Supplementation with a blend of krill and salmon oil is associated with increased metabolic risk in overweight men[1,2]

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ABSTRACT

Background: Krill is an increasingly popular source of marine n–3 (ω-3) PUFA that is seen as a premium product. However, to our knowledge, the effect of krill-oil supplementation on insulin sensitivity in humans has not been reported.

Objective: We assessed whether supplementation with a blend of krill and salmon (KS) oil [which is rich in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)] affects insulin sensitivity in overweight men.

Design: The design was a randomized, double-blind, controlled crossover trial. A total of 47 men with a mean ± SD age of 46.5 ± 5.1 y, who were overweight [body mass index (in kg/m^2) from 25 to 30] but otherwise healthy, received 5 1-g capsules of KS oil or a control (canola oil) for 8 wk and crossed over to another treatment after an 8-wk washout period. The primary outcome was insulin sensitivity assessed by using the Matsuda method from an oral-glucose-tolerance test. Secondary outcomes included lipid profiles, inflammatory markers, 24-h ambulatory blood pressure, and carotid artery intimamedia thickness.

Results: Unexpectedly, insulin sensitivity (per the Matsuda index) was 14% lower with the KS oil than with the control oil ($P = 0.049$). A mediation analysis showed that, after controlling for the likely positive effects of blood EPA and DHA (i.e., the omega-3 index), the reduction in insulin sensitivity after KS-oil supplementation was more marked [27% lower than with the control oil ($P = 0.009$)].

Conclusions: Supplementation with a blend of KS oil is associated with decreased insulin sensitivity. Thus, krill-oil supplementation in overweight adults could exacerbate risk of diabetes and cardiovascular disease. This trial was prospectively registered at the Australian New Zealand Clinical Trials Registry as ACTRN12611000602921. *Am J Clin Nutr* 2015;102:49–57.

Keywords: cholesterol, omega-3, insulin sensitivity, lipids, males, n–3 PUFA, nutraceutical

INTRODUCTION

The prevalence of obesity has increased rapidly in both developed and developing nations (1, 2). Obesity is linked to essential hypertension, dyslipidemia, and type 2 diabetes through pathologically reduced sensitivity to insulin (3). This insulin resistance is a common pathologic factor behind an oncoming "tsunami" of noncommunicable disease.

Lifestyle factors (such as diet and physical activity) influence insulin sensitivity both directly and through changes in adiposity. Although weight loss (4) and increased physical activity (5) improve insulin sensitivity, for a large proportion of the population, these goals are difficult to achieve. In contrast, small dietary modifications, such as supplementation with nutraceuticals, are easier to attain. Thus, if a dietary supplement can improve insulin sensitivity in at-risk groups, it may be possible to lower incidences of type 2 diabetes, cardiovascular disease, and other aspects of the metabolic syndrome.

Fish and krill oils principally contain the long-chain n–3 PUFAs EPA and DHA. Epidemiologic (6, 7) and animal (8, 9) evidence suggested that these n–3 PUFAs may improve insulin sensitivity and metabolic risk, but randomized controlled trials yielded conflicting results (10–14). Higher dietary (6) or plasma (7) n–3 PUFA concentrations are associated with lower risk of diabetes. In addition, a higher omega-3 index, i.e.,

$$(\text{EPA weight} + \text{DHA weight}) \times 100 \div \text{total red cell phospholipid weight} \quad (1)$$

which reflects dietary intake over a period of months, is associated with greater insulin sensitivity in children (15) and overweight men (16). In addition, it is clear that fish-oil supplementation reverses insulin resistance in the rat (8, 9, 17), and the G protein–coupled receptor 120 plays a key role (8).

A systematic review that included 11 randomized controlled trials and 618 participants concluded that n–3 PUFA supplementation did not influence insulin sensitivity (18). However, the individual trials were highly heterogeneous and included participants with and without type 2 diabetes and used a wide range of n–3 PUFA sources, doses, and control oils. Fish oil, in association with weight loss (10) or caloric restriction (11, 12), improved insulin sensitivity as did switching to a Mediterranean

[1] Supported by The Douglas Charitable Trust.

[2] Supplemental Figure 1 and Supplemental Table 1 are available from the "Supplemental data" link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

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diet (19), but it is difficult to isolate the specific effects of n–3 PUFA because of the complexity of these interventions. The replacement of nonoily fish in the diet with oily fish improved insulin sensitivity in a trial of 12 healthy older adults (13), but in a larger study of 162 adults, the addition of fish oil to a diet high in saturated or monounsaturated fat did not influence insulin sensitivity (14).

Krill is an increasingly popular source of marine n–3 PUFAs that differs from fish oil. Unlike fish oil that contains triglycerides, n–3 PUFAs in krill oil are predominantly in phospholipid form (20, 21). Also, krill oil contains astaxanthin, which is a carotenoid pigment and powerful antioxidant (22). Not surprisingly, differential effects of krill and fish oils have been described in mice (23). However, to our knowledge, the effect of krill-oil supplementation on insulin sensitivity in humans has not been reported. Therefore, we aimed to investigate whether supplementation with a blend of krill and salmon (KS)[5] oil would lead to changes in insulin sensitivity in a double-blind, randomized, controlled, crossover human trial.

METHODS

Ethics

Ethical approval was granted by the Central Regional Ethics Committee, New Zealand Ministry of Health (CEN/11/07/038). This trial was prospectively registered at the Australian New Zealand Clinical Trials Registry as ACTRN12611000602921. Written and verbal informed consent was obtained from all participants. This study was performed in accordance with all appropriate institutional and international guidelines and regulations for medical research in line with the principles of the Declaration of Helsinki.

Participants

Volunteers were recruited in 2012 by using advertisements in local newspapers that circulate freely in the central Auckland metropolitan area (New Zealand). Overweight [BMI (in kg/m^2): 25–30], middle-aged (35–55 y) men were eligible to participate. The study cohort represented a high-risk group that was likely to have early insulin resistance without metabolic decompensation to clinical disease (e.g., diabetes), which would make it easier to detect a potential insulin-sensitizing effect of KS oil. Note that only men were recruited so that the confounding effects of the menstrual cycle or oral contraceptives on insulin sensitivity (the primary outcome) could be avoided. Exclusion criteria were diabetes, hypertension (systolic blood pressure >145 mm Hg or diastolic blood pressure >95 mm Hg), known dyslipidemia, the use of tobacco, or the use of prescription medications that were likely to affect blood pressure, the lipid profile, or insulin sensitivity. Participants who were taking fish-oil or other n–3 supplements were asked to stop supplementation 4 wk before the first assessment and to maintain a similar diet and level of physical activity for the duration of the study.

[5] Abbreviations used: AV, anisidine value; BBI, Bang's blinding index; CRP, C-reactive protein; KS, krill and salmon; PV, peroxide value.

Random assignment and masking

The random assignment of participants to the treatment arm was done by using a computer random-number generation. The code was kept by a third party and was not released until after the statistical analysis. Both researchers and participants were blinded to the contents of capsules that were taken. To maintain the integrity of the trial evaluation, a statistical analysis was carried out on encoded data so that the data analyst was also blinded to treatment.

Study design

This study was a 24-wk randomized, double-blind, controlled crossover trial. Participants were randomly assigned to receive capsules with active treatment or a control for 8 wk (**Figure 1**). After an 8-wk washout period, participants switched to the other treatment for an additional 8 wk (Figure 1).

The active treatment contained krill oil (88%) and salmon oil (12%). This combined oil was 42.1% of phospholipids by weight, and each 1000-mg gelatin capsule contained 46 mg EPA and 31 mg DHA (**Table 1**). Participants were instructed to take 5 capsules as a single dose, once a day, with a glass of water, which equated to a daily supplementation with 400 mg n–3 PUFAs (including 230 mg EPA plus 154 mg DHA). The control intervention consisted of 1000 mg canola oil, which was also presented in a gelatin capsule that was minimally coated in fish oil (<5 μg) to match odor and flavor. Canola oil primarily contains oleic acid (Table 1), and there is no reliable evidence that a low dose of canola oil affects insulin sensitivity. Both KS- and control-oil capsules were manufactured by Nutrizeal Ltd., and their contents were verified independently by using gas chromatography (Table 1).

To establish their oxidative states, both trial oils were also analyzed for the peroxide value (PV) and anisidine value (AV) according to the European Pharmacopoeia methods (24). Average values were calculated on the basis of triplicate measures of oil pooled from 10 capsules. Assay characteristics were determined by using fish-oil capsules that were not used in this study. The PV had interassay and intra-assay CVs <3% and a lower limit of detection of 0.3 mEq/L, whereas the AV had inter- and intra-assay CV <4%. In addition, the Totox value was calculated as

$$(2 \times \mathrm{PV}) + \mathrm{AV} \qquad (2)$$

The KS oil had a PV below the lower limit of detection of 0.3 mEq/L and an AV of 11.0, which indicated a Totox value <11.6. The control oil (canola) had a PV of 3.1 mEq/L and an AV of 2.3, which indicated a Totox value of 8.5. Thus, both oils were relatively unoxidized and within international recommendations (25). Nonetheless, to minimize the oxidation of the oil before consumption, capsules were provided in sealed amber glass jars and participants were instructed to keep them in the refrigerator.

Clinical assessments

All participants were assessed on 4 occasions, immediately, before, and after each 8-wk intervention. Clinical assessments were carried out between 0700 and 0900 at the Maurice & Agnes Paykel Clinical Research Unit (Liggins Institute, University of Auckland) after an overnight fast and no strenuous activity over the previous 24 h.

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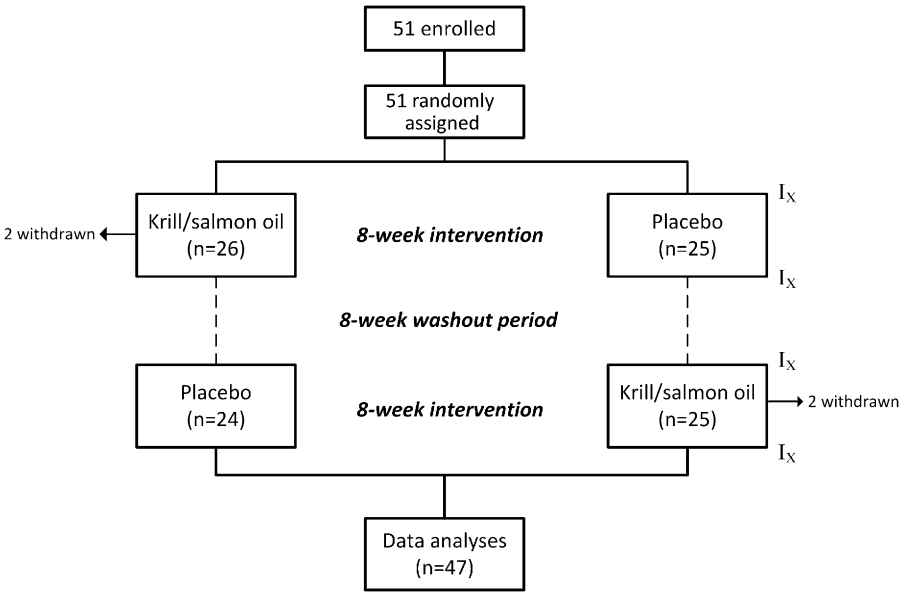

FIGURE 1 Summary of the study's recruitment process and trial execution. I_X indicates the timing of assessments. Two participants withdrew during the first treatment phase because of an allergic reaction to krill oil, whereas 2 subjects withdrew after crossover because of serious injury or engagement in marathon training.

The primary outcome was insulin sensitivity measured with a 75 g oral-glucose-tolerance test by using the Matsuda method (26). Blood samples were collected at 0, 30, 60, 90, and 120 min for glucose and insulin measurements. The Matsuda index has a strong correlation with the hyperinsulinemic euglycemic clamp (r = 0.77) (27) and excellent reproducibility during multiple measures (28). The oral disposition index (a measure of β cell function corrected for insulin sensitivity) (29) and the HOMA-IR were also calculated (30).

Fasting blood samples were used to assess other measures of metabolic disease risk, including uric acid, free fatty acid, and highly sensitive C-reactive protein (CRP) concentrations, as well as the lipid profile (triglyceride, total cholesterol, HDL-cholesterol, LDL-cholesterol, apolipoprotein A, and apolipoprotein B concentrations). An auxologic assessment included height measurement by using a Harpenden stadiometer. Weight and body composition were assessed by using whole-body dual-energy X-ray absorptiometry (Lunar Prodigy 2000; General Electric).

Twenty-four-hour ambulatory blood pressure monitoring was carried out before each clinical assessment. Participants were fitted with a Spacelabs 90207 or 90217 monitor (Spacelabs Medical Inc.) with each subject being assigned the same device model for all assessments. Measurements were performed every 20 min between 0700 and 2200 and every 30 min from 2200 to 0700. Only profiles with >14 daytime and >7 night-time recordings over a 24-h period were analyzed.

TABLE 1
Fatty acid concentrations in a control (canola oil) and krill and salmon oil determined by using gas chromatography–mass spectrometry[1]

	Control, mg/g oil	Krill and salmon oil, mg/g oil
16:0 (palmitic acid)	18.22 ± 1.45	81.94 ± 4.59
16:1n–7 (palmitoleic acid)	0.68 ± 0.03	11.98 ± 2.23
18:0 (stearic acid)	16.49 ± 0.76	16.18 ± 0.82
18:1n–7 (*cis*-vaccenic acid)	2.49 ± 0.15	5.60 ± 0.50
18:1n–9 (oleic acid)	611.19 ± 51.80	196.89 ± 33.92
18:2n–6 (linoleic acid)	31.21 ± 2.33	5.55 ± 0.87
18:3n–3 (α-linolenic acid)	41.74 ± 2.74	11.02 ± 0.49
18:3n–6 (γ-linolenic acid)	Nil	8.31 ± 0.34
20:0 (arachidic acid)	Nil	0.25 ± 0.01
20:1n–9 (eicosenoic acid)	Nil	0.36 ± 0.06
20:2n–6 (eicosadienoic acid)	Nil	0.23 ± 0.10
20:3n–6 (dihomo-γ-linolenic acid)	Nil	0.21 ± 0.04
20:4n–6 (arachidonic acid)	Nil	5.49 ± 0.67
20:5n–3 (EPA)	Nil	45.90 ± 4.07
22:5n–3 (docosapentaenoic acid)	Nil	2.40 ± 0.45
22:6n–3 (DHA)	Nil	30.76 ± 2.04

[1]All data are means ± SDs from an analysis in triplicate. The lower limit of detection was set at 0.1 mg/g oil; fatty acids with concentrations <0.1 mg/g are labeled "Nil." The krill and salmon oil was a blend of krill oil (88%) and salmon oil (12%).

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Carotid artery intimamedia thickness was also measured because it is a validated and reproducible parameter that is predictive of cardiovascular and cerebrovascular risks (31). Carotid intimamedia thickness was measured by using an M-Turbo ultrasound system (SonoSite) by the same trained investigator with longitudinal images attained by using a standard protocol (32). The right common carotid artery was scanned from both posterolateral and anterolateral views with digitally stored images analyzed by using computer-software automated calipers to measure the far wall (SonoCalctm v.4.1; SonoSite). Maximal thickness measurements from both views (~10 mm proximal to the carotid bulb) were used for comparative analysis with an intra-observer CV of 3.7% (16).

Lifestyle factors were recorded with an itemized food diary and physical activity recall. Three-day dietary records were collected before clinical assessments. Each dietary report encompassed an itemized nutritional intake recorded during 2 weekdays (Monday to Friday) and one weekend day. Nutritional intake was recorded by using standard household measures as well as the information from food labels when appropriate. Participants were instructed by a trained investigator who also reviewed all food records with each participant to address unclear descriptions, errors, omissions, or doubtful entries. Records were subsequently entered into Foodworks software (v6.0; Xyris Software) by the trained investigator. Physical activity was assessed by using the International Physical Activity Questionnaire (33), which covered the following 4 domains of physical activity: work related, transportation, housework/gardening, and leisure time.

Socioeconomic status was estimated from geocoded deprivation scores derived from current addresses by using the New Zealand Index of Deprivation 2006 (34). This index is based on household census data reflecting 9 aspects of material and social deprivation to divide New Zealand into tenths (scored 1–10) by residential address. A score of 1 represents the least-deprived areas, and a score of 10 represents the most-deprived areas. Scores are derived from units covering a small area, each reflecting ~90 people. Ethnicity was recorded by self-report by using a prioritized system such that, if multiple ethnicities were selected, the patient was assigned to a single category according to a hierarchical system of classification (order of priority was Maori, Pacific Island, Asian, and New Zealand European) (35).

Assays

Insulin concentrations were measured with an Abbott AxSYM system (Abbott Laboratories) by using a microparticle enzyme immunoassay with an interassay CV of 5.4%. Glucose, triglyceride, total cholesterol, HDL-cholesterol, LDL-cholesterol, apolipoprotein A, apolipoprotein B, free fatty acid, uric acid, and highly sensitive CRP concentrations were measured with a Hitachi 902 autoanalyzer (Hitachi High Technologies Corp.) by using an enzymatic colorimetric assay (Roche) with all CVs <3.2%.

Erythrocyte fatty acid analysis

Fatty acid profiles were analyzed via direct transesterification of the washed erythrocyte (red blood cell) fraction of blood followed by gas chromatography (36). Methanol:toluene (2 mL, 4:1 vol:vol) [containing 19:0 (20 mg/L) as an internal standard] was added to the sample. Acetyl chloride (200 μL) was added while mixed on a vortex and heated (1 h; 100°C). The tubes were cooled in water (5 min), had K_2CO_3 6% (5 mL) added, and were subsequently centrifuged (3000 × *g*; 5 min; 4°C). The upper toluene phase that contained fatty acid methyl esters was collected and stored in a gas chromatograph vial at −20°C for analysis.

Methylated fatty acid samples were analyzed with gas chromatography by using a fixed carbon-silica column (30 m × 0.25 mm, DB-225, J&W Scientific) (37). The gas chromatograph was equipped with a flame ionization detector, autosampler, and autodetector. Injector and detector ports were set at 250°C. Oven temperature was programmed at 170°C for 2 min and increased 10°C/min to 190°C, where it remained constant for 1 min. Then, the temperature increased 3°C/min to 220°C, which was maintained for a total run time of 30 min/sample. A split ratio of 10:1 and an injection volume of 3 μL were used. A known fatty acid mixture was compared with analyzed samples to identify peaks according to retention time; their concentrations were determined by using a 6890 Series gas chromatograph (Hewlett-Packard) with Chemstations Version A. 04.02 software (Chemstations Inc.). The omega-3 index was calculated as shown in Equation *1*.

Power and sample-size calculation

The power calculation was based on data from an Auckland population of adult men also overweight and of a similar age (38). On the basis of a mean (±SD) Matsuda index of 6.67 ± 3.73 (38), a total sample of 42 participants would have had ≥90% power at a 5% level of significance (2 sided) to detect a 20% change in the Matsuda index, assuming a correlation of 0.5 between measurements on the same subject. To allow for a 20% drop-out rate during the study, the recruitment target was 50 participants.

Safety assessment

All adverse events were recorded throughout the study, and participants were monitored for any adverse events such as gastrointestinal symptoms, allergy, and bleeding. A general medical examination and liver-function tests were also carried out. In addition, if hypertension or carotid atherosclerosis was identified during prescreening or the clinical assessment, an immediate referral was made to the general practitioner.

Treatment compliance

Participants were overissued with capsules by an amount unknown to them and asked to return all unused capsules. Treatment compliance was estimated from returned capsules.

Blinding success

During the final clinical assessment, all participants were asked if they were able to identify the capsules (i.e., KS or control oil) they took last. Their responses were noted, and the effectiveness of treatment blinding was assessed by using the Bang's blinding index (BBI) (39). In this study, blinding success was determined as per the following thresholds of Moroz et al. (40): BBI ≥0.2 (unblinded), > −0.2 but <0.2 (random guesses), and ≤−0.2 (opposite guesses).

Statistical analysis

Linear mixed regression models with a random-patient effect (SAS v.9.3; SAS Institute) were adopted to assess the main

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treatment effect. Models accounted for the treatment period (first leg or crossover). Regression models also adjusted for the baseline value of the outcome response to gain statistical efficiency and power (i.e., baseline data were included in the model as covariates). The interaction between the treatment and treatment period was also evaluated for each outcome response to assess the success of the washout. Variables of glucose homeostasis and inflammatory markers were log transformed to approximate normality. Physical activity, dietary intake, and n–3 PUFA concentrations were compared between treatments with paired *t* tests. All statistical tests were 2 tailed, and significance was maintained at 5%. Descriptive data are presented as means (± SDs); other data are presented as means (95% CIs) adjusted for treatment period and baseline values.

RESULTS

Participants

A total of 51 men were recruited into the clinical trial, but 4 subjects were subsequently excluded (Figure 1). Two participants dropped out of the trial soon after baseline because of allergic reaction to KS oil (unmasked by a third party). As a result, the intention-to-treat principle was not followed. We analyzed data on 47 participants (Figure 1) who were aged 46.5 ± 5.1 y and with BMI of 27.4 ± 1.8. The majority of participants (87%) were of European descent.

Primary outcome

Insulin sensitivity was 14% lower with KS-oil supplementation than with the control oil (Matsuda index: 4.57 compared with 5.33, respectively; $P = 0.049$) (**Table 2**). As previously reported (16), there was a positive association between the omega-3 index and insulin sensitivity at baseline. To investigate for possible effects of components of KS oil other than n–3 PUFAs, a mediation analysis was carried out by controlling for the effects of the omega-3 index on the primary outcome. This analysis showed that, after controlling for the likely positive effects of blood EPA and DHA (i.e., the omega-3 index), the reduction of insulin sensitivity after KS-oil supplementation was more marked [27% lower than with the control oil (4.04 compared with 5.53, respectively; $P = 0.009$)] (**Supplementary Figure 1**). When the omega-3 index was accounted for, there was no change in insulin sensitivity (compared with at baseline) with the control oil ($P = 0.88$), but participants experienced a 24%

TABLE 2
Primary and secondary outcomes in 47 overweight, middle-aged men after an 8-wk supplementation with a control (canola oil) or krill and salmon oil[1]

	Control	Krill and salmon oil	*P*
Primary outcome			
Insulin sensitivity (Matsuda index)	5.33 (4.73, 6.00)	4.57 (4.06, 5.14)	0.049
Secondary outcomes			
Anthropometric measures			
Weight, kg	89.0 (88.5, 89.5)	88.7 (88.2, 89.2)	0.45
Total body fat, %	28.0 (27.6, 28.3)	28.0 (27.7, 28.4)	0.82
Android fat:gynoid fat ratio	1.27 (1.25, 1.29)	1.28 (1.26, 1.30)	0.75
Glucose homeostasis			
HOMA-IR	1.85 (1.66, 2.07)	2.02 (1.80, 2.26)	0.26
Disposition index	5.37 (4.32, 6.67)	5.47 (4.39, 6.78)	0.87
Fasting glucose, mmol/L	5.30 (5.19, 5.40)	5.40 (5.30, 5.51)	0.092
Fasting insulin, mU/L	7.53 (6.67, 8.50)	8.22 (7.28, 9.27)	0.29
24-h ambulatory blood pressure			
Daytime systolic, mm Hg	126.2 (124.3, 128.1)	125.6 (123.7, 127.5)	0.61
Daytime diastolic, mm Hg	79.2 (77.9, 80.6)	78.9 (77.5, 80.3)	0.72
Night time systolic, mm Hg	110.7 (108.4, 112.9)	109.6 (107.3, 111.9)	0.42
Night time diastolic, mm Hg	66.6 (64.9, 68.4)	65.6 (63.8, 67.3)	0.36
Systolic dip, %	12.2 (10.6, 13.9)	12.6 (10.9, 14.2)	0.74
Diastolic dip, %	15.8 (13.7, 17.9)	16.7 (14.6, 18.8)	0.54
Carotid intimamedia thickness, mm	0.819 (0.801, 0.836)	0.843 (0.826, 0.861)	0.068
Other metabolic markers			
Free fatty acids, mmol/L	0.37 (0.32, 0.41)	0.39 (0.35, 0.44)	0.43
Uric acid, μmol/L	366 (355, 377)	364 (352, 375)	0.76
Highly sensitive CRP,[2] mg/L	1.01 (0.77, 1.31)	1.10 (0.84, 1.43)	0.54
Lipid profile			
Total cholesterol, mmol/L	5.17 (5.00, 5.33)	5.38 (5.21, 5.55)	0.11
LDL cholesterol, mmol/L	3.60 (3.44, 3.76)	3.74 (3.58, 3.90)	0.20
HDL cholesterol, mmol/L	1.15 (1.08, 1.23)	1.17 (1.09, 1.24)	0.79
Total cholesterol:HDL cholesterol	4.82 (4.52, 5.12)	4.79 (4.49, 5.09)	0.89
Triglycerides, mmol/L	1.14 (1.03, 1.25)	1.18 (1.07, 1.29)	0.64
Apolipoprotein A, mg/dL	162 (156, 168)	162 (157, 168)	0.93
Apolipoprotein B, mg/dL	104 (98, 110)	110 (104, 116)	0.053

[1]Results are from linear mixed regression models with a random patient effect. All values are means; 95% CIs in parentheses. Values were adjusted for treatment period and baseline values.
[2]CRP, C-reactive protein.

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reduction in the Matsuda index after taking KS oil ($P = 0.0008$) (Supplementary Figure 1).

Secondary outcomes

There were no significant differences between control and KS oils in the range of secondary outcomes assessed (Table 2). However, participants taking KS oil tended to have higher concentrations of apolipoprotein B than when taking the control oil ($P = 0.053$; Table 2). In addition, there was a trend toward an increase in carotid intimamedia thickness with KS oil ($P = 0.068$; Table 2).

Post hoc analyses

Within-group changes from baseline were assessed in post hoc analyses (**Supplemental Table 1**). Although there was no significant change in insulin sensitivity from baseline with the control oil ($P = 0.23$), participants experienced an 18% decrease in the Matsuda index ($P = 0.002$) while taking KS oil (Supplemental Table 1). The deterioration in insulin sensitivity after KS-oil supplementation was corroborated by the HOMA-IR that increased by 0.39 ($P = 0.016$) and fasting insulin that increased by 1.39 mU/L ($P = 0.048$; Supplemental Table 1). KS-oil supplementation was also associated with increases in carotid artery intimamedia thickness (+39 μm; $P = 0.001$) as well as apolipoprotein B (+8.02 mg/dL; $P = 0.009$), total cholesterol (+0.31 mmol/L; $P = 0.0004$), LDL cholesterol (+0.27 mmol/L; $P = 0.001$), and HDL cholesterol (+0.08 mmol/L; $P = 0.034$) concentrations (Supplemental Table 1). Note, however, that there were no differences between treatments when percentages of changes from baseline were compared.

Other adverse events

Apart from the described reports of allergy that led to the withdrawal of 2 participants, the main adverse event associated with the consumption of KS oil was a high incidence of eructation described as "fishy burps." Over the course of the trial, 22 participants (47%) reported that they experienced fishy burps while taking the KS oil compared with just one participant (2%) while taking the control oil ($P < 0.0001$).

Other adverse events were reported by participants while taking KS oil, including mild gastrointestinal symptoms ($n = 2$), increased bowel frequency ($n = 2$), and localized pimples ($n = 1$). Participants taking the control oil also reported mild gastrointestinal symptoms ($n = 1$) and increased bowel frequency ($n = 3$).

Compliance

The compliance of participants with the study protocol was high with 93.2% ± 5.8% of capsules being taken overall, although it varied in participants from 78.1% to 100%. Compliance was very similar in the first (93.9 ± 5.9%) and crossover (92.4 ± 8.1%) phases of the trial ($P = 0.21$), and it was not affected by treatment ($P = 0.61$), ethnicity ($P = 0.52$), age ($P = 0.27$), or socioeconomic status ($P = 0.93$).

Blinding

In participants who were last taking KS oil ($n = 23$), the majority ($n = 12$; 52%) correctly identified its content, whereas 4 subjects (17%) got it wrong, and 7 subjects (30%) answered that they did not know. For subjects taking the control oil ($n = 24$), there were 12 correct identifications (50%), 3 incorrect identifications (13%), and 9 subjects (38%) did not know. The BBI was 0.35 for the KS oil (95% CI: 0.04, 0.66) and 0.38 for the control oil (95% CI: 0.10, 0.65). On the basis of the thresholds of Moroz et al. (40), treatment blinding was not successful, with a considerable proportion of participants who correctly identified beyond chance the last treatment they were on. One participant intentionally cut a capsule to identify its content, but we are unaware of other subjects doing the same. Participants who reported fishy burps tended to be more likely to correctly identify the treatment than were those who did not experience them (OR: 3.54; 95% CI: 0.93, 13.51; $P = 0.065$), which suggested that this adverse effect contributed to unblinding. There were no differences in diet or physical activity during control- and KS-oil interventions (**Table 3**), which indicated that unblinding was not associated with a modification of important behaviors that influenced insulin sensitivity.

Dietary intake and physical activity

There was no evidence of dietary alterations or changes in physical activity while taking control- or KS-oil supplementation (Table 3). However, KS-oil supplementation increased blood concentrations of EPA by 60% ($P < 0.0001$) and DHA by 10% ($P = 0.025$), which led to a 21% difference in the omega-3 index between the 2 treatments (Table 3).

Washout period

There were no statistically significant interactions between the treatment and treatment period for any primary or secondary outcomes (data not shown). Thus, there was no observed carryover effect between treatment periods, and the washout phase was likely successful.

DISCUSSION

Contrary to our initial hypothesis, in this double-blind, controlled crossover trial there was a reduction in insulin sensitivity after supplementation with the KS blended oil than with the control oil (canola oil). There were no significant changes in body

TABLE 3
Physical activity, dietary intake, and n–3 PUFA concentrations in red blood cell phospholipids in 47 study participants receiving a control (canola oil) or krill and salmon oil[1]

	Control	Krill and salmon oil	*P*
Physical activity (Met-min/wk)	3635 ± 3747	3549 ± 2641	0.67
Total energy intake, kJ/d	8932 ± 2312	9102 ± 2985	0.51
Saturated fat intake, g/d	28.8 ± 11.5	28.9 ± 15.9	0.81
Fish meals per participant, *n*/wk	1.6 ± 1.1	1.5 ± 1.1	0.33
EPA, percentage weight/weight	1.54 ± 0.73	2.47 ± 0.62	<0.0001
DHA, percentage weight/weight	6.41 ± 1.53	7.06 ± 1.19	0.001
Omega-3 index	7.87 ± 2.10	9.53 ± 1.67	<0.0001

[1]Results from paired *t* tests. Data are means ± SDs. Omega-3 index represents the sum of EPA plus DHA concentrations (percentage weight/weight) in erythrocyte membrane fatty acids.

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composition, physical activity, or diet, each of which is a major lifestyle factor that is known to affect insulin sensitivity. Thus, our findings suggest that krill- and/or salmon-oil supplementation may increase risk of developing type 2 diabetes and cardiovascular disease.

In addition, a post hoc within-group analyses showed that there was an increase in LDL-cholesterol and apolipoprotein B concentrations as well as a subtle increase in carotid artery intima-media thickness after KS-oil supplementation, which suggested a more atherogenic lipid profile. However, because significant differences were not seen in the primary analyses, these findings must be interpreted with caution and need to be corroborated in other studies.

The control treatment in this study was canola oil, which conferred the advantage of matching the intervention for caloric density and fat content as well as reducing risk of unblinding. Canola oil is primarily made up of oleic acid, and there is no reliable evidence that oleic acid improves insulin sensitivity or the lipid profile. However, canola oil also contains phytosterols, and these may mediate a beneficial effect of high doses of canola oil (20–25 g/d) on serum triglycerides (41, 42) and cholesterol absorption (43). The dose of canola oil in this study was low (5 g/d), and we observed no effects on serum triglycerides or any other metabolic outcomes after canola-oil supplementation. Thus, it appears that canola oil had no metabolic effects in this study and was an appropriate control treatment.

The KS-oil treatment involved a 5-g/d intake, which equated to low-dose n–3 supplementation (400 mg/d) providing 80% of the daily intake of EPA and DHA recommended by the National Heart Foundation of Australia (44). Despite a relatively low dose of n–3 PUFA, KS-oil supplementation led to a 60% increase in EPA concentrations compared with those with the control, which was a magnitude similar to that in previous studies that showed metabolic and anti-inflammatory effects of n–3 PUFA supplementation (11, 13, 45). It seems unlikely that the n–3 PUFAs in the KS oil were the cause of the observed reduction in insulin sensitivity because, when the omega-3 index was controlled for, the adverse effect of KS oil on insulin sensitivity became more marked (Supplementary Figure 1). In addition, we showed that, in this cohort of overweight middle-aged men, the omega-3 index at baseline was associated with greater insulin sensitivity, lower nocturnal blood pressure, and lower free fatty acid and CRP concentrations, which suggested metabolic benefits to subjects with higher n–3 PUFA concentrations (16). Previous epidemiologic studies (6, 7) and clinical trials of fish-oil supplementation in healthy people have not supported an adverse metabolic effect of n–3 PUFA in men (10–12, 14). Oils derived from marine species are complex in their chemical composition, where n–3 PUFAs make up a fraction of their content. Thus, it is important to consider the composition of the trial oil because the potential benefits to insulin sensitivity of n–3 PUFAs appear to have been masked by the adverse effects of other compounds.

Because the trial oil was a blend of krill oil (88%) and salmon oil (12%), it was not possible to ascertain whether the metabolically adverse effect was due to krill oil, salmon oil, or both. However, it is probable that the compound(s) that mediated the metabolically adverse effect are in krill oil rather than salmon oil. First, as previously stated, prior interventional trials of fish-oil supplementation have not shown an impairment of insulin sensitivity (10–12, 14). Second, in a small study designed to compare the effects of krill and fish oil on plasma n–3 PUFAs, large but nonsignificant increases in fasting insulin (44%) and HOMA-IR (55%) were observed in the krill-oil group but not in the fish-oil group (46). Although this study was underpowered to detect a significant difference in fasting insulin or HOMA-IR between groups, these data also suggested a detrimental effect of krill oil on insulin sensitivity, which corroborated our findings.

Krill are crustaceans and are evolutionarily very distant from fish (i.e., vertebrates). Thus, the biologically active compounds present in krill and fish oils are likely to be distinct. Krill oil contains the carotenoid pigment astaxanthin that has antioxidant capacity (22) but which is unlikely to mediate the adverse metabolic effects observed. There are no data on the effects of astaxanthin on human metabolism, but in vitro evidence suggested that it may modulate peroxisome proliferator–activated receptor-γ (47). This is a nuclear receptor with important metabolic effects, and peroxisome proliferator–activated receptor-γ agonists are known to increase insulin sensitivity (48). In addition, astaxanthin supplementation has been shown to improve insulin sensitivity in animal models (49, 50).

Unlike fish oil in which fatty acids are predominantly incorporated into triglycerides, in krill oil, fatty acids are largely present as phospholipids. This difference is also unlikely to explain the observed reduction in insulin sensitivity. The KS oil used in this study was 42% phospholipids by weight, with the vast majority (76%) in the form of phosphatidylcholine. The health effects of dietary phospholipids are not well understood, but limited evidence suggested that a higher phosphatidylcholine fraction of membrane phospholipid is associated with greater insulin sensitivity (51). Thus, if supplementation with krill oil leads to a greater cell membrane phosphatidylcholine fraction, a reduction in insulin sensitivity would not be expected.

Nonetheless, the phospholipids present in krill oil are amphipathic, having hydrophilic and lipophilic properties. As a result, after extraction, krill oil may still contain amphipathic or water-soluble compounds (such as proteins) that would not be present in fish-oil supplements. We speculate that there are compounds present in krill oil that impair insulin sensitivity, and they are either not in fish or, if present in fish, are mostly excluded during oil extraction. The identification of these compounds could lead to a better understanding of the metabolic effects of marine oils that are not sourced from fish and possibly to processes that would exclude them from supplements.

Aside from fishy eructations, adverse events from the KS-oil supplementation were uncommon. However, 2 participants (4%) had an urticarial reaction to KS oil, which suggested a greater-than-expected incidence of allergy [crustacean allergy has a reported incidence of 1.6% (52)]. The presence of traces of protein in krill oil is a likely causative factor because tropomyosins were identified as the major allergens in Antarctic krill (53). In addition, krill tropomyosins show a significant homology to other crustaceans eaten as food, and immunoglobulin E against shrimp antigens was shown to cross-react with krill (54). Thus, people with a known allergy to shellfish, especially crustaceans, should avoid krill oil.

This randomized controlled crossover trial was robust and adequately powered to assess the effects of supplementation with the KS oil on insulin sensitivity. The major strengths of this study were the use of detailed metabolic assessments, recruitment of

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a cohort that was healthy but at increased metabolic risk, and careful assessment of compliance, physical activity, and diet, which did not differ between groups. In addition, participants, investigators, and the data analyst remained blinded to the identity of treatment oils until after the data analysis was complete.

Among the limitations, krill oil has a relatively low concentration of fatty acids, and thus, the dose of n–3 PUFAs provided by the KS supplement was relatively low at 400 mg/d. Because the trial oil was a blend of KS oil, it remains unclear as to whether the metabolically adverse effect was due to krill oil, salmon oil or both. In addition because of the chemical differences between krill oil and other marine oils, these data cannot be generalized to fish, calamari, or algal sources of n–3 PUFAs. Furthermore, we studied a relatively narrow range of individuals (overweight, middle-aged men who were living in a large urban center and mostly of European ethnicity), which may limit the wider applicability of our findings, particularly to women. Recent evidence that the effects of EPA and DHA on platelet aggregation and hemostatic markers are sex-specific raised the possibility that the metabolic effects of n–3 PUFAs could also differ according to sex (55).

In conclusion, this study provides evidence that supplementation with a blend of KS oil leads to a reduction in insulin sensitivity in overweight men. In addition a post hoc analysis suggests that this blended oil may also lead to a less-favorable lipid profile. The cause of these adverse effects is unclear and probably not associated with n–3 PUFAs but, rather, the likely effects of other chemical constituents of krill or salmon oil. We speculate that the adverse effects are more likely to be due to the krill-oil component and caution against the use of krill oil in individuals at increased risk of type 2 diabetes or cardiovascular disease, at least until there are more data on the metabolic effects of individual constituents of KS oil.

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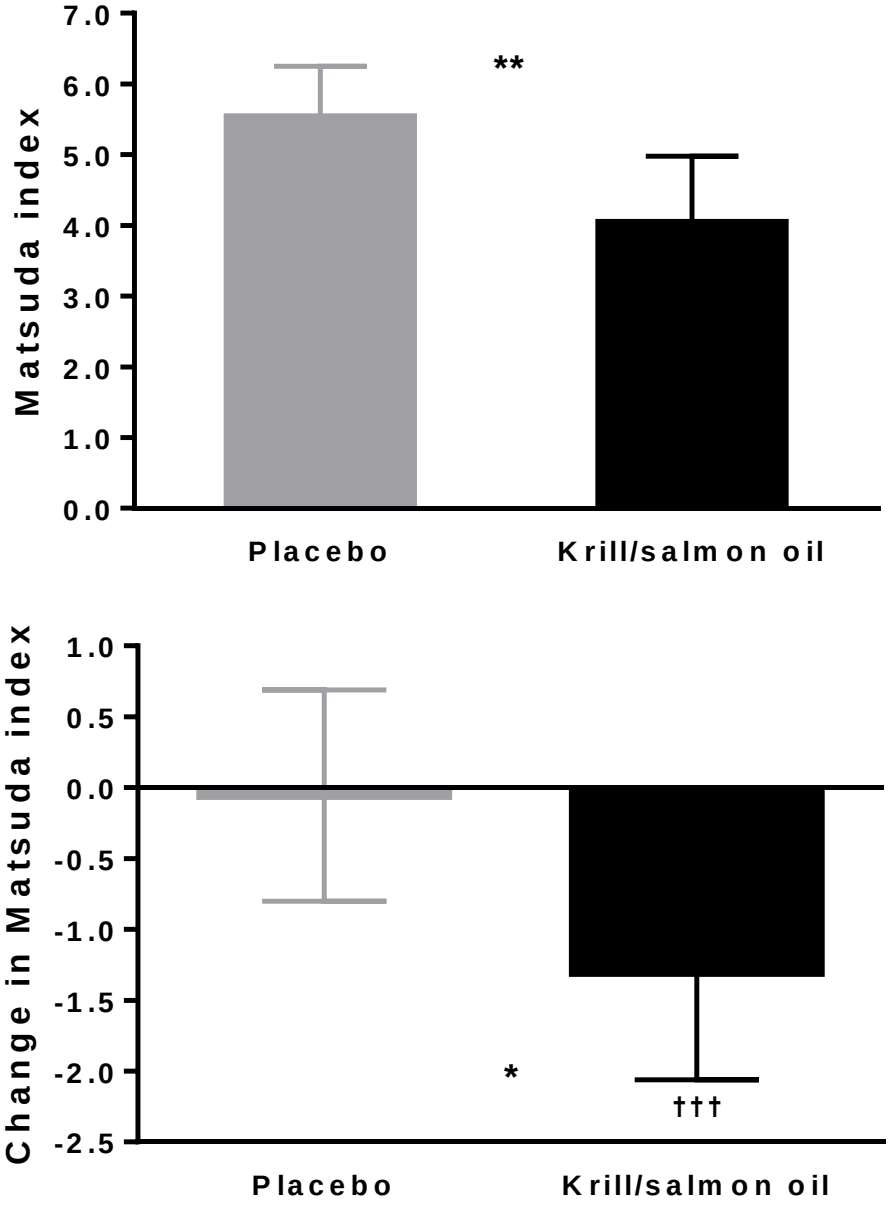
Online Supplemental Material

Supplemental Figure 1. Mediation analysis showing insulin sensitivity and change in insulin sensitivity as measured by the Matsuda method in 47 overweight middle-aged men, following a 8-week supplementation with control (canola oil) or krill/salmon oil. Data are means and 95% confidence intervals adjusted for treatment period, baseline values, and the participants' concentrations of EPA+DHA in erythrocyte membrane fatty acids. *p<0.05 and *p<0.01 for placebo vs krill/salmon oil; †††p<0.001 for the change from baseline associated with a particular treatment.



Online Supplemental Material

Supplemental Table 1. Changes in primary and secondary outcomes in comparison to baseline among 47 overweight middle-aged men, following an 8-week supplementation with control (canola oil) or krill/salmon oil.

		Control	Krill/salmon oil
Primary outcome	**Insulin sensitivity (Matsuda index)**	-0.38 (-1.00, 0.25)	-0.98 (-0.36, -1.61)**
Secondary outcomes	**Anthropometry**		
	Weight (kg)	0.43 (-0.09, 0.94)	0.12 (-0.40, 0.63)
	Total body fat (%)	0.08 (-0.29, 0.45)	0.14 (-0.23, 0.52)
	Android fat to gynoid fat ratio	-0.00 (-0.02, 0.02)	0.00 (-0.02, 0.02)
	Glucose homeostasis		
	HOMA-IR	0.16 (-0.16, 0.47)	0.39 (0.07, 0.70)*
	Disposition index	0.98 (-1.34, 3.29)	-0.40 (-2.90, 2.10)
	Fasting glucose (mmol/l)	-0.06 (-0.16, 0.05)	0.05 (-0.06, 0.15)
	Fasting insulin (mU/l)	0.70 (-0.67, 2.08)	1.39 (0.01, 2.77)*
	24-hour ambulatory blood pressure		
	Daytime systolic (mmHg)	0.19 (-1.72, 2.10)	-0.39 (-2.32, 1.55)
	Daytime diastolic (mmHg)	-0.13 (-1.50, 1.24)	-0.44 (-1.83, 0.94)
	Night time systolic (mmHg)	-0.03 (-2.28, 2.21)	-1.11 (-3.36, 1.16)
	Night time diastolic (mmHg)	0.09 (-1.66, 1.84)	-0.98 (-2.74, 0.78)
	Systolic dip (%)	0.24 (-1.39, 1.87)	0.60 (-1.05, 2.25)
	Diastolic dip (%)	-0.41 (-2.50, 1.67)	0.54 (-1.57, 2.65)
	Carotid-intima media thickness (mm)	0.007 (-0.016, 0.030)	0.039 (0.016, 0.062)**
	Other metabolic markers		
	Free fatty acids (mmol/l)	-0.02 (-0.06, 0.02)	0.01 (-0.04, 0.05)
	Uric acid (umol/l)	8.3 (-2.8, 19.3)	6.2 (-4.9, 17.3)
	CRP (mg/l)	0.45 (-0.50, 1.39)	0.19 (-0.76, 1.13)
	Lipid profile		
	Total cholesterol (mmol/l)	0.10 (-0.07, 0.26)	0.31 (0.14, 0.47)***
	LDL-C (mmol/l)	0.14 (-0.03, 0.30)	0.27 (0.11, 0.43)**
	HDL-C (mmol/l)	0.07 (-0.01, 0.14)	0.08 (0.01, 0.15)*
	Total cholesterol : HDL-C	-0.16 (-0.46, 0.14)	-0.18 (-0.48, 0.12)
	Triglycerides (mmol/l)	-0.05 (-0.16, 0.06)	-0.01 (-0.11, 0.10)
	Apolipoprotein A (mg/dl)	2.00 (-3.57, 7.57)	2.24 (-3.32, 7.81)
	Apolipoprotein B (mg/dl)	1.29 (-4.66, 7.23)	8.02 (2.07, 14.0)**

Results from linear mixed regression models with a random patient effect. Data are means and 95% confidence intervals adjusted for treatment period and baseline values. *p<0.05, **p<0.01, and ***p<0.001 for a change from baseline associated with a particular treatment.