Research Article

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Effects of krill oil intake on plasma cholesterol and glucose levels in rats fed a high-cholesterol diet

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Abstract

BACKGROUND: In this study, whole krill oil (WKO) and phospholipid-type krill oil (PKO) with different lipid composition were prepared. The effects of KO intake on plasma cholesterol and glucose levels in Wistar rats fed a high-cholesterol diet (HCD) were investigated.

RESULTS: WKO contained 37.63% triglycerides, 48.37% phospholipids, 13.54% free fatty acids and 0.66% cholesterol, whereas the corresponding values for PKO were 0.59, 69.80, 28.53 and 1.09% respectively. Meanwhile, PKO contained much more polyunsaturated fatty acids (PUFA, 37.76%) than WKO (28.36%). After 4 weeks of HCD consumption, plasma levels of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and glucose increased significantly, but that of high-density lipoprotein cholesterol (HDL-C) decreased significantly. The intake of PKO and WKO for 4 weeks caused a significant reduction in body weight gain and plasma levels of TC and LDL-C in HCD-fed rats. Compared with WKO, PKO was more effective in decreasing plasma TC and LDL-C levels.

CONCLUSION: PKO showed better overall cholesterol-lowering effects than WKO, which may be due to its higher *n*-3 PUFA levels.

Keywords: krill oil; *n*-3 polyunsaturated fatty acids; phospholipids; high-cholesterol-fed rat; cholesterol-lowering effect; glucose-lowering effect

INTRODUCTION

Antarctic krill (*Euphausia superba*) is an important fishery resource that is distributed in a wide circumpolar belt between the Antarctic continent and the polar front (http://www.fao.org/fishery/species/3393/en). A recent estimate of total *E. superba* biomass (post-larvae) was 379 Mt, with gross post-larval production ranging from 342 to 536 Mt year^{-1}.[1] According to the Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR), the catch limit for *E. superba* is currently 0.62 Mt year^{-1} (http://www.ccamlr.org/en/fisheries/krill-fisheries). The catch was actually 180 986 t in 2010–2011 according to the CCAMLR Statistical Bulletin (Vol. 24).

Antarctic krill is a rich source of high-quality protein, with an estimated protein content in the range 60–65% dry weight.[2] Similar to other animal proteins, the protein derived from krill is complete protein containing all nine of the indispensable amino acids required by humans.[2] The isoelectric precipitation technique was used recently to recover protein from krill.[3–5] The recovered protein had a low fluoride level and higher essential amino acid content and therefore better safety and nutritional value.

Apart from protein, oil is another main nutritional component of krill. It was reported that the total lipid content of krill ranged from 12 to 50% on a dry weight basis (http://themedicalbiochemistrypage.org/krilloil.php). Krill oil (KO) has attracted increasing attention in recent years owing to its health benefits. To date, inhibition of hepatic steatosis,[6,7] anti-inflammation,[8,9] antioxidation,[9,10] antihyperlipidaemic effect,[7,11] antihyperglycaemic effect,[7] modulation of the endocannabinoid system[12,13] and cardioprotective effect[14] due to KO have been observed.

Similar to fish oil (FO), KO contains high levels of *n*-3 polyunsaturated fatty acids (*n*-3 PUFA), particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).[2,10,15] However, most of the fatty acids (FA) in fish are incorporated into triglycerides (TG), whereas most of the FA in krill are incorporated into phospholipids

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(PL).[16] Some studies on humans and animals indicated that PL FA from KO had better bioavailability than TG FA from FO.[17,18] Rossmeisl *et al.*[19] found that dietary DHA and EPA in the form of marine fish PL are superior to TG with respect to the preservation of glucose homeostasis and the reversal of hepatic steatosis, adipocyte hypertrophy and low-grade white adipose tissue inflammation. Tandy *et al.*[7] found that PL containing saturated FA (SFA) also had pronounced lipid-lowering properties. In this situation, they suggested that PL *n*-3 PUFA (found in KO) may be more efficacious than TG *n*-3 PUFA. However, to date, there is a lack of direct evidence supporting the better lipid-lowering properties of PL *n*-3 PUFA originating from krill.

In this study, two types of KO with different lipid composition were prepared. The effects of KO intake on plasma lipid and glucose in Wistar rats fed a high-cholesterol diet were investigated. The aim of the study was to evaluate the difference in cholesterol- and glucose-lowing effects of the two types of KO.

MATERIALS AND METHODS

Raw material

Frozen *E. superba* was provided by Liaoning Province Dalian Ocean Fishery Group of Corporations (Dalian, China). It was lyophilised in a 2KBTES-55 freeze-dryer (VirTis Co., Gardiner, NY, USA) for 72 h. The dry krill was pulverised and stored at −20 °C until use.

Preparation of krill oil

In this study, two types of KO were extracted from dry krill powder using different organic solvents. Whole KO (WKO) was extracted (1:7 w/v) with hexane at 40 °C for 1.5 h, while phospholipid-type KO (PKO) was extracted (1:9 w/v) with ethanol at 50 °C for 1 h. Following centrifugation at 2000 × *g* for 10 min, the supernatant was collected. The organic solvent was removed by rotary evaporation at 40 °C and vacuum drying at 35 °C. The resultant oil was weighed and stored at −20 °C for further treatment. The oil recovery rate was calculated as follows:

$$\text{recovery rate (\%)} = (\text{oil yield of organic solvent extraction}/\text{oil yield of Soxhlet extraction}) \times 100$$

Soxhlet extraction

Total oils were extracted and determined by the Soxhlet method.[20] Briefly, 1 g of dry krill powder was wrapped in filter paper and placed in a Soxhlet extractor. Oil was extracted for 10 h using diethyl ether as solvent. Diethyl ether in the sample was subsequently evaporated by flushing with N_2 at 35 °C.

Thin layer chromatography with flame ionisation detection analysis

Lipid class was determined by thin layer chromatography with flame ionisation detection (TLC-FID) using an Iatroscan MK-6S analyser (Iatron Inc., Tokyo, Japan). Lipids were dissolved in chloroform at 10–20 mg mL^{-1}. Using a 10 µL syringe, 1 µL of lipid sample was spotted onto silica-coated quartz rods (SIII Chromarods, Iatron Inc.), then elution was performed with *n*-heptane/diethyl ether/formic acid (42:28:0.3 v/v/v). After development, the Chromarods were dried at 60 °C for 5 min and transferred into the Iatroscan, where each Chromarod was scanned by FID to detect and quantify the compounds separated on silica. The hydrogen flow rate was 160 mL min^{-1}, the air flow rate was 2000 mL min^{-1} and the scanning speed was 30 s per Chromarod burned. Data acquisition and processing were performed using i-Chromstar 6.3 integration software (SCPA, Bremen, Germany). Lipid standards were purchased from Sigma Chemical Co. (St Louis, MO, USA).

Preparation of fatty acid methyl esters

Saponification of samples was carried out according to a previously described method[21] with slight modification. Briefly, 100 mg of lipid sample was transferred into a Teflon-lined screw-capped vial containing 2.5 mL of 500 g L^{-1} KOH and 5 mL of 950 mL L^{-1} ethanol. After being tightly capped, the vial was heated in a water bath at 60 °C for 2 h with mild stirring. After incubation, the mixture was cooled quickly to room temperature in a cold water bath. The lipid phase (upper layer) containing the unsaponifiables was extracted six times with 3 mL of hexane each time.

After removal of the unsaponifiables, the remaining aqueous phase was adjusted to pH 2 with 6 mol L^{-1} HCl and extracted six times with 3 mL of hexane each time. The hexane extracts were combined and the solvent was removed by flushing with N_2 at 35 °C. The residue was dissolved in hexane at 10 mg mL^{-1}, then 2.5 mL of this solution was transferred into a Teflon-lined screw-capped vial. After the addition of 2 mL of freshly prepared methylation reagent (10 mL L^{-1} H_2SO_4 in high-performance liquid chromatography (HPLC)-grade methanol), the vial was heated in a water bath at 70 °C for 1 h. After incubation, the mixture was cooled and 1 mL of distilled water was added. The upper layer containing fatty acid methyl esters was collected and stored at −20 °C until analysis by gas chromatography/mass spectrometry (GC/MS).

Gas chromatography/mass spectrometry analysis

The fatty acid methyl esters were analysed by GC/MS using an Agilent 7890A GC/5975C MSD system equipped with an HP-5-MS capillary column (30 m × 0.25 mm × 0.25 µm) (Agilent Technologies, Palo Alto, CA, USA). Helium was used as the carrier gas at a constant pressure of 7.1 psi. The column temperature was held initially at 50 °C for 1 min, then raised to 170 °C at 50 °C min^{-1}, to 300 °C at 4 °C min^{-1} and to 320 °C at 40 °C min^{-1} and finally held at 320 °C for 3.6 min. The injection volume was 1 µL with a split ratio of 50:1. The MS was operated in EI mode (70 eV) with a 2 scan s^{-1} interval over the range *m/z* 50–550. The solvent delay was 4 min. The identification of fatty acid methyl esters was performed by comparison of relative retention times and mass spectra with authentic standards (37 Component FAME Mix, Supelco Inc., Bellefonte, PA, USA) and the NIST08 mass spectral database. The composition of fatty acids was calculated from their peak areas.

Animal experiment

The animal experiment was designed in accordance with the Regulations for the Administration of Affairs Concerning Experimental Animals approved by the State Council of the People's Republic of China. Forty-eight male Wistar rats (4 weeks old, weighing 90–120 g) were purchased from the Laboratory Animal Center of Dalian Medical University (Dalian, China). They were housed in plastic cages (three rats per cage) in a temperature-controlled room (23 ± 2 °C) on a 12/12 h light/dark cycle. All rats were fed adaptively for 2 week, after which they were randomly divided into two groups that received different diets.

- Group I. Normal control (NC) group: six rats fed a normal diet containing 9.8% water, 22.8% protein, 4% fat, 3.6% crude fibre and 6.68% ash (purchased from the Laboratory Animal Center of Dalian Medical University).
- Group II. High-cholesterol-fed (HCF) group: 42 rats fed a high-cholesterol diet (HCD, prepared by Beijing HFK Bioscience Co., Ltd, Beijing, China).

The HCD, consisting of 84.3% normal diet, 2.5% cholesterol, 10% lard and 5% egg yolk powder, was designed according to the formula reported by Suanarunsawat *et al*,[22] with some modifications.

After 4 weeks, blood samples were collected from the orbital sinus of 12 h-fasted rats under anaesthesia. The plasma was isolated and stored at −80 °C until performance of assays. The HCF group rats were then randomly divided into seven groups of six rats each.

- Group 1. Blank control (BC) group: administration of normal saline.
- Groups 2–4. Low-, mid- and high-dose WKO groups: administration of WKO at doses of 50, 200 and 400 mg kg^{-1} body weight (BW) respectively.
- Groups 5–7. Low-, mid- and high-dose PKO groups: administration of PKO at doses of 50, 200 and 400 mg kg^{-1} BW.

The supplements were given daily via intragastric administration for 4 weeks. The dose of oil used in animal experiments varies in the literature. In this study, the three levels of dose were selected according to the values reported by Zhu *et al*.[11] and Santos *et al*,[23] with slight modification. During the experiment, HCF group rats were still fed the HCD, while NC group rats were given saline as a placebo and still fed the normal diet. The differences in caloric and cholesterol intake between the BC group and the various WKO and PKO groups resulting from KO and saline consumption are listed in Table 1. After 4 weeks, blood samples were collected from the orbital sinus of 12 h-fasted rats under anaesthesia. The plasma was isolated and stored at −80 °C until performance of assays.

Plasma total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and glucose were measured using an ADVIA 2400 Clinical Chemistry System (Siemens, Tarrytown, NY, USA).

Table 1. Differences in caloric and cholesterol intake resulting from different types and doses of krill oil (KO) consumption between blank control (BC) and KO groups

Group[a]	Cholesterol (mg kg^{-1} body weight)	Energy (kcal kg^{-1} body weight)
BC	0	0
L-WKO	0.33	0.45
M-WKO	1.32	1.79
H-WKO	2.64	3.58
L-PKO	0.55	0.45
M-PKO	2.18	1.78
H-PKO	4.36	3.56

[a] L-WKO, low-dose whole krill oil (WKO) group; M-WKO, mid-dose WKO group; H-WKO, high-dose WKO group; L-PKO, low-dose phospholipid-type krill oil (PKO) group; M-PKO, mid-dose PKO group; H-PKO, high-dose PKO group.

Statistical analysis

Data are presented as mean ± standard deviation (SD). Statistical analysis was performed using SPSS 16.0 software (SPSS Inc., Chicago, IL, USA). Differences between means were evaluated by one-way analysis of variance (ANOVA) (*post hoc* test: least significant difference (LSD)) and *t* test. Comparisons that yielded $P < 0.05$ were considered significant.

RESULTS AND DISCUSSION

Lipid class and fatty acid composition

In this study, two solvents, hexane and ethanol, were used for preparation of two types of KO with different lipid class composition. Hexane is the most commonly used organic solvent in the lipid extraction industry owing to its strong affinity with oils and fats and its easy recovery (low vaporisation temperature).[24,25] However, as a less polar organic solvent, hexane is less efficient for extraction of polar lipids such as PL.[26] In contrast, ethanol, a polar solvent, is more often used for extraction of PL.[27] In this study the extraction method using hexane (for WKO) and ethanol (for PKO) achieved oil recoveries of 68.64 ± 0.04 and 66.67 ± 0.58% respectively.

TLC-FID analysis indicated that WKO contained 37.63 ± 4.41% TG, 48.37 ± 5.42% PL, 13.54 ± 1.37% free FA (FFA) and 0.66 ± 0.58% cholesterol (CHO), whereas PKO contained 0.59 ± 0.21% TG, 69.80 ± 1.94% PL, 28.53 ± 1.82% FFA and 1.09 ± 0.03% CHO. High PL level is a main feature of KO. Tandy *et al*.[7] reported that the KO supplied by Aker Biomarine ASA (Oslo, Norway) contained 23% TG, 6% FFA and 58% PL, while the KO extracted with hexane by Gigliotti *et al*.[10] contained predominantly PL (20–33%), polar non-PL (64–77%) and minor TG (1–3%). The polar non-PL was considered to be composed of CHO, mono- and diglycerides and red pigment, primarily astaxanthin.

The FA composition of WKO and PKO was analysed by GC/MS and is summarised in Table 2. WKO contained 28.36 ± 1.17% PUFA, 38.56 ± 1.79% monounsaturated FA (MUFA) and 32.19 ± 2.18% SFA, whereas the corresponding values for PKO were 37.76 ± 0.09, 29.38 ± 0.12 and 31.98 ± 0.13% respectively. The KO prepared in this study contained high levels of C14:0, C16:0, C16:1*n*-7, C18:1*n*-9, C20:5*n*-3 and C22:6*n*-3, which was consistent with results reported by Cripps *et al*.[28] By contrast, the KO prepared by Ali-Nehari *et al*.[15] using hexane extraction contained lower levels of EPA (9.41%) and DHA (3.74%). However, the KO supplied by Aker Biomarine ASA contained similar levels of PUFA (25%), EPA (12.5%) and DHA (7%)[7] to the WKO prepared in the present study.

In this study, PKO had a higher content of PUFA, particularly EPA and DHA, than WKO, which may be due to its higher PL content. As reported by Gigliotti *et al*,[10] krill PL contained much more total *n*-3 PUFA (47.4%), DHA (18.0%) and EPA (28.2%) but less SFA (23.5%) than krill TG (4.0, 1.1, 2.3 and 38.7% respectively).

Effects of high-cholesterol diet on body weight and plasma cholesterol and glucose levels

HCDs are widely used to generate a valid rodent model for the metabolic syndrome with hyperlipaemia, hyperglycaemia and obesity.[29] In this study, after 4 weeks of feeding, the plasma levels of TC, LDL-C and glucose of HCF rats increased significantly ($P < 0.01$) and the plasma HDL-C level of HCF rats decreased significantly ($P < 0.01$) (Table 3). In contrast, the plasma levels of TC, LDL-C and glucose of rats fed the normal diet for 4 weeks were almost unchanged, but the plasma HDL-C level of this group of

Table 2. Fatty acid composition of whole krill oil (WKO) and phospholipid-type krill oil (PKO)

Fatty acid[a]	Fatty acid content (%) WKO	PKO
C12:0	0.22 ± 0.01	0.15 ± 0.01
C14:1*n*-5	0.18 ± 0.01	0.10 ± 0.01
C14:0	11.97 ± 0.50	9.36 ± 0.31
C15:1*n*-5	0.18 ± 0.01	0.16 ± 0.01
C15:0	0.27 ± 0.06	0.36 ± 0.02
C16:1*n*-7	10.61 ± 093	6.40 ± 0.20
t-C16:1*n*-7	0.73 ± 0.07	0.65 ± 0.03
C16:0	18.46 ± 0.89	20.48 ± 0.09
C17:1*n*-7	0.13 ± 0.01	0.21 ± 0.01
C18:3*n*-6	0.29 ± 0.01	0.23 ± 0.02
t-C18:3*n*-3	2.68 ± 0.27	2.69 ± 0.17
C18:2*n*-6	3.76 ± 0.41	5.69 ± 1.40
C18:1*n*-9	24.07 ± 0.53	20.33 ± 0.41
t-C18:1*n*-5	0	0.30 ± 0.00
C18:0	1.27 ± 0.50	1.63 ± 0.29
C20:4*n*-6	0.25 ± 0.01	0.33 ± 0.01
C20:5*n*-3	13.61 ± 0.54	17.51 ± 0.66
C20:4*n*-3	0.35 ± 0.03	0.39 ± 0.02
C20:1*n*-9	1.78 ± 0.16	0.95 ± 0.03
C22:6*n*-3	7.12 ± 0.79	9.89 ± 0.40
C22:5*n*-3	0.30 ± 0.03	0.50 ± 0.03
C22:2*n*-6	0	0.52 ± 0.02
C22:1*n*-9	0.88 ± 0.04	0.29 ± 0.02
NFA	0.89 ± 0.08	0.88 ± 0.07
SFA	32.19 ± 2.18	31.98 ± 0.13
MUFA	38.56 ± 1.79	29.38 ± 0.12
PUFA	28.36 ± 1.17	37.76 ± 0.09

Values represent mean ± SD ($n = 3$).
[a] NFA, non-fatty acids; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

Table 3. Body weight (BW) gain and plasma total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and glucose levels in rats fed experimental diets

Parameter	Normal control (NC) group 0th week	4th week	High-cholesterol-fed (HCF) group 0th week	4th week
BW gain (g)		100.00 ± 9.49		123.21 ± 13.74$^{\#\#}$
TC (mmol L^{-1})	1.92 ± 0.21	1.54 ± 0.22	1.63 ± 0.22	2.30 ± 0.42$^{*,\#\#}$
LDL-C (mmol L^{-1})	0.17 ± 0.02	0.13 ± 0.04	0.14 ± 0.03	0.27 ± 0.06$^{*,\#\#}$
HDL-C (mmol L^{-1})	0.67 ± 0.07	0.56 ± 0.04*	0.63 ± 0.11	0.55 ± 0.12**
Glucose (mmol L^{-1})	3.39 ± 0.20	3.43 ± 0.48	3.62 ± 0.92	5.02 ± 1.05$^{*,\#\#}$

Values represent mean ± SD (six rats for NC group, 42 rats for HCF group). Significant differences between 0th week and 4th week were evaluated by paired-samples *t* test: $^{*}P < 0.05$; $^{**}P < 0.01$. Significant differences between NC and HCF groups after 4 weeks of treatment were evaluated by independent-samples *t* test: $^{\#\#}P < 0.01$.

Table 4. Effect of consumption of whole krill oil (WKO) and phospholipid-type krill oil (PKO) on body weight (g) of high-cholesterol-fed (HCF) rats

Group[a]	0th week	4th week	Body weight gain
NC	284.17 ± 12.81	319.17 ± 12.81	35.00 ± 8.94
BC	326.67 ± 14.72A	391.67 ± 24.83A	65.00 ± 13.04A
L-WKO	330.83 ± 15.30A	381.67 ± 7.53AB	50.83 ± 10.68B
M-WKO	323.33 ± 11.43AB	365.00 ± 16.83BC	41.67 ± 12.91B
H-WKO	323.33 ± 14.38AB	375.83 ± 21.08AB	52.50 ± 6.89AB
L-PKO	311.67 ± 14.02AB	352.50 ± 15.41C	40.83 ± 10.68B
M-PKO	317.52 ± 26.03AB	365.00 ± 26.83BC	47.50 ± 11.73B
H-PKO	305.83 ± 17.15B	351.07 ± 11.69C	45.83 ± 8.61B

Values represent mean ± SD (six rats for each group). Significant differences between different HCF groups were evaluated by one-way ANOVA (*post hoc* test: LSD): values in the same column with different letters are significantly different at $P < 0.05$.
[a] NC, normal control group; BC, blank control group; L-WKO, low-dose WKO group; M-WKO, mid-dose WKO group; H-WKO, high-dose WKO group; L-PKO, low-dose PKO group; M-PKO, mid-dose PKO group; H-PKO, high-dose PKO group.

rats decreased significantly ($P < 0.05$). Meanwhile, after 4 weeks of feeding, the BW gain and plasma levels of TC, LDL-C and glucose of HCF rats were significantly higher than those of NC group rats ($P < 0.01$), whereas the difference in plasma HDL-C level between HCF rats and NC group rats was not statistically significant. The results indicated that the HCF rats were suitable for *in vivo* assay of cholesterol- and glucose-lowering effects.

Effect of KO on body weight

As shown in Table 4, after 4 weeks of HCD, except for the H-PKO group, the difference in BW between other HCF groups was not statistically significant. In this situation, the effect of initial BW on BW gain could be excluded. After 4 weeks of treatment, the BW gains of BC, L-WKO, M-WKO, H-WKO, L-PKO, M-PKO and H-PKO group rats were 65.00 ± 13.04, 50.83 ± 10.68, 41.67 ± 12.91, 52.50 ± 6.89, 40.83 ± 10.68, 47.50 ± 11.73 and 45.83 ± 8.61 g respectively. In contrast, the BW gains of L-WKO, M-WKO, L-PKO, M-PKO and H-PKO group rats were significantly lower than that of BC group rats ($P < 0.05$). Zhu *et al.*[11] also found that the intake of KO significantly decreased the BW gain of high-fat-fed (HFF) rats ($P < 0.05$). However, Tandy *et al.*[7] reported that the intake of KO did not significantly decrease the BW gain of HFF mice. The difference in BW may affect serum lipid and serum lipoprotein. It has been reported that overweight or obesity always increases the risk of dyslipidaemia, which is characterised by increased TG levels, decreased HDL levels and abnormal LDL composition.[29] For humans, weight loss and exercise, even if they do not result in normalisation of BW, can improve this dyslipidaemia.[30]

Some studies on animals and humans have indicated that effects of long-chain *n*-3 PUFA on BW are independent of energy intake.[31–33] Tandy *et al.*[7] reported that the intake of KO did not affect the food intake of mice. Even so, the effect of food intake on BW gain could not be excluded, because the exact food intake was not recorded in this study. Besides suppression of appetite, LC *n*-3 PUFA may inhibit obesity through a number of potential effects, including improving circulation (which may facilitate nutrient delivery to skeletal muscle), changing gene expression (which may shift metabolism towards increased accretion of lean tissue), enhancing fat oxidation and energy expenditure and reducing fat deposition.[34]

Table 5. Effect of consumption of whole krill oil (WKO) and phospholipid-type krill oil (PKO) on plasma total cholesterol level (mmol L^{-1}) of high-cholesterol-fed (HCF) rats

Group[a]	0th week	4th week	Reducing rate (%)
NC	1.54 ± 0.22	1.18 ± 0.20	
BC	2.17 ± 0.23	2.03 ± 0.27	5.21 ± 11.28C
L-WKO	2.23 ± 0.28	1.54 ± 0.18**	29.58 ± 16.29AB
M-WKO	2.20 ± 0.43	1.50 ± 0.25**	30.50 ± 15.23AB
H-WKO	1.94 ± 0.28	1.54 ± 0.22**	19.97 ± 9.87BC
L-PKO	2.54 ± 0.40	1.52 ± 0.26**	39.68 ± 7.22A
M-PKO	2.64 ± 0.61	1.49 ± 0.15**	41.02 ± 14.31A
H-PKO	2.38 ± 0.30	1.48 ± 0.18**	37.60 ± 9.28A

Values represent mean ± SD (six rats for each group). Significant differences between 0th week and 4th week were evaluated by paired-samples *t* test: $^{**}P < 0.01$. Significant differences between different HCF groups were evaluated by one-way ANOVA (*post hoc* test: LSD): values in the same column with different letters are significantly different at $P < 0.05$.
[a]NC, normal control group; BC, blank control group; L-WKO, low-dose WKO group; M-WKO, mid-dose WKO group; H-WKO, high-dose WKO group; L-PKO, low-dose PKO group; M-PKO, mid-dose PKO group; H-PKO, high-dose PKO group.

Table 6. Effect of consumption of whole krill oil (WKO) and phospholipid-type krill oil (PKO) on plasma low-density lipoprotein cholesterol level (mmol L^{-1}) of high-cholesterol-fed (HCF) rats

Group[a]	0th week	4th week	Reducing rate (%)
NC	0.13 ± 0.04	0.11 ± 0.04	
BC	0.26 ± 0.06	0.25 ± 0.05	4.37 ± 20.23D
L-WKO	0.21 ± 0.04	0.16 ± 0.02*	22.81 ± 13.50CD
M-WKO	0.30 ± 0.16	0.14 ± 0.03*	49.01 ± 18.11AB
H-WKO	0.22 ± 0.09	0.14 ± 0.03*	36.42 ± 25.60BC
L-PKO	0.33 ± 0.11	0.13 ± 0.02**	56.32 ± 17.86A
M-PKO	0.36 ± 0.20	0.11 ± 0.04*	66.45 ± 15.03A
H-PKO	0.23 ± 0.06	0.12 ± 0.04**	48.07 ± 15.87AB

Values represent mean ± SD (six rats for each group). Significant differences between 0th week and 4th week were evaluated by paired-samples *t* test: $^{*}P < 0.05$; $^{**}P < 0.01$. Significant differences between different HCF groups were evaluated by one-way ANOVA (*post hoc* test: LSD): values in the same column with different letters are significantly different at $P < 0.05$.
[a]NC, normal control group; BC, blank control group; L-WKO, low-dose WKO group; M-WKO, mid-dose WKO group; H-WKO, high-dose WKO group; L-PKO, low-dose PKO group; M-PKO, mid-dose PKO group; H-PKO, high-dose PKO group.

Effect of KO on plasma levels of cholesterols

After 4 weeks, the plasma TC level of BC group rats was almost unchanged, whereas those of all KO group rats decreased significantly ($P < 0.01$) (Table 5). The reducing rates for BC, L-WKO, M-WKO, H-WKO, L-PKO, M-PKO and H-PKO group rats were 5.21 ± 11.28, 29.58 ± 16.29, 30.50 ± 15.23, 19.97 ± 9.87, 39.68 ± 7.22, 41.02 ± 14.31 and 37.60 ± 9.28% respectively. Except for the H-WKO group, all other KO groups showed significantly higher reducing rates than the BC group. The results indicated that WKO and PKO could reduce the plasma TC of hyperlipidaemic rats induced by HCD. Significant differences only existed between the PKO groups and the H-WKO group, despite higher reducing rates being observed in the PKO groups with respect to the WKO groups. Although the mid-dose group showed slightly higher reducing rate than the other two groups, no significant differences were observed between different doses among the WKO or PKO groups. Tandy *et al.*[7] and Zhu *et al.*[11] also found that the consumption of KO could significantly lower the serum TC level of HFF mice. In those two studies, the effects were not dose-dependent,[7,11] which was similar to our results.

After 4 weeks, the plasma LDL-C levels of all KO group rats decreased significantly ($P < 0.05$), whereas that of BC group rats was almost unchanged (Table 6). The reducing rates were 4.37 ± 20.23, 22.81 ± 13.50, 49.01 ± 18.11, 36.42 ± 25.60, 56.32 ± 17.86, 66.45 ± 15.03 and 48.07 ± 15.87% for BC, L-WKO, M-WKO, H-WKO, L-PKO, M-PKO and H-PKO group rats respectively. All KO groups except L-WKO showed significantly higher reducing rates than the BC group. The above results showed that WKO and PKO had the function of decreasing the plasma LDL-C of hyperlipidaemic rats induced by HCD. Although the PKO groups showed slightly higher reducing rates with respect to the WKO groups, significant differences only existed between the PKO groups and the L-WKO group. Meanwhile, the mid-dose group showed a slightly higher reducing rate than the other two groups among WKO or PKO groups, but a significant difference was only observed between the M-WKO group and the L-WKO group. Zhu *et al.*[11] also found that the serum LDL-C level of HFF rats decreased significantly after the intake of KO. However, the effects were also dose-independent.[11]

Apart from M-WKO group rats, the plasma HDL-C levels of all other group (including NC group) rats decreased significantly after 4 weeks ($P < 0.05$) (Table 7). Masella *et al.*[35] reported that the plasma HDL-C level of Wistar rats showed age-related changes. Generally, HDL1 increased with age while HDL2 and HDL3 decreased with age. HDL2 and HDL3 are major fractions, and their change may reflect the overall change in HDL. The reducing rates were 33.74 ± 7.03, 23.01 ± 16.57, −1.69 ± 14.22, 26.31 ± 12.14, 29.87 ± 8.22, 29.14 ± 17.13 and 26.95 ± 19.37% for BC, L-WKO, M-WKO, H-WKO, L-PKO, M-PKO and H-PKO group rats respectively. The results indicated that only M-WKO could inhibit the decline in plasma HDL-C level. Zhu *et al.*[11] found that the intake of KO did not change the serum HDL-C level of HFF rats.

After 4 weeks, the changes in plasma glucose level of BC and HCF group rats were not statistically significant (Table 8). The reducing rates were −17.88 ± 17.45, 14.66 ± 33.89, 25.34 ± 32.51, 17.90 ± 27.80, 8.50 ± 32.00, 15.66 ± 21.73 and −19.15 ± 23.60% for BC, L-WKO, M-WKO, H-WKO, L-PKO, M-PKO and H-PKO group rats respectively. Apart from L-PKO and H-PKO, all other KO groups showed significantly higher reducing rates than the BC group ($P < 0.05$). Tandy *et al.*[7] found that dietary KO supplementation significantly reduced the serum glucose level of HFF mice.

The *n*-3 PUFA, especially EPA and DHA, may be responsible for the cholesterol- and glucose-lowering effects of KO. A recent review article summarised that *n*-3 PUFA mainly exert their lipid-lowering effect through extensive regulation of lipid metabolism by inhibiting lipogenesis, promoting lipolysis and fatty acid oxidation and suppressing preadipocyte differentiation.[36] Martín de Santa Olalla *et al.*[37] summarised that *n*-3 PUFA mainly achieve their glucose-lowering function by improving insulin sensitivity. As described above, PKO contained more *n*-3 PUFA (particularly EPA and DHA) than WKO, which may contribute to its better lowering effects on plasma TC and LDL-C levels.

In addition to PUFA, two other components of KO, PL and astaxanthin, also showed lipid- and glucose-lowering effects in experimental animals.[7] Tandy *et al.*[7] found that PL containing SFA

Table 7. Effect of consumption of whole krill oil (WKO) and phospholipid-type krill oil (PKO) on plasma high-density lipoprotein cholesterol level (mmol L^{-1}) of high-cholesterol-fed (HCF) rats

Group[a]	0th week	4th week	Reducing rate (%)
NC	0.56 ± 0.04	0.38 ± 0.03	
BC	0.56 ± 0.07	0.38 ± 0.06**	33.74 ± 7.03A
L-WKO	0.56 ± 0.15	0.42 ± 0.058*	23.01 ± 16.57AB
M-WKO	0.42 ± 0.06	0.42 ± 0.05	−1.69 ± 14.22C
H-WKO	0.48 ± 0.10	0.39 ± 0.07**	26.31 ± 12.14B
L-PKO	0.59 ± 0.26	0.41 ± 0.06**	29.87 ± 8.22AB
M-PKO	0.61 ± 0.09	0.42 ± 0.05*	29.14 ± 17.13AB
H-PKO	0.61 ± 0.12	0.43 ± 0.07*	26.95 ± 19.37AB

Values represent mean ± SD (six rats for each group). Significant differences between 0th week and 4th week were evaluated by paired-samples *t* test: $^{*}P < 0.05$; $^{**}P < 0.01$. Significant differences between different HCF groups were evaluated by one-way ANOVA (*post hoc* test: LSD): values in the same column with different letters are significantly different at $P < 0.05$.
[a] NC, normal control group; BC, blank control group; L-WKO, low-dose WKO group; M-WKO, mid-dose WKO group; H-WKO, high-dose WKO group; L-PKO, low-dose PKO group; M-PKO, mid-dose PKO group; H-PKO, high-dose PKO group.

Table 8. Effect of consumption of whole krill oil (WKO) and phospholipid-type krill oil (PKO) on plasma glucose level (mmol L^{-1}) of high-cholesterol-fed (HCF) rats

Group[a]	0th week	4th week	Reducing rate (%)
NC	3.43 ± 0.48	3.22 ± 1.25	
BC	4.07 ± 0.77	4.71 ± 0.44	−17.88 ± 17.45B
L-WKO	5.06 ± 1.13	4.01 ± 1.02	14.66 ± 33.89A
M-WKO	5.90 ± 1.53	4.16 ± 1.16	25.34 ± 32.51A
H-WKO	5.39 ± 1.05	4.20 ± 0.86	17.90 ± 27.80A
L-PKO	4.97 ± 0.79	4.38 ± 1.05	8.50 ± 32.00AB
M-PKO	4.72 ± 0.63	3.98 ± 0.78	15.66 ± 21.73A
H-PKO	4.64 ± 0.57	5.43 ± 0.65	−19.15 ± 23.60B

Values represent mean ± SD (six rats for each group). Significant differences between 0th week and 4th week were evaluated by paired-samples *t* test. Significant differences between different HCF groups were evaluated by one-way ANOVA (*post hoc* test: LSD): values in the same column with different letters are significantly different at $P < 0.05$.
[a] NC, normal control group; BC, blank control group; L-WKO, low-dose WKO group; M-WKO, mid-dose WKO group; H-WKO, high-dose WKO group; L-PKO, low-dose PKO group; M-PKO, mid-dose PKO group; H-PKO, high-dose PKO group.

also had pronounced lipid-lowering properties. In this situation, they suggested that PL *n*-3 PUFA (found in KO) may be more efficacious than TG *n*-3 PUFA, despite a lack of direct evidence. Meanwhile, some studies on humans and animals indicated that PL FA had better bioavailability than TG FA.[17,18] The better bioavailability may also contribute to the better physiological functions of PL *n*-3 PUFA. PKO contained more PL than WKO, which may also account for its better lowering effects on plasma TC and LDL-C levels.

CONCLUSION

WKO and PKO were extracted from *E. superba* using hexane and ethanol respectively. PKO contained higher levels of PL and *n*-3 PUFA (particularly DHA and EPA) than WKO. A preliminary study on HCD-fed Wistar rats indicated that the consumption of WKO and PKO reduced BW gain and plasma TC and LDL-C levels. In contrast, PKO showed better functions than WKO, which may be due to its higher *n*-3 PUFA and PL levels. The exact mechanism behind the bioactivity of KO deserves further study.

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