"EVALUATION OF THE EFFECT OF NKO™ ON BIOMARKERS OF CHRONIC INFLAMMATION IN VIVO"

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Submitted by:

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1.0 SUMMARY

Neptune Krill Oil is a rich source of omega-3 and omega-9 fatty acids. In addition, Neptune Krill Oil has a high content in phospholipids, which are themselves rich in omega-3 fatty acids. Phospholipids are important in protecting membranes from toxic injury and free radical attack[1]. The composition of phospholipids in Neptune Krill Oil appears to be optimal to offer such protection. Furthermore, Neptune Krill Oil contains high quantities of the powerful biological antioxidants astaxanthin and canthaxanthin[2]. Astaxanthin has shown better antioxidant activity than alpha-tocopherol, beta-carotene, lycopene and lutein[2]. Neptune Krill Oil also contains a flavonoid with a novel molecular structure. Flavonoids have been studied for more than 60 years but had not been extracted until now from anything other than plants, vegetables, fruits and algae. The antioxidant activity of flavonoids has previously been shown[3]. Neptune Krill Oil is extremely resistant to oxidation, with peroxide values less than 0.1 units for more than 50 hours at 97.8°C.

Because of the remarkable complexity of the multiple bioactive components of Neptune Krill Oil, the unraveling of the exact mechanism of action is a very time consuming and multifactorial project, which is still ongoing. We speculate that it is based on the inhibition of prostacyclin (PGs, PGI_2) and thromboxane (TXs) and leukotrienes by interfering at the level of the cyclooxygenase or the lipoxygenase pathways. This works directly through the mechanism of pain in order to reduce inflammation and subsequently pain.

The effect of Neptune Krill Oil, on in joint pain, flexibility and stiffness was assessed at base line and at 7, 14 and 30-day follow-up visits. Successful demonstration of an enhanced effect

may provide a basis for clinical use of *Neptune Krill Oil* in patients with inflammatory disease. Subjects were randomly assigned to either *Neptune Krill Oil* or placebo administration. At baseline and at 7, 14 and 30-day follow-up visits blood was drawn for serum CRP analysis and patients were asked to answer the WOMAC questionnaire for an immediate response assessment.

2.0 INTRODUCTION

Inflammation is closely linked to the pathogenesis of atherosclerosis and joint disease and may be provoked by noninfectious (e.g., injury, smoking, diabetes, obesity) as well as infectious sources. Meanwhile, we should focus on identifying and treating subjects with evidence of chronic inflammation, whatever its cause, as they remain at increased risk for stroke, MI, and death. CRP appears to be a central player in the harmful effects of inflammation and an inexpensive screening test to assess inflammation-associated risk. However, values defining elevation of CRP will depend on multiple factors, including baseline cardiovascular risk, inflammatory or degenerative joint disease as determined by traditional and nontraditional risk factors. Patients with evidence of ongoing inflammation may derive specific benefit from anti-inflammatory. Thus, reduction of risk through weight loss, exercise, smoking cessation, β-blockers, diabetic control, and other therapies should be aggressively pursued. CRP may be useful in determining the relative benefit of more aggressive medical therapy for the individual patient with inflammatory disease.

The Western Ontario and McMaster (WOMAC) University Osteoarthritis index has been validated and is recommended for the assessment of treatment effects in patients with

osteoarthritis (OA) The WOMAC is the most commonly used disease-specific outcome instrument[4-11]. Even though it was initially developed for the assessment of pain, stiffness and function of daily living in the elderly with osteoarthritis it has recently been revised for younger and/or more active patients with knee injury and/or knee osteoarthritis[9].

3.0 OBJECTIVES:

- *To evaluate the effect of NKO™ on biomarkers of chronic inflammation*
- *To evaluate the effect of NKO™ on the quality of life of patients with arthritic disease*

4.0 HYPOTHESIS:

- *Neptune Krill Oil* is significantly more effective than placebo for the reduction of inflammation as measured by serum CRP
- *Neptune Krill Oil* is significantly more efficacious than Placebo for the management of pain in patients with rheumatoid arthritis and osteoarthritis.

5.0 METHODS:

Study Design:

This was a randomized, double blind, placebo controlled study. Patients was recruited from primary care physicians in Montreal Quebec.

Patients:

A total of 90 patients were recruited from primary care physicians in Quebec. Patients diagnosed with cardiovascular disease, rheumatoid arthritis or osteoarthritis and with increased levels of C-reactive protein >1.0mg/dl were eligible to participate in the study. . The following inclusion and exclusion criteria were applied to select the patients for the study.

Inclusion Criteria:

- Age between 30 and 75 years; both genders admissible.
- CRP > 1.0 mg/dl
- Patients were asked to stop use of all other "pain-killers" (except acetaminophen) the week prior to initiation of the trial for washout purposes.
- Intention to fully participate in study including attending physician appointments during trial.
- Signed consent form

Exclusion Criteria:

a) Gastro-intestinal Exclusions

- History of gastrointestinal perforation
- History of gastrointestinal hemorrhage
- Symptomatic peptic ulcer

b) Miscellaneous Exclusions

- Diabetes

- Subjects taking NSAIDs or COX-2 inhibitors (concomitantly)
- Subjects taking H2 inhibitors or proton pump inhibitors.
- Severe OA of the knee(s).
- Initiation of physical therapy or muscle conditioning within 3 months.
- Allergies to or a history of adverse reactions associated with seafood or Fish Oil use.
- Alcohol consumption exceeding 3 mixed drinks per day.
- Concurrent medical disease that could confound or interfere with the evaluation of pain.
- Participation in another clinical trial.
- Moderate or severe depression.
- Inability to complete study questionnaires.
- Women of childbearing age were required to have confirmed use of adequate contraception since their last menses and to agree to continue this practice during the study.
- Known or suspected or planned pregnancy

TREATMENT ADMINISTRATION:

A total of 90 patients, 45 per group, were randomly assigned by a computer-generated schedule into one of two groups; depending on the group patients are randomized in they will receive:

NKO™ 300mg per day

a. Neutral placebo 100mg

Patients will also receive a rescue analgesic medication, acetaminophen (325 mg tablets), which they will be allowed to take when required for severe pain throughout the trial. The maximum dose of acetaminophen allowed will be as recommended by the manufacturer; 1-2 capsules QID. All patients will be receiving background therapy with any other medications considered necessary by their physicians.

PRIMARY EFFICACY PARAMETER:

- *Biochemical measures:*
    - C-Reactive Protein (CRP)

SECONDARY EFFICACY PARAMETERS:

- *Western Ontario and McMaster (WOMAC) University Osteoarthritis index.*

STATISTICAL RATIONALE AND ANALYSIS:

A sample size of 45 patients / group will provide 80% power to detect a CRP reduction of 10% between any 2 treatment groups.

RESULTS:

TABLE 1. Patient Disposition by Group and Visit		Group	
		NKO 300 mg/day	Placebo
Visit	Baseline	45	45
	7 Days	45	45
	14 Days	44	43
	30 Days	44	43

TABLE 2. Patient Demographics			Group	
			NKO 300 mg/day	Placebo
Gender	Male	N	25	22
		%	55.6%	48.9%
	Female	N	20	23
		%	44.4%	51.1%
Age (years)	Mean		54.63	55.27
	Std Deviation		14.83	14.32
	Median		50.03	52.31
Acetaminophen use (prn)	Change Baseline – 30 days		-31.62%	-5.91%
	P-value between groups		0.012	
Concomitant Meds	P-value between groups		0.987	

TABLE 3. C – Reactive Protein (CRP) mg/dl by Group and Visit			NKO™ 300 mg/day	Placebo	P value (Between Groups)
Visit	Baseline	Mean	2.49	2.87	0.087
		Std Deviation	1.85	1.25	
		Median	2.28	2.83	
	7 Days	Mean	2.01	3.32	0.049
		% Change (Baseline - 7 days)	-19.3	15.7	
		Std Deviation	1.08	1.92	
		Median	1.95	3.26	
	14 Days	Mean	1.75	3.79	0.004
		% Change (Baseline - 14 days)	-29.7	32.1	
		Std Deviation	0.88	1.88	
		Median	1.86	4.02	
	30 Days	Mean	1.72	3.59	0.008
		% Change (Baseline - 30 days)	-30.9	25.1	
		Std Deviation	1.0	1.05	
		Median	1.69	3.44	
	P value (Within Groups) / Interaction		0.001	0.628	

TABLE 4. WOMAC Pain Scores by Group and Visit			Group		P value (Between Groups)
			NKO 300 mg/day	Placebo	
Visit	Baseline	Mean	3.39	3.07	0.539
		Std Deviation	.91	.60	
		Median	3.19	3.00	
	7 Days	Mean	2.41	2.78	0.052
		Std Deviation	.90	.61	
		Median	2.19	2.71	
	14 Days	Mean	2.52	3.26	0.003
		Std Deviation	.79	.67	
		Median	2.39	3.21	
	30 Days	Mean	2.09	3.05	0.009
		Std Deviation	.85	.59	
		Median	2.02	3.00	
	P value (Within Groups) / Interaction		0.002	0.138	

TABLE 5. Change in WOMAC Pain Scores / 100 by Group and Visit			Group		P value (Between Groups)
			NKO 300 mg/day	Placebo	
Visit	7 Days	Mean	-28.91	-9.44	0.050
		Std Deviation	18.70	26.98	
		Median	-25.00	-10.00	
		P-Value (Visit)	0.001	0.290	
	14 Days	Mean	-25.66	6.18	0.049
		Std Deviation	15.27	13.54	
		Median	-25.00	.00	
		P-Value (Visit)	0.022	0.208	
	30 Days	Mean	-38.35	-0.6	0.011
		Std Deviation	21.06	15.89	
		Median	-30.00	.00	
		P-Value (Visit)	0.001	0.610	

TABLE 6. WOMAC Stiffness Scores by Group and Visit			Group		P value (Between Groups)
			NKO 300 mg/day	Placebo	
Visit	Baseline	Mean	3.45	2.85	0.104
		Std Deviation	.95	.85	
		Median	3.48	3.02	
	7 Days	Mean	2.75	3.35	0.030
		Std Deviation	.84	.83	
		Median	2.48	3.10	
	14 Days	Mean	2.55	2.83	0.056
		Std Deviation	.79	.99	
		Median	2.50	3.00	
	30 Days	Mean	2.10	2.97	0.043
		Std Deviation	.85	.72	
		Median	2.00	3.01	
	P value (Within Groups) / Interaction		0.002	0.324	

TABLE 7. Change in WOMAC Stiffness Scores / 100 by Group and Visit			Group		P value (Between Groups)
			NKO 300 mg	Placebo	
Visit	7 Days	Mean	-20.29	17.54	0.001
		Std Deviation	24.31	29.88	
		Median	-25.00	25.00	
		P-Value (Visit)	0.004	0.127	
	14 Days	Mean	-26.09	-0.70	0.018
		Std Deviation	27.05	20.55	
		Median	-31.25	1.00	
		P-Value (Visit)	0.002	0.820	
	30 Days	Mean	-39.13	4.21	0.023
		Std Deviation	27.67	26.74	
		Median	-31.25	12.50	
		P-Value (Visit)	0.003	0.879	

TABLE 8. WOMAC Functional Impairment Scores by Group and Visit			Group		P value (Between Groups)
			NKO 300 mg/day	Placebo	
Visit	Baseline	Mean	3.34	2.98	0.105
		Std Deviation	.91	.41	
		Median	3.41	3.12	
	7 Days	Mean	2.58	2.94	0.023
		Std Deviation	.58	.37	
		Median	2.82	3.00	
	14 Days	Mean	2.36	2.65	0.021
		Std Deviation	.31	.36	
		Median	2.56	2.63	
	30 Days	Mean	2.14	2.78	0.135
		Std Deviation	.68	.44	
		Median	2.66	2.91	
	P value (Within Groups) / Interaction		0.018	0.138	

TABLE 9. Change in WOMAC Functional Impairment Scores / 100 by Group and Visit			Group		P value (Between Groups)
			NKO 300 mg/day	Placebo	
Visit	7 Days	Mean	-22.75	-1.34	0.008
		Std Deviation	10.59	5.86	
		Median	-20.53	1.55	
		P-Value (Visit)	0.005	0.750	
	14 Days	Mean	-29.34	-11.07	0.040
		Std Deviation	14.07	13.06	
		Median	-14.02	-6.15	
		P-Value (Visit)	0.016	0.094	
	30 Days	Mean	-35.93	-6.71	0.005
		Std Deviation	.9.69	7.34	
		Median	-20.47	-3.11	
		P-Value (Visit)	0.018	0.269	

CONCLUSION:

The results of the present study clearly indicate within a high level of certainty that NKO™ at a daily dose of 300mg may within a short time to reaction (7-14 days):

- **Significantly inhibit inflammation by reducing C - Reactive Protein (CRP)**
- **Alleviate symptoms caused by osteoarthritis and rheumatoid arthritis**

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