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 polyunsaturated 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...
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 acids, and glycrides. 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 RNasy 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).
2.4 Immunoblotting

Heart tissue was homogenized in T-PER buffer (Thermo Scientific, Rockford, IL, USA) containing protease (Roche, Norway) and phosphatase (Sigma, Norway) inhibitor cocktail. OGA inhibitor PUGNAc (250 μM, Sigma, Norway) was added to prevent the removal of O-GlcNAc as previously described [29]. Proteins were separated on 4-15% SDS- polyacrylamide gradient gel (Criterion TGX, Bio-Rad, Hercules, CA, USA) and transferred to PVDF membrane (Immobilon, Millipore, Bedford, MA, USA). Membranes were probed with primary antibodies directed against O-GlcNAc (RL-2, ab2739, Abcam, USA) or GAPDH (sc-32233, Santa Cruz Biotechnology, USA) as a loading control. Blots were visualized with enhanced chemiluminescence (Sigma, Norway) and the signal was detected with a Kodak imaging system (Kodak 1D Image Analysis Software, Scientific Imaging Systems, Rochester, NY, USA).

2.5 Statistical analysis

Values of blood pressure are presented as mean ± SD, whereas other data are given as mean ± SEM. Differences between HFD and HFD + CAL mice in body weight development during the initial 8 wk feeding period (Fig. 1a) were evaluated statistically by repeated measures ANOVA (Sigma Plot 13.0), whereas group differences in body and organ weights, blood pressure and gene expression after Ang II/saline infusion were analyzed by two-way ANOVA (with Holm-Sidak method as post-hoc test). Overall significance level was 0.05.

3 Results

3.1 Effect of Calanus oil on Ang II-induced changes on body and organ weight

In accordance with our previous observation [19], body weight gain over the initial 8 weeks feeding period was significantly attenuated in mice receiving dietary Calanus oil supplementation (HFD + CAL), as compared to mice receiving non-supplemented HFD (Fig. 1a). Following Ang II infusion, however, we observed a rapid decline in body weight, which was much more prominent in HFD compared to HFD + CAL mice (12% vs 6%, Fig. 1b). We also observed a small (3%), but statistically significant, decline in body weight in the HFD-fed group receiving saline, which could be related to stress caused by frequent blood pressure recordings over the last two weeks of the experiment. Interestingly, this decline in body weight was not seen in the HFD + CAL group.

The weights of perirenal and epididymal fat pads were reduced in saline-treated mice which had received dietary supplementation with Calanus oil, compared to mice that received non-supplemented HFD (Fig. 2). Membranes were probed with primary antibodies directed against O-GlcNAc (RL-2, ab2739, Abcam, USA) or GAPDH (sc-32233, Santa Cruz Biotechnology, USA) as a loading control. Blots were visualized with enhanced chemiluminescence (Sigma, Norway) and the signal was detected with a Kodak imaging system (Kodak 1D Image Analysis Software, Scientific Imaging Systems, Rochester, NY, USA).

Fig. 1. Body weight changes in high fat-fed mice before and after angiotensin II exposure. (a) Changes in body weight in mice receiving high fat diet either with no supplementation (HFD) or supplemented with 2% (w/w) Calanus oil (HFD + CAL) over the initial 8 wk feeding period. Mice were 5–6 weeks old at the start the experiment (n=24 in each group; *p < 0.05 vs. HFD). (b) Decline in body weight (in %) in HFD and HFD + CAL mice after exposure to Ang II (or saline). Note the more prominent Ang II-induced decline in body weight (p < 0.05) in mice not receiving Calanus oil supplementation. n=6 for HFD_Ang II treated mice, n=9–11 for the other groups. *p < 0.05 vs. before treatment; *p < 0.05 vs. HFD + CAL_Ang II.

Fig. 2. Effect of angiotensin II exposure on adipose tissue mass in high fat-fed mice with and without Calanus oil supplementation. Weight of perirenal (a) and epididymal (b) fat mass following 2 wk treatment with either saline or Ang II in mice receiving high fat diet, either with no supplementation (HFD) or supplemented with 2% (w/w) Calanus oil (HFD + CAL). *p < 0.05 vs. Calanus oil supplementation. n=8–12.

Values of blood pressure are presented as mean ± SD, whereas other data are given as mean ± SEM. Differences between HFD and HFD + CAL mice in body weight development during the initial 8 wk feeding period (Fig. 1a) were evaluated statistically by repeated measures ANOVA (Sigma Plot 13.0), whereas group differences in body and organ weights, blood pressure and gene expression after Ang II/saline infusion were analyzed by two-way ANOVA (with Holm-Sidak method as post-hoc test). Overall significance level was 0.05.
hand, infusion of Ang II did not cause any reduction of these fat depots in the Calanus oil-treated group (Fig. 2). Administration of Ang II also resulted in reductions in the weights of liver (14%), kidney (13%), and skeletal muscle (8%) in HFD mice not receiving Calanus oil (Fig. 3), but again this response was blunted in the Calanus oil-supplemented group.

Since Ang II is known to induce cardiac hypertrophy (both directly and in relation to increased blood pressure), we also examined heart mass in the various groups. As expected, treatment with Ang II led to a significant increase in heart weight (Fig. 3), but in this case there was no difference in the response between HFD and HFD+CAL mice (14% vs 12% increase). Dietary Calanus oil supplementation alone had no effect on heart weight (Fig. 3).

3.2. Effect of Calanus oil on Ang II-induced changes in blood pressure

Systolic and diastolic blood pressure (BP) remained stable during the initial part of the feeding period (week 3-8), with no differences between the HFD and HFD+CAL groups (Table 1). Ang II infusion produced a 17% increase in systolic BP and a 13% increase in diastolic pressure in HFD mice, whereas only a 5% (but still statistically significant) increase (was observed in the Calanus oil-supplemented group (Fig. 4)). In the absence of Ang II (i.e. in saline-treated mice) we observed no differences between the HFD and HFD+CAL groups with respect to either systolic or diastolic BP.

3.3. Effect of Calanus oil on Ang II-induced hypertrophy, fibrosis and inflammation

We also measured gene expression at the mRNA level of commonly used marker genes of hypertrophy (ANP, BNP, β-MHC), fibrosis (coll I-α1 and coll III-α1, TIMP and Fn-1) and inflammation (TNFα, IL-6) in cardiac muscle, as well as markers of fibrosis and inflammation in tissue samples from the descending aorta. In cardiac muscle Ang II infusion caused a marked increase in the expression of nearly all these genes. Supplementation with Calanus oil did not alter this response (Table 2). Fibrotic markers (coll I-α1 and coll III-α1, TIMP1 and Fn-1) in aortic samples were increased after administration of Ang II in mice receiving HFD, as well as in the HFD+CAL group (Fig. 5). Interestingly, Calanus oil-supplementation prevented the Ang II-induced increase of the inflammatory markers, TNFα and IL-6 (Fig. 5).

3.4. Effect of Calanus oil on cardiac O-GlcNAcylation

Since many of the physiological effects we observed in HFD mice treated with Calanus oil and/or Ang II may be affected by...
supplementation (HFD) or supplemented with 2% (w/w) Calanus oil (HFD

Table 1  

Systolic and diastolic blood pressure in high fat-fed mice with and without Calanus oil supplementation.

<table>
<thead>
<tr>
<th>Time (weeks of feeding)</th>
<th>HFD, systolic</th>
<th>HFD, diastolic</th>
<th>HFD + CAL, systolic</th>
<th>HFD + CAL, diastolic</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>109 ± 15</td>
<td>81 ± 7</td>
<td>83 ± 10</td>
<td>83 ± 10</td>
</tr>
<tr>
<td>4</td>
<td>111 ± 10</td>
<td>83 ± 7</td>
<td>114 ± 8</td>
<td>85 ± 8</td>
</tr>
<tr>
<td>5</td>
<td>110 ± 13</td>
<td>83 ± 11</td>
<td>109 ± 13</td>
<td>80 ± 9</td>
</tr>
<tr>
<td>6</td>
<td>113 ± 11</td>
<td>86 ± 11</td>
<td>112 ± 7</td>
<td>82 ± 8</td>
</tr>
<tr>
<td>7</td>
<td>100 ± 10</td>
<td>76 ± 7</td>
<td>105 ± 7</td>
<td>78 ± 5</td>
</tr>
<tr>
<td>8</td>
<td>112 ± 10</td>
<td>84 ± 10</td>
<td>113 ± 10</td>
<td>85 ± 9</td>
</tr>
</tbody>
</table>

Values of systolic and diastolic blood pressure (mmHg, mean ± SD, n=20–24) during the initial 8 wk feeding period in mice receiving high fat diet either with no supplementation (HFD) or supplemented with 2% (w/w) Calanus oil (HFD + CAL).

Table 2  

Myocardial mRNA expression in response to Ang II infusion in high fat-fed mice with and without Calanus oil supplementation.

<table>
<thead>
<tr>
<th></th>
<th>HFD Saline</th>
<th>HFD Ang II</th>
<th>HFD + CAL Saline</th>
<th>HFD + CAL Ang II</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANP</td>
<td>1.0 ± 0.12</td>
<td>5.59 ± 1.29*</td>
<td>0.75 ± 0.07</td>
<td>4.98 ± 0.78*</td>
</tr>
<tr>
<td>BNP</td>
<td>1.0 ± 0.15</td>
<td>3.21 ± 0.42*</td>
<td>1.04 ± 0.14</td>
<td>2.84 ± 0.29*</td>
</tr>
<tr>
<td>α-MHC</td>
<td>1.0 ± 0.03</td>
<td>0.94 ± 0.04</td>
<td>1.12 ± 0.03</td>
<td>0.93 ± 0.04</td>
</tr>
<tr>
<td>β-MHC</td>
<td>1.0 ± 0.12</td>
<td>3.21 ± 0.30*</td>
<td>1.03 ± 0.10</td>
<td>4.97 ± 1.17*</td>
</tr>
<tr>
<td>Agtr-1a</td>
<td>1.0 ± 0.07</td>
<td>0.82 ± 0.05</td>
<td>1.02 ± 0.05</td>
<td>0.89 ± 0.06</td>
</tr>
<tr>
<td>TNFα</td>
<td>1.0 ± 0.08</td>
<td>1.41 ± 0.24</td>
<td>1.28 ± 0.06</td>
<td>1.19 ± 0.11</td>
</tr>
<tr>
<td>IL-6</td>
<td>1.0 ± 0.37</td>
<td>4.61 ± 0.79*</td>
<td>1.05 ± 0.12</td>
<td>4.44 ± 1.65*</td>
</tr>
<tr>
<td>TIMP 1</td>
<td>1.0 ± 0.09</td>
<td>8.06 ± 1.72*</td>
<td>1.50 ± 0.12</td>
<td>5.51 ± 0.94*</td>
</tr>
<tr>
<td>Fn-1</td>
<td>1.0 ± 0.08</td>
<td>7.60 ± 3.25*</td>
<td>1.13 ± 0.08</td>
<td>6.32 ± 2.10*</td>
</tr>
<tr>
<td>Col Iα</td>
<td>1.0 ± 0.05</td>
<td>3.27 ± 0.54*</td>
<td>1.14 ± 0.03</td>
<td>2.87 ± 0.40*</td>
</tr>
<tr>
<td>Col IIIα</td>
<td>1.0 ± 0.07</td>
<td>3.27 ± 0.64*</td>
<td>0.92 ± 0.06</td>
<td>2.88 ± 0.40*</td>
</tr>
</tbody>
</table>

Mice received high fat diet either with no supplementation (HFD) or supplemented with 2% (w/w) Calanus oil (HFD + CAL). Values are normalized to HFD-Saline.

* p < 0.05 vs. corresponding saline group (n=8 in each group).

Interestingly, we observed a general increase in protein O-GlcNAcylation as a result of Calanus oil treatment, as demonstrated by increased O-GlcNAcylation level in the HFD + CAL group treated with saline (Fig. 6). Thus, the Ang II-induced increment in O-GlcNAcylation was clearly blunted in the HFD + CAL group.

The level of protein O-GlcNAcylation can be regulated by glutamine: fructose-6-phosphate aminotransferase (GFAT), the rate-limiting enzyme in the hexosamine biosynthesis pathway or by the enzymes catalyzing the addition or removal of the O-GlcNAc moiety from proteins, i.e. O-GlcNAc transferase (OGT) and O-GlcNAcase (OGA). No differences were detected between the HFD and HFD + CAL group, however, in the expression level of these genes (supporting data, Table S2).

4. Discussion

Calanus oil is a novel marine oil with potent anti-obesity and anti-inflammatory properties in rodents. In agreement with previous results [18,19] we found that mice given high fat diet (HFD) supplemented with Calanus oil gained less body weight over the initial 8 weeks feeding period than those receiving HFD without Calanus oil. The lower weight gain can be ascribed to reduced fat deposition, which in turn could be explained in terms of increased fat oxidation and/or decreased lipogenesis [32,33]. Although HFD feeding is supposed to activate the renin–angiotensin–aldosterone system [34], we observed no significant changes in systolic or diastolic blood pressure over the 8 weeks period prior to Ang II-infusion. However, this finding is in line with recent results by Calligaris et al. [35], showing no statistically significant differences in blood pressure between normal and obese (HFD-fed) C57BL/6 J mice after 8, 12 and 16 months of feeding. Likewise, Mark et al. [36] observed no increase in blood pressure in obese, leptin-deficient mice.

A striking observation in the present study was that acute treatment of diet-induced obese mice with Ang II, starting immediately after the initial 8 weeks feeding period, led to a marked decrease in body mass in comparison to saline-treated obese mice and, more importantly, that this effect was generally blunted in mice receiving dietary Calanus oil supplementation. Although Ang II is known to cause cachexia, the presently observed decline in body weight following Ang II infusion was quite remarkable. It should be noted, however, that the animals were obese and given a high fat diet. Thus, the results should not be compared with Ang II-induced weight loss in mice fed a standard chow.
4.1. Angiotensin II-induced tissue wasting is reduced by dietary Calanus oil.

Examination of the mechanisms behind the angiotensin II-induced body weight reduction was not within the scope of this study. However, Brink et al. [37] suggested already in 1996 that Ang II infusion produces weight loss in rats through a pressor-independent mechanism that includes a marked anorexigenic effect. In addition Cassis et al. [38] reported that low levels of Ang II infusion regulate body weight through mechanisms related to increased peripheral metabolism (reflected as increased surface temperature). Food intake was not recorded in this study, but we have previously documented that dietary supplementation with Calanus oil has no effect on food (energy) intake in high-fat fed mice [19]. Thus, one would assume that any reduction in food intake following Ang II exposure should be the same for HFD and HFD+CAL mice. Therefore, it is unlikely that the more pronounced body weight reduction in the HFD vs the HFD+CAL group could be explained by a stronger suppression of food intake. Interestingly, it has been shown that Ang II infusion leads to weight loss through increased protein breakdown and reduced protein synthesis (in addition to decreased appetite) through activation of the...
ubiquitin-proteasome pathway and increased apoptosis [40]. Zhang et al. [41] demonstrated that Ang II induced hepatic IL-6 and serum amyloid A production in the mouse which acted synergistically to disrupt insulin/IGF-1 signaling and promote skeletal muscle proteolysis. These pro-inflammatory mechanisms may in part have accounted for the reduction in body and organ (kidney, liver, skeletal muscle) weight reductions which occurred after Ang II exposure. In line with this view, we suggest that the lower body and organ weight reductions in the Calanus oil-treated group is related to the strong anti-inflammatory action of the oil, as demonstrated in previous studies from our group [18,19].

The largest reduction in organ weight following Ang II administration was observed in abdominal (perirenal and epididymal) adipose tissue. This result is in line with a recent paper by Cichello et al. [39], which showed that Ang II infusion in rats decreased body weight by wasting predominantly adipose tissue, most likely due to elevated energy expenditure via mitochondrial uncoupling (UCP3 protein activity). The mechanism by which Calanus oil supplementation led to loss of fat mass is, however, not clear. One possibility could be that it has an energy sparing effect by reducing Ang II-induced mitochondrial uncoupling in adipose tissue (as well as in skeletal muscle), e.g. via increased protein O-GlcNAcylation (see below) or anti-inflammatory mechanisms [18,19], but this possibility remains to be examined.

4.2. Angiotensin II-induced hypertension is reduced by dietary Calanus oil

Ang II treatment produced a clear increase in systolic blood pressure in HFD-fed mice not receiving Calanus oil, whereas the hypertensive response was significantly less in mice receiving dietary supplementation with Calanus oil. Also diastolic blood pressure increased in response to Ang II, but not in mice treated with Calanus oil. The mechanism by which Calanus oil attenuated the hypertensive response is not obvious, but several reports in the literature shows that n-3 PUFAs may play an important role in the regulation of vascular tone, either via interaction with intracellular calcium mechanisms [42] or via an antagonistic effect on the Ang II receptors [43]. We observed that Calanus oil supplementation effectively prevented the increase in TNFα and IL-6 expression in aortic tissue. Hence, it appears that dietary supplementation of HFD-fed mice with Calanus oil diminished the impact of the inflammatory response of Ang II. The attenuated inflammatory response in the aorta of the Calanus oil-treated group matches the lower rise in blood pressure in this group, but it could probably also be related to reduced release of pro-inflammatory cytokines from adipose tissue [18,19]. Finally, it should be mentioned that arterioles represent the primary vessels involved in the regulation of arterial blood pressure as well as organ blood flow. Hence, it would be of interest to find out whether Ang II-induced elevation in arteriolar vascular tone was related to increased arteriolar inflammation and, in particular, whether this process could be antagonized by dietary Calanus oil. For obvious reasons, however, we had to use arterial tissue for the mRNA expression analysis.

In line with the hypertensive response, myocardial mRNA expression of marker genes of hypertrophy (ANP, BNP, β-MHC) and fibrosis (col I-α1 and col III-α1, TIMP1 and Fn-1) was up-regulated, not only in the HFD group, but also in the HFD group receiving Calanus oil supplementation. Although the mRNA data indicates that Calanus oil had no effect on cardiac fibrosis, histological examination of cardiac tissue and/or assessment of collagen content are needed to firmly conclude that this was the case. Anyway, the increase in the level of gene expression was similar in both groups, irrespective of the pressor response, indicating that even a minor increase in blood pressure is capable of eliciting myocardial hypertrophy.

4.3. Dietary Calanus oil leads to post-translational protein modification

Protein O-GlcNAcylation is a post-translational modification of proteins in which a single beta-Acetyl-N-Glucosamine moiety is attached to serine and threonine through formation of an O-linked ester, similar to protein phosphorylation. The nature of this modification is controversial with respect to its impact on health, i.e. increased protein O-GlcNAcylation is linked to insulin resistance [44,45], but it has also been shown to be cardioprotective when induced before a challenge, such as ischemia-reperfusion or oxidative stress [46,47].

In the present study we observed that Ang II exposure caused a significant increase in the level of protein O-GlcNAcylation in heart tissue of mice receiving non-supplemented HFD. The interpretation of this finding is not obvious, but O-GlcNAcylation is believed to serve as an auto-protective alarm, mediating stress-induced signal transduction pathways augmenting stress tolerance [48]. In line with this view, Marsh et al. reported [21] that increased O-GlcNAcylation (in response to activation of the hexosamine biosynthesis pathway) blocked the hypertrophic response to Ang II in cardiomyocytes from diabetic mice. The same authors also showed that increased O-GlcNAcylation blocked the rise in intracellular calcium associated with Ang II stimulation of neonatal cardiomyocytes [22]. In this setting, it was interesting to note that Calanus oil supplementation per se also promoted a rise in cardiac protein O-GlcNAcylation in HFD-fed mice, which apparently was not further augmented by Ang II exposure. Whether this rise in protein O-GlcNAcylation may have contributed to reduce the Ang II-induced increase in blood pressure in Calanus oil-treated mice remains, however, to be seen. Finally, one could speculate that increased protein O-GlcNAcylation in response to Calanus oil took
place in other tissues in addition to the heart, and probably could explain the lower tissue wasting observed in adipose tissue, liver, kidney and skeletal muscle following Ang II infusion. Certainly, further research is required in order to determine how Calanus oil is increasing protein O-GlcNAcylation, and what the major protein targets for this modification might be.

4.4. Limitation

The main question raised in this study was to examine whether dietary Calanus oil could prevent Ang II-induced hypertension primarily in obese mice, but from an academic point of view it would certainly have been of interest to find out whether Calanus oil affects blood pressure also in non-obese mice fed a normal Chow diet. We believe that this question should be answered in a separate study, because the logistic challenges by including two more groups (Chow and Chow+Calanus oil) would require extra manpower to secure that procedures could be performed in a safe and timely manner, in particular the management of blood pressure measurements at close intervals after infusion of Ang II/saline.

Also, the non-invasive VPR tail-cuff method, which was used in the present study, does not provide the same accuracy as implanted pressure transducers and use of radiotelemetry or catheter-based direct blood pressure measurements. However, the tail-cuff method was required in order to cope with the high-throughput design of the current study.

5. Conclusions

In the present study we show for the first time that dietary Calanus oil has protective effects on the vascular system in obese mice by preventing the rise in systolic blood pressure following acute exposure to Ang II, suggesting that dietary intake of oil from the marine copepod Calanus finmarchicus could be a hypertension treatment option. In addition, Ang II-induced tissue wasting, especially of adipose tissue, was significantly reduced in mice receiving dietary Calanus oil, indicative of lower energy requirements for tissue preservation during the stress imposed by Ang II exposure. Apparently, the anti-hypertensive effect of the oil is related to its anti-inflammatory action in the vessel wall, and studies should be designed to determine whether targeting the inflammatory process in human obesity and diabetes could be a useful strategy for treatment and management of the accompanying hypertension. Also, the finding that gene expression (mRNA) for markers of structural remodeling, inflammation and fibrosis in the heart was not attenuated by Calanus oil treatment should be followed up with measurements of protein expression as well as cardiac functional measurements.

Disclosures

No conflicts of interest, financial or otherwise, are declared by the authors.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.jplefa.2016.03.006.

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