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Dietary Calanus oil antagonizes angiotensin II-induced hypertension and tissue wasting in diet-induced obese mice

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ABSTRACT

Background: We have recently shown that Calanus oil, which is extracted from the marine copepod *Calanus finmarchicus*, reduces fat deposition, suppresses adipose tissue inflammation and improves insulin sensitivity in high fat-fed rodents. This study expands upon our previous observations by examining whether dietary supplementation with Calanus oil could antagonize angiotensin II (Ang II)-induced hypertension and ventricular remodeling in mice given a high fat diet (HFD).
Methods: C57BL/6J mice were initially subjected to 8 weeks of HFD with or without 2% (w/w) Calanus oil. Thereafter, animals within each group were randomized for the administration of either Ang II (1 μg/kg/min) or saline for another two weeks, while still on the same dietary regimen.
Results: Ang II caused a marked decline in body and organ weights in mice receiving non-supplemented HFD, a response which was clearly attenuated in mice receiving Calanus oil supplementation. Furthermore, Ang II-induced elevation in blood pressure was also attenuated in the Calanus oil-supplemented group. As expected, infusion of Ang II produced hypertrophy and up-regulation of marker genes (mRNA level) of both hypertrophy and fibrosis in cardiac muscle, but this response was unaffected by dietary Calanus oil. Fibrosis and inflammation were up-regulated also in the aorta following Ang II infusion. However, the inflammatory response was blocked by Calanus oil supplementation. A final, and unexpected, finding was that dietary intake of Calanus oil caused a robust increase in the level of O-GlcNAcylation in cardiac tissue.
Conclusion: These results suggest that dietary intake of oil from the marine copepod *Calanus finmarchicus* could be a beneficial addition to conventional hypertension treatment. The compound attenuates inflammation and the severe metabolic stress caused by Ang II infusion. Although the present study suggests that the anti-hypertensive effect of the oil (or its n-3 PUFAs constituents) is related to its anti-inflammatory action in the vessel wall, other mechanisms such as interaction with intracellular calcium mechanisms or a direct antagonistic effect on Ang II receptors should be examined.
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1. Introduction

Visceral obesity is associated with a chronic, low-grade inflammation in adipose tissue with macrophage infiltration and increased production of inflammatory cytokines [1–3]. The release of cytokines from adipose tissue results in a systemic inflammation [3], which has been implicated in cardiovascular disease [4], such as endothelial dysfunction, hypertension, cardiac hypertrophy, atherosclerosis [4,5], as well as insulin resistance and its metabolic disorders [6]. Dietary intake of n-3 poly-unsaturated fatty acids (n-3 PUFAs) has received considerable attention as a preventive measure, based on a large number of experimental studies (including both cellular and animal models), demonstrating their potent anti-inflammatory action [7,8] and favorable effects on the cardiovascular system [9–11]. Epidemiological and clinical trials suggest that the anti-inflammatory properties of marine n-3 PUFAs might also confer cardioprotection in humans, although some controversy remains as to the efficacy of these fatty acids on reducing myocardial infarction, arrhythmia, cardiac and sudden death, or stroke [12–15].

A novel source of EPA and DHA for human consumption is oil from the marine copepod *Calanus finmarchicus*, which is the most abundant crustacean in the North Atlantic Ocean with annual

Abbreviations: Ang II, Angiotensin II; BP, Blood pressure; HFD, High fat diet; PUFAs, Polyunsaturated fatty acids

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production of several hundred million tonnes [17]. *Calanus finmarchicus* is harvested off the coast of Norway during early summer, using newly developed technology. The catch is pumped on board the fishing boat and immediately frozen for further land-based processing. The total annual harvest amounts to less than 0.01% of the annual growth in accordance with regulations by Norwegian fisheries management. The oil extracted from *C. finmarchicus* is ruby colored and slightly viscous, with $>86\%$ of the fatty acids present as wax esters bound predominantly to aliphatic long-chain monounsaturated alcohols [mostly 20:1(n-9) and 22:1 (n-11) alcohols], with minor amounts of free fatty acids, free fatty alcohols, and glycerides. The oil from *C. finmarchicus* also contains components that are not found, or found in very small quantities, in the majority of other marine oils, such as phytosterols and antioxidants (specifically, astaxanthin).

Obesity-related endothelial dysfunction and hypertension are associated with activation of the renin–angiotensin–aldosterone system, inflammation, reduced insulin metabolic signaling, and reduced nitric oxide production [16,17]. We recently reported that dietary supplementation with Calanus oil reduced intra-abdominal and hepatic fat deposition in mice during high-fat feeding [18,19]. In addition, the inflammatory level was markedly reduced, while systemic glucose tolerance was improved.

The current study expands upon our previous observations by examining the impact of Calanus oil on hypertension and cardiac remodeling in diet-induced obese mice challenged with two weeks of Ang II infusion in order to induce cardiovascular stress with hypertension.

Protein O-GlcNAcylation has been implicated in mediating many of the unfavorable effects of obesity, such as inflammation, oxidative stress and insulin resistance [20]. On the other hand, this process has also been implicated in mitigating the effect of Ang II on the development of cardiac hypertrophy [21,22]. Thus, we also examined the effect of Calanus oil treatment on myocardial protein O-GlcNAcylation in this model of both metabolic and cardiovascular stress.

In the present study we show for the first time that dietary Calanus oil has protective effects on the cardiovascular system in obese mice by preventing the rise in systolic and diastolic blood pressure following acute exposure to Ang II. In addition, Ang II-induced tissue wasting (cachexia) was significantly reduced in mice receiving dietary Calanus oil.

2. Materials and methods

2.1. Animals and study design

Diet-induced obese male mice were obtained by feeding C57BL/6J mice (Charles Rivers, Sulzfeld, Germany) a lard-based high fat diet (HFD #58V8, Test Diet, IPS Ltd, Notts, UK) containing 18%, 36% and 46% energy from protein, carbohydrate and fat, respectively. The mice (5–6 week old at the start of the feeding period) were randomly divided in two groups, one receiving HFD supplemented with 2% (w/w) Calanus oil (HFD+CAL), while the other received no supplementation (HFD). Addition of Calanus oil was compensated for by the removal of 2 g lard/100 g diet, so that the total fat content was unchanged and the diets remained isoenergetic. After an initial 8 weeks feeding period, both groups were further sub-divided into two groups, receiving 1 μg/kg/min Ang II (Calbiochem, Dramstadt, Germany) or saline for another two weeks via mini osmotic pumps (Alzet Micro-Osmotic Pump Model 1004, DURECT Corporation, ALZET Osmotic Pumps, Cupertino, CA, USA), while on the same dietary regimen. Body weight and blood pressure were measured weekly during the initial 8 weeks and every three days after Ang II administration. It should be noted that Ang II resulted in a marked reduction in body weight, and in some cases the weight loss was higher than 20%, resulting in sacrifice before the end of the experiment (humane endpoint).

The mice were treated in accordance to the guidelines on accommodation and care of animals formulated by the European Convention for the Protection of Vertebrate Animals for Experimental and Other Scientific purposes. All experiments were approved by the local authority of the National Animal Research Authority in Norway (FOTS id 4438/2012). All mice received food ad libitum, had free access to drinking water and were housed at 21 °C on a reversed light/dark cycle with 3–4 animals per cage.

At the end of the experiment, mice were killed by an overdose of pentobarbital, organs were carefully dissected out, weighed and either snap-frozen in liquid nitrogen or stored in RNA-protecting agents for later analysis.

2.2. Blood pressure measurement

Blood pressure (BP) was measured in conscious animals using the tail-cuff method (Kent Scientific, CODA- Torrington, CT, USA) [23]. A non-invasive tail-cuff method was chosen to include a large number of animals. Feng et al. [24] validated the volume-pressure recording (VPR) tail-cuff method by comparison to simultaneous radio-telemetry measurements and concluded that it provides accurate measurements over the physiological range of BP in mice. Furthermore, this method offers the highest degree of correlation with telemetry and catheter based direct BP measurements and is clearly the preferred tail-cuff sensor technology [25].

Mice were accustomed to the BP measurements during the first two weeks of the feeding period, and from week 3 BP was measured weekly under strictly controlled conditions, avoiding any external disturbance. A positive heat balance (in order to secure adequate tail perfusion) of the mice was maintained using a heated platform. BP recordings were based on 5 acclimatization cycles and 15 BP measurements and were accepted if the computer identified $>50\%$ successful readings based on predefined values for area under the pressure-volume curve created during release of pressure in tail cuff. BP increased following Ang II administration and reached a plateau after approximately one week. Towards the end of the two weeks treatment period, however, some of the animals showed a decline in BP. Due to this observation, as well as the loss of animals during the terminal phase, the BP values given in Figs. 1–4 are based on measurements performed on three consecutive days after BP had plateaued (day 6, 9 and 12).

2.3. Quantitative real-time PCR

mRNA expression in heart and aortic tissue (descending aorta) was determined using quantitative real-time PCR (qPCR). Samples were immersed in RNA later (Qiagen Hilden, Germany). Total RNA was extracted according to the RNeasy Fibrous Tissue kit Protocol (Qiagen, Hilden, Germany). cDNA was prepared using High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). qPCR was performed in an ABI PRISM 7900 HT Fast real-time thermal cycler as previously described [26]. Fast SYBR© Green master mix (Applied Biosystems, Foster City, CA, USA) was used. Based on its real-time efficiency and the Ct differences (Δ) between the different treatment groups the relative expression ratio of the target gene was calculated. The expression of the target genes was normalized to the most stable reference gene (GADPH, Cyclophilin, HPRT) based on testing by GeNorm [27] of possible reference genes, as described by others [28]. Primer sequences are shown in supporting data (Table S1).

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