

**Appendix IV.**

**Bioavailability of HiDHA® tuna oil (1)**

Yep YL, Li D, Mann NJ and Sinclair AJ. Bread enriched with microencapsulated tuna oil increases omega 3 fatty acids in humans. RMIT

# **Bread enriched with microencapsulated tuna oil increases plasma omega 3 fatty acids in humans**

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Guarantor: Professor Andrew J Sinclair

Short running title: MTO bread and plasma LC n-3 PUFA

1 **ABSTRACT**

2 **Objective:** The aim of this study was to determine the acute and chronic effects of low doses  
3 of long chain (LC) n-3 polyunsaturated fatty acids (PUFA)/(<100mg per day) on plasma LC  
4 n-3 PUFA levels, using a novel delivery form, bread containing microencapsulated tuna oil  
5 (MTO).

6 **Design:** Supplementation study with a randomised design.

7 **Setting:** RMIT University, Melbourne, Australia.

8 **Subjects:** Sixteen subjects aged 21-64 were recruited by advertisement at RMIT and  
9 Vegetarian Society of Victoria newsletters.

10 **Interventions:** Six omnivores (3 males, 3 females) participated in the acute study, which  
11 involved ingesting a prototype MTO bread containing ~ 80 mg of LC n-3 PUFA/4 slices.  
12 Plasma triacylglycerol fatty acid compositions were measured after an overnight fast and  
13 postprandially at 2 and 4 hours. Ten vegetarian subjects (9 males, 1 female) consumed MTO  
14 bread/6-8 slices/day containing ~ 60 mg of LC n-3 PUFA for three weeks in the chronic  
15 study. Fasting plasma total and phospholipid fatty acid compositions were measured at  
16 baseline and endpoint.

17 **Results:** In the acute study, proportion of 22:6n-3 and total n-3 PUFA in plasma  
18 triacylglycerol were significantly increased ( $p<0.05$ ). In the chronic study, the proportions of  
19 20:5n-3, 22:5n-3, 22:6n-6, total n-3 PUFA in plasma, and 22:6n-6 and total n-3 PUFA in  
20 plasma phospholipid fraction were significantly increased ( $p<0.05$ ) at the endpoint compared  
21 with the baseline. Ratio of n-6/n-3 in plasma and plasma phospholipid fraction were  
22 significantly decreased ( $p<0.05$ ) at the endpoint compared with the baseline.

23 **Conclusions:** This study showed that low dose LC n-3 PUFA, consumed as MTO-enriched  
24 bread, increased the LC n-3 PUFA levels in plasma of human subjects.

25

## 26 INTRODUCTION

27 The relationship between fish/fish oil intakes and human tissue long chain (LC) n-3  
28 polyunsaturated fatty acid (PUFA) accretion and subsequent manifestation of numerous  
29 health benefits is well documented in the literature. The Australian diet, typical of Western  
30 diets, contains low levels of LC n-3 PUFA which have been estimated to be between 100 to  
31 190 mg/day for adults (Sinclair et al, 1994). It is much lower than International Society for  
32 the Study of Fatty Acid and Lipids (ISSFAL) recommended dietary intakes 650 mg/day  
33 (Simopoulos et al. 1999).

34

35 The richest food sources of 20- & 22-carbon LC n-3 PUFA are fish and seafood. Minor  
36 amounts are also present in meats especially lean meat, offal, eggs, milk and dairy products.  
37 High consumption of these food sources elevates LC n-3 PUFA tissue levels (Bonaa et al,  
38 1992; Anttolainen et al, 1996; Mann et al, 1997). However, these conventional food sources  
39 may not be suitable or convenient for certain individuals or communities for various  
40 religious, ethical or personal (palatability, allergenicity) reasons. Ovolacto vegetarians obtain  
41 a limited amount of LC n-3 PUFA from eggs, milk and dairy products. Vegans must rely  
42 entirely on *in vivo* biosynthesis of these nutrients from the precursor alpha linolenic acid  
43 (18:3n-3), and the rate relies on the level of 18:3n-3 and its ratio to linoleic acid (18:2n-6) in  
44 the diet (Li et al, 1999a). However, using dietary 18:3n-3 is not as effective as direct  
45 consumption of LC n-3 PUFA from fish/fish oil for increasing tissue LC n-3 PUFA levels  
46 (Sanders and Roshanai 1983). Vegetarians have been demonstrated to have lower levels of  
47 LC n-3 PUFA in their platelets and plasma, which is associated with increased platelet  
48 activity and plasma 11-dehydro-thromboxane B<sub>2</sub> production (Li et al. 1999b).

49

50 One approach to improving the LC n-3 PUFA status of populations is to incorporate these  
51 important nutrients into frequently consumed processed foods. There have been a number of  
52 studies which have examined the effects of omega-3 enriched eggs (Jiang et al, 1993; Farrel,  
53 1998), milk (Visioli et al, 2000) and omega-3 enriched processed foods (Roche & Gibney,  
54 1994; Lovegrove et al, 1997; Sorensen et al, 1998) as means of improving tissue LC n-3  
55 PUFA levels. In general, these studies have used foods containing 290 mg to 600 mg LC n-3  
56 PUFA per serving, however there have been no studies looking at regular consumption of  
57 smaller doses of n-3 PUFA which if added to a variety of staple food may offer a more  
58 practical option without extensive change to habitual diets. Bread enriched with low level of  
59 microencapsulated tuna oil (MTO) (10 mg LC n-3 PUFA per slice) is currently available to  
60 Australian consumers. However, there is no data on the effect of low dose LC n-3 PUFA in  
61 processed foods on human n-3 PUFA status.

62

63 The aims of this study were: (1) to investigate the acute effects of a single dose of low level  
64 LC n-3 PUFA (~ 80 mg), as MTO-enriched bread on postprandial plasma LC n-3 PUFA  
65 levels; (2) to investigate the chronic effects of a daily dose of a lower level of LC n-3 PUFA  
66 (~ 60 mg), as MTO-enriched bread for three weeks on fasted plasma LC n-3 PUFA status.

67 We hypothesized that low dose MTO-enriched bread would improve plasma LC n-3 PUFA  
68 status.

69

## 70 **METHODS**

71 *Subjects:* Ethics approval was granted by the Human Research Ethics Committee of RMIT  
72 University, and all subjects gave their written consent before participating. Free-living and  
73 healthy volunteers, 12 males and 4 females (6 omnivores, 9 ovo-lacto vegetarians, 1 vegan)  
74 aged 21- 64 years were recruited from RMIT University and through advertisements in the

75 Vegetarian Society of Victoria newsletter. An omnivore was defined as someone who ate  
76 meat at least 5 times a week, an ovolacto vegetarian was defined as someone who ate no  
77 meat, not more than one fish meal and 3 eggs a week, and a vegan was defined as someone  
78 who ate meat, eggs and dairy products less than six times per year and had been following  
79 this diet for at least six months. The exclusion criteria were major medical illness, cigarette  
80 smoking, excessive alcohol intake and chronic use of aspirin or other anti-inflammatory  
81 drugs.

82

83 *Study design and diet:* The project consisted of two intervention studies; the acute and  
84 chronic effects of MTO-enriched bread on plasma LC n-3 PUFA status. In the acute study, 6  
85 omnivores (3males, 3 females) were advised to consume their usual foods for one week prior  
86 to the experiment day except one day before the study, when fish meals were not permitted.  
87 Subjects were given a single dose of LC n-3 PUFA (~ 80 mg) to observe changes in  
88 postprandial plasma LC n-3 PUFA levels at 2 and 4 hours. LC n-3 PUFA was delivered  
89 through 4 slices of prototype MTO-enriched bread (Bunge Cereal Foods Pty Ltd, Melbourne,  
90 Australia) together with 15 g of margarine (monounsaturated sunflower oil, Flora, Unilever  
91 Foods, Marrickville, NSW, Australia). The bread was eaten either lightly toasted or  
92 untoasted. Table spreads (jam, vegemite) and beverages (coffee, tea, orange juice) were also  
93 made available. After this meal, subjects were asked to refrain from consuming food except 1  
94 apple and water or zero calorie drinks, and to avoid any strenuous physical activity for the  
95 next 4 hours.

96

97 In the chronic study, 9 ovolacto vegetarians (8 males, 1 female) and 1 vegan (male) were  
98 instructed to consume 6 slices of commercially available MTO-enriched bread (containing 60  
99 mg of LC n-3 PUFA) daily either untoasted or toasted lightly with their habitual diet for three

100 weeks to investigate the changes in fasting plasma LC n-3 PUFA status. Foods rich in n-3  
101 PUFA, such as fish and all seafood, fish oil capsules, oat germ, wheat germ/germ oils,  
102 walnuts, linseeds and soy/linseed breads were excluded during the intervention period. The  
103 first loaf of enriched bread (toast sliced) was provided for subjects on their first blood  
104 sampling day, thereafter they purchased their own as required from local supermarkets. Each  
105 subject was required to fill out a semi-quantitative food frequency questionnaire recording his  
106 or her daily intake of fat-containing foods consumed during the 3-week intervention period.  
107 The questionnaire was primarily designed to estimate the habitual fat intake of each subject,  
108 specifically the LC n-3 PUFA and to check for compliance. Subjects were asked to avoid  
109 foods listed on the exclusion list, however, when it was not possible to adhere, details of food  
110 items and portion sizes of the 'prohibited foods' were to be declared. Subjects were advised  
111 to record the number of slices of bread consumed daily and to specify whether the slices were  
112 toasted or untoasted. Compliance for bread consumption was monitored by requesting  
113 subjects to present used bread packets and receipts at the end of the study period. All  
114 subjects were contacted once a week during the study period to check on their progress with  
115 bread consumption, to remind them to avoid exclusion foods and to generally maintain their  
116 interest and compliance.

117

118 *Blood specimen collections:* In the acute study, venous blood was drawn into a 9 mL EDTA  
119 vacuum tube from subjects between 0830 - 0900 hours after an overnight fast (baseline) and  
120 postprandially 2 and 4 hours following the ingestion 4 slices of prototype MTO-enriched  
121 bread. The sampling times were selected to coincide with peak plasma triacylglycerol (TAG)  
122 absorption (between 2-4 hrs postprandially) based on data from Agren et al (1996) and  
123 Dubois et al (1998). In the chronic study, subject's height, weight, percentage body fat, pulse  
124 and blood pressure were measured before bleeding. In the chronic study, venous blood was

125 collected into a 9 mL EDTA vacuum tube from subjects before (week 0) and after (week 3)  
126 the intervention period following a 12 hr overnight fast. Plasma was isolated by  
127 centrifugation at 3000 rpm for 10 mins at 4°C and stored at -80°C frozen in portions until  
128 analyses.

129

130 *Dietary assessment:* The diet records were analysed for total fat intake using Diet Version 4  
131 software (Xyris Software Pty Ltd, Highgate Hill, QLD, Australia) with NUTTAB 95  
132 database based on Composition of Food Australia (COFA). Estimation of the individual LC  
133 n-3 PUFA intake was based on published values of omega 3-containing food fatty acid  
134 concentrations (Sinclair et al, 1992; Mann et al, 1995; Quigley et al, 1995; Nichols et al, 1998  
135 and Sinclair et al, 1998).

136

137 *Plasma fatty acids:* Total lipid of plasma was extracted with chloroform : methanol (1 : 1,  
138 v/v), the plasma phospholipid (PL) and triacylglycerol (TAG) fractions were separated by  
139 thin-layer chromatography and the methyl esters of the fatty acids of total plasma, plasma PL  
140 and TAG fractions were prepared, fatty acid methyl ester were separated and quantified by  
141 gas-liquid chromatography as described previously (10).

142

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

148

149

**150 RESULTS**

151 Table 1 reports the fatty acid contents of prototype and commercial MTO-enriched breads  
152 and monounsaturated sunflower oil margarine. The total fat was found to be 2.9 g/100g  
153 bread. Total LC n-3 PUFA was 56 mg/100g and 24 mg/100g for prototype and commercial  
154 MTO-enriched breads, respectively. The prototype MTO-enriched bread contained 44 mg of  
155 22:6n-3, 8.9 mg of 20:5n-3 and 2.8 mg of 22:5n-3, and also 3.5 mg of AA per 100g. The  
156 commercial MTO-enriched bread contained 18 mg of 22:6n-3, 4.5 mg of 20:5n-3 and 1.5 mg  
157 of 22:5n-3, and also 1.9 mg of AA per 100g. There were no LC n-3 PUFA in  
158 monounsaturated sunflower oil margarine.

159

160 Table 2 shows the intake of major fatty acids intake in acute study. The mean intake of total  
161 fat was 19.1 g; 4.1 g from prototype MTO-enriched bread and 15 g from monounsaturated  
162 sunflower oil margarine. The mean intake of total LC n-3 PUFA was 79 mg; 22:6n-3 62 mg,  
163 20:5n-3 13 mg and 22:5n-3 4 mg.

164

165 In the acute study, the proportion of 22:6n-3 and total n-3 PUFA of plasma TAG increased in  
166 all the six subjects between 5% to 121% for total n-3 PUFA ( $p=0.0163$ ) and 22% to 153% for  
167 22:6n-3 ( $p=0.0147$ ) from baseline to 2 hours postprandial, respectively (Figures 1A and 1B).

168 The 20:5n-3 proportion of plasma TAG was also increased in all the six subjects between  
169 25% to 288% ( $p=0.14$ ) (Figure 1C).

170

171 Daily fat intakes of habitual diets and during the 3 weeks commercial MTO-enriched bread  
172 intervention period (experimental diet) in the chronic study are shown in Table 3. The mean  
173 daily intakes of total fat were 52 g/day and 55 g/day, PUFA were 20% and 23%, MUFA were  
174 42% and 40%, and SFA were 38% and 37% of total fat for habitual diet and experimental

175 diet, respectively. The daily intakes of total LC n-3 PUFA were 1 mg and 64 mg for habitual  
176 diet and experimental diet, respectively.

177

178 Physiological characteristics of the subjects in the chronic study are reported in the Table 4.

179 There were no significant changes in any measured physiological characteristics in the  
180 subjects during 3 weeks study period.

181

182 The proportion of total n-3 PUFA in the plasma increased between 1% to 42 % ( $p=0.001$ ),

183 while there was an increase in the proportion of 22:6n-3 for nine of the ten subjects

184 ( $p=0.006$ ), eight of ten for 20:5n-3 ( $p=0.0059$ ) and six of ten for 22:5n-3 ( $p=0.034$ ) at day 21

185 compared with day 0 (Figures 2A, 2B, 2C & 2D), respectively. Total plasma ratio of n-6 to n-

186 3 decreased in nine of ten subjects at day 21 compared with day 0 (Figure 2E).

187

188 In the plasma PL fraction, the proportions of total n-3 PUFA and 22:6n-3 increased in seven

189 and eight of ten subjects at day 21 compared with day 0, respectively ( $p$ -value was 0.03 and

190 0.0137) (Figure 3A & 3B). The 20:5n-3 proportion was raised in seven of ten subjects at day

191 21 compared with day 0 ( $p=0.119$ ) (Figure 3C). The ratio of n-6 to n-3 in plasma PL fraction

192 decreased in all the ten subjects after 3 weeks intervention ( $p=0.006$ ) (Figure 3D).

193

#### 194 **DISCUSSION**

195 The aim of the present study was to determine whether the consumption of bread enriched

196 with a low dose MTO would improve LC n-3 PUFA status in healthy individuals. To achieve

197 this, an acute study (single dose ingestion with 4 hr follow up) was conducted to establish the

198 feasibility of the use of a low intake of LC n-3 PUFA, delivered as MTO-enriched bread.

199 This was followed by a 3-weeks chronic study using commercial MTO bread as the LC n-3

200 PUFA source. We found that low dose MTO-enriched bread did improve plasma LC n-3  
201 PUFA status in both the acute and the chronic study.

202

203 Several biological markers have been employed as a measure of LC  $\omega$ -3 PUFA status in  
204 humans. Commonly in use are accessible tissues such as blood (plasma, platelets,  
205 erythrocytes, neutrophils), adipose tissue, breast milk and cheek cells. All have been reported  
206 to correlate reasonably well with dietary intake in prospective as well as dietary intervention  
207 trials (Brown et al, 1991; Hjartaker et al, 1997; Nelson et al, 1997; Vidgren et al, 1997 and  
208 Garland et al, 1998). The selection criteria for their use would then ultimately depend on the  
209 objectives and duration of the study. In the present study, where the main objective was to  
210 determine extent of dietary LC n-3 PUFA incorporation in general as opposed to specific  
211 organ function or pharmacological effects, plasma was selected because of its relative ease of  
212 sample collection and preparation. In the acute study, we monitored the plasma TAG fatty  
213 acid, not PL, since the plasma TAG can be acutely influenced by a single meal (Riboli et al  
214 1987).

215

216 In the acute experiments where early postprandial plasma was investigated, the level of LC n-  
217 3 PUFA incorporation was quantified in the nascent TAG fractions (location of newly  
218 absorbed LC n-3 PUFA following a meal). The acute study was primarily designed to  
219 ascertain that ingestion of low dose of LC n-3 PUFA in microencapsulated oil was  
220 bioavailable.

221

222 Dietary lipids are digested and absorbed rapidly after ingestion, following which, the longer  
223 chain fatty acids and monoacylglycerols are packaged into chylomicrons. These transport the  
224 absorbed TAG and other dietary lipids via the lymphatic system to the blood (Nelson &

225 Ackman, 1988). In this experiment, the plasma TAG at 2 and 4 hrs following the acute low  
226 dose of LC n-3 PUFA in MTO-enriched bread was used to represent the TAG in the  
227 chylomicrons since peak absorption generally occurs in the first 2-4 hrs after ingestion  
228 (Agren et al, 1996; Sanders et al, 1997). Additionally, it was also used to determine the  
229 feasibility of a novel delivery form, prototype bread enriched with the functional ingredient in  
230 the MTO form (80 mg LC n-3 PUFA). Twenty-grams of margarine was administered with  
231 the meal to boost chylomicron formation to attain adequate TAG for LC n-3 PUFA  
232 quantitation. The amount of fat intake selected was based on data from Dubois et al (1998).  
233 In that study, a 30 g fat meal increased serum TAG markedly within first 2 hrs and peaking 2-  
234 3 hrs following ingestion, while only a modest, though still significant rise, was observed in  
235 the 15 g fat meal, and no change occurred in the fat free meal during the 7 hr follow-up.

236

237 Having verified that a single low dose of LC n-3 PUFA (80 mg) in a novel delivery form  
238 (bread containing MTO) led to a significant increase in postprandial plasma LC n-3 PUFA  
239 levels, it was logical to follow on to a chronic feeding study involving a larger group of  
240 subjects. Hence the aim of the chronic study was to determine whether daily consumption of  
241 lower dose LC n-3 PUFA (approx. 60 mg) in 6 slices of enriched bread would elevate the  
242 fasting plasma LC n-3 PUFA content of healthy subjects.

243

244 Vegetarians (9 males and 1 female) were selected for this study because they do not normally  
245 consume LC n-3 PUFA-rich foods namely fish, seafood or other meat products. Therefore,  
246 any increase in LC n-3 PUFA status after the intervention period could be accurately  
247 attributed to the enriched bread without the need reliance on comprehensive diet records and  
248 LC n-3 PUFA database which is not available at the present time. This 3-week study  
249 demonstrated that daily consumption of low dose MTO-enriched bread (60 mg LC n-3

250 PUFA) enhanced the LC n-3 PUFA content of plasma lipids. The increase was significant in  
251 the plasma total lipids (18 % rise) as well as the PL fraction (12 % rise). All of the subjects  
252 had elevated total plasma LC n-3 PUFA levels after the test period. While this is a modest  
253 rise, the intake dose of 60mg LC n-3 PUFA is low by comparison with other studies (Gibney  
254 and Daly, 1994; Lovegrove et al, 1997; Wallace et al, 2000).

255

256 Saldeen et al (1998) more recently reported 50 % rise in plasma PL LC  $\omega$ -3 PUFA content in  
257 omnivorous subjects (n=9) after 2 weeks of consuming bread enriched with fish oil (non-  
258 encapsulated). The average LC n-3 PUFA intake of approx. 319 mg/day was five times the  
259 amount administered for the present study. Both studies, therefore, show that bread is a  
260 suitable matrix for delivery of LC n-3 PUFA to humans.

261

262 Microencapsulation technology is one of few strategies utilised by the food industry to  
263 protect sensitive PUFA against oxidation, thus preserving the LC n-3 PUFA concentration  
264 during processing and storage. In addition, microencapsulation masks the undesirable fishy  
265 odour and taste in the final product, and facilitates easy handling and storage. The  
266 bioavailability of LC n-3 PUFA in the MTO-enriched foods has been reported to be the same  
267 as n-3 PUFA in a capsule (Wallace et al, 2000). They found that there was no significant  
268 difference on platelet AA, 20:5n-3 and 22:6n-3 composition when 13 female subjects aged  
269 20-26 years consumed MTO-enriched foods (soup, biscuits and bread) containing 900 mg LC  
270 n-3 PUFA per day, equivalent to 3 g of tuna oil compared with age and sex matched 12  
271 subjects ingested three 1-gram tuna oil capsules per day. A similar dietary enrichment study  
272 (22 days) using microencapsulated fish oil in processed foods (as LC n-3 PUFA delivery  
273 vehicles) demonstrated elevated plasma 22:6n-3 and 20:5n-3 levels (greater than 11-fold and  
274 3-fold compared with habitual and control diets, respectively) in omnivores (n=9) (Lovegrove

275 et al, 1997). The level of LC n-3 PUFA consumption in their study however, was markedly  
276 higher than the present study by 10-fold at 1.4 g/day. Some of the food items were enriched  
277 with fish oil directly as opposed to the encapsulated form. The resulting dosage of LC n-3  
278 PUFA was achieved with 9 exchanges of control foods for the identical enriched variety,  
279 including 113 g mackerel pate.

280

281 Current Australian intake of dietary LC n-3 PUFA is approximately 100 to 190 mg/day, and  
282 recommendations for LC n-3 PUFA from 214 mg/day by British Nutrition Foundation (2000)  
283 to higher value 600 mg/day by ISSFAL (Simopoulos et al,1999). In order to bridge the gap  
284 between current intake at say 100 – 200 and recommended levels of 600 mg/day, the food  
285 industry may choose to enrich a variety of foods, all of which could then make a contribution.  
286 MTO facilitates the incorporation of LC n-3 PUFA into many food matrices. Since high  
287 levels of enrichment may raise the cost of the end products, a suitable minimal level is sought  
288 to meet consumer affordability without compromise on bioavailability and consequently  
289 health benefits. This study showed that as little as 60 mg/day was bioavailable, thus  
290 demonstrating the validity of MTO in bread as a delivery vehicle.

291

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Table 1. Fatty acid contents of prototype and commercial MTO-enriched bread, and monounsaturated sunflower oil margarine.

Fatty acid	Prototype MTO-enriched bread (mg/100g) <sup>a</sup>	Commercial MTO-enriched bread (mg/100g) <sup>a</sup>	Monounsaturated sunflower oil Margarine (g/100g) <sup>a</sup>
14:0	33	5	1.4
16:0	575	343	7.7
18:0	226	145	6.6
18:1	501	572	44.8
18:2n-6	1004	752	7.3
18:3n-3	41	107	1.7
20:4n-6	3.5	1.9	nd
20:5n-3	8.9	4.5	nd
22:5n-3	2.8	1.5	nd
22:6n-3	44	18	nd

<sup>a</sup>n=2, nd=not detected.

Table 2. Amount of major fatty acids ingested from prototype MTO-enriched meals in the acute study.

Fatty acid	Prototype MTO-enriched bread + Margarine <sup>a</sup>
16:0	2.1 g
18:0	1.6 g
18:1	10.3 g
18:2n-6	3.0 g
18:3n-3	0.6 g
20:5n-3	13 mg
22:5n-3	4 mg
22:6n-3	62 mg
Total LC n-3 PUFA	79 mg
Total fat	19.1 g <sup>a</sup>

<sup>a</sup>Analysed in duplicate, <sup>b</sup>4.1 g from prototype MTO-enriched bread and 15 g from monounsaturated sunflower oil margarine.

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

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

SFA=saturated fat, MUFA=monounsaturated fat, PUFA=polyunsaturated fat.

\*p<0.05

Table 4. Physiological characteristics of vegetarian subjects in chronic study.

	Day 0 (n=10)	Day 21 (n=10)
BMI (kg/m <sup>2</sup> )	22.9 ± 2.4	22.8 ± 0.9
Waist/hip ratio	0.84 ± 0.06	0.84 ± 0.02
Systolic BP (mmHg)	110 ± 7	117 ± 4
Diastolic BP (mmHg)	71 ± 5	71 ± 2
Pulse (bpm)	63 ± 7	66 ± 11
% Body fat	17.6 ± 5.0	17.2 ± 1.7

BMI=body mass index, BP=blood pressure.

**Legend**

Figure 1. Changes in composition of plasma TAG fraction 22:6n-3 (1A), total n-3 PUFA (1B) and 20:5n-3 (1C) after 6 subjects consumed a prototype MTO-enriched meal.

Pre = pre meal, Post 2 = postprandial 2 hours, Post 4 = postprandial 4 hours.

Figure 2. Changes in composition of total plasma n-3 PUFA (2A), 22:6n-3 (2B), 20:5n-3 (2C), 22:5n-3 (2D) and ratio of n-6/n-3 (2E) after 10 vegetarian subjects consumed commercial MTO-enriched bread for three weeks.

Figure 3. Changes in composition of plasma phospholipid total n-3 PUFA (3A), 22:6n-3 (3B), 20:5n-3 (3C) and ratio of n-6/n-3 (3D) after 10 vegetarian subjects consumed commercial MTO-enriched bread for three weeks.