

## **Appendix VI.**

### **Comparison of DHA rich fish oil to EPA rich fish oil**

Howe P. Cardiovascular trial to evaluate cardiovascular health benefits of docosahexaenoic acid rich fish oil supplement. Research Report for Clover Corporation, 1997.

# **NU-MEGA LIPIDS TRIAL**

## **FINAL REPORT**

to

**CLOVER CORPORATION Pty Ltd**

prepared by

*Peter R. C. Howe*

**CSIRO DIVISION OF HUMAN NUTRITION  
ADELAIDE**

**27th March, 1997**

<b><u>CONTENTS</u></b>	<b><u>Page</u></b>
Contents, appendices, abbreviations	2
Executive summary	3
Initial proposal (Annexure A of research agreement)	4
Progress	6
Results	7
Discussion	11
Conclusions and recommendations	12
References	13

### **Appendices**

Patient information sheet

Clinical data files

Laboratory data files

Specifications/analyses of supplements

### **ABBREVIATIONS**

BMI	body mass index
BP	blood pressure
DBP	diastolic blood pressure
DHA	docosahexaenoic acid
EPA	eicosapentaenoic acid
HDL	high density lipoprotein
LDL	low density lipoprotein
PUFA	polyunsaturated fatty acids
SBP	systolic blood pressure
TG	triglycerides

## EXECUTIVE SUMMARY

A clinical intervention trial was conducted by the Clinical Research Unit, CSIRO Division of Human Nutrition to compare the potential cardiovascular and anti-inflammatory benefits of the client's DHA-rich tuna fish oil (Nu-Mega) with those of a commercial MaxEPA-type fish oil.

A double-blind, crossover protocol was used. Adult male volunteers were randomised to take either supplement (eight 1g capsules/day) for 6 weeks then, after a 4 week washout period, to take the alternate supplement for a further 6 weeks. Initially and at the end of each intervention phase, blood pressure was measured in the clinic and by 24 hour ambulatory monitoring and a blood sample was taken for assessment of plasma lipids, platelet thromboxane production and monocyte cytokine production.

Thirty subjects completed the trial. With one exception, the supplements were well-tolerated, with fewer unfavourable comments about Nu-Mega oil than the comparator. They resulted in high circulating levels of  $\omega$ -3 fatty acids, with anticipated differential effects on EPA and DHA.

The average initial values for blood pressure and blood lipids were marginally elevated. Neither oil supplement affected total or HDL cholesterol and there were no clinically relevant changes in blood pressure. However, each supplement produced comparable reductions of both serum thromboxane production (40%) and plasma triglycerides (26%), with a modest (6%) elevation of LDL cholesterol. Effects of the supplements on cytokine production are still being analysed.

These findings demonstrate that a DHA-rich oil derived from Australian fish is as efficacious as imported EPA-rich fish oil in counteracting two major risk factors for cardiovascular disease, viz. hypertriglyceridaemia and thrombotic tendency, and they indicate that DHA can mediate these benefits. They warrant further evaluation of the appropriate doses for achieving optimal therapeutic benefits with minimal adverse effects in at risk individuals.

## INITIAL PROPOSAL - ANNEXURE A OF RESEARCH AGREEMENT

### CLINICAL TRIAL TO EVALUATE CARDIOVASCULAR HEALTH BENEFITS OF A DOCOSAHEXAENOIC ACID-RICH FISH OIL SUPPLEMENT

**Sponsor:** Mr. Hamish Drummond, Clover Corporation Pty Ltd

**Investigator:** A/Prof PRC Howe, CSIRO Division of Human Nutrition

**Brief title:** NU-MEGA LIPIDS TRIAL

**Objective:** To compare, in healthy volunteers, the bioavailability and cardiovascular health attributes of a DHA-rich tuna oil (Nu-Mega) and an EPA-rich oil (MaxEPA), taken as supplementary capsules.

**Rationale:** The cardioprotective effects of regular consumption of fish or fish oil are well established. These include reductions of blood pressure (BP), plasma triglycerides (TG), platelet aggregation and prevention of fatal arrhythmias. It is widely accepted that the long-chain omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), present in varying proportions in all fish oils, are the mediators of these beneficial effects. EPA, which predominates in fish oils obtained from the Northern hemisphere, has a key role as a substrate competing with arachidonic acid for the production of eicosanoids with a wide range of cardiovascular and inflammatory functions. However, recent studies in our Division suggest that DHA rather than EPA may be the principal active omega-3 fatty acid mediating a wide range of cardiovascular health benefits.

Typical doses of omega-3 fatty acids required to show benefits in short-term clinical trials have ranged upward from 2g/day for anti-thrombotic and TG-lowering effects or 3-4 g/day for blood-pressure reduction. This amounts to at least six 1g capsules/day of imported commercial fish oil supplements such as MaxEPA, containing 18% EPA and 12% DHA. On the other hand, consumption of as little as two meals of fish per week is thought to convey similar benefits. This may be attributable to the higher proportion of DHA to EPA in fish. Australian fish tend to be less oily than those of the Northern hemisphere. However, as the DHA/EPA ratio in their oil tends to be higher, it may yield comparable cardiovascular benefits at lower doses, thus reducing cost and increasing consumer acceptability.

**Approach:** We will compare the effects of taking MaxEPA or the sponsor's DHA-rich tuna oil, Nu-Mega (5%EPA and 25%DHA), on cardiovascular risk factors (BP, cholesterol, TG and thromboxane production) and markers of inflammation, interleukin 1b (IL1b) and tumor necrosis factor (TNFa), in normal healthy subjects. Based on the outcome, subsequent trials may be conducted to test indications for specific pathological conditions, such as hypertension, diabetes, arthritis or inflammatory bowel disease.

**Subjects:** Thirtytwo adult male volunteers will be recruited by advertisement. They will be fully informed of the nature of the study and screened by questionnaire with respect to the following exclusion criteria.

- Exclusions:** Uncontrolled hypertension (DBP >105 mmHg or SBP >160 mmHg); regular use of aspirin or NSAID; likelihood of commencing or requiring change of antihypertensive medication during the trial; risk of excessive bleeding; history of acute cardiac or renal disease, unstable angina or arrhythmia, diabetes, stroke, transient ischaemic attack; history of substance abuse.
- Location:** The study will be conducted in the Nutrition Research Clinic of the CSIRO Division of Human Nutrition at Kintore Avenue, Adelaide.
- Protocol:** A placebo-controlled, double-blind crossover design will be used. Subjects will visit the clinic on 3 occasions: initially and at the end of each treatment phase. At each visit, BP will be recorded seated and standing (average of 3 Dinamap readings taken at 1 minute intervals), a blood sample will be taken and a Spacelabs 90207 monitor will be fitted to record ABP every 20 min (day) or 40 min (night) for 24 hrs. Subjects will be randomly assigned to take eight 1g capsules/day of either Nu-Mega or MaxEPA for 6 wks, followed by a 4 wk washout period. They will then take the alternate oil for a further 6 wks. Oils conforming to NFA standards will be supplied by the sponsor in identical 1g soft gelatin capsules.
- Assays:** At the end of each treatment phase, platelet thromboxane production will be measured in serum as an index of anti-thrombotic potential. Total, HDL and LDL cholesterol and TG will also be measured in serum. Monocytes derived from blood samples initially and after 6 wks will be used to measure IL1b and TNFa production. Compliance will be assessed by counting returned capsules and from changes in serum fatty acid profiles.
- Outcomes:** The primary efficacy variables will be the within-individual differences in the 24-hr averaged SBP and/or DBP between the end of each intervention phase, serum TG and production of thromboxane. Inhibition of IL1b and TNFa production by isolated monocytes, an indicator of anti-inflammatory potential, is a secondary outcome measure.
- Statistics:** Data will be analysed on an intention-to-treat basis, with stratification of the variables used for subject blocking. Based on previous experience, we assume 80% power to detect a significant ( $p < 0.05$ ) within-individual change of at least 5 mmHg in the 24 hour averaged value for SBP.
- Reporting:** Data obtained for each subject will be maintained in confidential case report forms. Subjects will receive summaries of their individual results at the end of the trial. A report of the trial with detailed analyses of overall outcomes will be forwarded to the sponsor, with recommendations for publication.
- Approval:** Approval will be obtained from the CSIRO Human Ethics Committee. The National Food Authority will be notified of the study.
- Timetable:** August - patient recruitment, capsules supplied by Clover  
 October - randomisation of subjects  
 December - 1st treatment period completed  
 February, 97 - 2nd treatment period completed

March - data analysed, report submitted to Clover

### PROGRESS

- The research agreement with Clover Corporation was effected on 16th August, 1996.
- The CSIRO Human Ethics Committee was notified on 5th August, 1996 of modifications contained in the research proposal and approved the appended patient information sheet. The Australia New Zealand Food Authority was notified of the trial.
- Patients were recruited by advertisement in the Sunday Mail, 8th September, 1996. Screening visits were conducted in the following month with enrolment and randomisation completed by 15th October, when the first intervention phase commenced. This phase ended as scheduled on 20th December, 96. The second intervention phase finished as scheduled on 28th February, 97.
- CSIRO-based laboratory analyses were completed by 21st March, 97.  
In addition to the proposed analyses at the end of each treatment phase, pre-intervention blood samples taken at the first visit were also used for measurements of plasma lipids and platelet thromboxane production.
- Cytokine production by monocytes in blood samples obtained during the first intervention phase is being undertaken by Dr. M. James of Royal Adelaide Hospital. The cytokine assays have been temporarily delayed whilst quality control is being improved.
- With the exception of the cytokine data, which will be presented in a supplementary report, this report is a final presentation of the outcomes of this trial.

## RESULTS

### Subjects

A total of 32 adult males were recruited and randomised to commence on either treatment. Their screening data appears below. They were predominantly middle-aged and overweight, with casual BP readings ranging from high normal to moderate hypertension. None, however, were currently taking antihypertensive or hypolipidaemic medication.

Group A (commencing on supplement A)					Group B (commencing on supplement B)				
ID	BMI (kg/m <sup>2</sup> )	age (years)	SBP (mmHg)	DBP (mmHg)	ID	BMI (kg/m <sup>2</sup> )	age (years)	SBP (mmHg)	DBP (mmHg)
C01	34.4	45	168	109	C03	31.8	55	145	104
CO2	27.1	48	147	101	C04	29.2	57	142	96
CO5	29.4	50	146	93	C06	29.9	62	166	98
C07	27.8	60	158	106	C08	22.6	51	141	86
C09	23.9	57	155	88	C11	25.6	33	161	88
C10	34.4	68	159	94	C12	25.6	60	146	80
C13	28.7	58	166	86	C14	27.0	47	143	92
C15	32.4	47	136	77	C18	29.2	52	179	101
C16	28.3	66	157	93	C19	26.7	49	144	101
C17	26.1	49	155	93	C20	23.2	40	141	94
C21	24.9	40	134	92	C23	31.2	72	149	90
C22	23.6	46	152	97	C24	29.1	53	130	87
C25	26.0	39	137	86	C27	26.2	40	147	80
C26	30.8	62	156	80	C28	26.0	35	140	82
C30	30.6	33	152	75	C29	26.5	55	137	86
C32	28.5	43	140	88	C31	28.6	60	181	94
<b>mean:</b>	<b>28.5</b>	<b>50.7</b>	<b>151.1</b>	<b>91.1</b>	<b>mean:</b>	<b>27.4</b>	<b>51.3</b>	<b>149.5</b>	<b>91.2</b>

One subject (C10) withdrew due to ill health during the first intervention phase. Another subject who was initially randomised to supplement B (C11) withdrew prior to the final study visit due to an unexpected interstate transfer. Hence 15 subjects in each supplement group completed the study.

### Acceptability/tolerability of supplements

Subject C10 experienced stomach cramps and constipation whilst taking supplement A which necessitated his withdrawal from the trial. Numerous comments were made about taste and gastrointestinal responses, esp. reflux, particularly during the first intervention phase (see clinic data in appendix). The comments referred predominantly to supplement A (16 versus 7 for B).

### Compliance

Patients were issued with 3 containers (*nominally* containing 120 capsules each) for each intervention phase, thus providing 24 spare capsules. With the exception of known causes, capsule returns varied from 8 to 95, suggesting that the number of capsules actually packaged had only been approximated. However, the average return was 34, indicating a high overall level of compliance.

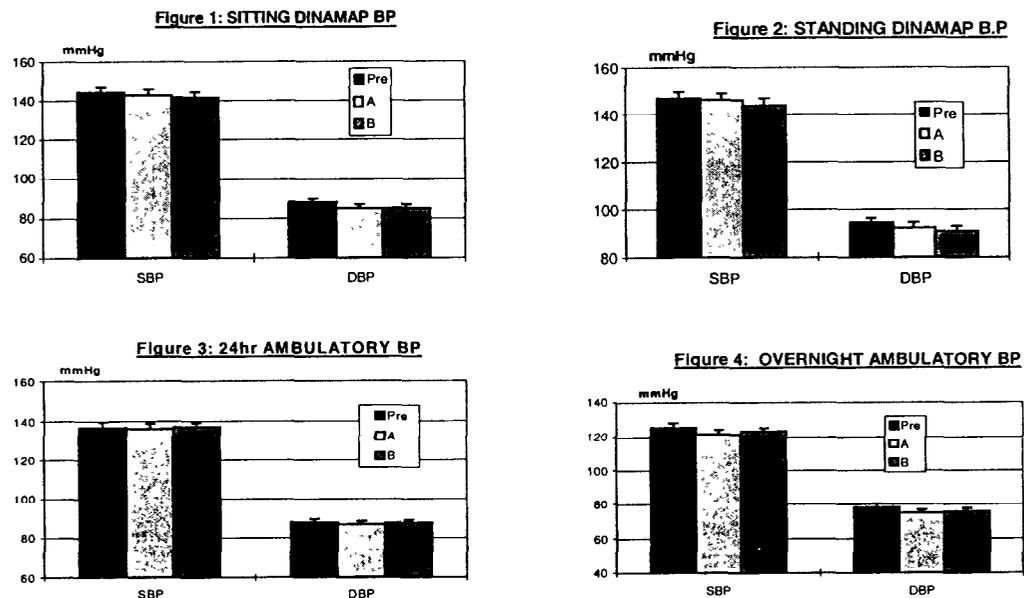
## Effects of supplementation on body weight

Mean BMI measurements at visit 1 for Groups A and B were  $28.2 \pm 0.8$  and  $27.5 \pm 0.7$  kg/m<sup>2</sup> respectively. There were no significant changes of body weight with either supplement.

## Effects of supplements on blood pressure

Treatment effects on BP were assessed from clinic measurements (average of four Dinamap readings), taken both seated and standing, and from 24 hour ambulatory monitoring, with readings taken every 20 minutes during the day or 40 minutes at night (11pm to 6 am).

Figs. 1-4 show means  $\pm$  SEM of BP measurements initially (pre) and whilst taking supplement A or B. Sitting clinic BP had fallen approx. 5/3 mmHg from screening values. Standing measurements reflect the postural rise in DBP. Both overestimate the 24 hour average obtained by ambulatory monitoring, due to the anticipated decline of BP whilst sleeping (nocturnal dipping).



The changes in BP tabulated below were derived by comparing measurements, within individuals, taken on each treatment phase either with each other (crossover) or with the respective pretreatment value.

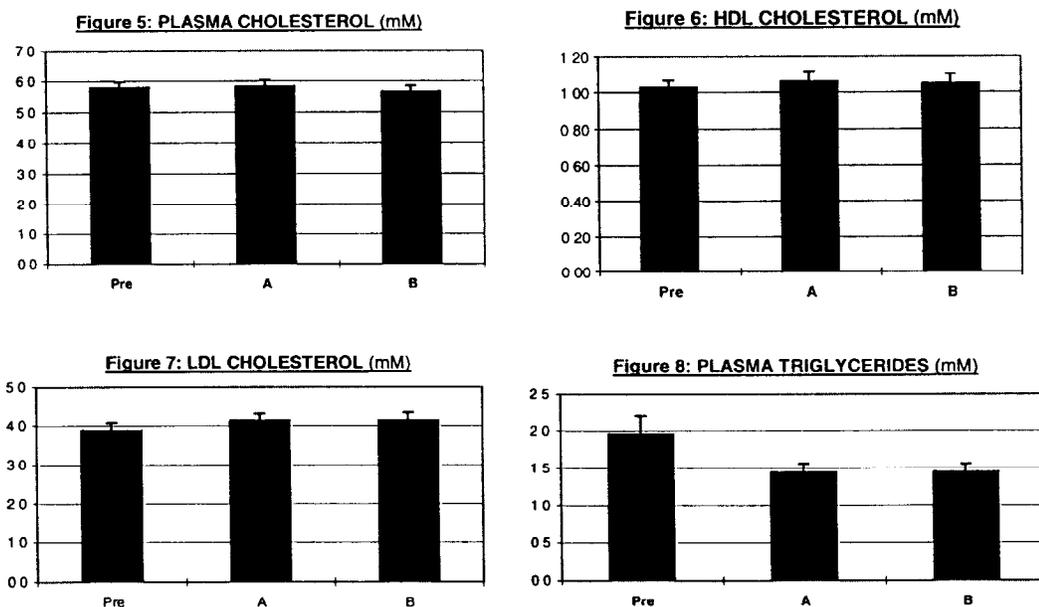
BP change (mmHg)	pretreatment → A	pretreatment → B	A → B (crossover)
seated SBP	-1.5 $\pm$ 1.4	-2.9 $\pm$ 1.6	-1.3 $\pm$ 1.5
DBP	-3.3 $\pm$ 1.3*	-3.2 $\pm$ 1.2*	0.1 $\pm$ 1.5
standing SBP	-0.8 $\pm$ 1.6	-2.9 $\pm$ 1.8	-2.1 $\pm$ 2.0
DBP	-2.3 $\pm$ 1.0*	-3.7 $\pm$ 1.2**	-1.4 $\pm$ 1.5
24 hr ambulatory SBP	-1.0 $\pm$ 1.8	0.1 $\pm$ 1.5	0.6 $\pm$ 1.5
DBP	-1.4 $\pm$ 1.2	-0.4 $\pm$ 1.0	0.7 $\pm$ 1.0
overnight SBP	-4.1 $\pm$ 2.3	-2.6 $\pm$ 1.8	0.4 $\pm$ 2.1
DBP	-3.4 $\pm$ 2.0	-2.5 $\pm$ 1.7	0.1 $\pm$ 1.5

\*p<0.05, \*\*p<0.01

There were significant reductions in clinic DBP with both supplements. However, these are probably temporal trends, which are commonly observed with repeated clinic measurements, reflecting progressive familiarity/comfort with the procedure. For this reason, comparisons are usually made with a simultaneous control (placebo-treated) group. The 24 hour ambulatory measurements are less susceptible to this artefact. Notably, neither the average 24 hour BP nor the average overnight BP were significantly affected by supplement. The primary outcome measure was the within-individual difference between supplements (crossover). Clearly, there was no difference between effects of supplements A and B on BP.

### Effects of supplement on plasma lipids

Total, HDL and LDL cholesterol and total TG were measured in plasma samples taken from each subject initially and after each intervention phase. Means  $\pm$  SEM of the values before and after each treatment appear in figs. 5- 8. Pretreatment cholesterol was mildly elevated (5.8 mM), with moderately high TG (1.96 mM).



The within-individual changes in lipid concentrations are tabulated below. Both supplements caused a highly significant decrease in TG (-26%) accompanied by a significant elevation of LDL cholesterol (6%), the magnitude being almost identical for each supplement. There were no significant changes in total or HDL cholesterol.

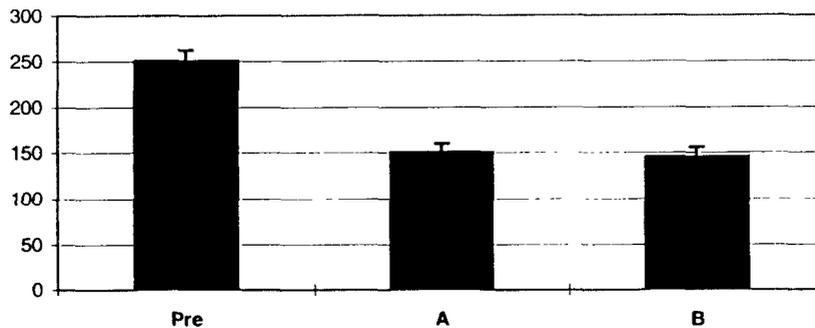
Change in lipids (mM)	pretreatment $\rightarrow$ A	pretreatment $\rightarrow$ B	A $\rightarrow$ B (crossover)
total cholesterol	0.05 $\pm$ 0.12	-0.14 $\pm$ 0.13	-0.01 $\pm$ 0.12
HDL cholesterol	0.03 $\pm$ 0.04	0.02 $\pm$ 0.04	-0.02 $\pm$ 0.04
LDL cholesterol	0.25 $\pm$ 0.10*	0.25 $\pm$ 0.08**	0.01 $\pm$ 0.10
TG	-0.51 $\pm$ 0.15**	-0.51 $\pm$ 0.17**	0.01 $\pm$ 0.06

\*p<0.05, \*\*p<0.01

### Effects of supplements on platelet thromboxane production

Maximally stimulated platelet thromboxane production was assessed by enzyme immunoassay of the stable product, thromboxane B<sub>2</sub>, in serum obtained from whole blood incubated for 1 hr at 37°C. As shown in Fig. 9, both supplements caused highly significant ( $p < 0.001$ ) reductions of thromboxane production: 40% and 42% for A and B respectively. Thus there was no difference in the crossover comparison between supplements A and B.

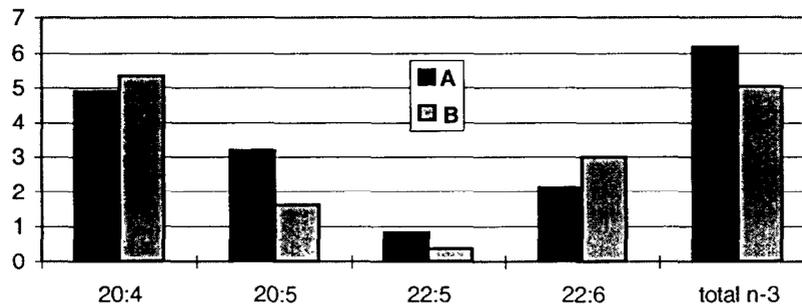
**Figure 9: SERUM THROMBOXANE B<sub>2</sub> (ng/ml)**



### Effects of supplements on plasma fatty acid profiles

Fig. 10 shows relative proportions of long chain PUFA present in plasma during treatment with A and B. The values are means for group A and B of plasmas taken at visit 2. The total for  $\omega$ -3 approximates that of the  $\omega$ -6 PUFA, arachidonic acid. As expected, the relative proportion of DHA/EPA was much greater with supplement B ( $1.9 \pm 0.1$ ) than with supplement A ( $0.7 \pm 0.04$ ).

**Figure 10: PLASMA FATTY ACIDS (% of total)**



### Identification/composition of supplements

At the conclusion of the trial, supplements A and B were identified as Epanoil (a commercial MaxEPA type of EPA-rich oil) and Nu-Mega tuna oil (the client's DHA-rich oil) respectively. The appendix contains information supplied by the client on the fatty acid, antioxidant and microbiological analyses for these oils, together with fatty acid analyses conducted in our laboratory on randomly sampled capsules. The relative proportion of DHA appears to be lower in our analysis of the capsules. However, the DHA/EPA ratio was essentially reversed between the two oils, i.e. 1/2.1 for supplement A (MaxEPA) and 3.2/1 for supplement B (Nu-Mega tuna oil), at least a sixfold difference.



## DISCUSSION

The purpose of this study was to compare the relative efficacy of the client's new DHA-rich tuna oil product (supplement B) with that of a typical commercial EPA-rich product, with respect to selected indices of clinical cardiovascular and anti-inflammatory benefits.

Recognition of the central role of eicosanoid mechanisms in the anti-inflammatory, anti-thrombotic and possibly antihypertensive effects of fish oil has led to the view that EPA is likely to be the primary mediator of these effects, by competing directly with arachidonic acid, the immediate precursor of eicosanoid synthesis<sup>1-3</sup>. For this reason, commercial interests have tended to focus on sources of EPA-rich fish oil. However, our research on dietary administration of purified fatty acids to experimental animals indicates that DHA may be more efficacious than EPA in reducing cardiovascular risk factors<sup>4</sup>. This is consistent with early observations on the relative cardiovascular benefits of consuming DHA-rich fish<sup>5-7</sup>. The recent introduction of DHA supplements during infant development has raised interest in sources of DHA-rich oil such as tuna. Studies are now being undertaken to evaluate other human health benefits of supplemental DHA in various forms, e.g. fish, fish oil, microalgal oil. However, there have been few reports of direct comparisons between DHA and EPA-rich supplements.

A primary objective of this study was to test the BP lowering potential of the DHA-rich oil. The most sensitive and reliable approach was to use 24 hour ambulatory BP monitoring<sup>8</sup>. This was supplemented with clinic BP measurements, taken both seated and standing. The clinic measurements of DBP were reduced by both fish oil supplements. However, this may simply reflect a commonly observed temporal regression of clinic BP readings. Indeed, the ambulatory measurements, which are less susceptible to this artefact, showed no significant change with supplement, although there was a trend for reduction of night-time BP. Meta-analyses indicate that the effective antihypertensive dose of  $\omega$ -3 fatty acids is  $>3\text{g/day}$ <sup>9,10</sup>, slightly higher than the dose used (approx 2.5g/day). However, as 3g of  $\omega$ -3 fatty acids from MaxEPA represents only 1g of DHA, the tuna oil supplement (B), yielding 2g of DHA/day, would have been expected to be efficacious were DHA the principal mediator of the antihypertensive effect of  $\omega$ -3 fatty acids. Clearly this was not the case. There was no indication of a differential effect between the EPA- and DHA-rich oils. The lack of effect of either oil can be attributed to the marginal dose. This dose may nevertheless be effective when combined with a low salt diet and/or in subjects on selected antihypertensive drug therapy<sup>11</sup>.

Plasma lipids, on the other hand, were affected by both supplements. Moreover, their effects were almost identical. Consistent with the established role of  $\omega$ -3 fatty acids in mildly hyperlipidaemic patients<sup>12</sup>, there was a substantial reduction of plasma TG and a small rise in LDL cholesterol with no change in total cholesterol. Average pretreatment plasma lipids were marginally elevated. In two subjects, however, plasma TG exceeded 4 mM; these were markedly reduced by both supplements.

The effects of DHA on plasma TG are controversial<sup>13-15</sup>. In a recent trial, a microalgal oil containing DHA (1.6 g/day) without EPA reduced TG by up to 22%<sup>13</sup> but in another trial, DHA-rich fish oil (1.5-1.8 g of DHA/day) did not affect plasma TG levels<sup>14</sup>. In yet another trial<sup>15</sup>, a DHA-rich fish diet was compared with EPA-rich fish oil and the microalgal DHA oil. The maximal reductions of fasting plasma TG were 27%, 26% and 17% respectively. Unlike the other supplements, the DHA oil failed to reduce postprandial TG but the dose of DHA

(1.7 g/day) was quite modest. Nevertheless, the fish diet, which was the most efficacious, provided only 0.4g of EPA and 0.7 g of DHA/day.

The current study appears to be unique in directly contrasting the effects of two fish oil preparations with similar total  $\omega$ -3 content but markedly different proportions of EPA and DHA. The equivalent efficacy suggests that DHA-rich tuna oil would be a suitable therapeutic option for the treatment of hypertriglyceridaemia. However, there are concerns about possible hyperglycaemic effects of fish oil supplementation in the most prevalent hypertriglyceridaemic condition, non-insulin dependent diabetes<sup>16,17</sup>. It will be important to establish that a minimum effective dose of the DHA-rich tuna oil will not adversely affect glycaemic control, as has been shown for Omacor, an EPA-rich oil now registered in Europe as a hypotriglyceridaemic agent<sup>18</sup>.

One of the first properties of fish oil postulated to contribute to prevention of cardiovascular mortality was the inhibition of platelet aggregation<sup>19</sup>. The significance of this antithrombotic effect was offset by concerns about increased risk of bleeding during fish oil supplementation, although concern has gradually declined with the use of moderate doses of fish oil<sup>17</sup>. However, we still need to determine the threshold antithrombotic dose and the relative efficacy of EPA and DHA. EPA competes directly with arachidonic acid as a substrate for thromboxane production, yielding the relatively inactive thromboxane A<sub>3</sub> whilst maintaining prostacyclin levels<sup>1-3</sup>. DHA can also inhibit thromboxane production by displacing arachidonic acid from precursor pools, inhibiting cyclooxygenase and possibly by blocking thromboxane receptors<sup>3</sup>.

The equal suppression of thromboxane production in the present study following consumption of equivalent amounts of EPA-rich and DHA-rich fish oil, resulting in a threefold difference in the plasma EPA/DHA ratio, strongly suggests that DHA can also counteract thromboxane production. It also implies that, like the consumption of DHA-rich fish<sup>5,6</sup>, the DHA-rich oil may help to reduce the risk of thrombosis. However, the dose of microalgal DHA oil which was found to lower plasma TG had no effect on thromboxane production<sup>13</sup>, emphasising the need to determine an adequate dose for each indication.

The effects of the fish oil supplements on cytokine production by monocytes are yet to be analysed. The cytokines being examined, viz. IL1 $\beta$  and TNF $\alpha$ , are mediators of inflammatory responses and their production is known to be inhibited by  $\omega$ -3 fatty acids<sup>20</sup>. Moreover, there is evidence that DHA may be more effective than EPA in suppressing inflammatory mechanisms<sup>21,22</sup>. The results should give an indication of the relative potential of EPA- and DHA-rich fish oils to suppress inflammation in chronic conditions such as psoriasis and rheumatoid arthritis.

## **CONCLUSIONS AND RECOMMENDATIONS**

The results indicate that neither Nu-Mega tuna oil nor the comparator oil are likely to improve blood pressure at the dose level used in this study. However, the DHA-rich Nu-Mega oil was equally effective as the EPA-rich oil in reducing plasma triglycerides and thrombotic potential, two significant cardiovascular risk factors.

Considering that triglyceride reduction is a registered therapeutic indication for EPA-rich oil, a dose/response relationship and safety profile (with particular reference to management of non-insulin dependent diabetes) should be established for Nu-Mega.

## REFERENCES

1. Gibson RA: The effect of diets containing fish and fish oils on disease risk factors in humans. *Aust NZ J Med* 1988;18:713-722.
2. Simopoulos AP: Omega-3 fatty acids in health and disease and in growth and development. *Am J Clin Nutr* 1991;54:438.
3. Kinsella JE, Lokesh B, Stone RA: Dietary n-3 polyunsaturated fatty acids and amelioration of cardiovascular disease: possible mechanisms. *Am J Clin Nutr* 1990;52:1-28.
4. McLennan PL, Howe, PRC, Abeywardena M *et al*: The cardiovascular protective role of Docosahexaenoic acid *Eur J Pharmacol* 1996;300:83-89.
5. Cobiac L, Clifton PM, Abbey M *et al*: Lipid, lipoprotein and haemostatic effects of fish vs fish oil n-3 fatty acids in mildly hyperlipidemic males. *Am J Clin Nutr* 1991;53:1210-1216.
6. Sanders, TAB, Hinds A: The influence of a fish oil high in docosahexaenoic acid on plasma lipoprotein and vitamin E concentrations and haemostatic function in healthy male volunteers. *Br J Nutr.* 1992;68:163-173.
7. Singer P, M Wirth, S Voigt *et al*: Blood pressure- and lipid lowering effect of mackerel and herring diet in patients with mild essential hypertension. *Atherosclerosis.* 1985;56: 223-235.
8. Lungershausen Y & Howe PR: Improved detection of a blood pressure response to dietary intervention with 24-hour ambulatory monitoring *Am J Hypertens* 1994;7:1115-1117.
9. Morris MC, Sacks F & Rosner B: Does fish oil lower blood pressure? A meta-analysis of controlled trials. *Circulation* 1993;88:523-533.
10. Appel LJ, Miller E, Seidler A & Whelton PK: Does supplementation of diet with fish oil reduce blood pressure? *Arch Int Med* 1993;153:1429-1438.
11. Howe PRC: Can we recommend fish oil for hypertension? *Clin Exp Pharm Physiol* 1995;22: 199-203.
12. Harris WS: Dietary fish oil and blood lipids. *Curr Opinion in Lipidol* 1996;7:3-7.
13. Conquer J & Holub BJ: Supplementation with an algae source of docosahexaenoic acid increases n-3 fatty acid status and alters selected risk factors for heart disease in vegetarian subjects. *J Nutr* 1996;126:3032-39
14. Hamazaki T, Sawazaki S, Asaoka E *et al*: Docosahexaenoic acid-rich fish oil does not affect serum lipid concentrations of normolipidaemic young adults. *J Nutr* 1996;126:2784.
15. Agren JJ, Hanninen O, Julkunen A *et al*: Fish diet, fish oil and docosahexaenoic acid rich oil lower fasting and postprandial plasma lipid levels. *Eur J Clin Nutr* 1996;50:765-771.
16. Harris WS: Do omega-3 fatty acids worsen glycemic control in NIDDM? *ISSFAL Newsletter.* 1996;3:6-9.
17. Leaf A: Health Claims: Omega-3 fatty acids and cardiovascular disease. *Nutr Rev* 1992;50: 150-154.
18. Mackness MI, Bhatnagar D, Durrington PN *et al*: Effects of a new fish oil concentrate on plasma lipids and lipoproteins in patients with hypertriglyceridaemia. *Eur J Clin Nutr.* 1994;48: 859-865.
19. Dyerberg J, Bang HO, Stofferson E *et al*: Eicosapentaenoic acid and prevention of thrombosis and atherosclerosis. *Lancet* 1978;2:117-119.
20. Grimble RF: Interaction between nutrients, pro-inflammatory cytokines and inflammation. *Clin Sci* 1996;91:121.
21. De Caterina R, Cybulsky M, Clinton S *et al*: The omega-3 fatty acid docosahexaenoate reduces cytokine-induced expression of proatherogenic and proinflammatory proteins in human endothelial cells. *Arterioscler Thromb.* 14: 1829-1836.
22. Robinson DR, Xu L, Tateno S *et al*: Suppression of autoimmune disease by dietary n-3 fatty acids. *J Lipid Res* 1993;34:1435-1444.

April 1, 1997

Mr Hamish Drummond  
Clover Corporation Pty Ltd  
PO Box 1133  
MONA VALE, NSW 2103

Dear Hamish,

I have pleasure in enclosing our report on the Nu-Mega Lipids Trial recently conducted in our Nutrition Research Clinic. As you are aware, the cytokine data from Mick James is still forthcoming but I was keen to provide you with our data at this stage.

The main findings are the reduction of triglycerides and inhibition of thromboxane production, in which the DHA-rich tuna oil was equally efficacious as the comparator.

I would be pleased to discuss any aspects of our findings or their interpretation with you.

With best wishes,

Yours sincerely,

**Peter R. C. Howe**  
Senior Principal Research Scientist

# **NU-MEGA LIPIDS TRIAL**

## **ADDENDUM TO FINAL REPORT**

from

**CSIRO DIVISION OF HUMAN NUTRITION**

to

**CLOVER CORPORATION LIMITED**

prepared by

*Peter R. C. Howe*

**1<sup>st</sup> July, 1997**

**(to be read in conjunction with the report dated 27<sup>th</sup> March 1997)**

## Overall Study Objective

To compare, in healthy volunteers, the bioavailability and cardiovascular health attributes of a DHA-rich tuna oil (Nu-Mega) and an EPA-rich oil (MaxEPA), taken as supplementary capsules.

## Specific objective

As a secondary outcome measure, to assess effects of the above supplements on production of pro-inflammatory cytokines, viz. interleukin 1b (IL1b) and tumor necrosis factor (TNFa). This objective was undertaken in collaboration with Dr Michael James, Royal Adelaide Hospital.

## Rationale

Purported health benefits of  $\omega$ -3 fatty acids extend from their role in early infant development through to the chronic cardiovascular and inflammatory disorders of middle age<sup>1</sup>. There is increasing evidence that  $\omega$ -3 supplementation can help alleviate inflammatory disorders such as arthritis, psoriasis, asthma, ulcerative colitis, autoimmune nephropathy and transplant rejection, but it is difficult to quantify the benefit. These disorders are characterised by excessive or inappropriate production of the pro-inflammatory cytokines, IL1b and TNFa, which accordingly provide an index for assessing anti-inflammatory activity<sup>2</sup>. Their production by monocytes in both healthy and affected subjects can be inhibited by  $\omega$ -3 supplementation<sup>3</sup>. Although the extent of inhibition has been correlated with monocyte EPA content<sup>4</sup>, the relative efficacy of EPA and DHA has not been established. This trial offered an opportunity for such a comparison.

## Experimental

Monocytes were isolated from fresh 20ml blood samples taken from 15 patients in each of the treatment groups at the 1<sup>st</sup> (pre-intervention) visit and again after 6 weeks (2<sup>nd</sup> visit). The IL1b and TNFa produced during lipopolysaccharide stimulation of the monocytes was measured using ELISA kits obtained from Cayman Chemical and Genzyme DuoSet respectively. The assays had been delayed to enable validation of these kits.

## Results

Figs 1 & 2 show mean values  $\pm$  SEM for monocyte production of IL1b and TNFa respectively in blood taken at each visit. Supplementation with both MaxEPA and NuMega caused significant reductions of IL1b; the respective within-subject reductions over 6 weeks of supplementation were  $4.8 \pm 1.5$  and  $2.6 \pm 1.0$  ng/ml. Statistical analysis (repeated measures ANOVA; SPSS) indicates that the effects of the two supplements were not significantly different. Thus the overall effect of  $\omega$ -3 supplementation was to reduce IL1b by 21% ( $P < 0.001$ ), an effect which was unrelated to within-individual changes in the ratio of EPA/DHA in plasma (see table). Although there was a strong correlation between IL1b and TNFa production at the pre-intervention visit, there was no consistent effect of  $\omega$ -3 supplementation on TNFa.

Fig 1

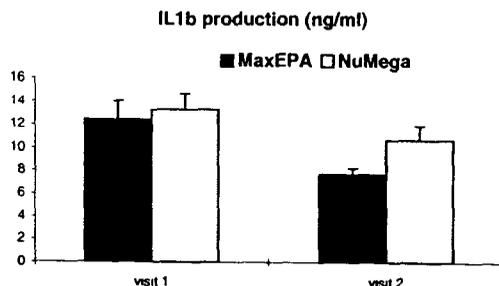
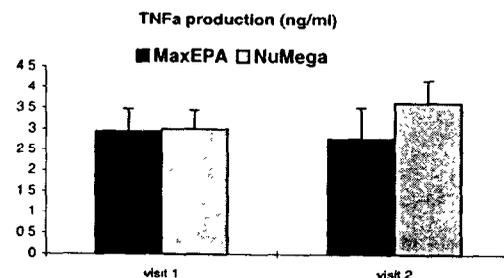


Fig 2



ID	treatment	IL-1b (ng/ml)			TNFa (ng/ml)			plasma f.a. at visit 2	
		visit 1	visit 2	change	visit 1	visit 2	change	total n-3%	DHA/EPA
C01	A-B	7.2	8.7	1.5	2.77	2.03	-0.74	7	0.676
C02	A-B	22.3	7	-15.3	8.83	2.27	-6.56	4.7	0.615
C05	A-B	9.03	7.9	-1.13	1.73	0.77	-0.96	4.7	0.593
C07	A-B	9.07	5.27	-3.8	3.23	1.47	-1.76	4.9	0.630
C09	A-B	7.73	11.13	3.4	1.1	4.07	2.97	7.2	0.465
C13	A-B	12.13	9	-3.13	2.8	3.7	0.9	5.1	0.704
C15	A-B	7.17	5.4	-1.77	1.4	3.23	1.83	4	1.059
C16	A-B	9.5	6.87	-2.63	1.4	1.8	0.4	8.4	0.600
C17	A-B	20	9.83	-10.17	5.33	3.7	-1.63	6.3	0.436
C21	A-B	15.12	8	-7.12	3.87	2.23	-1.64	6.1	0.958
C22	A-B	6.27	3.63	-2.64	1.57	0.43	-1.14	7.6	0.590
C25	A-B	16.1	9.77	-6.33	3.03	0.87	-2.16	6.7	0.788
C26	A-B	11.8	7.77	-4.03	1.17	1.2	0.03	6.3	0.647
C30	A-B	8.37	6.7	-1.67	2.03	1.77	-0.26	6.2	0.714
C32	A-B	25.1	8.3	-16.8	4.1	12.2	8.1	7.3	0.909
	MaxEPA	12.46	7.68	-4.77	2.96	2.78	-0.17	6.17	0.69
		1.54	0.51	1.45	0.53	0.73	0.81	0.32	0.04
		15	15	15	15	15	15	15	15
C03	B-A	14.4	9.9	-4.5	2.37	1.03	-1.34	4.5	1.150
C04	B-A	6.13	6.93	0.8	1.23	3.4	2.17	4.1	1.714
C06	B-A	16.4	13.5	-2.9	4.77	3.53	-1.24	5	2.615
C08	B-A	5.7	3.57	-2.13	0.63	4.13	3.5	3.7	1.833
C12	B-A	18.7	15	-3.7	2.13	1.63	-0.5	5.1	1.474
C14	B-A	17.1	18.9	1.8	2.9	4.13	1.23	4.1	1.714
C18	B-A	14.7	17.7	3	2.4	3.53	1.13	4.3	2.455
C19	B-A	3.8	7.13	3.33	0.97	4.83	3.86	5.4	2.063
C20	B-A	13.6	12.5	-1.1	3.97	7.37	3.4	5.4	1.632
C23	B-A	11.3	4.17	-7.13	3.8	0.87	-2.93	5.7	2.400
C24	B-A	19.4	9.37	-10.03	3.73	2.6	-1.13	5	1.611
C27	B-A	8.33	6.33	-2	1.3	1.9	0.6	4.7	2.000
C28	B-A	14.6	10.6	-4	5.93	6.2	0.27	6.9	1.864
C29	B-A	12.77	9.67	-3.1	2.63	1.4	-1.23	5.5	1.833
C31	B-A	22.5	15.2	-7.3	6.33	7.9	1.57	6.5	1.727
	NuMega	13.30	10.70	-2.60	3.01	3.63	0.62	5.06	1.87
		1.39	1.21	0.99	0.45	0.57	0.53	0.23	0.10
		15	15	15	15	15	15	15	15

### **Discussion**

The reduction of IL1b production by the  $\omega$ -3 supplements is consistent with their recognised anti-inflammatory effects. However, the results fail to show a predominant effect of either DHA or EPA. The extent of reduction was less than that reported in other studies, even in healthy subjects, where 40-85% reductions of IL1 have been reported<sup>3,4</sup>. Effects on TNF appear to be more variable, with no change seen in 3/6 studies<sup>3,4</sup>. Comparison with other studies indicates that both the dose and duration of treatment were adequate to demonstrate cytokine inhibition.

Our collaborators have obtained evidence that thromboxane A<sub>2</sub> facilitates cytokine production<sup>5</sup>. It is of interest, therefore, that MaxEPA and NuMega treatments reduced platelet thromboxane production by the same extent in this study. While the inhibition of IL1b could not be attributed specifically to either EPA or DHA, these fatty acids may have differential effects on other inflammatory mechanisms. For example, DHA has been shown to counteract cytokine-induced expression of cell adhesion molecules by human endothelial cells, a mechanism with major implications for the prevention of atherosclerosis<sup>6</sup>. Thus, in comparing the anti-inflammatory effects of EPA- and DHA-rich supplements, evaluation of additional indices may be appropriate.

### **References**

1. Simopoulos AP: Omega-3 fatty acids in health and disease and in growth and development. *Am J Clin Nutr* 1991;54:438.
2. Grimble RF: Interaction between nutrients, pro-inflammatory cytokines and inflammation. *Clin Sci* 1996;91:121.
3. Endres S: n-3 polyunsaturated fatty acids and human cytokine synthesis. *Lipids* 1996;31:S239-42.
4. Caughey GE, Pouliot M, Cleland L, James MJ: The effect on human tumor necrosis factor  $\alpha$  and interleukin 1 $\beta$  production of diets enriched in  $\omega$ -3 fatty acids from vegetable oil or fish oil. *Am J Clin Nutr* 1996;63:116-22.
5. Caughey GE, Pouliot M, Cleland L, James MJ: Regulation of tumor necrosis factor-alpha and IL-1 beta synthesis by thromboxane A<sub>2</sub> in nonadherent human monocytes. *J Immunol* 1997;158:351-8
6. De Caterina R, Cybulsky M, Clinton S *et al*: The omega-3 fatty acid docosahexaenoate reduces cytokine-induced expression of proatherogenic and proinflammatory proteins in human endothelial cells. *Arterioscler Thromb*. 14: 1829-1836.