	33 (41)	0.001 ↑
Fluids (serves)	5	1.2 (1.3)	1.2 (1.4)	0.422	3 (2)	3 (4)	0.686
Non-Core Foods (serves)*	10	3.7 (3.0)	5.0 (3.2)	0.005	0 (0)	3 (4)	0.063
Alcohol (serves)*	5	5.0 (0.0)	4.9 (0.6)	0.401	121 (100)	80 (99)	0.401
Sodium (mg)*	10	4.2 (4.0)	7.8 (3.3)	<0.001	31 (26)	54 (67)	<0.001 ↑
Saturated Fat (%Energy)*	5	2.2 (2.2)	4.1 (1.7)	<0.001	38 (31)	62 (77)	<0.001 ↑
Added Sugar (%Energy)*	10	9.8 (0.9)	9.8 (1.1)	0.716	117 (97)	77 (95)	0.716
Total HEIFA Score (mean, SD)	100	52.7 (8.1)	58.0 (8.6)	<0.001			

*Higher HEIFA scores indicate lower intake of these foods and nutrients. [^]Mann-Whitney test used for comparison between baseline and on ED HEIFA sub-scores. P<0.05 considered statistically significant. ↓ indicates significant decrease. ↑ indicates significant increase. ED – Elimination Diet, HEIFA – Healthy Eating Index for Australian Adults, SD – Standard Deviation, WFR – Weighed Food Record.

Table 2. Comparison of nutritional adequacy in baseline (n=121) and on ED WFRs (n=81), as per recommendations for macronutrients and micronutrients.

Macronutrients (AMDRs)	Mean Intake: % of Requirements (SD)		Proportion Meeting Requirements: n (%)			P-value	
	Baseline	On ED	Baseline	On ED			
%E from Carbohydrate	41 (9)	46 (9)	30 (25)	42 (52%)	<0.001		↑
%E from Fat	35 (8)	30 (8)	52 (43)	55 (68%)	<0.001		↑
%E from Protein	20 (5)	20 (5)	96 (79)	55 (68%)	0.067		
Fibre (AI)	97 (39)	91 (36)	40 (33)	24 (30%)	0.609		
Micronutrients (EARs)							
Thiamin	152 (77)	126 (52)	98 (81)	54 (67)	0.021		↓
Riboflavin	182 (80)	160 (78)	108 (89)	60 (74)	0.005		↓
Niacin	353 (122)	341 (129)	120 (99)	81 (100)	1.000		
Folate	171 (61)	135 (65)	107 (88)	54 (67)	<0.001		↓
Vitamin A	203 (169)	97 (101)	86 (71)	23 (28)	<0.001		↓
Vitamin C	356 (223)	239 (166)	115 (95)	71 (88)	0.057		
Calcium	87 (37)	67 (36)	38 (31)	11 (14)	0.004		↓
Iron	173 (73)	170 (59)	110 (91)	75 (93)	0.673		
Magnesium	126 (43)	117 (46)	84 (69)	53 (65)	0.552		
Phosphorus	248 (77)	226 (71)	120 (99)	81 (100)	1.000		
Potassium (AI)	103 (32)	100 (39)	62 (51)	33 (41)	0.143		
Zinc	147 (52)	143 (53)	95 (79)	66 (81)	0.607		
Sodium (UL)	90 (41)	60 (36)	84 (69)	74 (91)	<0.001		↑

P<0.05 considered statistically significant. ↓ indicates significant decrease. ↑ indicates significant increase. %E – % of Energy, AI – Adequate Intake, AMDR – Acceptable Macronutrient Distribution Range, EAR – Estimated Average Requirement, ED – Elimination Diet, SD – Standard Deviation, UL – Upper Limit, WFR – Weighed Food Record.

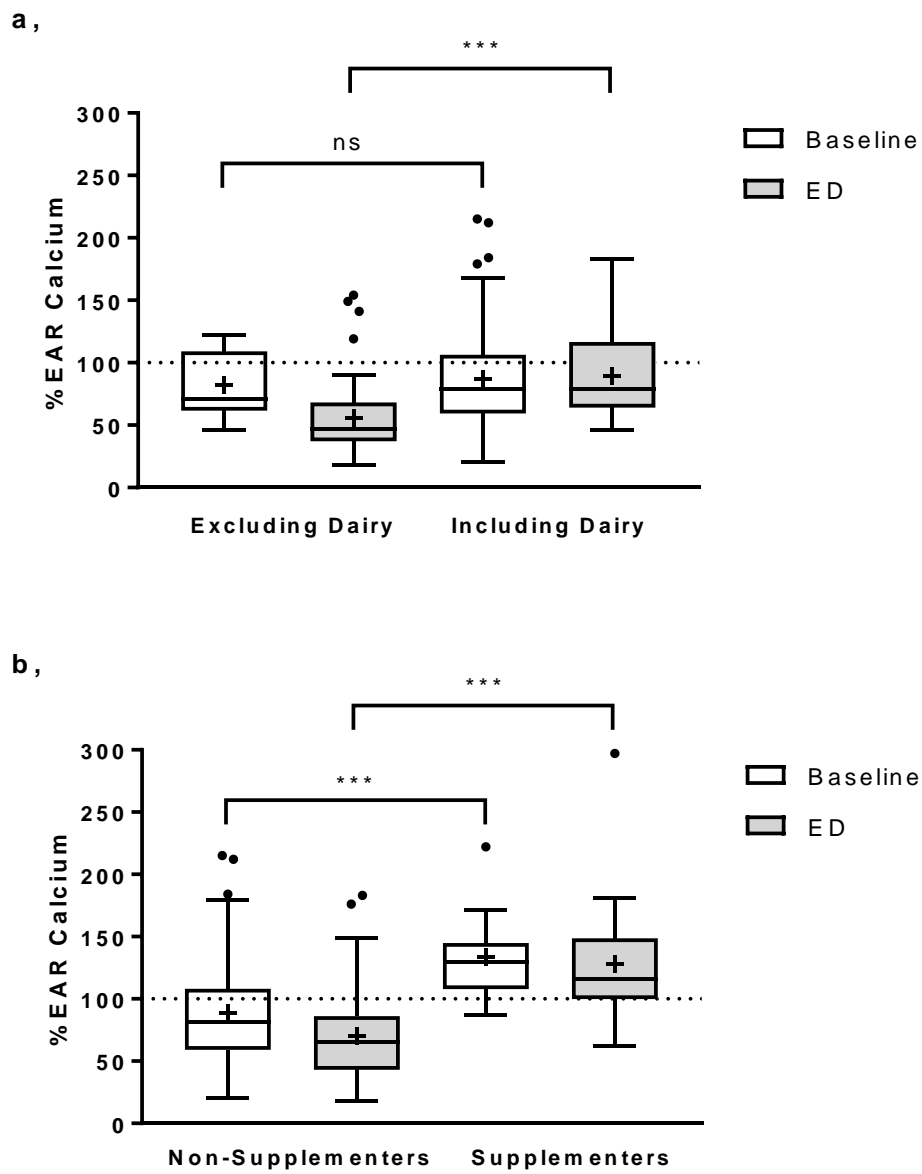


Figure 1. Effect of dairy exclusion and calcium supplementation on calcium intake, at baseline and on the Elimination Diet.

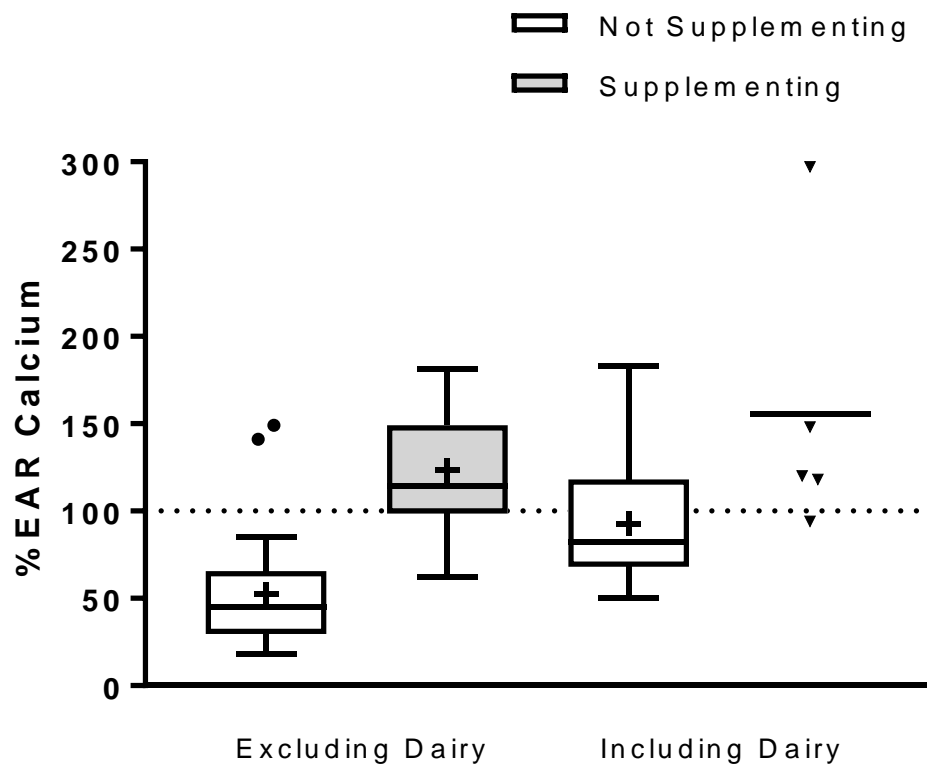
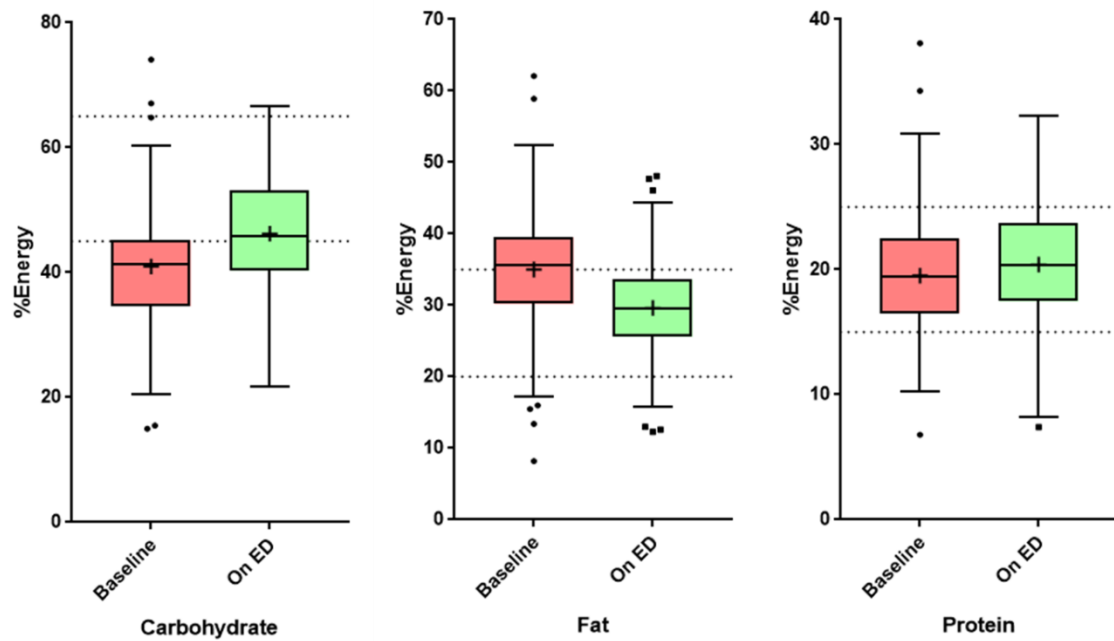


Figure 2. Comparison of calcium intake between four groups (\pm dairy and \pm supplements) on the Elimination Diet.

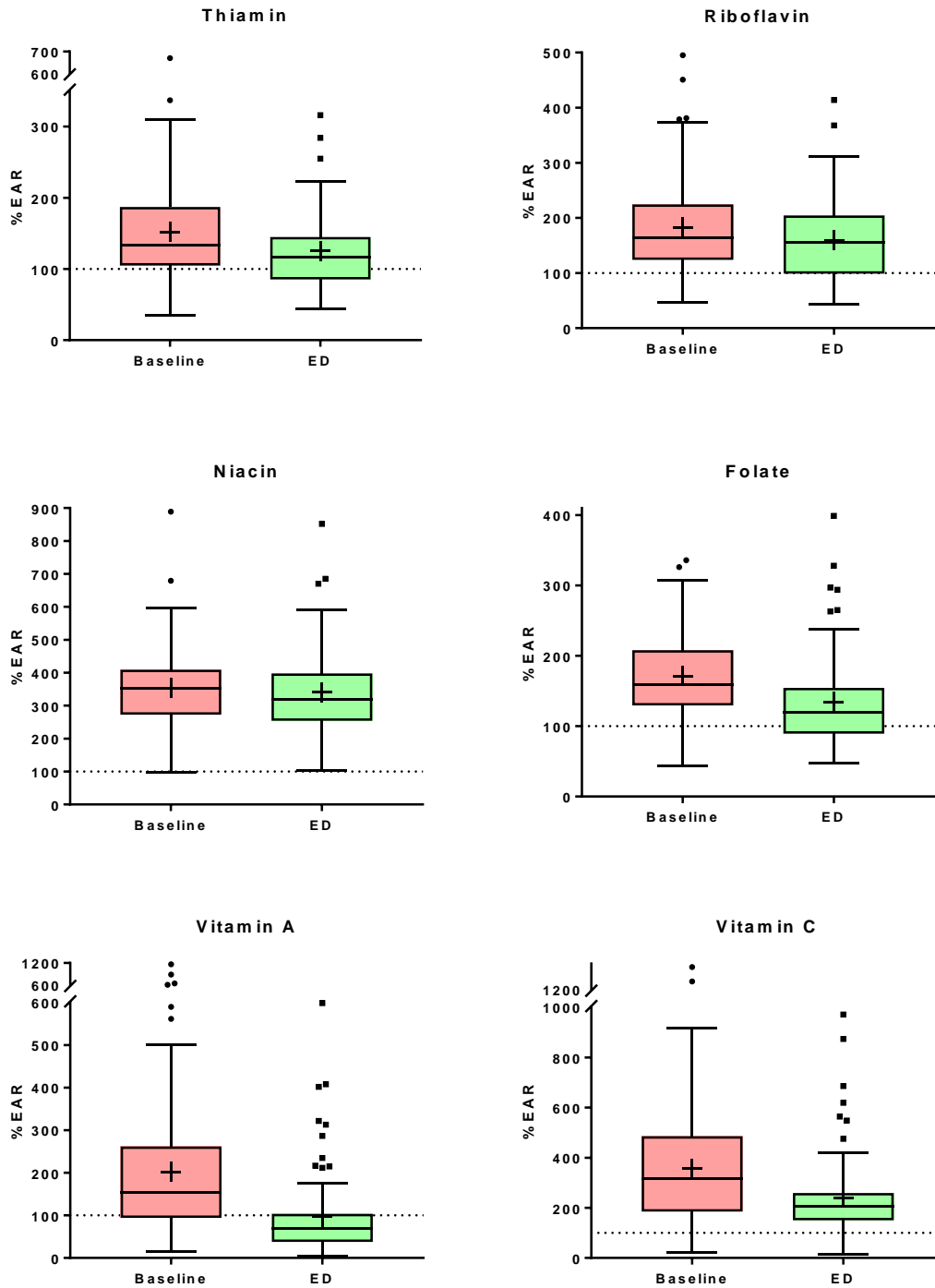
Supplementary Tables and Figures

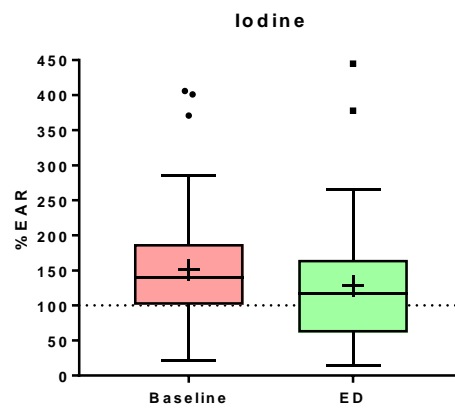
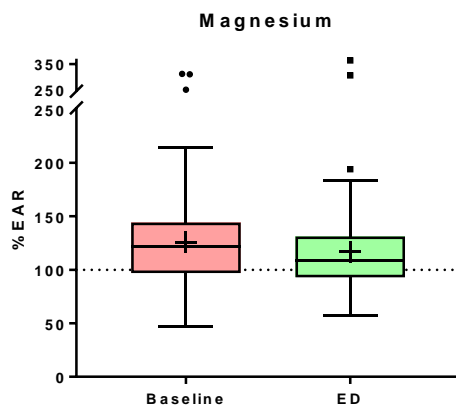
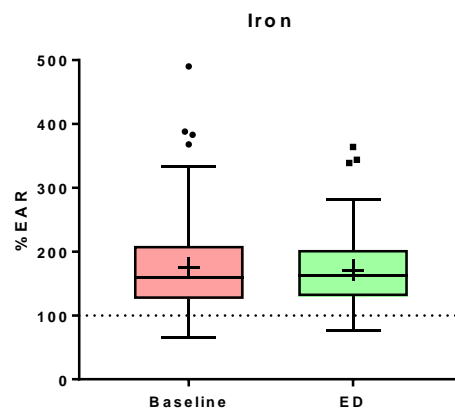
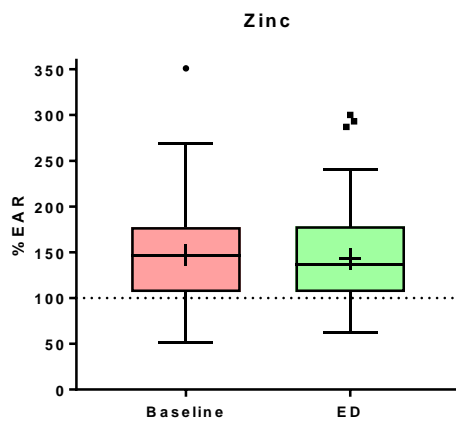
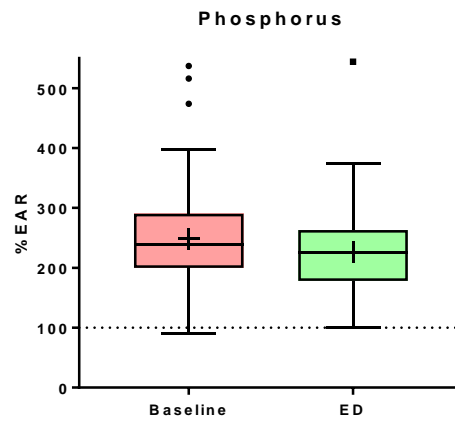
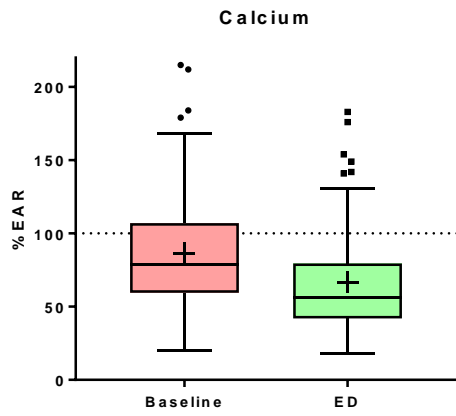
1. Box & Whiskers plots for carbohydrate, fat and protein, at baseline and on ED.

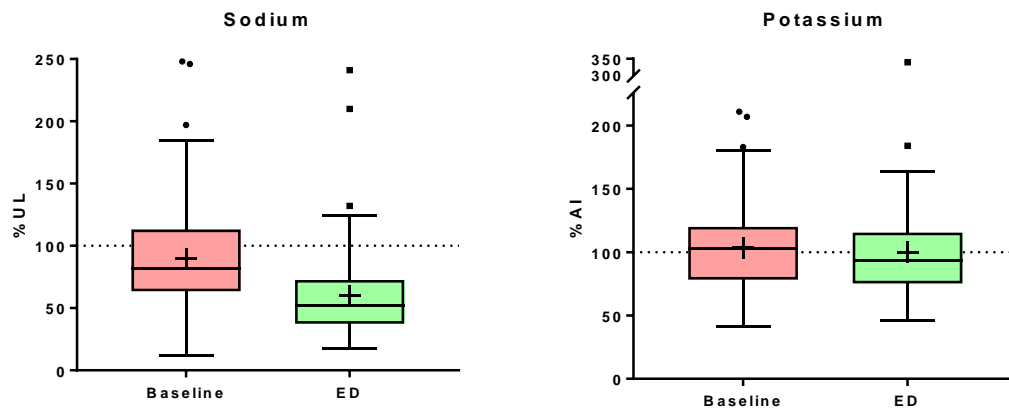


The ED resulted in better adherence to the AMDRs for carbohydrate and fat. AMDRs are represented as the dotted lines in each graph. Mean carbohydrate intake as a % of energy increased and mean fat intake as a % of energy decreased, so that more participants fell within the AMDR for both macronutrients. The majority of participants were within the AMDR for protein at both baseline and on ED.

2. Box & Whiskers plots for all micronutrients, at baseline and on ED.

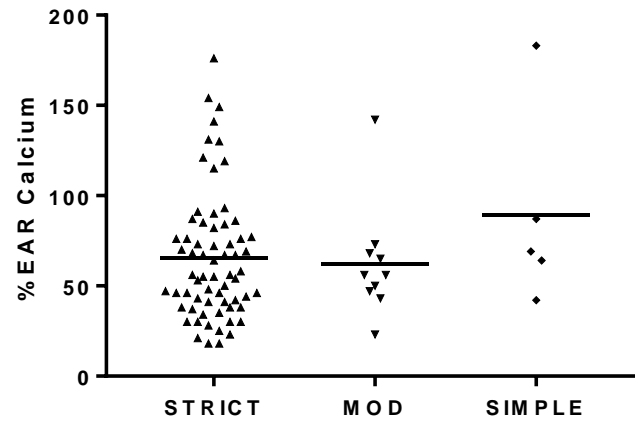






The ED resulted in decreases in intake for most micronutrients compared to baseline; however the majority of participants were still above the EAR for most micronutrients. There were significant decreases in the proportion of participants meeting the EAR on the ED compared to baseline for thiamin, riboflavin, folate, vitamin A, calcium and iodine. Iodine was excluded from this report as iodine content of foods can be varied, thus the FoodWorks results are likely unreliable. Mean intake was below the EAR on the ED for calcium and vitamin A. The proportion of participants exceeding the UL for sodium decreased on the ED compared to baseline.

3. Scatter plot of calcium intake by ED level.



Distribution of calcium intake (%EAR) was similar for each ED level (strict n=66, moderate n=10, simple n=5), thus the three groups were combined for the analyses in this report. The solid line indicates the mean calcium intake.

4. Use of dairy and calcium-fortified dairy alternatives, at baseline and on ED.

BASELINE	Proportion Consuming: n (%)	Mean Intake (serves/day)*
Milk	80 (66)	1.0
Yoghurt	40 (33)	0.5
Cheese	94 (78)	0.7
Soy Milk	29 (24)	0.9
Other Alternatives	6 (5)	0.5
Total	114 (94)	1.8
Proportion Adequate	20 (17)	-
ON ED	Proportion Consuming: n (%)	Mean Intake (serves/day)*
Milk	31 (38)	1.2
Yoghurt	13 (16)	0.5
Cheese	15 (19)	0.5
Soy Milk	17 (21)	1.4
Other Alternatives	20 (25)	0.9
Total	57 (70)	1.7
Proportion Adequate	10 (12)	-

*Mean intake in those consuming each type of dairy or alternative.

Decreases in the proportion of participants consuming milk, yoghurt and cheese were observed on the ED compared to baseline, due to a higher proportion of participants excluding dairy (68% vs 11%, respectively). Increases in the proportion of participants consuming 'other alternatives' (i.e. calcium-fortified rice milk) were observed on the ED compared to baseline, with an increased mean intake. Mean intake of calcium-fortified soy milk also increased. The proportion of participants having any dairy or alternatives decreased on the ED compared to baseline (70% vs 94%, respectively).

5. Use of calcium-fortified and unfortified rice milk on the ED.

Overall ED (n=81)	Proportion Consuming: n (%)	Mean Intake (serves/day)*
Fortified Only	20 (25)	0.9
Fortified and Unfortified	40 (49)	1.0
Excluding Dairy and Soy (n=34)		
Fortified Only	10 (29)	0.9
Fortified and Unfortified	25 (74)	1.1
Excluding Dairy and Soy and Not Supplementing (n=20)		
Fortified Only	5 (25)	0.6
Fortified and Unfortified	13 (65)	1.0

*Mean intake in those consuming each type of dairy alternative.

Twenty participants on the ED were drinking calcium-fortified rice milk, and another 20 participants were drinking unfortified rice milk. In those excluding dairy and soy on the ED, 10 participants were drinking calcium-fortified rice milk, with a further 15 participants drinking unfortified rice milk. There was no observed increase in intake of rice milk (calcium-fortified and unfortified) in those who were excluding dairy and soy, and not supplementing, compared to those on ED overall. Mean intake of rice milk was below the recommendations for intake of dairy and alternatives in each group.

Note: some rice milks were incorrectly identified as calcium-fortified or unfortified, due to a lack of information on brand used or incorrect calcium contents in FoodWorks.

Thus, these results should be used with caution.

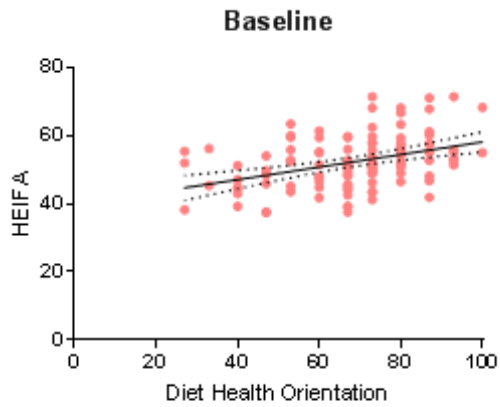
6. Effect of dairy exclusion and calcium supplementation, at baseline and on ED.

	Baseline (N=121)			On ED (N=81)		
	n (%)	Meeting EAR: n (%)	%EAR (SD)	n (%)	Meeting EAR: n (%)	%EAR (SD)
Excluding Dairy	13 (11)	4 (31)	82% (27%)	55 (68)	4 (7)	56% (30%)
Including Dairy	108 (89)	34 (31) ^a	87% (38%)	26 (32)	7 (27) ^b	90% (37%)
Not Supplementing	107 (88)	36 (34)	88% (38%)	47 (58)	8 (17)	70% (40%)
Supplementing*	14 (12)			34 (42)		
Without supps.		2 (14) ^c	74% (24%)		3 (9) ^c	62% (30%)
With supps.		13 (93) ^d	133% (34%)		26 (76) ^d	128% (43%)

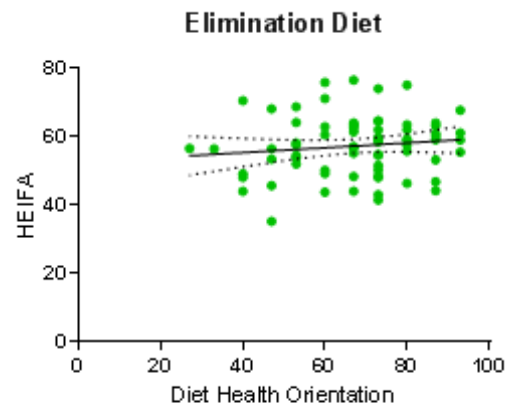
*Supplementers were participants taking a specific calcium and/or bone-strengthening supplement. ^a Non-significant compared to those excluding dairy. ^b Significant compared to those excluding dairy. ^c Non-significant compared to those not supplementing. ^d Significant compared to those not supplementing.

There was no difference in calcium intake between those excluding or including dairy at baseline, as those who were excluding dairy were drinking soy milk to replace cow's milk. Inclusion of dairy on the ED resulted in higher calcium intakes compared to those excluding dairy, as many of these participants were also excluding soy. There were no significant differences in calcium intake *from food* between participants who were and were not taking calcium supplements, at baseline and on ED. Total calcium intake, however, was significantly higher in those taking calcium supplements compared to those who were not, at baseline and on ED.

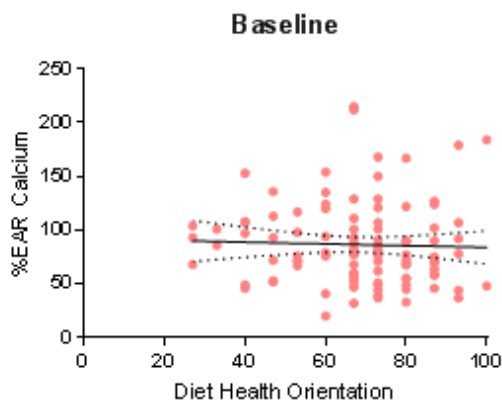
7. Correlation of HEIFA and calcium intake (%EAR) with the Diet Health Orientation score, at baseline and on ED.



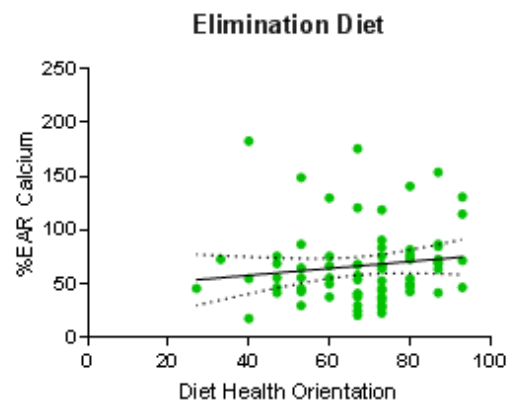
P for Correlation <0.05



Non-Significant



Non-Significant



Non-Significant

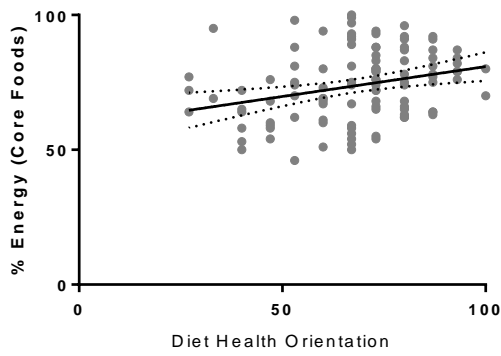
There was a significant correlation between the HEIFA and Diet Health Orientation scores at baseline, but this was not present on the ED. There was no correlation between calcium intake and the Diet Health Orientation score at baseline or on ED.

8. Contribution of core and non-core foods, and core and non-core ingredients to energy and calcium intake, at baseline and on ED.

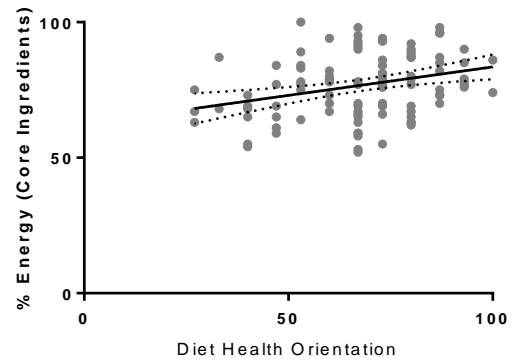
ENERGY		Baseline % (SD)	On ED % (SD)
Core Food	Core Ingredient	70 (13)	78 (14)
	Non-Core Ingredient	4 (4)	2 (4)
	Total	74 (13)	80 (14)
Non-Core Food	Core Ingredient	7 (8)	6 (8)
	Non-Core Ingredient	19 (11)	14 (11)
	Total	26 (13)	20 (14)
Total Core Ingredients		77 (11)	84 (12)
Total Non-Core Ingredients		23 (11)	16 (12)
CALCIUM		Baseline % (SD)	On ED % (SD)
Core Food	Core Ingredient	83 (13)	86 (15)
	Non-Core Ingredient	2 (2)	2 (4)
	Total	85 (12)	88 (14)
Non-Core Food	Core Ingredient	7 (10)	5 (11)
	Non-Core Ingredient	8 (7)	7 (9)
	Total	15 (12)	12 (14)
Total Core Ingredients		90 (8)	91 (11)
Total Non-Core Ingredients		10 (8)	9 (10)

Slightly more energy came from core foods on the ED compared to baseline (80% vs 74%, respectively), due to increases in energy from core ingredients of core foods and decreases in energy from non-core ingredients of non-core foods. Proportion of calcium from core foods was roughly equivalent between baseline and the ED (85% vs 88%, respectively). No statistical comparisons for level of significance were conducted.

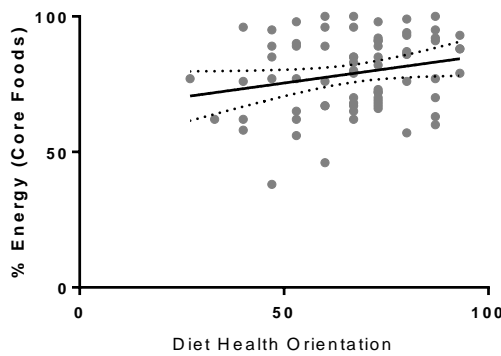
9. Correlation of %Energy from core foods and core ingredients with the Diet Health Orientation score, at baseline and on ED.



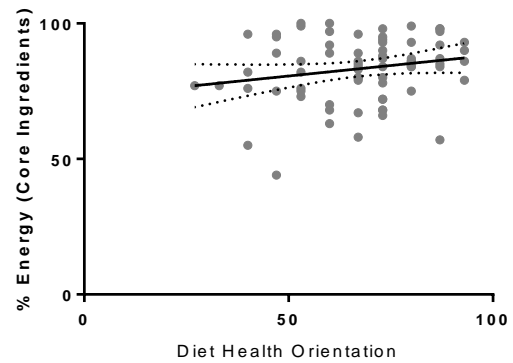
Baseline: P for Correlation <0.05



Baseline: P for Correlation <0.05



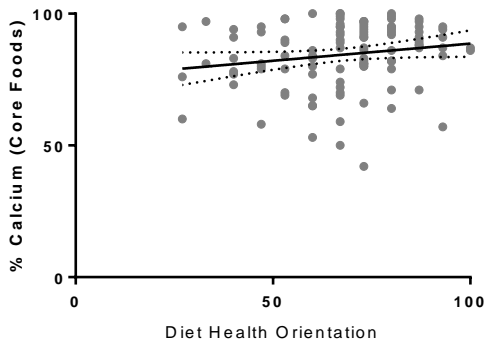
ED: non-significant



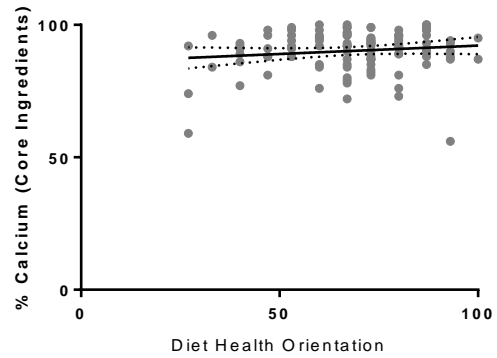
ED: non-significant

There was a significant correlation between energy from core foods and the Diet Health Orientation score, and energy from core ingredients and the Diet Health Orientation score, at baseline only. These correlations may not have existed on the ED due to the smaller sample size.

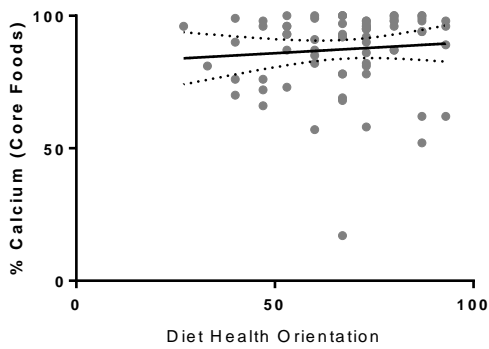
10. Correlation of %Calcium from core foods and core ingredients with the Diet Health Orientation score, at baseline and on ED.



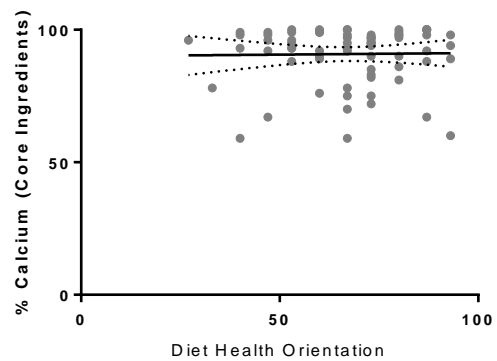
Baseline: P for Correlation <0.05



Baseline: non-significant



ED: non-significant



ED: non-significant

There was a significant correlation between calcium intake from core foods and the Diet Health Orientation score at baseline. There was no significant correlation with the Diet Health Orientation score and calcium intake from core ingredients at baseline, or core foods or core ingredients on the ED.