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Dietary krill oil (*Euphausia superba*) alleviates oxidative stress and DNA damages bio-markers in an experimental model of cafeteria diet-induced insulin resistance in rats

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Abstract

Chronic exposure to cafeteria-based diet has been shown to exert a number of adverse metabolic effects in both human and experimental studies. Indeed, krill oil has been the subject of extensive investigation regarding its possible beneficial effects on insulin resistance-related disorders. The present experiment aims to investigate the therapeutic effect of krill oil (*Euphausia superba*) in the modulation of metabolic disorders, oxidative stress and DNA oxidative damage markers in an experimental model of cafeteria diet-induced insulin resistance. A total of 30, 8-week male Wistar rats were divided into three equal groups: the control diet group (Control), the cafeteria diet group (CAF), and the cafeteria diet group enriched with krill oil at 2% (CAF-KO). After 8 weeks of the experiment, weight gain, adiposity index as well as plasma glucose, insulin, cholesterol and triglycerides were assayed. Insulin resistance was estimated using homeostasis model assessment (HOMA). In parallel, plasma and target tissues' (liver, pancreas, adipose tissue and muscle) pro-oxidant status were assessed by assaying thiobarbituric acid reactive substances (TBARS), lipid hydroperoxides (LPO), and isoprostanes (8-isoprostanes). The DNA oxidative damage was evaluated through measurement of the main product of its oxidation (8-OHdG). The 8-week cafeteria diet led to obesity, insulin resistance and dyslipidemia. Furthermore, this obesogenic diet increased the metabolic response to radical attack and DNA oxidation in both plasma and key tissues. Dietary krill oil displays remarkable health benefits by improving insulin resistance and dyslipidemia, which acts by preserving antioxidant mechanisms and protecting cellular components such as DNA and lipids from oxidative damage.

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Introduction

The link between health and food is more relevant than ever. Indeed, recent research confirms the important role of diet in the prevention of cardiovascular diseases and their risk factors, such as diabetes and obesity, which are a serious public health problem (Steinmetz and Potter, 1996) associated with metabolic abnormalities as a result of an overproduction of reactive oxygen species (ROS) (Bonnefont-Rousselot, 2013) likely to attack cellular targets promoting oxidative damage to lipids, proteins, nucleic acids, and can lead in particular to endothelial dysfunction and inflammatory processes (Hulsmans *et al.,* 2012). The association between the consumption of seafood and the beneficial effects on cardiovascular health has been reported in several epidemiological studies and clinical trials (Harris *et al.,* 2008; Eslick *et al.,* 2009). These effects are mainly attributed to their richness in long chain polyunsaturated fatty acids (n-3 PUFAs), especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), due to their anti-inflammatory, and antioxidant properties (Mas *et al.,* 2010; Richard *et al.,* 2009). However, fish with remarkable nutritional benefits are at present a limited resource due to the advent of new intensive fishing methods on the one hand, and the erosion of marine biodiversity on the other. Consequently, of increasing interest is the exploration and exploitation of new natural and alternative sources of antioxidant micronutrients that are readily available and can be harvested at low cost to facilitate the widespread production of food supplements fortified with antioxidant micronutrients (Shahidi, 2012).

Antarctic krill (*Euphausia superba*), a small crustacean found in the Southern Ocean, is the most abundant eukaryotic species in the oceans, whose biomass is estimated at 125–700 million tons (Fao, 2013) and is thus a convenient catch for commercial fisheries. Unlike traditional supplements based on omega-3 fatty acid compounds bound to triglycerides (fish oil and cod liver oil), or related to ethyl esters such as lipid-lowering drugs, krill oil has a high content of omega-3 fatty acids bound to phospholipids.

This form provides better bioavailability of n-3 PUFA, as well as their enhanced distribution in the various organs and their incorporation into cell membranes (Di Marzo, 2010). Furthermore, krill oil contains a carotenoid pigment of the xanthophylls astaxanthin family, with remarkable antioxidant activity acting in synergy with the EPA and DHA (Ulven, 2011). These effects are primarily mediated by increased production of antioxidant enzymes and the inhibition of the transcription of enzymes involved in the production of reactive oxygen species and nitrogen (free radicals) (Lay-Saw *et al.,* 2013).

The aim of this experimental study in a nutritional obesity model is to examine the effects of krill oil supplementation on markers of lipid peroxidation and oxidative DNA damage.

Materials and methods

Experimental protocol

The study was conducted on thirty male Wistar rats (Charles River Laboratories, France) aged 8 weeks and weighing 200 ± 20 g, kept in a cage maintained at a constant temperature of 24° C and a humidity of 60 ± 5% with 12 h of light provided daily. The animals had free access to water and food, while the Council of European Communities' advice regarding the protection and use of laboratory animals was followed (Council of European Communities, 1987).

Obesity was induced by a cafeteria-based diet, according to Darimont *et al.* (2004). This high caloric and hyperlipidic diet induced hyperphagia followed by obesity, with fast and quick installation in the Wistar rat model. The animals were divided into three groups of ten rats, with each group receiving one of the following diets over an 8 week period:

The control group received commercial standard chow (330 kcal/100 g), consisting of 5% fat, 25% protein and 70% carbohydrate.

The cafeteria group (CAF) consumed a cafeteria diet (420 kcal/100 g), composed of a standard 30 g mixture of sausage, biscuits, cheese, chips, chocolate, and peanuts in the respective proportions of 2: 2: 2: 1: 1: 1, according to Darimont *et al* (2004). This diet contained 26% protein, 27% carbohydrate and 47% fat.

The final CAF-KO group was submitted to the cafeteria diet enriched with 2% krill oil (Aker BioMarine ASA, Norway), administered by esophageal overfeeding.

Sacrifice, blood and organ collection
After 8 weeks on the diet, the rats from the three groups were weighed, anesthetized after 12 h of fasting by intraperitoneal injection of sodium pentobarbital solution 6.5% (0.1 ml/100 g body weight) and sacrificed. Blood was collected by abdominal aorta puncture in the presence of heparin and centrifuged for 10 min at 3000 rpm at 4 °C. The recovered plasmas were used on the same day of sacrifice for the biochemical parameter analyses of lipid peroxidation and oxidative DNA damage. In parallel, the organs such as the liver and pancreas, the visceral adipose tissue and gastrocnemius muscle were carefully collected, rinsed with 0.9% NaCl, dried and immediately weighed and stored at -70 °C for subsequent assays.

Biochemical parameter analysis
Plasma glucose levels were measured by enzymatic method according to Lowry and Passonneau (1972). The insulin concentration was determined by Mercodia Elisa kit (Cat No. 10125001) following the standard procedures. Commercial enzymatic colorimetric kits were utilized for the determination of plasma total cholesterol (TC) and triglycerides (TG) levels (Boehringer, Mannheim, Germany).

Pro-oxidant markers assay
The lipid peroxidation was estimated by measuring the thiobarbituric acid reactive substances (TBARS), the lipid hydroperoxides (LPO) and the isoprostanes (8-isoprostanes) content. The plasma TBARS levels were measured according to Ke and Woyewoda's (1979) method, and on the organs according to Genot's (1996) technique. Plasmatic and tissular LPO concentrations were determined according to the technique described in Eymard and Genot (2003).

The plasma and tissular 8-isoprostanes content was assayed by an Elisa kit (Abbexa Chemical, Cat No. 055154). Meanwhile, DNA oxidative damage was evaluated by measurement of the 8-hydroxy-2'-deoxyguanosine (8-OHdG) level in the plasma and studied tissues using an Elisa kit (Biorbyt, Cat No. 52606).

Statistical analysis
Statistical analysis was performed with Statistica for Windows (Version 5.1, Statsoft 97). All data are expressed as the mean ± SD of 10 rats per group.

After variance analysis, a single comparison of the two experimental groups' means was assessed by the Student's t-test. The means with symbols (*,#) were significantly different at $P < 0.05$ (* Control vs. CAF; # CAF vs. CAF-KO).

Results
Weight gain, adiposity index, glucose and lipid homeostasis
After 8 weeks of experiment, those rats submitted to the cafeteria diet (CAF) exhibited a significant increase in weight gain ($P < 0.0001$), as well as adiposity index ($P = 0.02$), compared to the rats in the Control group (Table.1). However, following supplementation with krill oil the rats in the CAF-KO group saw a significant decrease in weight gain (42%) and adiposity index (21%). Moreover, substitution of the standard diet with the CAF diet increased the insulinogenic index ($P = 0.01$) and HOMA-IR ($P = 0.0006$) significantly. Meanwhile, the CAF-KO group rats showed a reduction of the insulinogenic index (20%) and HOMA-IR (29%) compared to the Control group. The results of the lipid parameters showed an increase in plasma TC (80%) and TG (80%) content in the CAF rats compared to the Control group; whereas, dietary krill oil enrichment induced a significant decrease in TC ($P = 0.0004$) and TG ($P = 0.001$) concentrations in the CAF-KO group (Table. 1).

Table 1. Metabolic and hormonal data in control and experimental rats at sacrifice.

	Control	CAF	CAF-KO
Initial weight (g)	208 ± 2.3	205 ± 2.2	202 ± 2.8
Final weight (g)	282 ± 1.8	420 ± 3.8*	325 ± 1.6#
Weight gain (%)	36 ± 1.5	105 ± 2.5*	61 ± 1.8#
Visceral adipose weight (g)	6.45 ± 0.12	9.78 ± 0.18*	7.66 ± 0.15#
Muscle weight (g)	0.27 ± 0.01	0.32 ± 0.01*	0.30 ± 0.02
Adiposity index	22.5 ± 2.05	30.6 ± 2.18*	24.1 ± 1.15#
Plasma glucose (mM)	7.05 ± 0.20	8.16 ± 0.15*	7.35 ± 0.18#
Plasma insulin (ng / ml)	0.88 ± 0.10	1.25 ± 0.12*	1.05 ± 0.10
Insulinogenic index (µg / mol)	120 ± 8	162 ± 12*	130 ± 6#
HOMA-IR (mM.ng / ml)	6.72 ± 0.32	10.45 ± 0.69*	7.42 ± 0.45#
TC (mmol / L)	2.36 ± 0.12	4.25 ± 0.18*	3.18 ± 0.10#
TG (mmol / L)	2.15 ± 0.10	3.65 ± 0.16*	2.72 ± 0.15#

Data are expressed as the mean ± SD of 10 rats per group. After variance analysis, a single comparison of the means of the two experimental groups was assessed by the Student's t-test. The means with symbols (*,#) are significantly different at P < 0.05 (* Control vs. CAF, # CAF vs. CAF-KO).

Adiposity index: visceral adipose tissue weight/muscle weight (g /g)

Insulinogenic index: ratio between plasma insulin and glucose concentrations

HOMA-IR (insulin resistance index): product of glucose and insulin plasma levels.

Markers of oxidant status and DNA oxidative damage

Analysis of the lipid peroxidation and DNA oxidative markers shows that the plasma TBARS, LPO, 8-isoprostanes and 8-OHdG concentrations are significantly higher in the CAF group compared to the Control group, with a degree of significance of P < 0.0001 (Table. 2). However, supplementation with krill oil in the obese rats involved decreased levels of the plasma TBARS, LPO, 8-isoprostanes and 8-OHdG by 24%, 50%, 21% and 50%, respectively.

Table 2. Plasma markers of oxidant status and DNA oxidative damage in control and experimental rats.

	Control	CAF	CAF-KO
TBARS (µmol Eq MDA/l)	18.50 ± 1.2	32.25 ± 1.3*	24.68 ± 0.8#
LPO (µmol / l)	28.17 ± 0.25	42.33 ± 1.22*	36.22 ± 0.56#
8-isoprostanes (pg / ml)	124 ± 8.5	185 ± 12*	146 ± 9#
8-OHdG (pg / ml)	1.1 ± 0.1	3.6 ± 0.3*	1.8 ± 0.2#

Data are expressed as the mean ± SD of 10 rats per group. After variance analysis, a single comparison of the means of the two experimental groups was assessed by the Student's t-test. The means with symbols (*,#) are significantly different at P < 0.05 (* Control vs. CAF, # CAF vs. CAF-KO).

TBARS: thiobarbituric acid reactive substances; LPO: lipid hydroperoxides; 8-OHdG: 8-hydroxydeoxyguanosine.

Tissue TBARS, LPO, 8-isoprostanes and 8-OHdG content

A rise in the levels of TBARS (52%, 58%, 150%, 87%), LPO (135%, 117%, 66%, 70%), 8-isoprostanes (83%, 56%, 45%, 34%) and 8-OHdG (60%, 93%, 150%, 163%) is reported in the liver, pancreas, visceral adipose tissue and muscle, respectively, in the CAF group compared to the Control (Fig.1). Correspondingly, supplementing the CAF rats' diet with krill oil induced a reduction in TBARS

concentration (1.2, 1.5, 2, 1.6, -times), LPO (2, 1.8, 1.6, 1.7, times) in the liver, pancreas, visceral adipose tissue and muscle, respectively, and 8-isoprostanes with average reduction of (2.5 times) in the latter tissues.

The results of the analysis of target tissue DNA oxidation reveal a decrease in 8-OHdG levels in the liver and pancreas (1.5 times) and in the adipose tissue and muscle (2 times) in the obese rats supplemented with krill oil (Fig. 1).
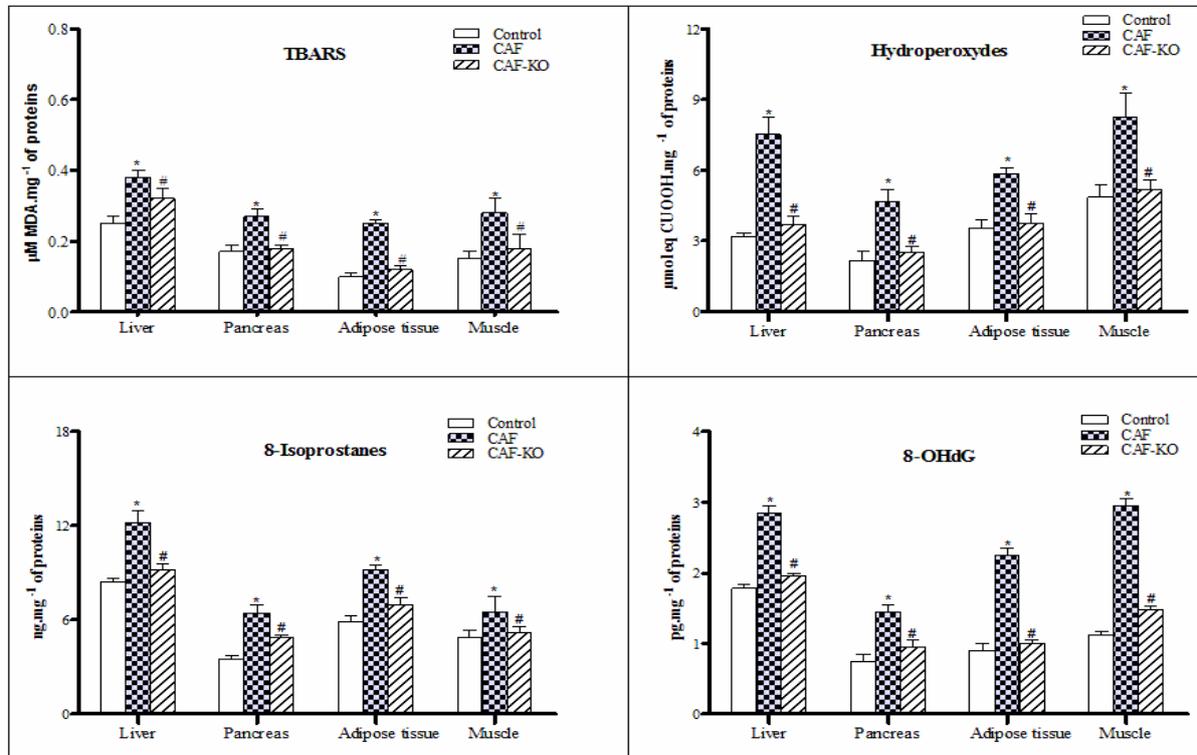

Fig. 1. Variations in tissue markers content of lipid peroxidation and DNA oxidative in experimental and control rats.

Data are expressed as the mean ± SD of 10 rats per group. After variance analysis, a single comparison of the means of the two experimental groups was assessed by the Student's *t*-test. The means with symbols (*,#) are significantly different at $P < 0.05$ (* Control vs. CAF, # CAF vs. CAF-KO).

Discussion

This study was conducted an animal model of nutritional obesity induced by the consumption of a cafeteria diet (Darimont *el al.,* 2004), in which we tested the effects of dietary krill oil naturally enriched in n-3 PUFA and antioxidants on the oxidative stress and oxidative DNA damage markers in obese rats. Our results show that in response to the cafeteria diet, the rats developed obesity characterized by hyperglycemia and dyslipidemia. Indeed, this obesogenic diet induces overeating, increased weight gain, and an alteration in carbohydrate and lipid homeostasis, all associated with increased insulin resistance (Flachs, 2005; Castro *et al.,* 2015). In addition, this diet stimulates lipogenesis in visceral

adipose tissue, favoring a chronic inflammatory state (Gauvreau *et al.,* 2011).

Our results show that supplementing obese rats' diet with krill oil induced a significant loss of body weight by reduction in fat mass and decrease in fat accumulation in the visceral adipose tissue. In addition, the krill oil included hypoglycemic effects reflecting an improvement in insulin sensitivity, and antilipemic effects characterized by a decrease in plasma TC and TG content, attributed to a reduction or an inhibition of liver and adipose tissue lipogenesis and a synthesis of the circulation of very low density lipoproteins (VLDL), resulting in a hypoglycemic effect or a stimulation of lipolysis (Oliver *et al.,* 2013).

These results demonstrate the benefits of krill oil in obese rats in terms of enhanced carbohydrate homeostasis and dyslipidemia management.

It is well established that oxidative stress is involved in cardiovascular complications associated with obesity. This can damage the cells and tissue not only by a direct attack of biomolecules (carbohydrates, proteins, phospholipids or DNA), but also via the production of the many by-products resulting from their degradation (Ramprasath *et al.,* 2013; Gardès-Albert *et al.,* 2013).

Our results highlight an increase in oxidative stress in the CAF group as compared to the Control by increased markers of lipid peroxidation TBARS, LPO and 8-isoprostanes, and oxidative damage of DNA in the plasma and target tissues (liver, pancreas, visceral adipose tissue and gastrocnemius muscle) compared to the Control group.

This oxidative stress is partly explained by hyperglycemia, the main source of reactive oxygen species production, hyperinsulinemia, lipogenesis and the reduction of antioxidant defenses (Grimstad *et al.,* 2012; Franco *et al.,* 2013).

Our study shows that the CAF-KO diet induced a decrease in lipid peroxidation and DNA oxidative damage markers in the plasma and studied target tissues compared to the CAF group. The reduction of lipid peroxidation and 8-OHdG induced an increase of tissular enzyme antioxidant defense, mediated by the combined action of the n-3 PUFA and astaxanthin contained in krill oil (Santocono *et al.,* 2006). Indeed, it has been shown that krill oil is able to limit the proteins, DNA and lipids' oxidative damage (Tripathi and Jena, 2009), and increase the activity and expression of tissue antioxidant enzymes (CAT, GSH-Px, and SOD) involved in the neutralization of free radicals caused by an obesogenic diet (Franco *et al.,* 2013).

Conclusion

Our study opens a path in understanding of metabolic and oxidative abnormalities observed in experimental obesity induced by the cafeteria diet, and highlights the benefits of krill oil supplementation in obese rats on the modulation of insulin resistance and atherogenic dyslipidemia, on the one hand, and the oxidative stress and DNA damage on the other. Krill oil offers the excellent bioavailability of antioxidant micronutrients and holds promising therapeutic benefits, justifying its value as a functional food in the severity reduction of the metabolic and oxidative alterations related to obesity.

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