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1 **ABSTRACT**

2

3 **Aim:** Claims have been made that the level of omega-6 fats in the diet are too high and that
4 this cannot be reduced without increasing the saturated fat intake. The aim of this study was
5 to design a diet within the framework of the Australian Guide to Healthy Eating which would
6 supply <2% energy (% E) from the omega-6 polyunsaturated fatty acid, Linoleic Acid,
7 compared with the 7-8 % E in the current Australian Diet.

8 **Methods:** Separate 7-day diet plans were designed using FoodWorks (version 2009) for
9 males (10,000 kilojoule (kJ)/day), and females (8,000 kJ/day). The reduction in dietary
10 omega-6 polyunsaturated fatty acid content was achieved by replacing standard plant-based
11 oils and spreads used in cooking and baking (canola and sunflower oils), with macadamia oil
12 and butter and restricting the intake of some processed foods. All diets complied with the
13 Australian Guide to Healthy Eating.

14 **Results:** We successfully designed diets which complied with the Australian Guide to
15 Healthy Eating and which had a linoleic acid content of 1.80 % E and 1.75 % E in females
16 and males respectively. In both cases, the omega-6:omega-3 ratio was reduced to 5.1:1,
17 compared to ~12:1 in the typical Australian diet and the saturated fat content was <10 % E.

18 **Conclusion:** These results suggest that reducing the linoleic acid content of the diet can be
19 readily achieved within the boundaries set by the Australian Guide to Healthy Eating, without
20 an increase in saturated fat intake.

21

1 INTRODUCTION

2 The omega-3 (n-3) and omega-6 (n-6) polyunsaturated fatty acids (PUFA) represent two
3 **biologically important** fatty acids in the typical Australian diet. The **most commonly**
4 **consumed n-3 fatty acids in the Australian diet** are alpha linolenic acid (ALA,18:3 n-3),
5 which is found mainly in plants, and the n-3 long chain PUFA (LCPUFA), eicosapentaenoic
6 acid (EPA) and docosahexaenoic acid (DHA), which are found mainly in fish and seafood.¹

7 **The n-3 LCPUFA can be synthesised from the short-chain precursor, ALA, in vivo, however**
8 **the efficiency of this conversion in humans is generally poor, and the majority of n-3**
9 **LCPUFA must be derived pre-formed from the diet.**² The main n-6 PUFA are linoleic acid
10 (LA) and arachidonic acid (AA). LA is found at high levels in many vegetable oils and
11 spreads and LA-rich oils are widely used in the manufacture of processed and convenience
12 foods.¹

13
14 Whilst both the n-6 and n-3 PUFA are essential fatty acids for human health, they each have
15 distinct biological functions. The n-6 PUFA give rise to pro-inflammatory eicosanoids and
16 promote adipose tissue deposition, whereas the n-3 PUFA have anti-inflammatory effects and
17 reduce adipogenesis and lipogenesis.³ As a result, an excess consumption of n-6 PUFA
18 relative to n-3 PUFA over a period of time promotes inflammation and increases adipogenic
19 activity, and has negative effects on cardiovascular and metabolic health.⁴ **Conversely, the**
20 **beneficial effects of decreasing the n-6/n-3 PUFA ratio of the diet on a range of chronic**
21 **conditions, including rheumatoid arthritis and cardiovascular disease, have been reported in a**
22 **number of large randomised controlled trials (for review see,⁵).** The imbalance between n-3
23 and n-6 PUFA also has implications for PUFA metabolism, since the n-6 and n-3 PUFA
24 compete with one another for conversion to their long-chain derivatives and incorporation
25 into tissues.² Consequently, dominance of n-6 over n-3 PUFA limits the endogenous

1 conversion of plant-derived ALA to EPA and DHA and the incorporation of pre-formed n-3
2 LCPUFA obtained from the diet into tissue membranes, which is necessary for their
3 beneficial biological effects.⁶

4

5 The diets of early humans are thought to have contained equal amounts of n-6 and n-3 PUFA
6 ⁷ However, since the industrial revolution there has been a substantial shift in dietary fatty
7 acid composition and in most western countries, including Australia, the typical diet now
8 contains between 10 and 20 fold more n-6 PUFA than n-3 PUFA.^{1, 8-9} This shift has been
9 driven in part by recommendations from health agencies as early as the 1950s to reduce the
10 intake of saturated fat in the diet to lower the risk of cardiovascular disease. This, coupled
11 with the emergence of margarines as cheap substitutes for butter and cheap vegetable oils as
12 an alternative to animal fats, resulted in the wide-spread replacement of traditional animal-
13 based products used in cooking and baking, which were high in saturates and low in PUFA,
14 with vegetable-based oils and spreads, which are lower in saturates but contain high levels of
15 n-6 PUFA.¹⁰ These oils and spreads remain substantially cheaper and more widely available
16 than animal-based fats and are used in large quantities in most processed and fast foods.
17 Thus, the increased reliance on these convenience foods compared to home-cooked meals has
18 contributed to further increases in dietary n-6 PUFA intake. In contrast the n-3 PUFA intake
19 across this same period has remained relatively constant, resulting in an increasing
20 dominance of n-6 PUFA over n-3 PUFA in the modern diet and there is growing concern
21 about the potential health implications of this trend.¹⁰⁻¹²

22

23 The balance between n-6 and n-3 PUFA in the diet is likely to be particularly important in
24 Australia, since the majority of Australians eat very little fish, and despite recommendations
25 being in place for over 5 years to increase fish consumption to at least 2 meals per week the

1 average Australian consumes less than one fish meal per fortnight.¹³ It has therefore been
2 argued that reduced n-6 PUFA consumption, in the context of a balanced and nutritionally
3 complete diet, will have beneficial effects on the n-3 LCPUFA status of individuals, and thus
4 confer metabolic and cardiovascular benefits.³ However, this approach has been complicated
5 by the fact that current recommendations by a number of health agencies, including the
6 National Heart Foundation, are that the population should further increase their consumption
7 of n-6 PUFA to optimise cardiac health.¹⁴ Furthermore, commentators have suggested that it
8 is not possible to significantly reduce n-6 PUFA intake without increasing the saturated fat
9 intake to levels in excess of recommendations (*unpublished observations, 2011*).

10

11 The aim of this study was to design a diet within the framework of the Australian Guide to
12 Healthy Eating (AGHE)¹⁵ which would supply $\leq 2\%$ energy (% E) from LA compared with
13 the 7-8 % E in the current typical Australian diet,¹ whilst maintaining the saturated fat intake
14 at below 10 % E.

15

16 METHODS

17 All dietary analyses were conducted using the latest AUSNUT food composition tables,
18 developed as part of the National Children's Nutrition and Physical Activity Survey, which
19 contains nutrient values for more than 4200 foods, beverages and supplements in the FoodWorks
20 Program (VERSION 2009, Xyris software, Australia). LA, ALA and n-3 LCPUFA content of
21 foods was obtained from the Australian RMIT Fatty Acids database within the FoodWorks
22 program. The estimated energy requirements were calculated using the NRV Equation within
23 the FoodWorks program and assumed an age of 35 and a very light level of physical activity.
24 Separate diet plans were designed for males (~10,000 kJ/day) (based on weight 75 kg, height
25 175 cm), and females (~8,000 kJ/day) (based on weight 65 kg, height 165 cm). Both male

1 and female diets consisted of breakfast, lunch, dinner and two snacks for a total of 7 days and
2 on any given day, the same foods/meals were included for both males and females. The
3 higher energy content in the male diet was achieved by increasing the size of bread and cereal
4 serves in accordance with the AGHE.

5
6 The male and female diets were designed based on foods and meals which are regularly
7 consumed amongst Australians, including a variety of breads, cereals, grains, fruit,
8 vegetables, fish, chicken and red meat and in line with the AGHE.¹⁶ All foods and ingredients
9 were readily available in Australian supermarkets. The changes in n-6 PUFA content were
10 achieved principally by changing the oils and spreads used in food preparation and limiting
11 the consumption of processed foods

12
13 The diet was designed to include popular main meals obtained from a recent population-
14 based survey conducted in Australia¹⁷ and to include a selection of convenience foods and
15 recipes which contained relatively low LA levels. Recipes for home-made sweets and dinners
16 were included in the diet plans. Evening meals were planned based on the top 10 dinners
17 consumed within Australian households with the inclusion of two fish meals (non-fatty white
18 fish fillet and tuna mornay) per week.¹⁷ The availability of food products and brands used in
19 the dietary plans was established by supermarket visits in the metropolitan area.

20
21 The diets were designed to supply $\leq 2\% \text{ E}$ from LA (5.4 g/day for male and 4.3 g/day for
22 female). The $2\% \text{ E}$ target was selected since empirical equations derived to explain the
23 interrelationship between dietary PUFA intake and tissue n-6 and n-3 PUFA levels¹⁸ suggest
24 that decreasing intake to $2\% \text{ E}$ would enable Australians to achieve a target n-3 status for
25 cardio protection without increasing their current n-3 LCPUFA intakes.¹ Since standard

1 vegetable-based oils and spreads supply a large percentage of the LA in the typical Australian
2 diet, macadamia oil (LA, 1-3 % ALA; 1-2 % of total fatty acids) and butter (LA, 0.2 % ALA;
3 0.1 % of total fatty acids), both of which contain very low total PUFA levels in addition to
4 low amounts of LA, were used as alternatives in cooking and baking. Processed foods with
5 high LA content were substituted with lower LA alternatives.

6

7 To illustrate the impact of using different oils in cooking and baking on dietary fatty acid
8 composition, the effect of substituting the macadamia oil and butter in the diet with two
9 widely used plant-based oils, canola (Gold n Canola© spread and canola oil) and sunflower
10 (Flora© spread and sunflower oil), was evaluated using FoodWorks.

11

12 All values of dietary content are expressed as % E. For all fatty acids, macronutrients and
13 micronutrients, the average intake across the 7 days of the diet were determined. For the
14 substitution experiment, LA levels obtained using the 3 different oils were compared using a
15 one-way ANOVA. The Duncan's multiple range test was used post-hoc to identify significant
16 differences between groups. A *p* value of < 0.05 was considered statistically significant.

17

18 RESULTS

19 A diet was designed for a 7-day period for both males and females based on the framework
20 set by the AGHE and achieved our aim of supplying <2 % E LA. Examples of a single day of
21 the diet for males and females are shown in **Table 1**. On any given day the foods/meals
22 included for males and females were similar, however in order to demonstrate more of the
23 range of foods included over the 7-day period, we chose to present an example of the diet for
24 two different days (one weekday for males and one weekend day for females). The average
25 LA intakes across the 7 days of the diet of were 1.80 % E (range, 1.60-2.00 % E) and 1.75 %

1 E (range, 1.50-1.95 % E) in females and male respectively. The average daily energy intake
2 was 8175 kJ for females and 9952 kJ for males. The n6/n3 ratio in the low LA diet was 5.1 :
3 1 in both males and females.

4
5 The macronutrient composition of the diet complied with that specified in the AGHE (i.e. 15-
6 25 % E protein, 45-65 % E carbohydrate, 25-30 % E total fat) in both males and females
7 (Table 2). The average daily saturated fat content of the diet was 8.2 % E in both males and
8 females, which was well below <10% E set within the guidelines. Both male and female diets
9 also supplied sufficient dietary fibre (Table 2) and key micronutrients (iron, vitamin C,
10 vitamin D, vitamin E, vitamin A, potassium, magnesium, calcium, phosphorous, zinc, iodine,
11 calcium) to comply with current Dietary Guidelines and Nutrient Reference Values (Table
12 3).^{19,15} Iron intakes for the females were, on average, 75% of RDIs, but were well above the
13 estimated average requirements specified by the NHMRC (Table 3).^{19,15}

14
15 Substituting the butter and macadamia oils in the low LA diet with oils more commonly used
16 in cooking and baking in the typical Australian diet (canola and sunflower oil), without any
17 other dietary modifications, was associated with a significant increase in the LA content of
18 the diet, both in g/day and as % E, as assessed by one-way ANOVA. In males, the
19 substitution of macadamia oil and butter with canola or sunflower-based oils and spreads
20 resulted in a significant increase in the average daily intake of LA from 1.75 % E in the low
21 LA diet to 2.96 % E and 5.55 % E for the canola and sunflower diets respectively,
22 representing a 1.7 and 3 fold increase in LA levels. Similar results were found in females
23 (Table 4). The LA content of the sunflower-based diet was also significantly higher than that
24 of the canola-based diet in both males and females (Table 4).

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DISCUSSION

The data provided in this study provides a useful insight into a dietary strategy which could counteract the increasing LA intakes in typical Western diets. We have shown that it is possible to reduce the LA content of the diet from 7-8 % E to <2 % E by simply substituting common vegetable-based oils and spreads (canola and sunflower) with low LA alternatives, in this case macadamia oil and butter, for cooking, baking and use as spreads. The dramatic impact of high n-6 PUFA spreads and oils on the LA intake was highlighted by comparing the fatty acid composition of three diets which varied only in the types of oils/spreads which were used. As such, our study suggests that it would be possible to achieve a substantial shift in the fatty acid profile of the Australian diet simply by replacing LA-rich oils and spreads with low-PUFA alternatives and avoiding high n-6 PUFA convenience foods. Importantly, the decrease in n-6 PUFA intake was achieved with adherence to the AGHE and recommended daily intakes and/or estimated average requirements of key macro and micronutrients and without an accompanying increase in saturated fat intake. Indeed saturated fat levels were well below 10 % of total energy intake.

Replacing sunflower or canola oils and spreads with macadamia oil and butter significantly reduced the LA content of the diet, and reduced the LA:ALA ratio to ~5:1 in both males and females, compared to ~12:1 in the typical Australian diet.¹ Whilst there have been previous studies in which macadamia oil and/or macadamia nuts have been used as sources of dietary MUFA,²⁰⁻²² the diet in the current study is, to the best of our knowledge, the first to use macadamia oil substitution specifically to lower dietary n-6 PUFA intake. Macadamia oil was specifically chosen for this study due to its very low PUFA content (LA, 1-3 % ALA, 1-2 % of total fatty acids).²³ Additionally its mild aroma and sensory properties make it suitable for

1 use in cooking and baking.²⁴ Other oils that are very low in LA are appearing on the market
2 that may also be useful in reducing the LA content of the diet, including high oleate
3 sunflower oils.

4
5 Whilst there is limited data to support it, one of the principal arguments promoted against
6 decreasing the n-6 PUFA content of the diet is that this could not be achieved without an
7 accompanying increase in dietary saturated fat content (*unpublished observations*). However,
8 this study has demonstrated that it is possible to significantly reduce the LA content of the
9 diet whilst maintaining saturated fat intake of <10% E, in accordance with current health
10 recommendations.^{14-15,19} This was achieved by substituting the n-6 PUFA with oils/spreads
11 which contained high levels of monounsaturated fatty acids (MUFA), rather than saturates.
12 The potential health benefits of high MUFA diets have been investigated in previous clinical
13 trials.²⁵⁻²⁶ In one of these randomised controlled trials, high MUFA diets based on olive or
14 peanut oils were found to be more effective at reducing serum triglyceride concentrations and
15 reducing cardiovascular risk than the American Heart Association/National cholesterol
16 education program step II diet (low total fat), even though both diets contained the same
17 amounts of saturates.²⁵

18
19 Previous studies have also reported that increasing dietary MUFA intake through increased
20 consumption of macadamia oil²² or macadamia nuts²⁰ resulted in significant reductions in
21 total and LDL cholesterol after 4 weeks. This effect occurred despite the fact that there was
22 no effect of these dietary interventions on plasma n-3 or n-6 PUFA concentrations, and that
23 the diets were matched for total energy and PUFA content. In a separate study by Curb and
24 colleagues (2000), a macadamia diet providing 37 % E as fat, largely in the form of MUFA,
25 produced comparable decreases in total and LDL cholesterol and increases in HDL

1 cholesterol after 4 weeks compared with the Step I diet of the American Heart Association,
2 which had a significantly lower total fat content.²¹ Despite these promising findings, studies
3 of the health benefits of macadamia oil and of high-MUFA diets in general have been limited,
4 and it will be important to evaluate the potential benefits of high MUFA vs high PUFA diets
5 in future randomised controlled trials.

6

7 LA is an essential fatty acid which must be derived from the diet, and there have been
8 previous suggestions that an excessive reduction in LA intake could result in essential fatty
9 acid deficiency. The results from both animal and human studies have led to estimates that a
10 daily intake of 1-2% E of is required to prevent deficiency.²⁷ However, earlier studies suggest
11 that this figure may be in excess of actual requirements; in rats, the tissue content of highly
12 unsaturated fatty acids (HUFA) are maintained with intakes as low as 0.3% E of both
13 essential PUFAs (LA and ALA) and no physiological signs of fatty acid deficiency were
14 noted at this level of intake.² Whilst these animal studies suggest that the level of LA in the
15 low-LA diet is highly unlikely to be associated with any physiological symptoms of essential
16 fatty acid deficiency, additional studies in humans are required to confirm this. In addition,
17 most Australians already consume some n-3 LCPUFA as part of their normal diet, mainly
18 from fish, red meat and eggs.¹

19

20 One of our core objectives in this study was to design a diet consisting of foods which could
21 readily be obtained from the supermarket at a reasonable cost, and to select dishes previously
22 shown to be popular amongst Australian families. However, we are aware that long-term
23 maintenance of our diet is complicated by the fact that the majority of processed foods and
24 take-away foods contain high levels of n-6 PUFA-based oils, and largely need to be avoided
25 in order to achieve the LA targets.¹ Thus, shifting the n-6/n-3 balance in the Australian diet

1 on a population level will require either avoidance of take-way and processed foods by
2 consumers, combined with a switch in their choice of spreads and oils, or changes within the
3 food industry, in particular the adoption of lower LA oils by the fast-food industry,
4 restaurants and in the production of processed foods. The versatility of macadamia and high
5 oleate sunflower oils could be seen as an opportunity for the food industry to market a
6 product with added health benefits, provided that sufficient evidence is obtained to allow
7 such health claims to be made. A major challenge to enacting such changes comes from the
8 current guidelines release by a number of prominent health agencies, including the National
9 Heart Foundation of Australia, which include recommendations to further *increase* n-6
10 PUFA intake in order to achieve optimal health benefits.¹⁴

11
12 It is important to make the point that even Australians who do not consume fish still obtain
13 ~100 mg/day of n-3 LCPUFA from other dietary sources, in particular meat and eggs.¹
14 Indeed, data from the National Nutrition Survey conducted in 1995 showed that, on average,
15 meat contributed almost as much as seafood to the n-3 LCPUFA intake of Australians.^{1,13}
16 Furthermore, the results of previous studies suggest that lowering the level of LA in the diet
17 whilst maintaining the same intake of meat and eggs is likely to translate into significant
18 improvements in n-3 LCPUFA status of the population and the associated health benefits.¹⁸ It
19 is also clear, however, that the level of n-3 and n-6 PUFA in red meat and eggs varies
20 considerably depending on the composition of the animal feed, and it will be important to
21 examine strategies for reducing the n-6/n-3 PUFA ratios in these products in the future.

22
23 In summary, this study clearly shows that it is possible to substantially reduce dietary LA
24 intake by simply replacing the oils and spreads commonly used in food preparation with
25 macadamia oil and butter. Macadamia oil is widely available in the domestic market in

1 Australia and is priced similarly to olive oil, making it a viable alternative to high n-6 PUFA
2 plant-based oils. Similarly, butter is cheap and readily available and we have shown that it is
3 possible to use butter in the place of polyunsaturated margarines whilst still adhering to
4 recommendation of no more than 10% E saturated fat. Since our diets have been designed to
5 comply with the AGHE, we have ensured that they are nutritionally complete. In addition, we
6 have focussed on ensuring that the diets are practically orientated in order to be acceptable to
7 the general Australian population. Whilst, this study is an important first step to designing a
8 low LA diet for Australians, further studies which examine the effect of our diet on n-3
9 LCPUFA status, blood lipids and clinical measures of cardiovascular and metabolic health
10 will be important in order to test its effectiveness in conferring health benefits.

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Table 1 Examples a single day of the diet for males and females. In order to demonstrate more of the range of foods included over the 7-day period, this table presents an example of the diet for two different days (one weekday for males and one weekend day for females), however on any given day the same foods were used for males and females.

	MALE		FEMALE	
Breakfast	Sanitarium weet-bix	4 biscuits	Wholemeal bread	2 slices
	Milk (reg fat)	200 ml	Butter	2 tsp
	Raw sugar	1 tsp	Tomato, grilled	2 medium
	Coffee with red fat milk		Mushroom, cooked in oil	1 cup
	Raw sugar	1 tsp	Macadamia oil	2 tsp
			Coffee with red fat milk	
Morning Tea	Granny smith apple	1	Chocolate cake*	1 serve
	Low fat fruit yoghurt	200 g tub	Coffee with red fat milk	
	Coffee with red fat milk		Raw sugar	1 tsp
	Raw sugar	1 tsp		
Lunch	Wholemeal bread	2 slices	Wholemeal bread	2 slices
	Leg ham	2 slices	Leg ham	20 g
	Tomato	1 medium	Tomato	1 medium
	Cucumber	6 slices	Cheddar cheese	40 g
	Lettuce	2 small leaves		
	Carrot	¼ cup	Fruit salad	1 cup
Afternoon Tea	Macadamia shortbread*	2 serves	Low fat fruit yoghurt	200 g
	Banana, Cavendish	1 medium	Fruit loaf	2 slices
	Coffee with red fat milk		Butter	2 tspn
	Raw sugar	1 tsp		
Dinner	Spaghetti Bolognese	1 serve	Beef casserole	1 serve
	Pasta, white wheat flour based	2 cup	White rice	1 cup cooked
	Parmesan cheese	2 tbsp grated	Red wine	2 (150 ml) glasses
	Garlic bead	60 g		
	Red wine	2 (150 ml) glasses		
Dessert	Fruit salad in natural juice	1 cup	Canned peaches in natural juice	1 cup
	Yoghurt (reg fat)	200 g	Reduced fat custard	200 ml
Total Energy	9400 kJ		8214 kJ	
Total n-6 PUFA	4.96g (1.95 % E)		4.49 g (2.02 % E)	

Table 2 Average nutritional content of the low LA diet across the 7 day period for females and males

	Female		Male	
	Average	Range	Average	Range
Energy Dietary Fibre (kJ)	8175	7945-8685	9952	8907-11515
Carbohydrate (% E)	45.3%	41.0-50.4%	46.4%	38.9-52.4%
Protein (% E)	16.7%	13.2-18.2%	16.9%	13.4-20.4%
Total Fat (% E)	27.2%	22.1-31.5%	26.6%	23.7-30.1%
Saturated Fat (% E)	8.2%	4.2-13.8%	8.2%	4.6-13.5%
LA (% E)	1.80%	1.6-2.0%	1.75%	1.50-1.95%
ALA (% E)	0.35%	0.31-0.38%	0.34%	0.32-0.36%
total n-3 LCPUFA (% E)	0.14%	0.01-0.40%	0.19%	0.06-0.45%
Dietary Fibre (g)	32.0	26.0-43.0	36.0	28.0-48.0

**Data obtained from AusNut 2007 via FoodWorks Program,*

Table 3 Average micronutrient content of the low LA diet across the 7 day period for females and males

	Female			Male		
	Average	Range	% RDI	Average	Range	% RDI
Iron(mg)	11.2	6 - 14.9	75%	14.0	8.5 - 18.6	175 %
Vitamin C (mg)	178	68.0 - 3823	397%	190	68.0 -3 83	421%
Vitamin D (ug)	3.32	1.71 - 6.76	97%	4.5	2.5 - 9.9	95%
Vitamin E (mg)	13.1	8.5 - 19.9	187%	13.6	9.1 - 20.3	136%
Vitamin A (ug)	1331	588 - 2097	190%	1431	652 - 2159	159%
Folate (ug)	441	263 - 574	110%	507	317 - 658	127
Potassium (mg)	4166	3652 -4800	150%	4581	3993 -5435	120%
Magnesium (mg)	337	275 - 404	105%	396	313 - 494	95%
Calcium (mg)	1010	758 - 1119	101%	1206	923 -1533	120%
Phosphorous (mg)	1560	1290 -1816	156%	1960	1566 -2455	195%
Zinc (mg)	11.6	8.37 -15.21	145%	13.9	9.9 - 17.4	99%
Iodine (ug)	108	68.8 - 161	85%	135	111 - 189	94%

**Data obtained from AusNut 2007 via FoodWorks Program, RDI=recommended daily intake*

Table 4 The effect of replacing macadamia oil with canola and sunflower oils on average daily LA content in males and females

	FEMALE			MALE		
	Macadamia	Canola	Sunflower	Macadamia	Canola	Sunflower
g/day	3.99 ± 0.16 ^a	7.08 ± 0.56 ^b	13.5 ± 1.7 ^c	4.71 ± 0.20 ^a	7.87 ± 0.32 ^b	14.7 ± 1.2 ^c
% E	1.80 ± 0.07 ^a	3.22 ± 0.26 ^b	6.15 ± 0.78 ^c	1.74 ± 0.06 ^a	2.96 ± 0.17 ^b	5.55 ± 0.52 ^c

Data expressed as mean SEM. Different superscripts denote values which are significantly different by one way ANOVA ($p < 0.05$)

For Peer Review