JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2009, 57, 9339–9345 9339
DOI:10.1021/jf9016042

Dietary Krill Oil Supplementation Reduces Hepatic Steatosis, Glycemia, and Hypercholesterolemia in High-Fat-Fed Mice

Sally Tandy,[†] Rosanna W. S. Chung,[†] Elaine Wat,[†] Alvin Kamili,[†] Kjetil Berge,[‡] Mikko Griinari,[§] and Jeffrey S. Cohn*,[†]

[†]Nutrition and Metabolism Group, Heart Research Institute, Sydney, Australia, [‡]Aker Biomarine, Oslo, Norway, and [§]Clanet Oy, Helsinki, Finland

Krill oil (KO) is rich in n-3 fatty acids that are present in phospholipids rather than in triglycerides. In the present study, we investigated the effects of dietary KO on cardiometabolic risk factors in male C57BL/6 mice fed a high-fat diet. Mice (n = 6−10 per group) were fed for 8 weeks either: (1) a nonpurified chow diet (N); (2) a high-fat semipurified diet containing 21 wt % buttermilk + 0.15 wt % cholesterol (HF); (3) HF supplemented with 1.25 wt % KO (HFKO1.25); (4) HF with 2.5 wt % KO (HFKO2.5); or (5) HF with 5 wt % KO (HFKO5.0). Dietary KO supplementation caused a significant reduction in liver wt (i.e., hepatomegaly) and total liver fat (i.e., hepatic steatosis), due to a dose-dependent reduction in hepatic triglyceride (mean ± SEM: 35 ± 6, 47 ± 4, and 51 ± 5% for HFKO1.25, -2.5, and -5.0 vs HF, respectively, $P < 0.001$) and cholesterol (55 ± 5, 66 ± 3, and 71 ± 3%, $P < 0.001$). Serum cholesterol levels were reduced by 20 ± 3, 29 ± 4, and 29 ± 5%, and blood glucose was reduced by 36 ± 5, 34 ± 6, and 42 ± 6%, respectively. Serum adiponectin was increased in KO-fed animals (HF vs HFKO5.0: 5.0 ± 0.2 vs 7.5 ± 0.6 μg/mL, $P < 0.01$). These results demonstrate that dietary KO is effective in improving metabolic parameters in mice fed a high-fat diet, suggesting that KO may be of therapeutic value in patients with the metabolic syndrome and/or nonalcoholic fatty liver disease.

KEYWORDS: Krill oil; n-3 fatty acids; phospholipids; cholesterol; liver; hepatomegaly; hepatosteatosis; triglyceride

INTRODUCTION

The health-promoting effects of dietary long-chain n-3 fatty acids (FAs), eicosapentanoic acid (EPA, 20:5) and docosahexanoic acid (DHA, 22:6), present in fatty fish and fish oil (FO), have been extensively documented. In addition to an ability to reduce plasma lipid levels in animals (*1*) and humans (*2*), they have been shown to prevent arrythmias, decrease blood pressure, and reduce platelet aggregation (*3*). Dietary supplementation with n-3 FAs is thus beneficial for the prevention and/or treatment of cardiovascular disease (CVD) (*4*) and possibly other inflammatory and neurological disorders (*5*, *6*). Increased consumption of EPA and DHA may also be of clinical significance in the prevention and reversal of insulin resistance (*7*).

Krill oil (KO), extracted from Antarctic krill (*Euphausia superba*), has a unique chemical composition. Like FO, it is rich in long-chain n-3 FAs; however, the n-3 FAs are present in the form of phospholipid (PL) rather than triglyceride (TG). This may be biologically and therapeutically significant, since (a) PL FAs are well-absorbed by the intestine (*8*) and (b) they are readily incorporated into cell membranes (*9*). Dietary PLs, in particular those originating from soy and safflower, have consistently been shown to reduce plasma and liver lipid levels in experimental animals (*10*). Recent work from our laboratory has demonstrated that the addition of dairy milk PLs to the diet at a level of 2.5% by weight (wt %) can similarly reduce cholesterol and TG levels in high-fat-fed C57BL/6 mice (*11*). In addition to a high PL content, KO contains several endogenous antioxidants including astaxanthin, a strong lipid-soluble antioxidant. Astaxanthin is responsible for the deep red color of KO. It preserves KO against oxidation and has the potential to provide additional health-promoting properties. (*12*−*14*). It is this unique biomolecular composition that is thought to be responsible for KO's anti-inflammatory, analgesic, and hypolipidemic properties in human subjects (*15*−*17*).

Although clinical work has provided evidence that dietary KO supplementation has beneficial metabolic effects, there is a lack of basic research supporting its health-promoting properties. There is also little information available concerning its mechanism of action. The present study was therefore carried out to investigate the dose-dependent effects of dietary KO supplementation on cardiovascular and metabolic parameters in C57BL/6 mice fed a high-fat diet. Unlike the majority of FO studies carried out in mice, KO was investigated as an addition of fat to the diet rather than a substitution, mimicking the situation in humans given a dietary supplement. Furthermore, we investigated KO doses of 1.25, 2.5, and 5.0% by weight [equivalent to 0.24, 0.49, and 0.98 wt % or 0.55, 1.10, and 2.19% by energy (en %) EPA + DHA]. This range of doses mimics the average intake of n-3 FAs of humans in Western countries of about 0.7 en % (*18*) and is in

*To whom correspondence should be addressed. Tel: (612)8208-8906. Fax: (612)9565-5584. E-mail: cohnj@hri.org.au.

Published on Web 09/17/2009

contrast to the high doses of n-3 FAs investigated in previous rodent studies (i.e., > 3% en EPA + DHA) (*19*).

MATERIALS AND METHODS

Animals and Diets. Experiments were approved by the Animal Welfare Committee of the Sydney South West Area Health Service. Six week old male C57BL/6 mice were obtained from the Australian Resources Centre (Perth, Australia). They were housed in standard cages (five mice per cage) at a constant temperature of 20 °C with a 12 h light/dark cycle. They were allowed ad libitum access to diet and water. After 1 week of acclimatization, they were divided into five groups (two cages for each group). One group (n = 10) was fed a normal nonpurified diet (N), that is, normal mice pellets (Specialty Feeds, Glen Forrest, Western Australia). The nonpurified diet was composed of wheat, lupins, barley, soya meal, fish meal, mixed vegetable oils, canola oil, sodium chloride, calcium carbonate, dicalcium phosphate, magnesium oxide, and a vitamin and trace mineral premix. It contained 4.6% total fat, 4.8% crude fiber, and 19% protein. A second group (HF, n = 6) was fed a high-fat diet containing 21 wt % butterfat and 0.15 wt % cholesterol (SF00-219, Specialty Feeds, WA). This semipurified diet contained (g/kg) the following: casein, 195; DL-methionine, 3; sucrose 341; wheat starch, 154; cellulose, 50; clarified butter, 210; calcium carbonate, 17.1; sodium chloride, 2.6; potassium citrate, 2.6; potassium dihydrogen phosphate, 6.9; potassium sulfate, 1.6; AIN93G trace minerals, 1.4; choline chloride (65%), 2.5; vitamins, 10; cholesterol, 1.5. The three remaining groups (designated HFKO1.25, HFKO2.5 and HFKO5.0, n = 10 in each group) received the HF diet supplemented with 1.25, 2.5, and 5 wt % of KO, respectively. Food intake and body weight were recorded weekly. The KO (Superba Krill Oil) was prepared and analyzed by Nutrizeal (Nelson, New Zealand) and supplied by Aker Biomarine ASA (Oslo, Norway).

Tissue Processing. The mice were fed diets for 8 weeks. The food was removed the night (i.e., 12 h) before sacrifice. The mice were exsanguinated by heart puncture under methoxyflurane anesthesia. The blood was allowed to clot, and the serum was separated by centrifugation (1500*g*, 10 min). Sera were aliquoted and stored frozen (−80 °C) until analysis. The livers were immediately excised, weighed, and divided into small pieces (100−150 mg) for storage at −80 °C (for lipid and protein analysis) or in RNAlater Solution (Albion, Austin, TX) (for gene expression analysis). Epididymal, inguinal, and perirenal fat pads, as well as the quadriceps muscle from one leg, were excised, weighed, divided, and stored as described for liver samples.

Biochemical Analyses. The blood glucose was measured in whole blood with a glucose meter (Accu-Chek Integra, Roche Diagnostics). The serum insulin was measured by enzyme-linked immunosorbent assay (ELISA; Ultra Sensitive ELISA Kit, Crystal Chem Inc.). The serum TG, total cholesterol, and free fatty acid (NEFA) concentrations were measured by enzymatic methods, using GPO-PAP and CHOD-PAP (Roche Diagnostics) and Wako NEFA C (Wako Pure Chemicals, Osaka, Japan) kits, respectively. The serum PL levels were measured enzymatically, using Wako Phospholipids C kits (Wako Pure Chemicals). The serum apoA-I concentration was measured by competitive ELISA, whereby goat antimouse apoA-I polyclonal antibody was used for detection and goat antirabbit IgG HRP-conjugated was used for quantification (Biodesign International, Saco, ME). Total liver lipids were determined gravimetrically after extraction by the method of Bligh and Dyer (*20*). Individual hepatic lipids were quantitated enzymatically (as described above) after solubilization in isopropanol.

Protein Quantification. The liver tumor necrosis factor α (TNFα) protein was measured by ELISA (Quantikine mouse TNFα Immunoassay) after extraction of protein from liver. In brief, approximately 100 mg of liver was homogenized in 250 μL of buffer containing 20 mM Tris-HCL, 100 mM NaCl, 50 mM NaF, 5 mM $Na_4P_20_7$, and 1% Triton-X 100. The protease inhibitor cocktail (Roche, Complete Protease Inhibitor Cocktail Tablets, 1 tablet in 5 mL of buffer) was added immediately before use. Samples were incubated on ice for 15 min before centrifugation (15 min, 4°, 12000 rpm). The supernatant was removed and assayed for protein content (BCA protein assay reagent, Thermo Fisher Scientific, Inc.)

Gene Expression Analysis. Hepatic mRNA levels were measured by real-time polymerase chain reaction (PCR). Total RNA was isolated by selective binding to a silica gel-based membrane following lysis and homogenization of liver samples in a denaturing guanidine thiocyanate buffer (RNeasy kit, Qiagen). RNA (100 ng) was reverse transcribed into cDNA using random primers provided with the iScript cDNA Synthesis Kit (Bio-Rad). Selected genes were amplified using iQ SYBR Green Supermix (Bio-Rad) in an iCycler system (Bio-Rad) with 20 pmol of both forward and reverse primers. PCR conditions were as follows: 1 cycle of 95 °C for 3 min, 50 cycles of 95 °C for 30 s, 55−60 °C for 30 s and 72 °C for 30 s, followed by 1 cycle of 95 °C for 1 min. The purity of PCR products was assessed by melt curve analysis. Relative gene expression was calculated by normalizing cycle threshold (Ct) values for genes of interest with Ct values for cyclophilin using the $\delta-\delta$ Ct method. Primer sequences were as follows: ACC (forward, 5′-TTCTGAATGTGGCTATCAAGACTGA-3′; reverse, 5′-TGCTGGGTGAACTCTCTGAACA-3′); ACO (forward, 5′- TTTGTTGTCCCTAT CCGTGAGA-3′; reverse, 5′-CCGATATCC-CCAACAGTGATG-3′); CPT1a (forward, 5′- AACCCAGTGCCT-TAACGATG-3′; reverse, 5′-GAACTGGTGGCCA ATGAGAT-3′); cyclophilin (forward, 5′-TGGAAGAGCACCAAGACAGACA-3′; reverse, 5′-TGCCGGAGTCGACAATGAT-3′); FAS (forward, 5′- ATCCTGG-AACGAG AACACGATCT-3′; reverse, 5′-AGAGACGTGTCACTCC-TGGACTT-3′); HMGCoA reductase (forward, 5′-TGTGGCCAGGA-GTTTGGTGACTGA-3′; reverse, 5′-TAAGATTCAACAACTCTGCT-GACC-3′); LDL-R (forward, 5′-CTGTGGGCTCCATAGGCTATCT-3′; reverse, 5′- GCGGTCCAGGGTCATCTTC-3′); LIPE (forward, 5′-TCAACCGACCAGGAGTGCTC-3′; reverse, 5′-CTCTGGGTCTAT-GGCGAATC-3′); MCAD (forward, 5′- GCTCGTGAGCACATTGAA-AA-3′; reverse, CATTGTCCAAAAGCCAAACC-3′); MGLL (forward, 5′-CATGGACTTAGGGCCTGAAA-3′; reverse, GGAGGCTGTTCC-CCTATCTC-3′); PPAR-α (forward, 5′- ATTCGGGTGAAGCTG-3′; reverse, CTGGCATTTGTTCCG-3′); SCD-1 (forward, 5′-GATAGAG-CAAGTCCCCGTTG-3′; reverse, 5′- CCTGCATTAACCCCCTTCAC-3′); SAA1 and 2 (forward, 5′- GCGAGCCTACACTGACATGA-3′; reverse, 5′-TTTTCTCAGCAGCCCAGACT-3′); SREBP-1c (forward, 5′-GAGGATAGCCAGGTCAAAGC-3′; reverse, 5′- GCAGGTCAGA-CACAGAAAGG-3′); SREBP-2 (forward, 5′- GCGTTCTGGAGAC-CATGGA-3′; reverse, 5′-ACAAAGTTGCTCTGAAAACAAATCA-3′); TNFα (forward, 5′-GCTACGACGTGGGCTACA-3′; reverse, 5′-CCCTCACACTCAGATCATCTTCT-3′).

Statistical Analysis. All values are means ± standard errors of the mean (SEMs). Prism 4 for Windows (version 4.0c, GraphPad Software, Inc.) was used for statistical analysis. Significant differences between N and HF-fed mice were assessed by Student's t-test and between HF- and HFKO-supplemented mice by analysis of variance (ANOVA), followed by Tukey's test for significance. A probability of $P < 0.05$ was considered to be statistically significant.

RESULTS

The KO was a clear, dark red, viscous oil with a light shrimp odor. It contained 25 wt % polyunsaturated FAs, of which 12.5 wt % was EPA and 7 wt % was DHA. The KO was 23 wt % TG, 6 wt % FFA, and 58 wt % PL [51 wt % was phosphatidylcholine (PC), 6 wt % was phosphatidylethnolamine (PE), and 1 wt % was lysophosphatidylcholine (lysoPC)]. About 80% of total KO n-3 FAs was in the form of PL. The astaxanthin content was 201 mg/100 g.

The addition of different amounts of KO to the HF diet did not affect the amount of food consumed each day (**Table 1**). The average daily food intake per mouse in the HF groups was about 2.5 g/day. The food intake by the N group was significantly greater (26%) than the HF group ($P < 0.01$). At the commencement of the study, no significant difference existed between mean body weights. After 8 weeks of diet feeding, HF mice weighed significantly more than N animals ($P < 0.001$) and had gained significantly more weight ($P < 0.001$). There was no significant difference between the final mean body weight or the total weight gain of HF and HFKO groups (**Table 1**).

The HF diet resulted in a significant increase in liver weight (i.e., hepatomegaly). The mean liver weight was 56 ± 4% higher

Table 1. Body Weight and Food Intake of Mice Fed the Experimental Diets[a]

	N	HF	HFKO1.25	HFKO2.5	HFKO5.0
initial wt (g)	18.1 ± 0.3	18.5 ± 0.8	18.0 ± 0.3	18.2 ± 0.6	18.3 ± 0.8
final wt (g)	24.6 ± 0.3	31.7 ± 0.9$^{###}$	31.0 ± 0.9	31.8 ± 0.7	32.4 ± 1.0
wt gain (g)	6.5 ± 0.4	13.3 ± 1.1$^{###}$	13.0 ± 0.9	13.6 ± 0.7	14.1 ± 0.6
food intake (g animal^{-1}· day^{-1})	3.4 ± 0.1	2.7 ± 0.2$^{##}$	2.4 ± 0.1	2.7 ± 0.2	2.4 ± 0.1

[a] Values represent means ± SEMs (n = 6−10 mice per group). Mice were fed diets for 8 weeks. No significant differences were observed between HF and HFKO groups by ANOVA followed by Tukey's test for significance. Significant differences between N and HF by Student's t-test were as follows: $^{##}P < 0.01$, and $^{###}P < 0.001$.

Table 2. Tissue Weight of Mice Fed the Experimental Diets[a]

	N	HF	HFKO1.25	HFKO2.50	HFKO5.0
liver					
(g)	0.99 ± 0.03	1.54 ± 0.09$^{###}$	1.26 ± 0.04^{b}	1.26 ± 0.03^{b}	1.27 ± 0.06^{a}
(g/100 g body wt)	4.15 ± 0.07	5.04 ± 0.17$^{###}$	4.26 ± 0.11^{b}	4.04 ± 0.10^{c}	4.09 ± 0.09^{c}
adipose tissue					
(g)	0.56 ± 0.02	2.21 ± 0.21$^{###}$	2.30 ± 0.14	1.93 ± 0.15	2.24 ± 0.38
(g/100 g body wt)	2.39 ± 0.09	7.24 ± 0.53$^{###}$	7.60 ± 0.32	6.23 ± 0.41	7.07 ± 1.05
quadricep muscle					
(g)	0.14 ± 0.01	0.15 ± 0.01	0.14 ± 0.01	0.15 ± 0.01	0.16 ± 0.01
(g/100 g body wt)	0.59 ± 0.03	0.50 ± 0.02	0.47 ± 0.01	0.49 ± 0.02	0.51 ± 0.03

[a] Values represent means ± SEMs (n = 6−10 mice per group). Mice were fed diets for 8 weeks. Data are expressed as grams of tissue or as grams of tissue per 100 g of body wt. Adipose tissue comprised total fat from three depots: inguinal, renal, and epididymal. Significant difference between N and HF by Student's t-test: $^{###}P < 0.001$. Significant difference between HF- and HFKO-supplemented animals by ANOVA followed by Tukey's test for significance. $^{a}P < 0.05$, $^{b}P < 0.01$, and $^{c}P < 0.001$.

in the HF as compared to the N group (**Table 2**, $P < 0.001$). KO supplementation (at all doses) significantly reduced the mean liver weight (i.e., by 18 ± 3, 18 ± 2, and 17 ± 3% as compared to HF for the HFKO1.25, -2.5, and -5.0 groups, respectively). When expressed relative to body weight, the liver weight ratios were 22 ± 2% higher in HF than N ($P < 0.001$). KO supplementation significantly reduced the liver weight ratio in all HFKO groups. When compared to HF, the reduction was 15 ± 2, 20 ± 2, and 19 ± 2% in the three HFKO groups, respectively. Adipose tissue, comprising fat from three different depots (epididymal, perirenal, and inguinal) was significantly greater in HF than N animals (2.9 ± 0.5 fold, $P < 0.001$). There was no significant difference between any of the HF-fed groups. The quadriceps muscle mass, expressed in grams or grams per 100 g body wt, did not vary significantly between dietary groups. (**Table 2**).

As shown in **Figure 1A**, the beneficial effect of KO on HF diet-induced hepatomegaly was associated with a significant, dose-dependent decrease in total hepatic fat content (i.e., by 38 ± 6, 48 ± 4, and 50 ± 4% for HFKO1.25, -2.5, and -5.0, respectively). The measurement of liver TG, cholesterol, and PL (**Figure 1B−D**) revealed that hepatic steatosis in HF-fed animals was due to a significant accumulation of liver TG and cholesterol but not PL. HF diet increased liver TG and cholesterol 3.2 ± 0.4 ($P < 0.001$) and 5.0 ± 0.3 ($P < 0.01$)-fold, respectively, as compared with N animals. KO supplementation resulted in a reduction in liver TG and cholesterol, HF vs HFKO5.0, by 51 ± 12% ($P < 0.01$), and TC, by 71 ± 11.3% ($P < 0.01$).

The HF diet caused a significant increase in serum TG, cholesterol, and PL levels (**Table 3**). KO supplementation, on the other hand, significantly reduced serum cholesterol (i.e., 20 ± 3%, $P < 0.05$; 29 ± 4%, $P < 0.01$; and 29 ± 5%, $P < 0.01$, for HFKO1.25, -2.5, and -5.0, respectively) and PL (i.e., 15 ± 3%, $P < 0.05$; 23 ± 3%, $P < 0.001$; and 27 ± 2%, $P < 0.001$) in a dose-dependent fashion. Serum TG levels in KO-supplemented animals had a tendency to be elevated with respect to HF animals, although this did not reach significance.

Neither serum glucose, insulin, nor adiponectin levels were significantly different in N vs HF mice. However, KO supplementation decreased serum glucose in the case of HF vs HFKO5.0 by 43 ± 1.8% ($P < 0.01$) and increased serum adiponectin levels, HF vs HFKO5.0, by 50 ± 18% ($P < 0.001$). Serum insulin levels were not affected by KO supplementation (**Table 3**). The HOMA index [glucose (mmol/L) × insulin (μIU/mL)/22.5] tended to be lower with KO supplementation, although this difference was not statistically significant (data not shown).

To determine whether reduction in hepatic steatosis mediated by KO supplementation was associated with a reduction in hepatic inflammation, the level of TNFα protein was quantified by ELISA and was found to be significantly elevated in HF animals with respect to N (8 ± 2-fold, N vs HF, $P < 0.05$). KO supplementation resulted in a significant decrease at all doses (i.e., 78 ± 7%, $P < 0.01$; 82 ± 12%, $P < 0.01$; and 85 ± 7%, $P < 0.01$, for HFKO1.25, -2.5, and -5.0, respectively) (**Figure 2**). Analysis of hepatic TNFα mRNA expression revealed a similar effect. HF-fed mice had levels some 5 ± 1-fold greater than N ($P < 0.01$). KO supplementation reduced HF diet-induced TNFα expression significantly by 58 ± 7% in the case of HFKO5.0.

To shed light on the mechanism or mechanisms by which dietary KO was able to affect lipid metabolism in the liver, the mRNA level of a number of genes affecting hepatic FA and cholesterol metabolism was determined (**Table 4**). The expression of three genes associated with FA synthesis was significantly decreased in KO-supplemented animals. Fatty acid synthase (FAS) was reduced by 62 ± 5% ($P < 0.001$), acetyl-CoA carboxylase (ACC) was reduced by 53 ± 6% ($P < 0.01$), and stearoyl-CoA desaturase-1 (SCD-1) was reduced by 66 ± 7% ($P < 0.001$) in the HFKO5.0 v HF diet group. The level of expression of two genes affecting FA catabolism was significantly lower in HFKO. Monoglyceride lipase (MGLL) was reduced in HFKO5.0 by 53 ± 5% ($P < 0.001$), while the expression of hormone-sensitive lipase (LIPE) was reduced by 39 ± 7%

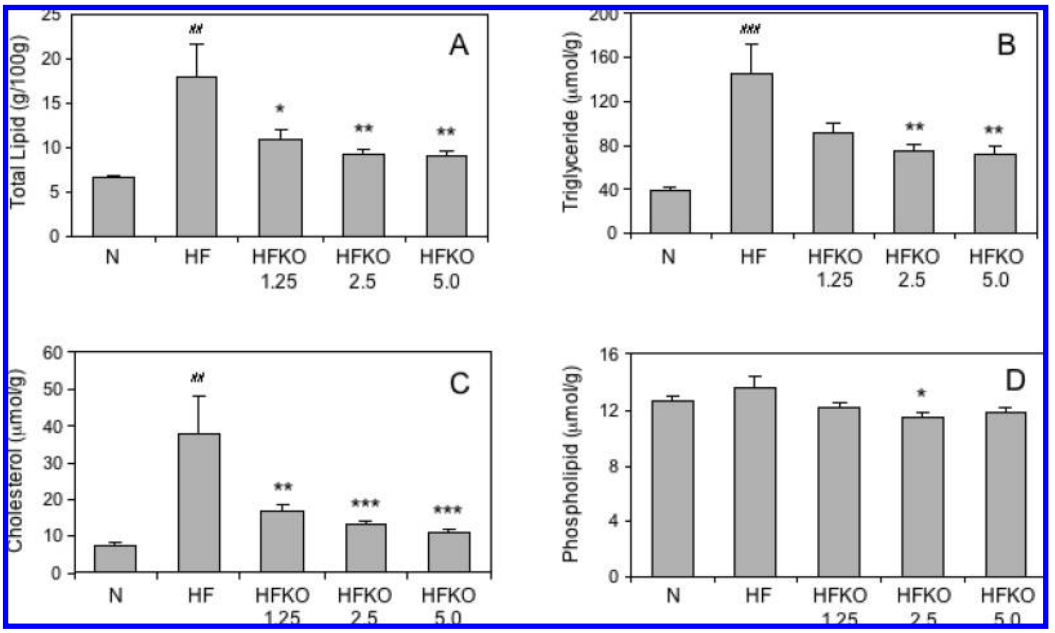

Figure 1. Effect of dietary KO supplementation on hepatic steatosis in high-fat-fed mice: (**A**) total liver fat, (**B**) TG, (**C**) cholesterol, and (**D**) PL. Results represent means ± SEMs ($n = 6-10$ mice per group). Mice were fed diets for 8 weeks. Significant difference between N- and HF-supplemented animals by Student's t-test. $^{\#\#}P < 0.01$, and $^{\#\#\#}P < 0.001$. Significant difference between HF- and HFKO-supplemented animals by ANOVA followed by Tukey's test for significance. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

Table 3. Serum Lipid, Glucose, Insulin and Adiponectin Levels in Mice Fed Experimental Diets[a]

	N	HF	HFKO1.25	HFKO2.5	HFKO5.0
TG (mmol/L)	0.4 ± 0.03	0.6 ± 0.06$^{\#\#}$	0.8 ± 0.08	0.8 ± 0.09	0.8 ± 0.06
cholesterol (mmol/L)	2.6 ± 0.1	4.3 ± 0.3$^{\#\#\#}$	3.4 ± 0.1^{a}	3.0 ± 0.1^{b}	3.0 ± 0.2^{b}
PL (mmol/L)	1.3 ± 0.1	1.7 ± 0.1$^{\#\#\#}$	1.5 ± 0.1^{a}	1.3 ± 0.1^{c}	1.3 ± 0.1^{c}
NEFA (m/eq/L)	0.6 ± 0.04	0.8 ± 0.06	0.8 ± 0.04	0.7 ± 0.06	0.7 ± 0.03
glucose (mmol/L)	11.5 ± 0.6	13.1 ± 1.6	8.3 ± 0.6^{b}	8.7 ± 0.8^{a}	7.5 ± 0.8^{b}
insulin (ng/mL)	1.1 ± 0.3	0.7 ± 0.1	0.6 ± 0.1	0.8 ± 0.1	0.7 ± 0.1
adiponectin (μg/mL)	4.7 ± 0.2	5.0 ± 0.1	6.5 ± 0.3	6.5 ± 0.4	7.5 ± 0.6*

[a] Values represent means ± SEMs ($n = 6-10$ mice per group). Mice were fed diets for 8 weeks. Significant difference between N and HF by Student's t-test: $^{\#\#}P < 0.01$, and $^{\#\#\#}P < 0.001$. Significant difference between HF- and HFKO-supplemented animals by ANOVA followed by Tukey's test for significance. $^{a}P < 0.05$, $^{b}P < 0.01$, and $^{c}P < 0.001$.

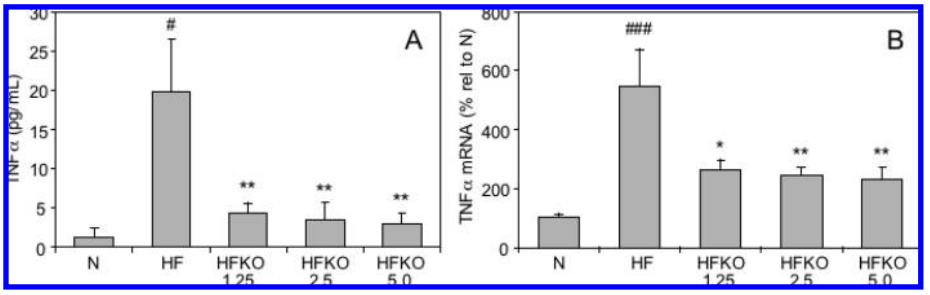

Figure 2. Effect of dietary KO supplementation on hepatic TNFα expression. (**A**) TNFα protein, quantified by ELISA, and (**B**) TNFα mRNA expressed as a ratio of cyclophilin and then as a percentage of values in N mice. Results represent means ± SEMs ($n = 6-10$ mice per group). Mice were fed diets for 8 weeks. Significant difference between N- and HF-supplemented animals by Student's t-test. $^{\#}P < 0.05$, and $^{\#\#\#}P < 0.001$. Significant difference between HF- and HFKO-supplemented animals by ANOVA followed by Tukey's test for significance. $^{*}P < 0.05$, and $^{**}P < 0.01$.

($P < 0.05$) in HFKO2.5 animals. Two genes promoting FA oxidation were examined, and mRNA levels of acyl-CoA oxidase (ACO) were unaffected by KO supplementation, although the expression of carnitine palmitoyl transferase-1a (CPT1a) was reduced significantly by 40 ± 6% ($P < 0.05$) in HFKO2.5 animals. Finally, two genes involved in hepatic cholesterol metabolism, 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCoA R), and cholesterol uptake, low-density lipoprotein receptor (LDL-R), were investigated. Both genes were down-regulated at higher KO doses. HMGCoA R expression was reduced by 40 ± 5% ($P < 0.01$). LDL-R was reduced by 47 ± 4% ($P < 0.05$) in HFKO2.5 relative to HF animals. The expression of the three transcription factors, sterol regulatory element binding protein-1c (SREBP-1c), SREBP-2, and PPAR-α, was reduced by KO supplementation, particularly at higher doses, that is, 2.5 and 5.0%.

DISCUSSION

The present study has shown that dietary KO supplementation significantly reduced hepatomegaly, hepatic steatosis, and

Table 4. Expression of Hepatic Genes Controlling FA and Cholesterol Metabolism in Mice Fed Experimental Diets[a]

	N	HF	HFKO1.25	HFKO2.50	HFKO5.0
FA synthesis and uptake					
FAS	109 ± 15	44 ± 9$^{\#\#}$	27 ± 4	13 ± 1^{c}	17 ± 2^{c}
ACC	104 ± 10	67 ± 10$^{\#\#}$	50 ± 5	28 ± 3^{c}	32 ± 4^{b}
SCD1	106 ± 11	232 ± 43$^{\#\#}$	154 ± 18	55 ± 8^{c}	78 ± 16^{c}
CD36	101 ± 5	232 ± 28$^{\#\#\#}$	256 ± 11	131 ± 13	219 ± 38
FA oxidation					
ACO	103 ± 9	122 ± 18	137 ± 10	81 ± 9	102 ± 12
MCAD	102 ± 7	117 ± 17	96 ± 7	73 ± 5^{a}	91 ± 11
CPT1a	103 ± 8	118 ± 16	118 ± 8	75 ± 7^{a}	85 ± 8
TG catabolism					
MGLL	102 ± 7	146 ± 17$^{\#}$	114 ± 9	50 ± 4^{c}	68 ± 7^{b}
LIPE (HSL)	109 ± 14	174 ± 26$^{\#}$	148 ± 12	104 ± 11^{a}	115 ± 14
cholesterol synthesis and uptake					
HMG-CoA R	105 ± 11	45 ± 4$^{\#\#\#}$	41 ± 4	25 ± 2^{b}	27 ± 2^{a}
LDL-R	105 ± 10	58 ± 11$^{\#\#}$	60 ± 7	31 ± 2^{a}	38 ± 4
transcription factors					
SREBP-1c	120 ± 22	133 ± 43	84 ± 12	50 ± 6^{a}	61 ± 9^{a}
SREBP-2	104 ± 10	59 ± 6$^{\#\#}$	52 ± 3	37 ± 3^{b}	39 ± 4^{a}
PPAR-α	104 ± 10	124 ± 23	105 ± 13	63 ± 5^{a}	81 ± 9^{a}

[a] Values represent mRNA levels expressed as a ratio relative to cyclophilin. These values were then expressed as a percentage of those in N mice. Results represent means ± SEMs (n = 6−10 mice per group). Mice were fed diets for 8 weeks. Significant difference between N and HF by Student's t-test: $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, and $^{\#\#\#}P < 0.001$. Significant difference between HF- and HFKO-supplemented animals by ANOVA followed by Tukey's test for significance. $^{a}P < 0.05$, $^{b}P < 0.01$, and $^{c}P < 0.001$.

hypercholesterolemia in C57BL/6 mice fed a high-fat diet. In addition, KO supplementation significantly reduced fasting blood glucose and increased serum adiponectin levels. These data indicate that dietary KO has a beneficial effect on lipid and glucose metabolism in experimental mice with diet-induced obesity and insulin resistance.

At least three KO components could be responsible either individually or in combination for the marked improvements observed in lipid and glucose metabolism. They are (a) the n-3 FAs, (b) the PL, or (c) the astaxanthin. Numerous studies have documented the beneficial effects of n-3 FAs when added to the diet in the form of TG (i.e., n-3:TG). Certain studies in mice have documented the ability of n-3:TG to reduce plasma and liver lipid levels (*21*−*25*), others have reported the ability of n-3:TG to increase insulin sensitivity (*26*, *27*), while others have described the anti-inflammatory effects of n-3:TG, either in the liver (*28*−*30*) or in the adipose tissue (*31*, *32*). Most of these studies used relatively high doses of FO (i.e., 10% or more FO by energy, equivalent to > 3% en EPA + DHA), and virtually all of them substituted fat in the diet with n-3:TG, rather than adding n-3:TG to the diet in the form of a dietary supplement. The present study is thus unique, considering that beneficial effects of KO were observed at a level of 1.25 wt % KO, equivalent to only 0.55% en EPA + DHA, and occurred in diets where the n-3:PLs of KO were added to a background diet high in saturated fat and cholesterol (i.e., 21 wt % butterfat + 0.15 wt % cholesterol).

A novel feature of KO is its high PL content (i.e., 58 wt % PL, comprising 51% PC, 6% PE, and 1% lysoPC). Dietary PLs, particularly those of plant origin containing polyunsaturated FAs, are known to reduce plasma and liver lipid levels in experimental animals (*10*). Recent work in our laboratory has shown that PLs containing saturated FAs can also have pronounced lipid-lowering properties. The lipid-lowering properties of dietary PLs are therefore not entirely dependent on the degree of saturation of their component FAs. The base moiety and hence the type or class of PL in the diet (i.e., the presence of PC in KO) appears to be an important factor. These data raise the possibility that n-3:PL or n-3:PC (found in KO) may be more efficacious than n-3:TG (found in FO)−a supposition that needs to be verified in future studies.

It is possible that astaxanthin in KO also made a contribution to the lipid- and glucose-lowering effects observed in the present study. The KO contained 201 mg/100 g of total astaxanthin (192 mg/100 g esterified and 9 mg/100 g free). The astaxanthin content was therefore 0, 2.5, 5.0, and 10 mg/100 g in the HF, HFKO1.25, HFKO2.5, and HFKO5.0 diets, respectively. On average, mice consumed 2.5 g of diet/day and therefore received 0, 62.5, 125, and 250 μg of astaxanthin per day−equivalent to doses of 0, 2.5, 5.0, and 10.0 mg/kg/day. A dose of 50 mg/kg/day astaxanthin has been shown in spontaneously hypertensive rats to increase plasma adiponectin, reduce fasting blood glucose, and improve insulin sensitivity (*13*). In a second study in obese mice fed a high-fat diet, 6 and 30 mg/kg/day doses of astaxanthin reduced liver weight, liver TG, and plasma TG concentration (*14*). Together, these data indicate that astaxanthin at doses as low as 5−10 mg/kg/day can have beneficial effects on lipid and glucose metabolism in experimental animals.

It is now well-recognized that dietary fats influence plasma and liver lipid metabolism by regulating the expression of key enzymes responsible for lipid synthesis and catabolism. Polyunsaturated FAs (both n-3 and n-6) activate peroxisome proliferater-activated receptor-α (PPARα) by binding directly to this transcription factor, leading to stimulation of hepatic FA oxidation. At the same time, PUFA inhibit hepatic FA synthesis by suppressing SREBP-1 nuclear abundance through suppression of SREBP-1c gene transcription and enhancement of proteosomal SREBP-1 degradation and mRNA-SREBP-1c mRNA decay (*33*). In the present study, KO supplementation resulted in a marked reduction in hepatic TG levels and a significant decrease in the expression of SREBP-1c-regulated enzymes affecting FA synthesis in the liver, namely, FAS, ACC, and SCD-1. This was associated with decreased levels of SREBP-1c mRNA. The mRNA level of PPARα enzymes affecting FA oxidation (ACO and CPT-1a) also tended to be reduced by KO. Furthermore, PPARα levels were lower at higher doses of KO. A possible explanation for this lack of hepatic PPAR-α activation is that the relatively low levels of KO used in the present study were sufficient to diminish SREBP-1c-mediated effects but not sufficient to activate PPARα. Support for this concept can be found in the work of Nakatani et al. (*34*), who fed FO for 1 week to mice at different doses (10−60% en). At the lowest dose (10% by energy or 4.4% by weight FO), SREBP-1c target genes were significantly reduced, but PPARα target genes were not significantly increased. Higher doses resulted in statistically significant increases in PPARα-regulated genes in the absence of further decreases in SREBP-1c. Interestingly, only at very high doses (> 30% en FO) were statistically significant decreases observed in body weight and fat mass, suggesting that changes in adipose tissue mass due to dietary n-3 FAs are dependent on PPARα activation. Consistent with this concept, lack of PPARα activation in the present study was associated with an absence of statistically significant changes in adipose tissue mass.

It has been suggested by Kim et al. (*22*) that SREBP-1c affects the basal expression of cholesterogenic genes, and consistent with this concept, KO supplementation was associated with a significant decrease in HMGCoA-reductase and LDL-R mRNA levels.

SREBP-1c is only a weak activator of cholesterogenic gene expression, however, while SREBP-2 is a more selective activator. Because hepatic SREBP-2 mRNA levels were also reduced by KO, it is quite likely that changes in SREBP-2 expression and activation mediated the changes in cholesterogenic gene expression. Irrespective of the transcriptional factor that was involved, reduced cholesterol synthesis in the liver was probably responsible for the significant decrease in hepatic and serum cholesterol levels in KO-supplemented animals.

A large body of evidence has been presented showing that supplementation of the diet with n-3 FAs reduces plasma TG levels in animals and in humans (*35*, *36*). The hypotriglyceridemic effect of n-3 FAs is due to reduced hepatic TG synthesis and a decrease in the secretion of TG-rich very low-density lipoprotein (VLDL) from the liver. A TG-lowering effect was not, however, observed in the present study. There are two possible reasons: (a) relatively low doses of KO did not lead to a significant activation of PPARα, which led to absence of a PPARα-mediated reduction in serum TG, and/or (b) the addition rather than the substitution of KO to the HF diet meant that increased calories in the KO-supplemented diets obscured beneficial effects of KO on serum TG.

We have found that KO supplementation significantly reduced serum glucose levels in HF-fed mice in a dose-dependent manner, while increasing serum adiponectin, particularly in mice fed 5.0 wt % KO. The ability of dietary n-3 FAs to affect adipose tissue by increasing adiponectin mRNA expression and secretion in mice has been demonstrated in a number of studies (*27*, *31*, *37*, *38*). The finding that KO supplementation results in increased circulating adiponectin levels is significant considering that this adipocytokine increases insulin sensitivity in the liver and skeletal muscle by enhancing tissue fat oxidation. Adiponectin has also been shown to have important anti-atherogenic and anti-inflammatory properties (*39*, *40*).

Hepatic steatosis or fatty liver is a serious health concern in affluent societies, and its incidence is increasing at an alarming rate (*41*, *42*). Because drug treatments have proven inadequate, there is great interest in different foods and food components with the potential to prevent and/or delay the development of fatty liver disease. The present results suggest that KO might be useful in this regard. KO supplementation had a pronounced effect on hepatomegaly and hepatic steatosis in high-fat-fed mice by significantly reducing total liver mass and total liver lipid (both TG and cholesterol). Hepatic inflammation was also reduced as evidenced by a marked reduction in liver TNFα at both the mRNA and the protein levels (**Figure 2**).

It is significant that the beneficial effects of KO supplementation observed in the present study parallel the metabolic activity of KO observed in clinical studies. For example, Bunea et al. have demonstrated a benefit of KO supplementation on plasma lipid levels in patients with hypertriglyceridemia and hypercholesterolemia (*16*). When KO was compared with FO, KO (1−3 g/day) was found to be significantly more effective than FO at reducing plasma glucose, LDL and TG levels, as well as at increasing the plasma concentration of high-density lipoprotein (HDL)-cholesterol. A beneficial effect of KO has also been demonstrated in the management of premenstrual syndrome and dysmenorrheal (*15*), while more recently, KO was shown to significantly inhibit inflammation in patients with increased levels of C-reactive protein (CRP) (*17*). These human studies are encouraging; however, they need to be replicated in different patient groups. Furthermore, the apparent ability of KO to be more effective than FO in reducing inflammation and/or plasma lipid levels needs further evaluation.

In conclusion, our study has demonstrated that KO has the ability to significantly improve lipid and glucose metabolism in mice fed a high-fat diet. KO supplementation was particularly efficacious in lowering hepatic levels of TG and cholesterol, thereby alleviating diet-induced hepatomegaly and hepatic steatosis. While caution should be exercised in extrapolating the present results to humans, they support the concept that dietary KO may be of therapeutic benefit in patients with metabolic syndrome and/or individuals with NAFLD.

ABBREVIATIONS

ACC, acetyl-CoA carboxylase; ACO, acyl-CoA oxidase; CD36, cluster of differentiation 36; CPT, carnitine palmitoyl transferase; CRP, C-reactive protein; CVD, cardiovascular disease; DHA, docosahexanoic acid; EPA, eicosapentanoic acid; FA, fatty acid; FAS, fatty acid synthase; FO, fish oil; HDL, high-density lipoprotein; HF, high-fat-fed mice; HFKO, high-fat-fed mice supplemented with krill oil; HMGCoA, 3-hydroxy-3-methylglutaryl-coenzyme A; KO, krill oil; LDL-R, low-density lipoprotein receptor; LIPE, hormone-sensitive lipase; MGLL, monoglyceride lipase; N, normal diet-fed mice; NAFLD, non-alcoholic fatty liver disease; PL, phospholipid; PPARα, peroxisome proliferater-activated receptor-α; SCD-1, stearoyl-CoA desaturase-1; SREBP, sterol regulatory element binding protein; TG, triglyceride; TNFα, tumor necrosis factor α; VLDL, very low-density lipoprotein.

ACKNOWLEDGMENT

The expert assistance of Kim Berry and Herbert Ayala in maintaining our experimental animals was very much appreciated.

LITERATURE CITED

(1) Harris, W. S. n-3 fatty acids and serum lipoproteins: animal studies. *Am. J. Clin. Nutr.* **1997**, *65*, 1611S–1616S.
(2) Eslick, G. D.; Howe, P. R.; Smith, C.; Priest, R.; Bensoussan, A. Benefits of fish oil supplementation in hyperlipidemia: A systematic review and meta-analysis. *Int. J. Cardiol.* **2009**, *136*, 4–16.
(3) Breslow, J. L. n-3 fatty acids and cardiovascular disease. *Am. J. Clin. Nutr.* **2006**, *83*, 1477S–1482S.
(4) Harris, W. S.; Miller, M.; Tighe, A. P.; Davidson, M. H.; Schaefer, E. J. Omega-3 fatty acids and coronary heart disease risk: Clinical and mechanistic perspectives. *Atherosclerosis* **2008**, *197*, 12–24.
(5) Cleland, L. G.; James, M. J.; Proudman, S. M. The role of fish oils in the treatment of rheumatoid arthritis. *Drugs* **2003**, *63*, 845–853.
(6) Boudrault, C.; Bazinet, R. P.; Ma, D. W. Experimental models and mechanisms underlying the protective effects of n-3 polyunsaturated fatty acids in Alzheimer's disease. *J. Nutr. Biochem.* **2009**, *20*, 1–10.
(7) Fedor, D.; Kelley, D. S. Prevention of insulin resistance by n-3 polyunsaturated fatty acids. *Curr. Opin. Clin. Nutr. Metab. Care* **2009**, *12*, 138–146.
(8) Cansell, M.; Nacka, F.; Combe, N. Marine lipid-based liposomes increase in vivo FA bioavailability. *Lipids* **2003**, *38*, 551–559.
(9) Lemaitre-Delaunay, D.; Pachiaudi, C.; Laville, M.; Pousin, J.; Armstrong, M.; Lagarde, M. Blood compartmental metabolism of docosahexaenoic acid (DHA) in humans after ingestion of a single dose of [^{13}C]DHA in phosphatidylcholine. *J. Lipid Res.* **1999**, *40*, 1867–1874.
(10) Cohn, J. S.; Wat, E.; Kamili, A.; Tandy, S. Dietary phospholipids, hepatic lipid metabolism and cardiovascular disease. *Curr. Opin. Lipidol.* **2008**, *19*, 257–262.
(11) Wat, E.; Tandy, S.; Kapera, E.; Kamili, A.; Chung, R. W.; Brown, A.; Rowney, M.; Cohn, J. S. Dietary phospholipid-rich dairy milk extract reduces hepatomegaly, hepatic steatosis and hyperlipidemia in mice fed a high-fat diet. *Atherosclerosis* **2009**, *205*, 144–150.
(12) Naito, Y.; Uchiyama, K.; Aoi, W.; Hasegawa, G.; Nakamura, N.; Yoshida, N.; Maoka, T.; Takahashi, J.; Yoshikawa, T. Prevention of diabetic nephropathy by treatment with astaxanthin in diabetic db/db mice. *Biofactors* **2004**, *20*, 49–59.

(13) Hussein, G.; Nakagawa, T.; Goto, H.; Shimada, Y.; Matsumoto, K.; Sankawa, U.; Watanabe, H. Astaxanthin ameliorates features of metabolic syndrome in SHR/NDmcr-cp. *Life Sci*. **2007**, *80*, 522–529.
(14) Ikeuchi, M.; Koyama, T.; Takahashi, J.; Yazawa, K. Effects of astaxanthin in obese mice fed a high-fat diet. *Biosci., Biotechnol., Biochem*. **2007**, *71*, 893–899.
(15) Sampalis, F.; Bunea, R.; Pelland, M. F.; Kowalski, O.; Duguet, N.; Dupuis, S. Evaluation of the effects of Neptune Krill Oil on the management of premenstrual syndrome and dysmenorrhea. *Altern. Med. Rev*. **2003**, *8*, 171–179.
(16) Bunea, R.; El Farrah, K.; Deutsch, L. Evaluation of the effects of Neptune Krill Oil on the clinical course of hyperlipidemia. *Altern. Med. Rev*. **2004**, *9*, 420–428.
(17) Deutsch, L. Evaluation of the effect of Neptune Krill Oil on chronic inflammation and arthritic symptoms. *J. Am. Coll. Nutr*. **2007**, *26*, 39–48.
(18) Kris-Etherton, P. M.; Taylor, D. S.; Yu-Poth, S.; Huth, P.; Moriarty, K.; Fishell, V.; Hargrove, R. L.; Zhao, G.; Etherton, T. D. Polyunsaturated fatty acids in the food chain in the United States. *Am. J. Clin. Nutr*. **2000**, *71*, 179S–188S.
(19) Harris, W. S. n-3 fatty acids and serum lipoproteins: animal studies. *Am. J. Clin. Nutr*. **1997**, *65*, 1611S–1616S.
(20) Bligh, E. G.; Dyer, W. J. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol*. **1959**, *37*, 911–917.
(21) van Vlijmen, B. J.; Mensink, R. P.; van't Hof, H. B.; Offermans, R. F; Hofker, M. H.; Havekes, L. M. Effects of dietary fish oil on serum lipids and VLDL kinetics in hyperlipidemic apolipoprotein E*3-Leiden transgenic mice. *J. Lipid Res*. **1998**, *39*, 1181–1188.
(22) Kim, H. J.; Takahashi, M.; Ezaki, O. Fish oil feeding decreases mature sterol regulatory element-binding protein 1 (SREBP-1) by down-regulation of SREBP-1c mRNA in mouse liver. A possible mechanism for down-regulation of lipogenic enzyme mRNAs. *J. Biol. Chem*. **1999**, *274*, 25892–25898.
(23) Sekiya, M.; Yahagi, N.; Matsuzaka, T.; Najima, Y.; Nakakuki, M.; Nagai, R.; Ishibashi, S.; Osuga, J.; Yamada, N.; Shimano, H. Polyunsaturated fatty acids ameliorate hepatic steatosis in obese mice by SREBP-1 suppression. *Hepatology* **2003**, *38*, 1529–1539.
(24) Ide, T. Interaction of fish oil and conjugated linoleic acid in affecting hepatic activity of lipogenic enzymes and gene expression in liver and adipose tissue. *Diabetes* **2005**, *54*, 412–423.
(25) Qi, K.; Fan, C.; Jiang, J.; Zhu, H.; Jiao, H.; Meng, Q.; Deckelbaum, R. J. Omega-3 fatty acid containing diets decrease plasma triglyceride concentrations in mice by reducing endogenous triglyceride synthesis and enhancing the blood clearance of triglyceride-rich particles. *Clin. Nutr*. **2008**, *27*, 424–430.
(26) Neschen, S.; Morino, K.; Dong, J.; Wang-Fischer, Y.; Cline, G. W.; Romanelli, A. J.; Rossbacher, J. C.; Moore, I. K.; Regittnig, W.; Munoz, D. S.; Kim, J. H.; Shulman, G. I. n-3 Fatty acids preserve insulin sensitivity in vivo in a peroxisome proliferator-activated receptor-alpha-dependent manner. *Diabetes* **2007**, *56*, 1034–1041.
(27) González-Périz, A.; Horrillo, R.; Ferré, N.; Gronert, K.; Dong, B.; Morán-Salvador, E.; Titos, E.; Martínez-Clemente, M.; López-Parra, M.; Arroyo, V.; Clària, J. Obesity-induced insulin resistance and hepatic steatosis are alleviated by omega-3 fatty acids: a role for resolvins and protectins. *FASEB J*. **2009**, DOI: 10.1096/fj.08-125674.
(28) González-Périz, A.; Planagumá, A.; Gronert, K.; Miquel, R; López-Parra, M.; Titos, E.; Horrillo, R.; Ferré, N.; Deulofeu, R.; Arroyo, V.; Rodés, J.; Clària, J. Docosahexaenoic acid (DHA) blunts liver injury by conversion to protective lipid mediators: protectin D1 and 17S-hydroxy-DHA. *FASEB J*. **2006**, *20*, 2537–2539.
(29) Schmöcker, C.; Weylandt, K. H.; Kahlke, L.; Wang, J.; Lobeck, H.; Tiegs, G.; Berg, T.; Kang, J. X. Omega-3 fatty acids alleviate chemically induced acute hepatitis by suppression of cytokines. *Hepatology* **2007**, *45*, 864–869.
(30) Todoric, J; Löffler, M.; Huber, J.; Bilban, M.; Reimers, M.; Kadl, A.; Zeyda, M.; Waldhäusl, W.; Stulnig, T. M. Adipose tissue inflammation induced by high-fat diet in obese diabetic mice is prevented by n-3 polyunsaturated fatty acids. *Diabetologia* **2006**, *49*, 2109–2119.
(31) Saraswathi, V.; Gao, L.; Morrow, J. D.; Chait, A.; Niswender, K. D.; Hasty, A. H. Fish oil increases cholesterol storage in white adipose tissue with concomitant decreases in inflammation, hepatic steatosis, and atherosclerosis in mice. *J. Nutr*. **2007**, *137*, 1776–1782.
(32) Huber, J.; Löffler, M.; Bilban, M.; Reimers, M.; Kadl, A.; Todoric, J.; Zeyda, M.; Geyeregger, R.; Schreiner, M.; Weichhart, T.; Leitinger, N.; Waldhäusl, W.; Stulnig, T. M. Prevention of high-fat diet-induced adipose tissue remodeling in obese diabetic mice by n-3 polyunsaturated fatty acids. *Int. J. Obes*. (*London*) **2007**, *31*, 1004–1013.
(33) Jump, D. B.; Botolin, D.; Wang, Y.; Xu, J.; Christian, B.; Demeure, O. Fatty acid regulation of hepatic gene transcription. *J. Nutr*. **2005**, *135*, 2503–2506.
(34) Nakatani, T.; Kim, H. J.; Kaburagi, Y.; Yasuda, K.; Ezaki, O. A low fish oil inhibits SREBP-1 proteolytic cascade, while a high-fish-oil feeding decreases SREBP-1 mRNA in mice liver: Relationship to anti-obesity. *J. Lipid Res*. **2003**, *44*, 369–379.
(35) Goldberg, R. B.; Sabharwal, A. K. Fish oil in the treatment of dyslipidemia. *Curr. Opin. Endocrinol., Diabetes Obes*. **2008**, *15*, 167–174.
(36) Davidson, M. H. Mechanisms for the hypotriglyceridemic effect of marine omega-3 fatty acids. *Am. J. Cardiol*. **2006**, *98*, 27i–33i.
(37) Flachs, P.; Mohamed-Ali, V.; Horakova, O.; Rossmeisl, M.; Hosseinzadeh-Attar, M. J.; Hensler, M.; Ruzickova, J.; Kopecky, J. Polyunsaturated fatty acids of marine origin induce adiponectin in mice fed a high-fat diet. *Diabetologia* **2006**, *49*, 394–397.
(38) Itoh, M.; Suganami, T.; Satoh, N.; Tanimoto-Koyama, K.; Yuan, X.; Tanaka, M.; Kawano, H.; Yano, T.; Aoe, S.; Takeya, M.; Shimatsu, A.; Kuzuya, H.; Kamei, Y.; Ogawa, Y. Increased adiponectin secretion by highly purified eicosapentaenoic acid in rodent models of obesity and human obese subjects. *Arterioscler., Thromb., Vasc. Biol*. **2007**, *27*, 1918–1925.
(39) Kadowaki, T.; Yamauchi, T.; Kubota, N.; Hara, K.; Ueki, K.; Tobe, K. Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. *J. Clin. Invest*. **2006**, *116*, 1784–1792.
(40) Lu, J. Y.; Huang, K. C.; Chang, L. C.; Huang, Y. S.; Chi, Y. C.; Su, T. C.; Chen, C. L.; Yang, W. S. Adiponectin: A biomarker of obesity-induced insulin resistance in adipose tissue and beyond. *J. Biomed. Sci*. **2008**, *15*, 565–576.
(41) Manco, M.; Bottazzo, G.; DeVito, R.; Marcellini, M.; Mingrone, G.; Nobili, V. Nonalcoholic fatty liver disease in children. *J. Am. Coll. Nutr*. **2008**, *27*, 667–676.
(42) Amarapurkar, D.; Kamani, P.; Patel, N.; Gupte, P.; Kumar, P.; Agal, S.; Baijal, R.; Lala, S.; Chaudhary, D.; Deshpande, A. Prevalence of non-alcoholic fatty liver disease: Population based study. *Ann. Hepatol*. **2007**, *6*, 161–163.

Received May 14, 2009. Revised manuscript received July 15, 2009. Accepted August 26, 2009. The present research was supported by financial funding from Aker BioMarine ASA.