

## Original Article

# Bread enriched with microencapsulated tuna oil increases plasma docosahexaenoic acid and total omega-3 fatty acids in humans

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The aim of this study was to determine the acute and chronic effects of low doses of long chain (LC) n-3 polyunsaturated fatty acids (PUFA) (<100 mg per day) on plasma LC n-3 PUFA levels using a novel delivery form; bread containing microencapsulated tuna oil (MTO). Six omnivores (three men and three women) participated in the acute study, which involved ingesting a prototype MTO bread containing approximately 80 mg of LC n-3 PUFA/four slices. Plasma triacylglycerol fatty acid compositions were measured after an overnight fast and postprandially at 2 and 4 h. In the chronic study, 10 vegetarian subjects (nine men and one woman) consumed MTO bread at six to eight slices/day (comprising 60 mg of LC n-3 PUFA) as the only dietary source of these PUFA for three weeks. Fasting plasma total and phospholipid fatty acid compositions were measured at baseline and endpoint. In the acute study, the proportions of 22:6 n-3 and total n-3 PUFA in plasma triacylglycerol were significantly increased ( $P < 0.05$ ). In the chronic study, the proportions of 20:5 n-3, 22:5 n-3, 22:6 n-3, total n-3 PUFA in plasma, and 22:6 n-3 and total n-3 PUFA in plasma phospholipid fractions were significantly increased ( $P < 0.05$ ) at the endpoint compared with the baseline. This study showed that a low dose of LC n-3 PUFA, consumed as MTO-enriched bread, was bioavailable, as measured by an increase in LC n-3 PUFA levels in the plasma of human subjects.

**Key words:** microcapsule, n-3 fatty acid, tuna oil.

## Introduction

The relationship between fish/fish oil intakes and human tissue long chain (LC) n-3 polyunsaturated fatty acid (PUFA) accretion and subsequent manifestation of numerous health benefits has been well documented. The Australian diet, typical of Western diets, contains low levels of LC n-3 PUFA, which have been estimated to be between 100 and 190 mg/day for adults.<sup>1</sup> It is much lower than the International Society for the Study of Fatty Acid and Lipid's (ISSFAL) recommended dietary intake of 650 mg/day.<sup>2</sup>

The richest food sources of 20- and 22-carbon LC n-3 PUFA are fish and seafood. Minor amounts are also present in meats, especially lean meat, offal, eggs, milk and dairy products. High consumption of these food sources elevates LC n-3 PUFA tissue levels.<sup>3–5</sup> However, these conventional food sources may not be suitable or convenient for certain individuals or communities for various religious, ethical or personal (palatability, allergenicity) reasons. Ovolacto vegetarians obtain a limited amount of LC n-3 PUFA from eggs, milk and dairy products. Vegans must rely entirely on *in vivo* biosynthesis of these nutrients from the precursor alpha-linolenic acid (18:3 n-3), and the rate relies on the level of

18:3 n-3 and its ratio to linoleic acid (18:2 n-6) in the diet.<sup>6</sup> However, using dietary 18:3 n-3 is not as effective as direct consumption of LC n-3 PUFA from fish/fish oil for increasing tissue LC n-3 PUFA levels.<sup>7</sup> Vegetarians have been demonstrated to have lower levels of LC n-3 PUFA in their platelets and plasma, which is associated with increased platelet activity and plasma 11-dehydro-thromboxane B<sub>2</sub> production.<sup>8</sup>

One approach to improving the LC n-3 PUFA status of populations is to incorporate these important nutrients into frequently consumed processed foods. There have been a number of studies that have examined the effects of omega-3-enriched eggs,<sup>9,10</sup> milk<sup>11</sup> and processed foods<sup>12–15</sup> as a means of improving tissue LC n-3 PUFA levels. In general, these studies have used foods containing 290–600 mg LC

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Accepted 12 February 2002

n-3 PUFA per serving; however, there have been no studies looking at regular consumption of smaller doses of n-3 PUFA which, if added to a variety of staple foods, may offer a more practical option without extensive change to habitual diets. Bread enriched with a low level of microencapsulated tuna oil (MTO) (10 mg LC n-3 PUFA per slice) is currently available to Australian consumers. However, there is no data on the effect of low dose LC n-3 PUFA in processed foods on human n-3 PUFA status.

The aims of this study were: (i) to investigate the acute effects of a single dose of low level LC n-3 PUFA (approximately 80 mg from MTO-enriched bread) on postprandial plasma LC n-3 PUFA levels; and (ii) to investigate the chronic effects of a daily dose of a lower level of LC n-3 PUFA (approximately 60 mg from MTO-enriched bread) for three weeks on fasted plasma LC n-3 PUFA status. We hypothesised that low dose MTO-enriched bread would improve plasma LC n-3 PUFA status.

## Materials and methods

### Subjects

Ethics approval was granted by the Human Research Ethics Committee of RMIT University, and all subjects gave their written consent before participating. Free-living and healthy volunteers, (12 men and four women; six omnivores, nine ovo-lacto vegetarians and one vegan) aged 21–64 years were recruited from RMIT University and through advertisements in the Vegetarian Society of Victoria newsletter. An omnivore was defined as someone who ate meat at least five times a week, an ovo-lacto vegetarian was defined as someone who ate no meat, not more than one fish meal and three eggs a week, and a vegan was defined as someone who ate meat, eggs and dairy products less than six times per year and had been following this diet for at least six months. The exclusion criteria were major medical illness, cigarette smoking, excessive alcohol intake and chronic use of aspirin or other anti-inflammatory drugs.

### Study design and diet

The project consisted of two intervention studies; the acute and chronic effects of MTO-enriched bread (using Clover Corporation Driphorm 25) on plasma LC n-3 PUFA status. In the acute study, six omnivores (three men and three women) were advised to consume their usual foods for one week prior to the experiment day, except one day before the study when fish meals were not permitted. Subjects were given a single dose of LC n-3 PUFA (approximately 80 mg) to observe changes in postprandial plasma LC n-3 PUFA levels at 2 and 4 h. LC n-3 PUFA was delivered through four slices of prototype MTO-enriched bread (Bunge Cereal Foods, Melbourne, Australia) together with 20 g of monounsaturated sunflower oil margarine (Flora; Unilever Foods, Marrickville, NSW, Australia). The bread was eaten either lightly toasted or untoasted. Table spreads (jam, vegemite) and beverages (coffee, tea, orange juice) were also made available. After this meal, subjects were asked to refrain from consuming food, except one apple and water or zero

calorie drinks, and to avoid any strenuous physical activity for the next 4 h.

In the chronic study, nine ovo-lacto vegetarians (eight men and one woman) and one vegan (male) were instructed to consume six slices of commercially available MTO-enriched bread (containing 60 mg of LC n-3 PUFA; using Clover Corporation Driphorm 25) daily, either untoasted or toasted lightly, with their habitual diet for three weeks to investigate the changes in fasting plasma LC n-3 PUFA status. Foods rich in n-3 PUFA, such as fish and all seafood, fish oil capsules, oat germ, wheat germ/germ oils, walnuts, linseeds and soy/linseed breads were on the subjects' exclusion list during the intervention period. The first loaf of enriched bread (sliced toast loaf) was provided for subjects on their first blood sampling day, thereafter they purchased their own as required from local supermarkets. Each subject was required to fill out a semiquantitative food frequency questionnaire recording his or her daily intake of fat-containing foods consumed during the 3 week intervention period. The questionnaire was primarily designed to estimate the habitual fat intake of each subject, specifically the LC n-3 PUFA, and to check for compliance. Subjects were asked to avoid foods listed on the exclusion list; however, when it was not possible to adhere, details of food items and portion sizes of the 'prohibited foods' were to be declared. Subjects were advised to record the number of slices of bread consumed daily and to specify whether the slices were toasted or untoasted. Compliance for bread consumption was monitored by requesting subjects to present used bread packets and receipts at the end of the study period. All subjects were contacted once a week during the study period to check on their progress with bread consumption, to remind them to avoid exclusion foods and to generally maintain their interest and compliance.

### Blood specimen collections

In the acute study, venous blood was drawn into a 9 mL ethylenediaminetetraacetic acid (EDTA) vacuum tube from subjects between 08:30 and 09:00 h after an overnight fast (baseline) and postprandially (2 and 4 h following the ingestion four slices of prototype MTO-enriched bread). The sampling times were selected to coincide with peak plasma triacylglycerol (TAG) absorption (between 2 and 4 h postprandially), based on data from Agren *et al.*<sup>16</sup> and Dubois *et al.*<sup>17</sup> In the chronic study, each subject's height, weight, percentage body fat, pulse and blood pressure were measured before bleeding. In the chronic study, venous blood was collected into a 9 mL EDTA vacuum tube from subjects before (week 0) and after (week 3) the intervention period, following a 12 h overnight fast. Plasma was isolated by centrifugation at 1000 g for 10 min at 4°C and stored frozen in portions at –80°C until analysis.

### Dietary assessment

The diet records were analysed for total fat intake using Diet Version 4 software (Xyris Software, Highgate Hill, QLD, Australia) with the NUTTAB 95 database based on Composition of

Food Australia (COFA). Estimation of the individual LC n-3 PUFA intake was based on published values of omega-3-containing food fatty acid concentrations.<sup>18–22</sup>

#### Plasma fatty acids

Total plasma lipids were extracted with chloroform : methanol (1:1 v/v). The plasma phospholipid (PL) and TAG fractions were separated by thin-layer chromatography and the methyl esters of the fatty acids of total plasma, plasma PL and TAG fractions were prepared. Fatty acid methyl esters were separated and quantified by gas-liquid chromatography, as described previously.<sup>23</sup>

#### Statistical analyses

The data analyses were performed using a STATVIEW software program (Abacus Concepts, Berkeley, CA, USA). ANOVA with repeated measures was used to determine the effect of MTO on plasma LC n-3 PUFA status. The values were reported as mean  $\pm$  SD in all the results tables and mean  $\pm$  SEM in all the graphs, unless otherwise specified. *P*-values less than 0.05 were considered to be significant.

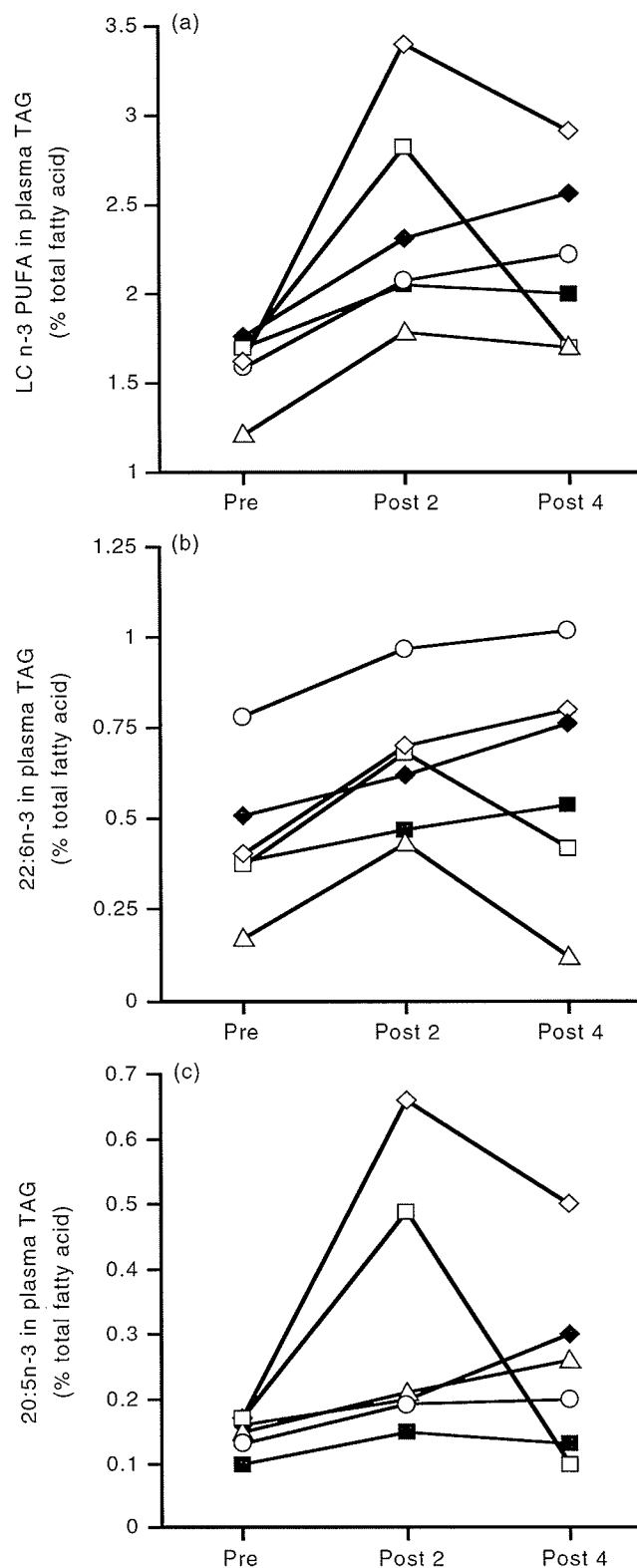
#### Results

The total fat content of prototype MTO-enriched bread was 2.9 g/100 g bread. The prototype MTO-enriched bread contained 44 mg of 22:6 n-3, 8.9 mg of 20:5 n-3 and 2.8 mg of 22:5 n-3, and also 3.5 mg of arachidonic acid (AA) per 100 g. In the monounsaturated sunflower oil margarine, the  $\alpha$ -linolenic acid (ALA) content was 255 mg/20 g of margarine, and there were no LC n-3 PUFA in this product.

In the acute study, subjects consumed four slices of prototype bread and 20 g of margarine. The total fat contributed by each food was 4.1 g from the bread and 15 g from the margarine. The mean intake of fatty acids from the bread–margarine meal was: total LC n-3 PUFA, 79 mg; 22:6 n-3, 62 mg; 20:5 n-3, 13 mg; 22:5 n-3, 4 mg; and 18:3 n-3, 298 mg.

Changes in the proportions of docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and total LC n-3 PUFA in plasma TAG for the individual subjects in the acute study are shown in Fig. 1. The total LC n-3 PUFA proportion of plasma TAG increased in all six subjects by between 5 and 121% for total n-3 PUFA ( $P = 0.0163$ ), from  $0.81 \pm 0.47$  to  $1.22 \pm 0.68$  mg/100 mL from baseline to 2 h postprandially (Fig. 1a). The 22:6 n-3 proportion in plasma TAG increased in all six subjects by between 22 and 153% ( $P = 0.0147$ ), from  $0.43 \pm 0.37$  to  $0.73 \pm 0.56$  mg/100 mL from baseline to 2 h postprandially (Fig. 1b). The 20:5 n-3 proportion of plasma TAG was also increased in all six subjects by between 25 and 288%; however, this was not significant ( $P = 0.14$ ) (Fig. 1c).

The commercial MTO-enriched bread contained 18 mg of 22:6 n-3, 4.5 mg of 20:5 n-3 and 1.5 mg of 22:5 n-3, and also 1.9 mg of AA per 100 g. Daily fat intakes of habitual diets and during the 3 weeks of the commercial MTO-enriched bread intervention period (experimental diet) in the



**Figure 1.** Changes in the composition of the plasma triacylglycerol fraction. (a) Total long chain (LC) n-3 polyunsaturated fatty acids (PUFA), (b) 22 : 6n-3 and (c) 20 : 5n-3 after consumption of a microencapsulated tuna oil-enriched meal. Post 2, postprandial 2 h; post 4, postprandial 4 h; pre, pre-meal.

chronic study are shown in Table 1. The mean daily intakes for the habitual and experimental diets were: total fat, 52 and 55 g/day; PUFA, 20 and 23%; monounsaturated fatty acids (MUFA), 42 and 40%; and saturated fatty acids (SFA), 38 and 37% of total fat, respectively. The daily intakes of total LC n-3 PUFA were 1 and 64 mg for the habitual and experimental diets, respectively. The daily intake of ALA was 1900 mg for the habitual diet and 670 mg for the experimental diet.

The physiological characteristics of the subjects in the chronic study were as follows: body mass index (BMI),  $22.9 \pm 2.4$  kg/m<sup>2</sup>; waist/hip ratio,  $0.84 \pm 0.06$ ; percentage body fat,  $17.6 \pm 5.0\%$ ; pulse,  $63 \pm 7$  b.p.m.; systolic blood pressure,  $110 \pm 7$  mmHg; and diastolic blood pressure,  $71 \pm 5$  mmHg. There were no significant changes in any physiological characteristics measured in the subjects during the 3 week study period.

In the chronic study, the proportion of 20:5 n-3, 22:5 n-3, 22:6 n-3 and total LC n-3 PUFA in the total plasma lipids increased significantly after the 3 week study (Table 2). The increase in total LC n-3 PUFA was from 1 to 42% ( $P = 0.001$ ), while there was an increase in the proportion of 22:6 n-3 for nine of the 10 subjects ( $P = 0.006$ ), in eight of the 10 for 20:5 n-3 ( $P = 0.0059$ ) and in six of the 10 for 22:5 n-3 ( $P = 0.034$ ) at day 21 compared with day 0 (Table 2).

**Table 1.** Daily fat and LC n-3 PUFA intake of habitual diet and during the 3 week commercial MTO-enriched bread intervention period in vegetarians

	Habitual diet ( <i>n</i> = 10)	Experimental diet ( <i>n</i> = 10)
Total fat (g)	52 ± 25	55 ± 26
SFA (% total fat)	38 ± 8	37 ± 7
MUFA (% total fat)	42 ± 5	40 ± 5
PUFA (% total fat)	20 ± 6	23 ± 5
Total LC n-3 PUFA (mg/day)	1 ± 0	64 ± 8*

\* $P < 0.05$ . LC, long chain; MTO, microencapsulated tuna oil; MUFA, monounsaturated fat; PUFA, polyunsaturated fat; SFA, saturated fat.

The concentration of 22:6 n-3 and total n-3 PUFA in the plasma increased significantly from 2.78 to 3.43 mg/100 mL and from 5.73 to 6.80 mg/100 mL, respectively.

In the plasma PL fraction, the proportions of 20:5 n-3, 22:6 n-3 and the total LC n-3 PUFA increased significantly over the 3 week study (Table 2). The increase in total LC n-3 PUFA and in the plasma PL 22:6 n-3 occurred in seven and eight of the 10 subjects at day 21 ( $P$  values were 0.03 and 0.0137, respectively) compared with day 0 (Table 2). The 20:5 n-3 proportion in this fraction was raised in seven of the 10 subjects at day 21 compared with day 0 ( $P = 0.119$ ). The concentration of 22:6 n-3 and total n-3 PUFA in the plasma PL increased significantly from 2.32 to 2.66 mg/100 mL and from 4.25 to 4.74 mg/100 mL, respectively.

## Discussion

The aim of the present study was to determine whether the consumption of bread enriched with a low dose of MTO would improve LC n-3 PUFA status in healthy individuals, as measured by plasma levels of these PUFA. To achieve this, an acute study (single dose ingestion with 4 h follow up) was conducted to establish the feasibility of the use of a low intake of LC n-3 PUFA, delivered as MTO-enriched bread. This was followed by a 3 week chronic study using commercial MTO bread as the LC n-3 PUFA source. We found that low dose MTO-enriched bread did improve plasma LC n-3 PUFA status in both the acute and the chronic study. In other words, the LC n-3 PUFA in the MTO-enriched bread was bioavailable.

Several biological markers have been employed as a measure of LC n-3 PUFA status in humans. Commonly in use are accessible tissues such as blood (plasma, platelets, erythrocytes, neutrophils), adipose tissue, breast milk and cheek cells. All have been reported to correlate reasonably well with dietary intake in prospective as well as dietary intervention trials.<sup>24–28</sup> The selection criteria for their use would then ultimately depend on the objectives and duration of the study. In the present study, the main objective was to determine extent of dietary LC n-3 PUFA incorporation in

**Table 2.** Plasma fatty acid composition in vegetarians consuming MTO-containing bread in the chronic study†

Fatty acid	Total plasma lipids		Phospholipid fraction		<i>P</i> -value	
	Week 0	Week 3	Week 0	Week 3	Total	PL
16:0	21.23 ± 1.30	21.38 ± 1.58	27.87 ± 1.04	27.78 ± 1.81	0.331	0.412
18:0	6.48 ± 0.70	6.47 ± 0.72	13.63 ± 1.39	13.81 ± 1.42	0.484	0.188
18:1 n-9	20.32 ± 2.10	19.70 ± 2.96	9.89 ± 0.86	9.63 ± 1.12	0.174	0.137
18:2 n-6	30.88 ± 3.80	30.95 ± 4.98	24.60 ± 2.68 <sup>a</sup>	23.64 ± 2.90 <sup>b</sup>	0.457	0.024
18:3 n-3	0.76 ± 0.26	0.66 ± 0.19	0.25 ± 0.12	0.21 ± 0.07	0.139	0.085
20:3 n-6	1.74 ± 0.34	1.82 ± 0.34	3.65 ± 0.78	3.76 ± 0.79	0.010	0.147
20:4 n-6	5.79 ± 1.32	5.78 ± 1.08	9.87 ± 1.80	9.69 ± 1.69	0.484	0.328
20:5 n-3	0.54 ± 0.30 <sup>a</sup>	0.61 ± 0.33 <sup>b</sup>	0.72 ± 0.37 <sup>a</sup>	0.82 ± 0.50 <sup>b</sup>	0.002	0.047
22:5 n-3	0.49 ± 0.16 <sup>a</sup>	0.53 ± 0.15 <sup>b</sup>	1.01 ± 0.27	1.02 ± 0.31	0.014	0.405
22:6 n-3	0.99 ± 0.29 <sup>a</sup>	1.25 ± 0.21 <sup>b</sup>	2.13 ± 0.54 <sup>a</sup>	2.45 ± 0.30 <sup>b</sup>	0.002	0.007
Total LC n-3 PUFA	2.02 ± 0.66 <sup>a</sup>	2.40 ± 0.56 <sup>b</sup>	3.86 ± 0.93 <sup>a</sup>	4.30 ± 0.95 <sup>b</sup>	0.0001	0.006

†Percentage of total fatty acids. Values are expressed as mean ± SD for 10 subjects. Statistical significance (paired *t*-test):  $P < 0.05$ . Significant differences between values within the same row are indicated by different superscript letters (<sup>a,b</sup>). LC, long chain; PL, phospholipid; PUFA, polyunsaturated fatty acid.

general as opposed to specific organ function or pharmacological effects, and plasma was therefore selected because of its relative ease of sample collection and preparation. In the acute study, we monitored plasma TAG fatty acids, not PL, as plasma TAG can be acutely influenced by a single meal.<sup>29</sup> In the chronic study, we monitored the LC n-3 PUFA in total plasma lipids (fatty acids from cholesteryl esters, TAG, free fatty acids and PL) and in plasma PL. The reason for this was that while most of the LC n-3 PUFA in human plasma are found in the PL fraction, there is a finite amount of these PUFA in the other lipid classes.<sup>30</sup>

In the acute experiments where early postprandial plasma was investigated, the level of LC n-3 PUFA incorporation was quantified in the nascent TAG fractions (location of newly absorbed LC n-3 PUFA following a meal). The acute study was primarily designed to ascertain whether an ingested low dose of LC n-3 PUFA in microencapsulated oil was bioavailable.

Dietary lipids are digested and absorbed rapidly after ingestion, following which, the longer chain fatty acids and monoacylglycerols are packaged into chylomicrons. These transport the absorbed TAG and other dietary lipids via the lymphatic system to the blood.<sup>31</sup> In this experiment, the plasma TAG at 2 and 4 h following the acute low dose of LC n-3 PUFA in MTO-enriched bread was used to represent the TAG in the chylomicrons as peak absorption generally occurs in the first 2–4 h after ingestion.<sup>16,32</sup> Additionally, it was also used to determine the feasibility of a novel delivery form, prototype bread enriched with the functional ingredient in the MTO form (80 mg LC n-3 PUFA). Twenty-grams of margarine was administered with the meal to boost chylomicron formation to attain adequate TAG for LC n-3 PUFA quantification. The amount of fat intake selected was based on data from Dubois *et al.*<sup>17</sup> In that study, a meal containing 30 g fat increased serum TAG markedly within the first 2 h and peaked 2–3 h following ingestion, while only a modest, though still significant, rise was observed in the 15 g fat meal, and no change occurred in the fat-free meal during the 7 h follow-up.

Having verified that a single low dose of LC n-3 PUFA (80 mg) in a novel delivery form (bread containing MTO) led to a significant increase in postprandial plasma LC n-3 PUFA levels, it was logical to follow on to a chronic feeding study involving a larger group of subjects. Hence the aim of the chronic study was to determine whether daily consumption of a lower dose of LC n-3 PUFA (approximately 60 mg) in six slices of enriched bread would elevate the fasting plasma LC n-3 PUFA content of healthy subjects.

Vegetarians (nine men and one woman) were selected for this study because they do not normally consume foods containing LC n-3 PUFA, namely fish, seafood, meat or meat products, and they have significantly lower levels of LC n-3 PUFA in the plasma than omnivores.<sup>6,8</sup> Therefore, any increase in LC n-3 PUFA status after the intervention period could be accurately attributed to the enriched bread without the need for reliance on comprehensive diet records and the LC n-3 PUFA database, which was not available at

the time. This 3 week study demonstrated that daily consumption of low dose MTO-enriched bread (60 mg LC n-3 PUFA/day) enhanced the LC n-3 PUFA content of plasma lipids. The increase was significant in the plasma total lipids (18% rise) as well as the PL fraction (12% rise). All of the subjects had elevated total plasma LC n-3 PUFA levels after the test period. While this is a modest rise, the intake dose of 60 mg LC n-3 PUFA is low by comparison with other studies.<sup>13,15,33</sup> During the intervention period, the subjects consumed a diet low in ALA acid (0.6 g/day). This intake was significantly lower than their usual ALA acid intake (1.9 g/day) and also lower than the usual intake of Australians (1.9 ± 1.7 g/day). It is possible that dietary ALA could contribute to plasma LC n-3 PUFA levels; however, only two studies have reported increases in plasma DHA levels following ALA-rich diets. Mest *et al.* reported that 30 mL of linseed oil daily (equivalent to 20 g ALA/day) for 4 weeks increased the plasma PL DHA proportions from 3.9 to 7.1%,<sup>34</sup> while Ezaki *et al.* reported that an additional intake of ALA of 3 g/day significantly increased plasma DHA levels after 10 months, but not after 4 months.<sup>35</sup> Therefore, it seems unlikely that the low amount of ALA consumed during the 3 week intervention (0.6 g/day) would have contributed to the increase in the plasma DHA reported in the present study.

Saldeen *et al.*<sup>36</sup> more recently reported a 50% rise in plasma PL LC n-3 PUFA content in omnivorous subjects ( $n = 9$ ) after 2 weeks of consuming bread enriched with fish oil (non-encapsulated). The average LC n-3 PUFA intake of approximately 319 mg/day was five times the amount administered for the present study. Both studies therefore show that bread is a suitable matrix for delivery of LC n-3 PUFA to humans.

Microencapsulation technology is one of few strategies utilised by the food industry to protect sensitive PUFA against oxidation, thus preserving the LC n-3 PUFA during processing and storage. In addition, microencapsulation masks any possible undesirable fishy odour and taste in the final product, and facilitates easy handling and storage. The bioavailability of LC n-3 PUFA in the MTO-enriched foods has been reported to be the same as n-3 PUFA in a capsule.<sup>15</sup> These authors found that there was no significant difference in platelet AA, 20:5 n-3 and 22:6 n-3 composition when 13 female subjects aged 20–26 years consumed MTO-enriched foods (soup, biscuits and bread) containing 900 mg LC n-3 PUFA per day, equivalent to 3 g of tuna oil, compared with 12 age- and sex-matched subjects who ingested three 1-g tuna oil capsules per day. A similar dietary enrichment study (22 days) using microencapsulated fish oil in processed foods (as LC n-3 PUFA delivery vehicles) demonstrated elevated plasma 22:6 n-3 and 20:5 n-3 levels (greater than 11-fold and 3-fold compared with habitual and control diets, respectively) in omnivores ( $n = 9$ ).<sup>13</sup> The level of LC n-3 PUFA consumption in their study, however, was markedly higher than the present study by more the 20-fold at 1.4 g/day. Some of the food items were enriched with fish oil directly, as opposed to the encapsulated form. The resulting

dosage of LC n-3 PUFA was achieved with nine exchanges of control foods for the identical enriched variety, including 113 g mackerel pate.

The current Australian intake of dietary LC n-3 PUFA is approximately 100–190 mg/day, and recommendations for LC n-3 PUFA range from 214 mg/day by the UK Department of Health<sup>37</sup> to the higher value of 650 mg/day by ISSFAL.<sup>2</sup> In order to bridge the gap between current intake, at around 100–200 mg/day, and the recommended levels of 650 mg/day, the food industry may choose to enrich a variety of foods, all of which could then make a contribution. MTO facilitates the incorporation of LC n-3 PUFA into many food matrices. Because high levels of enrichment may raise the cost of the end products, a suitable minimal level is sought to meet consumer affordability without compromise on bioavailability and, consequently, health benefits. This study showed that as little as 60 mg/day was bioavailable, thus demonstrating the validity of MTO in bread as a delivery vehicle.

**Acknowledgements.** We acknowledge the Clover Research Corporation of Australia for their contribution of a postgraduate scholarship for Yolande Yep and Bunge Defiance for financial support for the project.

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