Exercise training induces insulin-sensitizing PAHSAs in adipose tissue of elderly women

Marie Brezinova, Tomas Cajka, Marina Oseeva, Marek Stepan, Klara Dadova, Lenka Rossmeislova, Milos Matous, Michaela Siklova, Martin Rossmeisl, Ondrej Kuda

**Article Info**

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**Abstract**

Adverse effects of aging can be delayed with lifestyle interventions. We examined how exercise training (ET) alone or combined with omega-3 polyunsaturated fatty acid (PUFA) affects serum and adipose tissue (AT) lipids in older women. Fifty-five sedentary older women were included in the physical activity program and given either sunflower (Placebo) or wax esters-rich (Calanus) oil capsules for 4 months. Serum and subcutaneous abdominal AT samples were acquired while maximum rates of oxygen consumption (VO2 max), insulin sensitivity (hyperinsulinemic-euglycemic clamps) and comprehensive lipidome profiles were determined before and after the study.

ET increased VO2 max in both groups. Lipidomics profiling revealed unusual serum triacylglycerols and phospholipids with ether-bound alcohols in the Calanus group, while ET generally induced shorter-chain triacylglycerols in AT, suggesting increased de novo lipogenesis. The latter was positively associated with whole-body insulin sensitivity. Unexpectedly, insulin-sensitizing lipokines from the family of branched palmitic acid esters of hydroxy stearic acid (PAHSAs) were elevated in both serum and AT after ET, while PAHSAs-containing triacylglycerols were detected in AT.

ET stimulated beneficial changes in AT, including PAHSAs synthesis. Although the added value of omega-3 PUFA supplementation was not proven, our discovery can help understand the nature of the metabolic benefits of exercise.

1. Introduction

Aging is associated with redistribution of adipose tissue (AT), characterized by increased visceral and ectopic fat deposition, which may be independent of changes in body weight due to concomitant decreases in muscle mass (sarcopenia) [1]. These changes are then related to an increased risk of metabolic diseases such as type 2 diabetes and cardiovascular disease [1]. Thus, AT dysfunction appears to be one of the important contributors to impaired metabolic status in the elderly; it is characterized by altered lipid storage, impaired de novo lipogenesis (DNL) and lipolysis, and increased pro-inflammatory state due to changes in innate immunity [2,3]. Circulating pro-inflammatory cytokines secreted by AT have been suggested to promote sarcopenia in the elderly, and obesity was the main factor explaining poorer physical performance in older adults with metabolic syndrome [4].

In the elderly, lifestyle interventions based on the increased physical activity are primarily aimed to improve muscle function and/or cardiovascular fitness, but recent data suggest that AT may also contribute to the beneficial effects of exercise on systemic inflammation and overall health [5]. Accordingly, it has been shown that exercise-induced lipokines increasing muscle fatty acid (FA) uptake are produced in brown AT [6], while transplantation of AT from mice subjected to exercise training (ET) into their sedentary counterparts improved glucose homeostasis of the recipients [5,7]. Moreover, AT was identified as a source of branched FA esters of hydroxy FA (FAHFA), i.e. a growing family of endogenous lipids with documented anti-inflammatory and insulin-sensitizing effects at the systemic level [8–10], whose regulation and relevance to the beneficial effects of exercise are currently unknown.

The pro-inflammatory phenotype may also be affected by natural
substances such as omega-3 polyunsaturated fatty acids (PUFA) and specialized pro-resolving mediators [11–13]. Omega-3 PUFA supplementation reduced RT and systemic inflammation in obese non-diabetic subjects [13], and it could represent a potential strategy for the treatment/prevention of sarcopenia through increased muscle protein synthesis [14]. Calanus oil represents a novel source of omega-3 PUFA, which is unique in its combination of PUFA and alcohols [15]. Moreover, in dietary obese mice, supplementation of omega-3 PUFA-containing wax esters from Calanus oil ameliorated AT dysfunction more effectively than the same dose of omega-3 PUFA administered as ethyl esters [16,17].

Thus, the objective of this study was to evaluate in older sedentary individuals the effect of ET alone, or in combination with Calanus oil, on serum and AT lipidome and its relationship to insulin sensitivity as well as other clinical parameters.

2. Materials and methods

2.1. Study design

This work is based on the clinical study EXODYA (Effect of Exercise training and Omega-3 fatty acids on metabolic health and Dysfunction of Adipose tissue in elderly; NCT number: NCT03386461), and focuses primarily on the presentation of lipidomics data and their association with clinical parameters. Briefly, fifty-five healthy sedentary women aged 65–80 were enrolled in the physical activity program (i.e. ET) that consisted of supervised combined aerobic (mainly nordic walking, moderate intensity 60–85% VO2 peak) and resistance training (mainly functional muscle training adapted for elderly and stretching) for 1 h, 3 times a week for 4 months. Details of the study including changes in other anthropometric parameters and the function of cardiovascular system and muscle will be published elsewhere. During the study duration, subjects were taking either 5 capsules of Calanus oil (Calanus) or sunflower oil (Placebo). The dose of omega-3 PUFA in the Calanus group was approximately 230 mg EPA and DHA per day. All measurements, procedures and sample collection were performed at week 0 (before) and week 16 (after), on an outpatient basis, after an overnight fast. All subjects were taking either 5 capsules of Calanus oil (Calanus) or sunflower oil (Placebo). The dose of omega-3 PUFA in the Calanus group was approximately 230 mg EPA and DHA per day. All measurements, procedures and sample collection were performed at week 0 (before) and week 16 (after), on an outpatient basis, after an overnight fast (10–12 h) fasting with water ad libitum. At both visits, serum, red blood cells (RBC), subcutaneous abdominal AT samples, and anthropometric parameters were acquired. Measurement of maximum oxygen consumption (VO2 max) and hyperinsulinemic-euglycemic clamps (HEC) parameters were acquired. Measurement of maximum oxygen consumption (VO2 max) and hyperinsulinemic-euglycemic clamps (HEC) were performed as before [18,19]. Fifty paired serum samples and 46 paired AT samples were successfully processed through the lipidomics and metabolomics pipelines. False discovery rate (FDR) was set to FDR < 0.1. Multiple comparisons (Sidak’s test) were used to compare the groups and to test the effect of ET. For simplicity, the factors were referred to as “Exercise” (E), “Diet” (D), and their interaction (I).

3. Results

3.1. Exercise improved physical fitness and insulin sensitivity

We aimed to reveal whether ET, alone or combined with omega-3 PUFA supplementation, could affect adiposity and whole-body parameters of physical fitness and glucose metabolism. Although we did not observe significant changes in body weight, BMI, fat mass and fat free mass in response to either ET or ET with omega-3 PUFA, physical fitness was improved by ET regardless of omega-3 PUFA supplementation, as indicated by changes in VO2 max (Table 1). Moreover, whole body insulin sensitivity, evaluated as glucose disposal rate during the HEC (i.e. M-value), was improved by ET, with statistically significant effect in the Calanus group (Table 1).

Table 1
Clinical characteristics of the participants.

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Calanus</th>
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<tbody>
<tr>
<td><strong>Before</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>70.0 ± 3.7</td>
<td>70.4 ± 3.7</td>
</tr>
<tr>
<td>BMI (kg m⁻²)</td>
<td>27.1 ± 4.1</td>
<td>26.7 ± 3.9</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>71.9 ± 13.1</td>
<td>71.0 ± 12.8</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>26.5 ± 8.7</td>
<td>25.4 ± 8.0</td>
</tr>
<tr>
<td>Fat free mass (kg)</td>
<td>45.5 ± 5.2</td>
<td>45.6 ± 5.6</td>
</tr>
<tr>
<td>VO2 max (L min⁻¹)</td>
<td>19.5 ± 3.0</td>
<td>22.0 ± 3.2</td>
</tr>
<tr>
<td>Fasting glucose (mM)</td>
<td>5.73 ± 0.62</td>
<td>5.54 ± 0.49</td>
</tr>
<tr>
<td>M (mg kg⁻¹ min⁻¹)</td>
<td>5.38 ± 1.91</td>
<td>5.78 ± 2.29</td>
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<tr>
<td><strong>After</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>71.0 ± 3.7</td>
<td>4.2 ± 3.7</td>
</tr>
<tr>
<td>BMI (kg m⁻²)</td>
<td>27.1 ± 4.1</td>
<td>3.8 ± 2.7</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>71.5 ± 10.6</td>
<td>71.5 ± 11.7</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>26.9 ± 8.0</td>
<td>26.7 ± 7.9</td>
</tr>
<tr>
<td>Fat free mass (kg)</td>
<td>44.6 ± 3.2</td>
<td>44.8 ± 4.7</td>
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<tr>
<td>VO2 max (L min⁻¹)</td>
<td>20.0 ± 4.5</td>
<td>22.1 ± 4.2</td>
</tr>
<tr>
<td>Fasting glucose (mM)</td>
<td>5.46 ± 0.48</td>
<td>5.52 ± 0.56</td>
</tr>
<tr>
<td>M (mg kg⁻¹ min⁻¹)</td>
<td>5.46 ± 2.39</td>
<td>6.37 ± 2.36</td>
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* Statistically significant effect of ET (before vs. after); two way repeated measures ANOVA; n = 23; means ± SD.
anti-inflammatory lipid mediators

Since no public LC-MS/MS data were available for the Calanus oil, and to completely assess intervention-induced changes in serum and AT lipidome, we first explored the lipid composition of capsules using a methodology focused on intact lipids. Placebo capsules contained sunflower oil, mainly linoleic and oleic acids bound in triacylglycerols (TAG), while the Calanus capsules consisted of long-chain FA and alcohols, including wax esters. We have identified six abundant long-chain wax esters (Table S1); characterization of one representative is included in Fig. 1.

3.3. Calanus oil did not affect ET–induced changes in the production of anti-inflammatory lipid mediators

We aimed to explore, whether ET alone or in combination with Calanus supplementation could be linked to changes in the production of various lipid mediators in AT. Calanus oil contains omega-3 PUFA, and thus we explored a hypothesis that supplemented omega-3 PUFA could serve as substrates for the synthesis of omega-3 PUFA-derived anti-inflammatory specialized pro-resolving mediators (SPMs) [12]. Although we detected SPMs in several samples, we were unable to obtain a complete profile for the entire cohort. Therefore, we focused on the SPM precursors, monohydroxylated PUFA (Fig. 2). Using targeted lipidomics, we found that eicosanoids, represented by 5-, 12- and 15-hydroxyeicosatetraenoic acids, were affected by ET in both serum and AT (Fig. 2A,C). Docosanoids, represented by 7-, 14-, and 17-hydroxydocosahexaenoic acids, were relatively scattered before the intervention and consolidated to lower levels after the intervention in both serum and AT (Fig. 2B,D), suggesting the selective effects of the intervention in individuals with higher baseline docosanoid levels.

3.5. Insulin sensitivity correlated with short-chain TAGs in AT after ET

Next, we took advantage of the clinical characteristics comprising metabolic parameters of the participants and calculated correlations between the clinical and metabolomics data. We focused our attention on the metabolic status induced by ET at the end of the study, regardless of dietary supplementation. The strongest correlations were observed between the M value (a measure of whole-body insulin sensitivity; see above) and AT metabolite profiles (Fig. 4). Thus, M values positively correlated with short/medium-chain TAGs containing 38 to 48 carbons and 0 to 3 double bonds, which could serve as markers of DNL in AT [30,31], and also with diacylglycerols (DAGs) and ether-containing phosphatidylethanolamines (PEe). On the contrary, the M value correlated negatively with ether-containing phosphatidylcholines (PCE), TAGs containing 18:1 (oleic acid) and long-chain sphingomyelins.

3.6. Serum and AT levels of insulin-sensitizing PAHSAs were elevated by exercise

The presence of strong positive correlations between short-chain TAGs and whole-body insulin sensitivity has led us to explore potential changes in the levels of novel antidiabetic FAHFA mediators, in particular those from the palmitic acid esters of hydroxystearic acid (PAHSA) family [8], which could be a part of the mechanistic link between ET and increased insulin sensitivity. Except for 12/13-PAHSA, the other four PAHSA regioisomers as well as their total levels were significantly elevated by Calanus oil supplementation (Table S2), the omega-3 index (sum of EPA and DHA in erythrocyte membranes expressed as a percentage of total erythrocyte FA [28]) showed no difference between the Calanus and Placebo group (from 5.1 ± 1.4 to 6.1 ± 1.7 and from 5.0 ± 1.0 to 6.4 ± 1.2%, respectively). This suggests that Calanus oil supplementation had no additional benefits in terms of increasing bioavailability of omega-3 PUFA.

Metabolite profiles in AT were affected by ET only. Although this effect was less pronounced when compared to serum (Fig. 3A), several metabolites could be linked to the intrinsic metabolic pathways of AT. Thus, tissue levels of arginine, previously linked to the activation of lipolysis and fat mass reduction [29] (Table S3), were significantly elevated. Interestingly, a sequence of three TAGs 46:0, 48:0, and 50:0 was also elevated (Fig. 3D), suggesting activation of de novo lipogenesis in AT by exercise [30,31].

3.2. Calanus oil is rich in free FA and polyunsaturated wax esters

Fig. 1. An example of long-chain wax ester extracted from Calanus oil. A) Extracted ion chromatogram of FA 18:4-WE 40:5 (m/z 600.571), B) MS1 spectrum showing presence of [M + NH4]+ and [M + Na]+ adducts, C) MS/MS spectrum of precursor ion m/z 600.571 acquired at a normalized collision energy (NCE) of 20%.

Next, we have measured serum and AT metabolomic profiles using LIMEX, a targeted and untargeted workflow combining the lipidome, metabolome and exposome. Annotated data were processed via MetaBioAnalyzer Time Series module [26]. Venn diagrams revealed that serum metabolome was primarily affected by the type of dietary supplementation (Placebo vs. Calanus) while AT metabolome was affected by ET (Fig. 3A and B). A complete overview of statistically significant metabolites is presented as Tables S2 and S3.

3.4. Serum and AT metabolome were affected by diet and exercise, respectively

Serum levels of lipids containing ether-bound FA, e.g. unusual TAGs 58:2e and 60:3e, and phosphatidylcholine (PC) 36:6e (Fig. 3C) were elevated in the Calanus group, and we have also identified 15 unique TAGs with ether-bound alkyls (Table S4). This suggests that fatty alcohols from the Calanus oil were efficiently incorporated into circulating lipids, especially TAGs. Although there were many glycerophospholipid species containing omega-3 PUFA (especially DHA) significantly elevated by Calanus oil supplementation (Table S2), the omega-3 index (sum of EPA and DHA in erythrocyte membranes) was also elevated (Fig. 3D), suggesting activation of de novo lipogenesis in AT by exercise [30,31].

3.7. TAG estolides, which represent the FAHFA metabolic reservoir, were elevated by ET

Based on the recent discovery of FAHFA-containing triacylglycerol estolides (FAHFA-TG or TAG EST) in AT [32] and our unpublished
we further explored the lipidomic data and successfully identified 22 metabolites from the TAG EST family (Table S6). Total levels of TAG EST tended to be higher after ET, as documented by two TAG EST (Fig. 6A). Furthermore, we explored the relationship between the level of whole-body insulin sensitivity (i.e. M value) at the end of study, regardless of supplementation, and different TAG EST by calculating the respective correlations. It was revealed that TAG EST 68:1 had the strongest positive correlation with the M value (Fig. 6B), further confirming the potential role of elevated PAHSAs levels in ET-induced changes in insulin sensitivity.

### 4. Discussion

The aim of this part of the EXODYA project was to analyze the effects of ET on serum and AT metabolome and lipidome in elderly women, and to explore potential synergy between ET and omega-3 PUFA supplementation in these effects. The analysis of anthropometric and biochemical parameters showed a beneficial effect of ET on whole-body fitness, but failed to prove an additive effect of a low dose of omega-3 PUFA. There could be several reasons for this finding. The form of omega-3 PUFA used in this study was Calanus oil, a natural lipid extract from the marine copepod *Calanus finmarchicus* [15], with a
unique combination of FA, fatty alcohols and wax esters. Although we detected elevated serum levels of lipids with ether-bound alkyls, thus proving bioavailability of Calanus oil, there was no increase in the omega-3 index and PUFA-derived lipid mediators in the Calanus group. Therefore, it is possible that higher doses of Calanus oil are needed to significantly increase the omega-3 index and to serve as an adequate source of omega-3 PUFA when compared to e.g. re-esterified TAGs \[18\]. The dose of 230 mg EPA and DHA per day provided by Calanus oil capsules was on the lower border of recommended dietary intake \[33\]; apparently, despite its unique composition, Calanus oil as vehicle could not boost the bioavailability of omega-3 PUFA at this low dose of supplementation. Furthermore, we were able to detect markers of Calanus oil in serum but not in AT, which further suggests that the dose was not high enough for the omega-3 PUFA to enter the slow-turning lipid pool in AT.

This interpretation is further supported by the measurement of eicosanoids and docosanoids in serum and AT samples, where we observed the effect of ET but not the expected increase in SPMs in Calanus oil-supplemented participants. The levels of anti-inflammatory docosanoids were either unchanged or even tended to decrease, primarily in subjects with high baseline docosanoid levels, which was possibly due to suppression of low-grade inflammation in response to ET. This

**Fig. 4.** An overview of moderate to strong correlations between the M value and AT metabolites. Metabolites from different lipid classes are visualized in a network using nodes of different shape and color, while the strength of their positive (pink lines) or negative (purple lines) correlations with the M value (selected on the basis of Pearson’s correlation coefficient $r \geq 0.5$, $p \leq 0.05$, and false discovery rate FDR $< 0.1$) is indicated by lines of different thickness. A number X:Y in each frame denotes the number of carbons and the number of double bonds in the corresponding metabolite; “e” suffix marks ether species. Tabular overview in Table S5.

**Fig. 5.** PAHSA levels in serum (nM) and AT samples (pmol/g). A) Serum levels of PAHSA regioisomers. B) PAHSA levels in AT. Mixed effects models: EI, statistically significant effect of exercise & factor interaction (exercise/diet); *, significant effect of exercise (before vs after, multiple comparisons); $n = 12–23$; bars are means ± SEM.
hypothesis is supported by a recent study that failed to demonstrate additive effects of omega-3 PUFA supplementation combined with resistance training in older men [34].

Metabolomics and lipidomics analysis of serum samples from overnight fasting patients revealed that the serum profiles were affected mainly by Calanus oil supplementation, reflecting probably the short-term changes in serum lipid composition after consuming oil capsules. The involvement of the liver and intestine in the wax ester metabolism and systemic availability of EPA and DHA is unknown. However, the difference between incorporation of EPA and DHA into very low-density lipoprotein TAGs and chylomicrons in postprandial period has been documented in humans [35], suggesting a possible partitioning of FA into different lipid pools within the liver before hepatic TAG synthesis and systemic availability. Regardless of these aspects, we were able to describe several novel and unusual lipids including ether TAGs and phospholipids, which were enriched in serum of Calanus oil-supplemented participants. However, these specific markers of Calanus oil intake (e.g. ether lipids, stearidonic acid 18:4 n-3) were not observed in AT of these subjects. On the other hand, there was a clear pattern of TAGs with short acyl chains, which was associated with the effect of exercise in AT. Interestingly, TAGs enriched in palmitic acid and myristic acid were previously linked with increased DNL from carbohydrates both in the liver and AT [30,31,36,37]. Thus, the presence of these particular TAGs in AT of subjects undergoing the ET regimen in our current study suggests a previously unrecognized relationship between the effects of exercise and the induction of this particular metabolic pathway in AT. The mechanism of this induction is not clear, but could be secondary to ET-induced improvements in insulin sensitivity of AT.

Importantly, the ET-induced changes in whole-body insulin sensitivity provided the highest correlations with AT metabolites. Specifically, we found a long list of short-chain TAGs, potentially related to DNL, which positively correlated with the M value at the end of the study, regardless of supplementation. On the other hand, the M value correlated negatively with long-chain polyunsaturated TAGs and ether-containing PCs. While the DNL pathway could be linked to beneficial changes in AT during exercise [38], the role and negative associations of various PCe species with the M value is puzzling due to the lack of information on the biological effects of PCe.

It is now well documented that AT produces a family of FAHFA lipid mediators such as PAHSAs that exert potent anti-inflammatory and insulin-sensitizing effects while improving glucose metabolism in AT [8,9,39,40]. Here we show that all PAHSA regioisomers except one were elevated in response to ET. This effect could be linked to improved glucose metabolism in AT due to exercise and thus contribute to changes in whole-body insulin sensitivity in the elderly. To this point, it has been shown that a single dose of PAHSA improved insulin sensitivity in aged, glucose-intolerant chow-fed mice [8]. The PAHSAs levels are regulated by multiple factors, depending also on the nutritional status and anatomical location of the fat depot [8]. Recently, a FAHFA-containing metabolic reservoir of TAG EST was discovered [32]. When we explored the levels of TAG EST in AT samples from the participants in our study, the total levels of these lipids tended to increase in response to ET, and the levels of TAG EST 68:1 positively correlated with the M values after the ET. In contrast to previous work by Yore et al. [8], positive correlations between the M value and various PAHSAs before and after ET were weak ($r < 0.4$). This was most probably because the range of values of various anthropometric parameters and the M values within our cohort was relatively narrow and more compact than the cohort of insulin-sensitive and diabetic patients reported before [8,10].

In conclusion, our data suggest that ET stimulates beneficial metabolic changes in AT, including the synthesis of PAHSAs and TAG EST. Although the added value of omega-3 PUFA supplementation in terms of these effects has not been demonstrated, our discovery of ET-induced positive metabolic changes in AT lipidome, linked to increased production of potent anti-inflammatory and insulin-sensitizing lipid mediators, could improve our understanding of the mechanisms underlying the metabolic benefits of exercise. While the above changes were uncovered in the elderly subjects participating in the specific ET regimen, it is possible that they represent a general phenomenon associated with the effects of exercise regardless of its type or age of the target population.

**Transparency document**

The Transparency document associated with this article can be found, in online version.

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**Declaration of competing interests**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

**Author contributions**


