Oxylipin profiles and levels vary by skeletal muscle type, dietary fat and sex in young rats

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Novelty bullets: points that summarize the key findings in the work:	Rat soleus (SO) compared to red (RG) and white gastrocnemius (WG) muscles have a higher number and greater mass of oxylipins., Oxylipins generally reflect diet effects on PUFA in all muscles, but there are notable exceptions., Oxylipins in SO and RG are higher in females.
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Title: Oxylin profiles and levels vary by skeletal muscle type, dietary fat and sex in young rats

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Abstract

PUFA-derived bioactive lipid mediators called oxylipins have been shown to influence muscle growth, inflammation and repair in select muscles. Since individual oxylipins have varying effects and potencies, broad profiling in differing muscle types is required to further understand their overall effects. In addition, diet and sex are key determinants of oxylipin levels. Therefore, to provide comprehensive data on oxylipin profiles in rat soleus (SO), red gastrocnemius (RG), and white gastrocnemius (WG) muscles, female and male weanling Sprague-Dawley rats were provided control or experimental diets enriched in n-3 (ω -3) or n-6 (ω -6) PUFA for 6 weeks. Free oxylipin analysis by HPLC/MS/MS revealed that SO muscle had 25% more oxylipins and 4-13 times greater oxylipin mass than WG muscle. Dietary n-3 PUFA, α -linolenic acid, EPA, and DHA, each increased n-3 oxylipins derived directly from their precursors and several that were not direct precursors, while reducing arachidonic acid derived oxylipins. Dietary linoleic acid had few effects on oxylipins. Oxylipins with a sex effect were higher in females in SO and RG. Oxylipins generally reflected the effects of diet and sex on PUFA, but there were exceptions. These fundamental oxylipin profile data provide groundwork knowledge and context for future research on muscle oxylipin functions.

Novelty

- Rat soleus (SO) compared to red (RG) and white gastrocnemius (WG) muscles have a higher number and greater mass of oxylipins.
- Oxylipins generally reflect diet effects on PUFA in all muscles, but there are notable exceptions.

- Oxylipins in SO and RG are higher in females.

Keywords: oxylipins, skeletal muscle, n-3 PUFA, n-6 PUFA, sex, eicosanoids, soleus, white gastrocnemius, red gastrocnemius

Draft

Introduction

Oxylipins are bioactive lipids that mediate many of the biological effects of fatty acids. They are formed from PUFA via cyclooxygenase (COX), lipoxygenase (LOX), and cytochrome P450 (CYP) enzymes to produce over 200 known mediators that regulate such diverse functions such as cell growth and repair, inflammation, blood clotting, sleep behavior and hemodynamics (Gabbs et al. 2015). Although there is a large literature on fatty acids and much is known about individual oxylipins, studies on overall oxylipin profiles have just recently been emerging due to the greater availability of HPLC/MS based methods that allow simultaneous analysis of multiple bioactive compounds. Such overall oxylipin profiling is important because oxylipins can have both synergistic and opposing effects, and the combined effects of individual oxylipins are what determine their overall biological effects.

Oxylipins in muscle have been studied less than some other tissues, but individual oxylipins have been shown to have important functions in muscle. Some of these functions include exercise-induced fatty acid uptake into muscle (Stanford et al., 2018), inflammatory responses during muscle injury and recovery (Markworth et al. 2016, 2020; Giannakis et al. 2019), and muscle growth and repair (Markworth and Cameron-Smith 2013; Ho et al. 2017; Giannakis et al. 2019; Markworth et al. 2020). Nieman et al. (2014) showed that 80 oxylipins increase in plasma after exercise and that some were associated with oxidative stress, suggesting that there may be changes in muscle oxylipin profiles with exercise. Recently this was demonstrated with oxylipin profiling of human skeletal muscle (specifically *m. vastus lateralis*), which showed that oxylipins derived via all three pathways were elevated following exercise (Vella et al. 2019). The *m. vastus lateralis* is

composed of an approximately equal mix of type I and type II fibres (Delp and Duan 1996; Gosker et al. 2007), but it is not known whether oxylipin profiles differ in different types of muscle. It has been shown that there are differences in the CYP-derived oxylipins in intimal vs medial smooth muscle cells (Thomson et al. 2015), which are composed primarily of type II fibres (Eddinger and Meer 2007). Therefore, the first objective of this study was to examine the oxylipin profile of rat soleus (SO) with predominantly type I fibres, red gastrocnemius (RG) with similar proportions of type I and II fibres, and white gastrocnemius (WG) with primarily type II fibres (Delp and Duan 1996).

Although dietary PUFA affect oxylipin profiles in tissues by changing their fatty acid composition, the oxylipin profile does not completely reflect tissue fatty acid composition, necessitating the direct analysis of oxylipins (Leng et al. 2017; Ferdouse et al. 2019a). With respect to muscle, we have reported that dietary n-3 (α -linolenic acid (ALA), EPA, and DHA) and n-6 (linoleic acid (LA)) PUFA alter the oxylipin profile in rat heart (Ferdouse et al. 2019a). Dietary arachidonic acid (AA), EPA and DHA were shown to alter oxylipins derived from these PUFA in mouse skeletal muscle, but the type of muscle analyzed was not indicated (Naoe et al. 2019). Incubation of myoblast cells with AA also has been shown to increase synthesis of COX oxylipins (Markworth and Cameron-Smith 2013). These studies show that dietary PUFA alter oxylipins and fatty acids in different muscles, and therefore potentially can alter muscle functions. Therefore, the second objective of this study was to examine the effects of the predominant (and essential) dietary PUFA (ALA and LA), as well as EPA and DHA (major fish oil n-3

PUFA) on the oxylipin profiles in SO, RG and WG skeletal muscles with differing proportions of type I and II muscle fibres.

Sex is another variable that affects oxylipins, as shown in multiple rat tissues, including liver, kidney, brain, adipose, heart and spleen (Leng et al. 2017; Mendonça et al. 2018; Ferdouse et al. 2019b, 2019a; Pauls et al. 2020). More than half of oxylipins in the rat heart display a sex effect, with >95% being higher in female hearts (Ferdouse et al. 2019a). Additionally, muscles exhibit sex dimorphism in size and function (da Boit et al. 2018; Miotto et al. 2018; Haynes et al. 2020; Stock et al. 2020) and in fibre type distributions (Suzuki and Yamamuro 1985; English et al. 1999; Daniels et al. 2008; Haizlip et al. 2015), so it is likely that there also will be sex differences in oxylipins in skeletal muscle and between muscle types with differing proportions of type I and II fibres. The third objective, therefore, was to examine the effect of sex on oxylipins in rat SO, RG and WG muscles in this study.

Materials and Methods

Rats and diet

The rats and diets utilized in this study have been reported previously in detail (Leng et al. 2017, 2018) (Supplementary Table S1). Briefly, after 1 week of acclimation, 6 male and 6 female weanling Sprague-Dawley rats were allocated to 6 diets after staff not associated with the diet assignment had randomly placed the cages on the racks. All researchers were aware of group allocations throughout the study. Cages were distributed by diet and sex groups across and between cage racks both vertically and horizontally. The diets were based on the AIN93G diet except that they contained 10g instead of 7g

oil/100g diet, and the source of oil differed from the AIN93G. In the control diet, adequate levels of the essential fatty acids, LA and ALA, and high levels of MUFA were achieved using a blend of different oils. In the experimental diets the oil blends were altered so that the MUFA was replaced by 3g/100g diet with different PUFA including ALA, EPA, DHA, LA, and a mixture of LA and ALA that maintained the n-3/n-6 fatty acid ratio of the control. The levels of saturated and unsaturated fatty acids were similar across all diets. Rats were weighed every 2 weeks for 6 weeks before termination in random order under isoflurane anesthesia. All animal procedures were performed in accordance with the Canadian Council for Animal Care guidelines and approved by the University of Manitoba Animal Care Committee. Rats were housed singly in solid bottom cages at a room temperature of 23°C and a 12 hour light/dark cycle. Wooden blocks and commercial rodent bedding were included in the cages for enrichment. Following termination, the right hind leg was removed through the hip joint and frozen immediately in liquid nitrogen and stored at -80°C until analysis. At time of analysis the leg was thawed on ice (30-60 min) while in a cold room at 4°C, skin and tendons were removed and SO, RG, and WG muscles were dissected out and promptly frozen in liquid nitrogen and stored at -80°C prior to analysis.

Oxylin analysis

Samples of SO, RG, and WG were selected in random order before homogenization using a Bead-Ruptor homogenizer (Omni International Inc.) in Tyrode's salt buffer at pH 7.6. The amount of muscle homogenate (400µL containing 70mg muscle) needed for optimum analysis was determined from a dose-response curve.

Samples were adjusted to pH 3 with concentrated HCl after addition of antioxidant and deuterated internal standards (Cayman Chemical, MI, USA) as described (Aukema et al. 2016; Monirujjaman et al. 2017; Leng et al. 2017). Free oxylipins were extracted using Strata-X SPE columns (Phenomenex, CA, USA) preconditioned with methanol and pH 3 water. After sample loading, columns were washed with 10% methanol and pH 3 water, dried with hexane, followed by sample elution with methanol. Samples were dried through sodium sulfate and then under nitrogen and resuspended in the initial mobile phase (water/acetonitrile/acetic acid, 70/30/0.02 v/v/v) for HPLC/MS/MS (Shimadzu Nexera XR coupled with a QTRAP 6500 (Sciex, ON)). Oxylipins were quantified using the stable isotope dilution method (Hall and Murphy 1998). Oxylipins scanned but below the level of detection (<3 times baseline) or detected but below the level of quantification (>3 to <5 times baseline) are reported in Supplementary Table S2. Complete details of the methods are found in (Aukema et al., 2016; Leng et al., 2017; Monirujjaman et al., 2017).

Fatty acid analysis

Samples of 250µL of tissue homogenate (containing 43.75mg tissue) were selected in a random order and analyzed for fatty acids. Dipentadecanoyl phosphatidylcholine as internal standard and BHT antioxidant were added prior to Folch extraction (Folch et al. 1957) of total lipids. Since phospholipid is the main source of PUFA for free oxylipin synthesis, the total phospholipid fraction was separated by TLC using a mobile phase of heptane/isopropyl/acetic acid (60/40/3, v/v/v) (Aukema et al. 1992), and methylated using 6% sulfuric acid in methanol (Fair et al., 2004). The derived

fatty acid methyl esters were analyzed by gas chromatography as described (Aukema et al. 1992; Sankaran et al. 2004).

Statistical analysis

The sample size used for oxylipin and fatty acid analyses was based on published literature examining the same outcomes in other tissues of the same rats (Leng et al. 2017, 2018; Mendonça et al. 2018; Cayer et al. 2019; Ferdouse et al. 2019b, 2019a; Pauls et al. 2020). Normally distributed data or data that could be normalized by log transformation were analyzed by 2-way ANOVA for the main effects of diet and sex and for interaction (simple) effects between diet with sex, followed by Tukey's post hoc comparisons using SAS software (SAS Institute Inc., Cary, NC, USA). A Shapiro-Wilk approach was used to test normality and data that could not be normalized were analyzed by the Kruskal-Wallis test. Outliers were removed if they were outside the range of the median $\pm 3S_n$ (the symmetric group on n elements) (Rousseeuw and Croux 1993; Jones 2019), and samples were removed if more than one third of the sample contained outliers. Oxylipin means were summed and a pooled standard deviation was calculated, using Cohen's formula, for comparisons of oxylipin totals using Prism software version 6.07 (GraphPad) (Cohen 1988). Groups were reported as mean $\pm$ standard error with significance set at $P < 0.05$.

Results

General findings

Rats were healthy throughout the study and at the end of the feeding period males weighed more, but there were no effects of dietary PUFA on body weights (Leng et al. 2017, 2018).

Oxylipins in different muscle types

Out of 160 oxylipins scanned for by HPLC/MS/MS, a total of 74 were detected in at least one type of muscle. The number of oxylipins was higher in SO (74) compared to either RG (64) or WG (59) muscle tissue (Fig. 1-3). From a mass perspective, these differences were even greater: ng oxylipins/g tissue were 4-13 times higher in soleus compared to WG muscle, with the RG oxylipin mass amount generally in between (Fig. 4). By number, at least one-half and one-third of oxylipins were formed via the LOX and CYP pathways, respectively, and <10% were formed via the COX pathway (Supplementary Tables S3-5); by mass, over 85% were derived via the LOX pathway (Supplementary Figure S1).

The proportions of oxylipins derived from n-3 (47-49%) and n-6 (51-53%) PUFA were similar across the three muscle types (Fig. 1-3). Of the detected n-3 oxylipins, 41-46%, 29-34%, 20-23% and 3% were derived from DHA, EPA, ALA and docosapentaenoic acid (DPA_{n-3}), respectively, and of the n-6 oxylipins, 63-69%, 26-33% and 3-6% were derived from AA, LA, and dihomo- γ -linolenic acid (DGLA), respectively (Fig. 1-3). The distributions of oxylipin masses were similar to the oxylipin numbers for n-3 oxylipins, but not for n-6 oxylipins, where LA oxylipins made up more of the total n-6 oxylipin mass (82-87%) and AA and DGLA oxylipins made up less (13-18% and $\leq 0.20\%$, respectively), across the muscle types (Supplementary Tables S3-5).

Dietary PUFA effects on muscle n-3 and n-6 oxylipins

Overall, dietary ALA, EPA and DHA increased the oxylipins directly derived from their corresponding PUFA in all muscle types (Fig. 1-3). In addition, a small number of n-3 oxylipins that were not directly derived from the dietary PUFA were also elevated. For example, 12-hydroxy-eicosapentaenoic acid (HEPE) derived from EPA was also higher in SO and WG muscle of ALA- and DHA-fed rats (Fig. 1 & 3). Additionally, one DHA derived oxylipin in the SO muscle of ALA-fed rats and three DHA derived oxylipins in the SO muscle of EPA-fed rats were higher (Fig. 1). Further, the one DPA_{n-3} oxylipin scanned for and detected was higher in all muscle types of EPA-fed rats (Fig. 1-3). In contrast to the n-3 PUFA, higher dietary LA did not significantly alter any n-3 oxylipins in any muscle type (Fig 1-3).

With respect to n-6 oxylipins, there were lower AA oxylipins in muscles from rats provided dietary ALA, EPA and DHA diets when compared to those provided the control diet (Fig. 1-3), with the overall effects being greater in order of DHA $\geq$ EPA $\geq$ ALA. The number of AA oxylipins in either or both sexes that were lower in SO, RG and WG muscles, respectively, were 37%, 50% and 47% in DHA-fed rats, 22%, 50% and 37% in EPA-fed rats and 22%, 23% and 16% in ALA-fed rats, when compared to rats given the control diet (Fig. 1-3). Higher levels of dietary LA had few effects on AA oxylipins, with no apparent pattern of effects in any of the muscle types (Fig. 1-3). There were also few differences in LA oxylipins, with no apparent pattern, except possibly in SO muscle, where the LA oxylipins, 9- and 13-hydroxy-octadecadienoic acid (HODE) were elevated with higher dietary LA (Fig. 1).

Comparison of oxylipins with fatty acids

Similar to oxylipins, muscle fatty acids mostly reflected the dietary n-3 PUFA composition, with higher levels of each n-3 fatty acid in all muscle types from rats provided their corresponding fatty acids in the diet (Fig. 1-3, Supplementary Tables S6-8). However, there were exceptions, as dietary ALA also increased EPA and DPA_{n-3}, and dietary EPA also increased ALA and DPA_{n-3} in SO and WG muscle, despite not observing such increases in their corresponding oxylipins (except 12-HEPE) (Fig. 1 & 3). Similarly, EPA was reduced by dietary LA, but EPA oxylipins were not in these two muscle types (Fig. 1 & 3). In contrast, n-3 PUFA and their oxylipin patterns were similar in the RG muscle (Fig. 2).

Dietary n-6 PUFA changes also tended to reflect oxylipins, with dietary n-3 PUFA reducing AA levels in all muscle types, and dietary LA having few effects on either LA or AA (Fig. 1-3, Supplementary Tables S6-8). Dietary DHA resulted in lower AA levels compared to dietary EPA and ALA (Fig. 1-3). Exceptions to this included higher LA with no change in LA oxylipins in RG, and no change in LA with increased 9- and 13-HODE in SO, in rats provided the LA diet (Fig. 1 & 2).

These changes in muscle oxylipins and PUFA were reflected in the overall distributions of muscle oxylipins and PUFA. Examples of distributions in RG muscle from rats provided control, ALA and LA diets are shown in Fig. 5, and all muscle types and diets data shown in Supplementary Tables S9-11. These distributions show that the effect of increasing dietary PUFA on the overall proportion of its corresponding oxylipin was often greater than its effect on its corresponding PUFA. For example, ALA makes up

4% of the total PUFA precursor pool, while the ALA oxylipins make up 26% of the total oxylipin pool in RG of rats provided the ALA diet (Fig. 5). Similar effects are observed for EPA and LA, but less so for DHA (Fig. 5 and Supplementary Tables S9-11).

Sex effects

Sex differences in oxylipins were observed in all three muscle types, with 8-28% of oxylipins displaying either a main effect of sex or a simple effect due to diet by sex interactions (Fig. 6). In SO and RG, oxylipins displaying a sex effect were almost always higher in females, especially in RG. Four oxylipins in SO and 8 oxylipins in RG displayed a main effect of sex, all of which were higher in females (Fig. 6). In addition, 1 of 2 oxylipins that displayed an interaction with diet and a simple effect of sex in at least 1 diet were higher in females in SO muscle, while 9 of 10 oxylipins with a simple effect of sex in at least 1 diet were higher in females in RG muscle (Fig. 6). On the other hand, the effect of sex in WG was split between males and females, with 5 oxylipins higher in males (all simple effects in 1 or 2 diets) and 5 oxylipins higher in females (1 main sex effect and 4 simple effects in at least 1 diet) (Fig. 6).

In contrast to the predominance of higher oxylipins in females, PUFA precursors with a sex effect in RG muscles were higher in males, with gamma linolenic acid (GLA) and AA displaying this as a main effect (Supplementary Table S7). In SO muscle, the PUFA precursor ALA was higher in males (main effect) and DPA_{n-3} was higher in females (simple effect in EPA diet) (Supplementary Table S6), but no oxylipins derived from these PUFA displayed sex differences (Supplementary Table S3). In WG muscle, main sex effects were seen in ALA, DPA_{n-3}, and DGLA, which were higher in males,

while DHA was higher in females, but these sex differences also were largely not reflected in their respective oxylipins (Supplementary Table S8).

Discussion

This study provides fundamental data on comprehensive oxylipin profiles in differing muscle types, the effects of dietary PUFA (ALA, EPA, DHA, LA, and LA+ALA) and of sex. The proportions of oxylipins derived from n-6 and n-3 PUFA were similar across SO, RG and WG muscle types. While AA oxylipins represented the highest number of oxylipins, LA oxylipins contributed most to oxylipin mass across all three muscle types similar to what has been reported for *m. vastus lateralis* muscle (Vella et al. 2019) and in rat heart, a tissue largely comprised of muscle (Ferdouse et al. 2019a). Compared to WG muscle, 25% more oxylipins were detected in SO muscle and mass of oxylipins ranged from 4-13 times higher in SO.

Similar to the heart, DHA oxylipins made up the majority of n-3 oxylipins, but in contrast to the heart in which approximately one-third of oxylipins were n-3 derived (Ferdouse et al. 2019a), in skeletal muscle about half were n-3 oxylipins. Also similar to the heart, dietary n-3 PUFA reduced AA oxylipins, but had little effect on other n-6 oxylipins in skeletal muscle (Ferdouse et al. 2019a). In heart, EPA oxylipins were higher not only in the EPA diet group, but also in ALA and DHA diet groups (Ferdouse et al. 2019a). Yet, in skeletal muscle we found that only one EPA oxylipin (12-HEPE) exhibits this same effect in SO and WG. Another effect unobserved in skeletal muscle is the LA+ALA diet affecting ALA or EPA oxylipins, which was observed in heart (Ferdouse et al. 2019a).

The implications of the higher number and mass of oxylipins in SO (type I muscle) compared to WG (type II) and RG muscle (type I & II) remains to be delineated. Oxylipins have been implicated in exercise-induced increases in fatty acid uptake in muscle, muscle injury and recovery, muscle growth and repair and in exercise associated oxidative stress (Markworth and Cameron-Smith 2013; Nieman et al. 2014; Ho et al. 2017; Stanford et al. 2018; Giannakis et al. 2019; Vella et al. 2019; Markworth et al. 2020). Oxylipins are bioactive lipids that mediate their effects via activation of cell surface G protein-coupled receptors and intracellular effectors (Gabbs et al. 2015). One such muscle effector known to be activated by oxylipins is the transcription factor, PPAR (Naruhn et al. 2010; Yamada et al. 2014). In particular, PPAR δ and α are involved in lipid metabolism and mitochondrial biogenesis, and are expressed in high amounts in skeletal muscle tissue, especially in type I fibres with elevated oxidative capacity (Wang et al. 2004; Barquissau et al. 2017). This is consistent with the higher levels of oxylipins in SO muscle, but much still needs to be understood regarding the roles of oxylipins in muscle types.

In response to dietary n-3 PUFA, oxylipins derived directly from their n-3 PUFA (ALA, EPA, DHA) precursor increased the most. In addition, all dietary n-3 PUFA reduced AA derived oxylipins, with more oxylipins being reduced by DHA than by EPA or ALA. These effects are similar to what has been observed in other tissues in these rats (Leng et al. 2017, 2018; Mendonça et al. 2018; Cayer et al. 2019; Ferdouse et al. 2019b, 2019a; Pauls et al. 2020), and in skeletal muscle of unspecified type in mice (Naoe et al. 2019). On the other hand, effects on oxylipins that were not directly derived from the dietary n-3 PUFA differed between tissues and muscle types. In this regard, SO muscle

was similar to kidney and adipose in which dietary ALA and/or EPA also increased DHA oxylipins (Leng et al. 2018; Cayer et al. 2019), while RG and WG did not, similar to heart, liver, brain and spleen (Leng et al. 2018; Ferdouse et al. 2019a, 2019b; Pauls et al. 2020). For dietary LA effects on LA oxylipins, SO was similar to the heart and most other tissues in which dietary LA increased LA oxylipins (Ferdouse et al. 2019a). RG and WG, however, were more similar to brain which was the only other tissue in which dietary LA had no effect on LA oxylipins (Ferdouse et al. 2019b). The effect of dietary LA on EPA but not EPA oxylipins also has been observed in rat heart, kidney and liver (Leng et al. 2017; Ferdouse et al. 2019a). Taken together, this demonstrates that while many of the effects of dietary PUFA on oxylipins are similar between muscle types and across tissues, there are some unique effects both across tissues and between muscle types.

These dietary effects on oxylipins may have implications for muscle function, growth and recovery from injury. Oxylipins derived from AA, which were reduced with dietary n-3 PUFA feeding in the current study, specifically prostaglandins, can increase skeletal muscle growth (Markworth and Cameron-Smith 2013) and stimulate muscle specific stem cells (Ho et al. 2017). Prostaglandins are involved in the inflammatory response of muscle tissue in response to tissue injury; however another type of AA oxylipin group called lipoxins are involved in resolving inflammation (Markworth et al. 2016). Further, resolvins, protectins, and maresins formed from EPA and DHA have anti-inflammatory effects in muscle (Markworth et al. 2016). In SO and WG muscle in the current study, the DHA diet increased 14- and 17-hydroxy-docosaheptaenoic acid (HDoHE), markers of D series resolvins, protectins and maresins, and increased 18-HEPE (precursor for E series

resolvins) in RG and WG. However, these resolvins, protectins and maresins were not present at detectable levels in muscle, as compared to other tissues in these rats in which several of these mediators were detected, including liver (Leng et al. 2017), heart (Ferdouse et al. 2019a) brain (Ferdouse et al. 2019b), and spleen (Pauls et al. 2020). Resolvins in particular may be of interest in muscle since injection of both resolvin D1 and resolvin D2 have been shown to improve muscle recovery following injury and decrease inflammation in mouse tibialis anterior muscle (Giannakis et al. 2019; Markworth et al. 2020). The LA oxylipins 13- and 9-HODE, which were increased by both EPA and LA diets in SO, have also been found to be related to post-exercise oxidative stress (Nieman et al. 2014). Further, although diet effects on 12,13-dihydroxy-octadecenoic acid (DiHOME) were not observed in the current study, this LA-derived oxylipin increases fatty acid uptake into mouse skeletal muscle and into skeletal muscle cell culture, where it increases mitochondrial respiration and may be beneficial for exercise endurance (Stanford et al. 2018). Hence, the changes in oxylipins in response to diet are likely to impact muscle function and repair.

Previous findings in other tissues of these rats revealed that oxylipins were generally higher in one sex predominantly, depending on the tissue. Oxylipins were generally higher in males in liver, kidney and brain (Leng et al. 2017; Ferdouse et al. 2019b), and higher in females in heart, spleen and adipose (Mendonça et al. 2018; Ferdouse et al. 2019a; Pauls et al. 2020). In the current study we show that there were generally fewer oxylipins that displayed a sex effect when compared to some other tissues, and thus there was not a strong sexual dimorphism in this regard. However, those displaying a sex effect were generally higher in females in SO and RG muscle, which is

similar to the heart (Ferdouse et al. 2019a). To our knowledge, although sex differences in 12,13-DiHOME have been observed in plasma following exercise (Stanford et al. 2018), there have been no reports on sex differences in skeletal muscle oxylipins. It is possible that the higher muscle oxylipin levels in females may reflect differences in muscle fibre type, since the relative proportions and sizes of muscle fibre types vary in multiple species (human, mouse, rabbit) when comparing intact females to males or to ovariectomized females (Suzuki and Yamamuro 1985; English et al. 1999; Daniels et al. 2008). In mouse SO, it has been reported that females have a lower proportion of type IIa fibres compared to males (Haizlip et al. 2015). Herein, we showed that the muscle with the highest content of type I and lowest content of type II fibres (SO) has the highest levels of oxylipins, possibly explaining the higher levels of oxylipins in females. Further studies examining the reasons for and implications of the sex differences in muscle oxylipins are warranted.

The results herein also confirm the findings in other tissues which demonstrate that although dietary PUFA effects on oxylipins generally are reflective of overall effects on tissue PUFA, this is not always the case and cannot be assumed. For example, dietary ALA increased EPA and DHA in SO and WG muscle, despite not increasing their corresponding oxylipins (except 12-HEPE). Also, in RG muscle of LA-fed rats, LA levels were higher but LA oxylipin levels were not, while LA was not altered in SO, but LA-derived 9- and 13-HODE were increased. Similarly, the effect of increasing dietary PUFA on the overall proportion of its corresponding oxylipin often was greater than its effect on that PUFA in muscle. Further, sex differences in oxylipins mostly did not reflect

sex differences in PUFA. Thus, the many conclusions about oxylipins based on PUFA data alone in the literature will need to be re-examined.

It should be noted that an animal model may not create generalizable findings for humans since lipid metabolism differs between species; however, we may expect sex, diet and muscle type differences in human oxylipin profiles. Although these results may not be generalizable to a human model, the use of different muscle types and dietary PUFA allows for a more thorough analysis that may accelerate human clinical trials. Additionally, exercise has reported short-term influences on the overall oxylipin profile in human muscle tissue (Vella et al. 2019), but the effect of exercise was not included in the current analysis. Such studies, as well as gene and protein expression analyses of oxylipin synthesizing enzymes would be warranted in future studies. Another limitation of the current study is that although this analysis captures muscle types, and the fibre composition of these have been described (Delp and Duan 1996; Gosker et al. 2007), we did not directly analyze fibre type and it is possible that these may have been influenced by diet (Mizunoya et al. 2013). Future research using immunohistochemistry coupled with laser capture microdissection may confirm fibre type-specific differences in oxylipin profiles through fibre isolation (Morissette et al. 2014).

Conclusions

The current study provides fundamental baseline data on oxylipins in differing muscle types that will guide future research into muscle oxylipins and function. Oxylipin levels are higher in SO compared to WG muscle and are higher in SO and RG muscles in females compared to males. Emerging literature is beginning to demonstrate the

importance of the oxylipin profile in muscle growth, repair, exercise, and inflammation. Dietary PUFA, especially n-3 PUFA, have profound effects on the muscle oxylipin profile and would be expected to alter the functions of these muscles. Such dietary manipulations therefore have potential for altering muscle function in health and disease.

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Competing Interests Statement

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Author Contributions

A.L.P. wrote the manuscript and, with V.W., dissected muscle, conducted experiments, and collected and analyzed data following diet treatment and rat termination by T.W. and S.L. T.A.D. was consulted for muscle dissection. H.M.A designed the study, supervised data collection, analysis and writing, and obtained the funding. All authors revised and approved of the final manuscript.

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Data Availability

Data underpinning the work are available from the corresponding author upon request.

Draft

References

- Aukema, H.M., and Holub, B.J. 1989. Effect of dietary supplementation with a fish oil concentrate on the alkenylacyl class of ethanol- amine phospholipid in human platelets. *J. Lipid Res.* **30**: 59–64.
- Aukema, H.M., Winter, T., Ravandi, A., Dalvi, S., Miller, D.W., and Hatch, G.M. 2016. Generation of Bioactive Oxylipins from Exogenously Added Arachidonic, Eicosapentaenoic and Docosahexaenoic Acid in Primary Human Brain Microvessel Endothelial Cells. *Lipids* **51**(5): 591–599. Springer Berlin Heidelberg. doi:10.1007/s11745-015-4074-0.
- Aukema, H.M., Yamaguchi, T., Takahashi, H., Celi, B., and Holub, B.J. 1992. Abnormal lipid and fatty acid compositions of kidneys from mice with polycystic kidney disease. *Lipids* **27**(6): 429–435. doi:10.1007/BF02536384.
- Barquissau, V., Ghandour, R.A., Ailhaud, G., Klingenspor, M., Langin, D., Amri, E.Z., and Pisani, D.F. 2017. Control of adipogenesis by oxylipins, GPCRs and PPARs. *Biochimie* **136**: 3–11. doi:10.1016/j.biochi.2016.12.012.
- da Boit, M., Tommasi, S., Elliot, D., Zinellu, A., Sotgia, S., Sibson, R., Meakin, J.R., Aspden, R.M., Carru, C., Mangoni, A.A., and Gray, S.R. 2018. Sex Differences in the Associations between L-Arginine Pathway Metabolites, Skeletal Muscle Mass and Function, and their Responses to Resistance Exercise, in Old Age. *J. Nutr. Heal. Aging* **22**(4): 534–540. doi:10.1007/s12603-017-0964-6.
- Cayer, L.G.J., Mendonça, A.M., Pauls, S.D., Winter, T., Leng, S., Taylor, C.G., Zahradka, P., and Aukema, H.M. 2019. Adipose tissue oxylipin profiles vary by anatomical site and are altered by dietary linoleic acid in rats. *Prostaglandins*

- Leukot. Essent. Fat. Acids **141**(October 2018): 24–32. Elsevier Ltd.
doi:10.1016/j.plefa.2018.12.004.
- Cohen, J. 1988. Statistical Power Analysis for the Behavioral Sciences Second Edition. Lawrence Erlbaum Associates, New York.
- Daniels, D.W., Tian, Z., and Barton, E.R. 2008. Sexual dimorphism of murine masticatory muscle function. Arch. Oral Biol. **53**(2): 187–192.
doi:10.1016/j.archoralbio.2007.09.006.
- Delp, M.D., and Duan, C. 1996. Composition and size of type I, IIA, IID/X, and IIB fibers and citrate synthase activity of rat muscle. J. Appl. Physiol. **80**(1): 261–270.
doi:10.1152/jappl.1996.80.1.261.
- Eddinger, T.J., and Meer, D.P. 2007. Myosin II isoforms in smooth muscle: Heterogeneity and function. Am. J. Physiol. - Cell Physiol. **293**(2).
doi:10.1152/ajpcell.00131.2007.
- English, A.W., Eason, J., Schwartz, G., Shirley, A., and Carrasco, D.I. 1999. Sexual Dimorphism in the Rabbit Masseter Muscle: Myosin Heavy Chain Composition of Neuromuscular Compartments. Cells Tissues Organs **164**(4): 179–191. Karger Publishers. doi:10.1159/000016658.
- Fair, D.E., Ogborn, M.R., Weiler, H.A., Bankovic-Calic, N., Nitschmann, E.P., Fitzpatrick-Wong, S.C., and Aukema, H.M. 2004. Nutrient Metabolism — Research Communication Renal Disease Progression After 1 and 3 Weeks in Han : SPRD- cy. J. Nutr. **134**(6): 1504–1507.
- Ferdouse, A., Leng, S., Winter, T., and Aukema, H.M. 2019a. Dietary n-6 and n-3 PUFA alter the free oxylipin profile differently in male and female rat hearts. Br. J. Nutr.

122(3): 252–261. doi:<https://doi.org/10.1017/S0007114519001211>.

Ferdouse, A., Leng, S., Winter, T., and Aukema, H.M. 2019b. The Brain Oxylipin Profile Is Resistant to Modulation by Dietary n-6 and n-3 Polyunsaturated Fatty Acids in Male and Female Rats. *Lipids* **54**(1): 67–80. doi:10.1002/lipd.12122.

Folch, J., Lees, M., and Sloane, G.H. 1957. A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* **226**(1): 497–509.

Gabbs, M., Leng, S., Devassy, J.G., Monirujjaman, M., and Aukema, H.M. 2015. Advances in Our Understanding of Oxylipins Derived from Dietary PUFAs. *Adv. Nutr.* **6**(5): 513–540. doi:10.3945/an.114.007732.

Giannakis, N., Sansbury, B.E., Patsalos, A., Hays, T.T., Riley, C.O., Han, X., Spite, M., and Nagy, L. 2019. Dynamic lipid mediator changes support macrophage subtype transitions during muscle regeneration. *Nat Immunol* **20**(5): 626–636. doi:10.1038/s41590-019-0356-7.

Gosker, H.R., Zeegers, M.P., Wouters, E.F.M., and Schols, A.M.W.J. 2007. Muscle fibre type shifting in the vastus lateralis of patients with COPD is associated with disease severity: A systematic review and meta-analysis. *Thorax* **62**(11): 944–949. doi:10.1136/thx.2007.078980.

Haizlip, K.M., Harrison, B.C., and Leinwand, L.A. 2015. Sex-Based Differences in Skeletal Muscle Kinetics and Fiber-Type Composition. *Physiology* **30**(1): 30–39. American Physiological Society. doi:10.1152/physiol.00024.2014.

Hall, L.M., and Murphy, R.C. 1998. Electrospray mass spectrometric analysis of 5-hydroperoxy and 5-hydroxyeicosatetraenoic acids generated by lipid peroxidation of red blood cell ghost phospholipids. *J. Am. Soc. Mass Spectrom.* **9**(5): 527–532.

doi:10.1016/S1044-0305(98)00013-0.

- Haynes, E.M.K., Neubauer, N.A., Cornett, K.M.D., O'connor, B.P., Jones, G.R., and Jakobi, J.M. 2020. Age and sex-related decline of muscle strength across the adult lifespan: A scoping review of aggregated data. *Appl. Physiol. Nutr. Metab.* **45**(11): 1185–1196. doi:10.1139/apnm-2020-0081.
- Ho, A.T. V, Palla, A.R., Blake, M.R., Yucel, N.D., Wang, Y.X., Magnusson, K.E.G., Holbrook, C.A., Kraft, P.E., Delp, S.L., and Blau, H.M. 2017. Prostaglandin E2 is essential for efficacious skeletal muscle stem-cell function, augmenting regeneration and strength. *Proc. Natl. Acad. Sci. U. S. A.* **114**(26): 6675–6684. doi:10.1073/pnas.1705420114.
- Jones, P.R. 2019. A note on detecting statistical outliers in psychophysical data. *Attention, Perception, Psychophys.* **81**(5): 1189–1196. *Attention, Perception, & Psychophysics.* doi:10.3758/s13414-019-01726-3.
- Leng, S., Winter, T., and Aukema, H.M. 2017. Dietary LA and sex effects on oxylipin profiles in rat kidney, liver, and serum differ from their effects on PUFAs. *J. Lipid Res.* **58**(8): 1702–1712. American Society for Biochemistry & Molecular Biology (ASBMB). doi:10.1194/jlr.m078097.
- Leng, S., Winter, T., and Aukema, H.M. 2018. Dietary ALA, EPA and DHA have distinct effects on oxylipin profiles in female and male rat kidney, liver and serum. *J. Nutr. Biochem.* **57**: 228–237. Elsevier Inc. doi:10.1016/j.jnutbio.2018.04.002.
- Markworth, J.F., Brown, L.A., Lim, E., Floyd, C., Larouche, J., Castor-Macias, J.A., Sugg, K.B., Sarver, D.C., Macpherson, P.C.D., Davis, C., Aguilar, C.A., Maddipati, K.R., and Brooks, S. V. 2020. Immunoresolvents support skeletal myofiber

regeneration via actions on myeloid and muscle stem cells. *JCI Insight* **5**(18): e137713. doi:10.1172/jci.insight.137713.

Markworth, J.F., and Cameron-Smith, D. 2013. Arachidonic acid supplementation enhances in vitro skeletal muscle cell growth via a COX-2-dependent pathway. *Am. J. Physiol. Physiol.* **304**(1): C56–C67. doi:10.1152/ajpcell.00038.2012.

Markworth, J.F., Maddipati, K.R., and Cameron-Smith, D. 2016. Emerging roles of pro-resolving lipid mediators in immunological and adaptive responses to exercise-induced muscle injury. *Exerc. Immunol. Rev.* **22**: 110–134. Available from <http://www.embase.com/search/results?subaction=viewrecord&from=export&id=L611842412>.

Mendonça, A.M., Cayer, L.G.J., Pauls, S.D., Winter, T., Leng, S., Taylor, C.G., Zahradka, P., and Aukema, H.M. 2018. Distinct effects of dietary ALA, EPA and DHA on rat adipose oxylipins vary by depot location and sex. *Prostaglandins Leukot. Essent. Fat. Acids* **129**: 13–24. Churchill Livingstone. doi:10.1016/j.plefa.2017.12.004.

Miotto, P.M., Mcglory, C., Holloway, T.M., Phillips, S.M., and Holloway, G.P. 2018. Sex differences in mitochondrial respiratory function in human skeletal muscle. *Am J Physiol Regul Integr Comp Physiol* **314**: 909–915. doi:10.1152/ajpregu.00025.2018.-Mitochondrial.

Mizunoya, W., Iwamoto, Y., Shirouchi, B., Sato, M., Komiya, Y., Razin, F.R., Tatsumi, R., Sato, Y., Nakamura, M., and Ikeuchi, Y. 2013. Dietary fat influences the expression of contractile and metabolic genes in rat skeletal muscle. *PLoS One* **8**(11): 1–14. doi:10.1371/journal.pone.0080152.

- Monirujjaman, M., Devassy, J.G., Yamaguchi, T., Sidhu, N., Kugita, M., Gabbs, M., Nagao, S., Zhou, J., Ravandi, A., and Aukema, H.M. 2017. Distinct oxylipin alterations in diverse models of cystic kidney diseases. *Biochim. Biophys. Acta - Mol. Cell Biol. Lipids* **1862**(12): 1562–1574. Elsevier. doi:10.1016/j.bbalip.2017.08.005.
- Morissette, M.P., Susser, S.E., Stammers, A.N., O'Hara, K.A., Gardiner, P.F., Sheppard, P., Moffatt, T.L., and Duhamel, T.A. 2014. Differential regulation of the fiber type-specific gene expression of the sarcoplasmic reticulum calcium-ATPase isoforms induced by exercise training. *J. Appl. Physiol.* **117**(5): 544–555. American Physiological Society. doi:10.1152/jappphysiol.00092.2014.
- Naoe, S., Tsugawa, H., Takahashi, M., Ikeda, K., and Arita, M. 2019. Characterization of lipid profiles after dietary intake of polyunsaturated fatty acids using integrated untargeted and targeted lipidomics. *Metabolites* **9**(10). doi:10.3390/metabo9100241.
- Naruhn, S., Meissner, W., Adhikary, T., Kaddatz, K., Klein, T., Watzer, B., Müller-Brüsselbach, S., and Müller, R. 2010. 15-Hydroxyeicosatetraenoic acid is a preferential peroxisome proliferator-activated receptor β/δ agonist. *Mol. Pharmacol.* **77**(2): 171–184. doi:10.1124/mol.109.060541.
- Nieman, D.C., Shanely, R.A., Luo, B., Meaney, M.P., Dew, D.A., and Pappan, K.L. 2014. Metabolomics approach to assessing plasma 13- and 9-hydroxy-octadecadienoic acid and linoleic acid metabolite responses to 75-km cycling. *Am. J. Physiol. Integr. Comp. Physiol.* **307**(1): R68–R74. doi:10.1152/ajpregu.00092.2014.
- Pauls, S.D., Ragheb, M., Winter, T., Leng, S., Taylor, C.G., Zahradka, P., and Aukema,

- H.M. 2020. Spleen Oxylin and Polyunsaturated Fatty Acid Profiles are Altered by Dietary Source of Polyunsaturated Fatty Acid and by Sex. *Lipids* **55**(3): 261–270. doi:10.1002/lipd.12235.
- Rousseeuw, P.J., and Croux, C. 1993. Alternatives to the Median Absolute Deviation. *J. Am. Stat. Assoc.* **88**(424): 1273–1283.
- Sankaran, D., Lu, J., Bankovic-Calic, N., Ogborn, M.R., and Aukema, H.M. 2004. Modulation of renal injury in pcy mice by dietary fat containing n-3 fatty acids depends on the level and type of fat. *Lipids* **39**(3): 207–214. doi:10.1007/s11745-004-1221-7.
- Stanford, K.I., Lynes, M.D., Takahashi, H., Baer, L.A., Arts, P.J., May, F.J., Lehnig, A.C., Middelbeek, R.J.W., Richard, J.J., So, K., Chen, E.Y., Gao, F., Narain, N.R., Distefano, G., Shettigar, V.K., Hirshman, M.F., Ziolo, M.T., Kiebish, M.A., Tseng, Y.H., Coen, P.M., and Goodyear, L.J. 2018. 12,13-diHOME: An Exercise-Induced Lipokine that Increases Skeletal Muscle Fatty Acid Uptake. *Cell Metab.* **27**(5): 1111–1120. Cell Press. doi:10.1016/j.cmet.2018.03.020.
- Stock, M.S., Oranchuk, D.J., Burton, A.M., and Phan, D.C. 2020. Age-, sex-, and region-specific differences in skeletal muscle size and quality. *Appl. Physiol. Nutr. Metab.* **45**(11): 1253–1260. doi:10.1139/apnm-2020-0114.
- Suzuki, S., and Yamamuro, T. 1985. Long-term effects of estrogen on rat skeletal muscle. *Exp. Neurol.* **87**(2): 291–299. doi:10.1016/0014-4886(85)90219-5.
- Thomson, S., Edin, M.L., Lih, F.B., Davies, M., Yaqoob, M.M., Hammock, B.D., Gilroy, D., Zeldin, D.C., and Bishop-Bailey, D. 2015. Intimal smooth muscle cells are a source but not a sensor of anti-inflammatory CYP450 derived oxylin. *Biochem.*

Biophys. Res. Commun. **463**(4): 774–780. Elsevier Ltd.

doi:10.1016/j.bbrc.2015.06.012.

Vella, L., Markworth, J.F., Farnfield, M.M., Maddipati, K.R., Russell, A.P., and Cameron-Smith, D. 2019. Intramuscular inflammatory and resolving lipid profile responses to an acute bout of resistance exercise in men. *Physiol. Rep.* **7**(13): 1–13. doi:10.14814/phy2.14108.

Wang, Y.X., Zhang, C.L., Yu, R.T., Cho, H.K., Nelson, M.C., Bayuga-Ocampo, C.R., Ham, J., Kang, H., and Evans, R.M. 2004. Regulation of muscle fiber type and running endurance by PPAR δ . *PLoS Biol.* **2**(10). doi:10.1371/journal.pbio.0020294.

Yamada, H., Oshiro, E., Kikuchi, S., Hakozaiki, M., Takahashi, H., and Kimura, K.I. 2014. Hydroxyeicosapentaenoic acids from the Pacific krill show high ligand activities for PPARs. *J. Lipid Res.* **55**(5): 895–904. doi:10.1194/jlr.M047514.

Fig. 1. Soleus (SO) n-3 and n-6 oxylipins, and their precursor PUFA in rats provided α -linolenic acid (ALA), EPA, DHA, linoleic acid (LA), and LA+ALA supplemented diets compared to control diets. *Denotes no primary standard and therefore not quantified. Means $\pm$ SEM and statistical analysis data are shown in Supplementary Table S3 for oxylipins and Supplementary Table S6 for fatty acids. AA, arachidonic acid; DGLA, dihomo- γ -linolenic acid; DiHDoPE, dihydroxy-docosapentaenoic acid; DiHETE, dihydroxy-eicosatetraenoic acid; DiHETrE, dihydroxy-eicosatrienoic acid; DiHODE, dihydroxy-octadecadienoic acid; DiHOME, dihydroxy-octadecenoic acid; DPA, docosapentaenoic acid_{n-3}; EpETE, epoxy-eicosatetraenoic acid; EpETrE, epoxy-eicosatrienoic acid; EpODE, epoxy-eicosadienoic acid; EpOME, epoxy-octadecenoic acid; HDoHE, hydroxy-docosahexaenoic acid; HEPE, hydroxy-eicosapentaenoic acid; HETE, hydroxy-eicosatetraenoic acid; HETrE, hydroxy-eicosatrienoic acid; HHTrE, hydroxy-heptadecatrienoic acid; HODE, hydroxy-octadecadienoic acid; HOTrE, hydroxy-octadecatrienoic acid; HX, heptoxilin; k, keto; OxoETE, oxo-eicosatetraenoic acid; OxoODE, oxo-octadecadienoic acid; OxoOTrE, oxo-octadecatrienoic acid; PG, prostaglandin; PD, protectin D; PL, phospholipid; PUFA, polyunsaturated fatty acid; tet, tetranor; TriHOME, trihydroxy-octadecenoic acid; TX, thromboxane.

Fig. 2. Red gastrocnemius (RG) n-3 and n-6 oxylipins, and their precursor PUFA in rats provided α -linolenic acid (ALA), EPA, DHA, linoleic acid (LA), and LA+ALA supplemented diets compared to control diets. *Denotes no primary standard and therefore not quantified. Means $\pm$ SEM and statistical analysis data are shown in

Supplementary Table S4 for oxylipins and S7 for fatty acids. AA, arachidonic acid; DGLA, dihomo- γ -linolenic acid; DiHDoPE, dihydroxy-docosapentaenoic acid; DiHETE, dihydroxy-eicosatetraenoic acid; DiHETrE, dihydroxy-eicosatrienoic acid; DiHOME, dihydroxy-octadecenoic acid; DPA, docosapentaenoic acid_{n-3}; EpETE, epoxy-eicosatetraenoic acid; EpETrE, epoxy-eicosatrienoic acid; EpODE, epoxy-eicosadienoic acid; EpOME, epoxy-octadecenoic acid; HDoHE, hydroxy-docosahexaenoic acid; HEPE, hydroxy-eicosapentaenoic acid; HETE, hydroxy-eicosatetraenoic acid; HETrE, hydroxy-eicosatrienoic acid; HHTrE, hydroxy-heptadecatrienoic acid; HODE, hydroxy-octadecadienoic acid; HOTrE, hydroxy-octadecatrienoic acid; k, keto; OxoETE, oxo-eicosatetraenoic acid; OxoODE, oxo-octadecadienoic acid; OxoOTrE, oxo-octadecatrienoic acid; PG, prostaglandin; PL, phospholipid; PUFA, polyunsaturated fatty acid; TriHOME, trihydroxy-octadecenoic acid.

Fig. 3. White gastrocnemius (WG) n-3 and n-6 oxylipins, and their precursor PUFA in rats provided α -linolenic acid (ALA), EPA, DHA, linoleic acid (LA), and LA+ALA supplemented diets compared to control diets. *Denotes no primary standard and therefore not quantified. Means $\pm$ SEM and statistical analysis data are shown in Supplementary Table S5 for oxylipins and S8 for fatty acids. AA, arachidonic acid; DGLA, dihomo- γ -linolenic acid; DiHETE, dihydroxy-eicosatetraenoic acid; DiHETrE, dihydroxy-eicosatrienoic acid; DiHOME, dihydroxy-octadecenoic acid; DPA, docosapentaenoic acid_{n-3}; EpETE, epoxy-eicosatetraenoic acid; EpETrE, epoxy-eicosatrienoic acid; EpODE, epoxy-eicosadienoic acid; EpOME,

epoxy-octadecenoic acid; HDoHE, hydroxy-docosahexaenoic acid; HEPE, hydroxy-eicosapentaenoic acid; HETE, hydroxy-eicosatetraenoic acid; HETrE, hydroxy-eicosatrienoic acid; HHTrE, hydroxy-heptadecatrienoic acid; HODE, hydroxy-octadecadienoic acid; HOTrE, hydroxy-octadecatrienoic acid; k, keto; OxoETE, oxo-eicosatetraenoic acid; OxoODE, oxo-octadecadienoic acid; OxoOTrE, oxo-octadecatrienoic acid; PG, prostaglandin; PL, phospholipid; PUFA, polyunsaturated fatty acid; TriHOME, trihydroxy-octadecenoic acid.

Fig. 4. Rat muscle total oxylipin mass in soleus (SO), red gastrocnemius (RG), and white gastrocnemius (WG) in male and female rats provided control, alpha-linolenic acid (ALA), EPA, DHA, linoleic acid (LA), and LA+ALA diets. Values with differing letters within graphs are significantly different from each other ($P < 0.05$). Black bars represent female rats, while grey bars represent male rats.

Fig. 5. Comparison of oxylipin and phospholipid PUFA profiles in red gastrocnemius (RG) of rats provided control, alpha-linolenic acid (ALA), and linoleic acid (LA) diets. Male and female summed means were used for analysis. For other diet and muscle data and data separated by sex see Supplementary Tables S9-11.

Fig. 6. Oxylipins with significant sex differences in rat soleus (SO), red gastrocnemius (RG), and white gastrocnemius (WG) in rats provided α -linolenic acid (ALA), EPA, DHA, linoleic acid (LA), and LA+ALA supplemented diets compared to control diets. Data are displayed as fold differences by comparing the higher mean oxylipin level to the

lower mean oxylipin level between sexes. Oxylipins higher in males are presented as gray bars to the left and those higher in females are presented as black bars to the right. Bars without diet labels indicate main sex effects, and bars with diet labels indicate simple sex effects that were significant for only that diet. Data and statistics are shown in Supplementary Tables S3-5. *PD1 could not be calculated as it has zero value in EPA female group; a value of 1 was arbitrarily assigned to this ratio. ALA, α -linolenic acid; LA, linoleic acid.

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SO n-3 Oxylipins										
Diet Sex	ALA		EPA		DHA		LA		LA+ALA	
	F	M	F	M	F	M	F	M	F	M
ALA oxylipins										
9-HOTrE										
9-OxoOTrE										
13-HOTrE										
9,10-EpODE*										
12,13-EpODE										
12,13-DiHODE										
15,16-EpODE*										
15,16-DiHODE*										
EPA oxylipins										
5-HEPE										
8-HEPE										
9-HEPE										
11-HEPE										
12-HEPE										
15-HEPE										
14,15-EpETE										
14,15-DiHETE										
17,18-EpETE										
18-HEPE										
DPA oxylipins										
17k-DPA										
DHA oxylipins										
PD ₁ *										
4-HDoHE										
7-HDoHE										
8-HDoHE										
10-HDoHE										
11-HDoHE										
13-HDoHE										
14-HDoHE										
16-HDoHE										
17-HDoHE										
4k-DHA										
16,17-EpDoPE										
16,17-DiHDoPE										
19,20-EpDoPE										
19,20-DiHDoPE										
20-HDoHE										

Legend

Shading indicates differences (P<0.05):

Higher than control and all other groups

Higher than control and 2-3 other groups

Higher than control and/or 1 other group

Lower than control and all other groups

Lower than control and 2-3 other groups

Lower than control and/or 1 other group

SO n-6 Oxylipins										
Diet Sex	ALA		EPA		DHA		LA		LA+ALA	
	F	M	F	M	F	M	F	M	F	M
AA oxylipins										
PGD ₂										
PGE ₂										
6k-PGF _{1α}										
PGF _{2α}										
TXB ₂										
12-HHTrE										
5-HETE										
5-OxoETE										
8-HETE										
9-HETE										
11-HETE										
12-HETE										
12-OxoETE										
tet-12-HETE										
15-HETE										
15-OxoETE										
8,15-DiHETE										
HXB ₃ *										
5,6-EpETrE										
8,9-EpETrE										
8,9-DiHETrE										
11,12-EpETrE										
11,12-DiHETrE										
14,15-EpETrE										
14,15-DiHETrE										
16-HETE										
20cooh-AA										
LA oxylipins										
9-HODE										
9-OxoODE										
13-HODE										
13-OxoODE										
9,10,13-TriHOME										
9,12,13-TriHOME										
9,10-EpOME										
9,10-DiHOME										
12,13-EpOME										
12,13-diHOME										
DGLA oxylipins										
8-HETrE										
15-HETrE										

SO Precursor PL PUFA										
Diet Sex	ALA		EPA		DHA		LA		LA+ALA	
	F	M	F	M	F	M	F	M	F	M
n-3 PUFAs										
ALA										
EPA										
DPA										
DHA										
n-6 PUFAs										
AA										
LA										
DGLA										

RG n-3 Oxylipins										
Diet Sex	ALA		EPA		DHA		LA		LA+ALA	
	F	M	F	M	F	M	F	M	F	M
ALA oxylipins										
9-HOTrE										
9-OxoOTrE										
13-HOTrE										
9,10-EpODE*										
12,13-EpODE										
15,16-EpODE*										
EPA oxylipins										
5-HEPE										
8-HEPE										
9-HEPE										
11-HEPE										
12-HEPE										
15-HEPE										
14,15-EpETE										
14,15-DiHETE										
17,18-EpETE										
18-HEPE										
DPA oxylipins										
17k-DPA										
DHA oxylipins										
4-HDoHE										
7-HDoHE										
8-HDoHE										
10-HDoHE										
11-HDoHE										
13-HDoHE										
14-HDoHE										
16-HDoHE										
17-HDoHE										
16,17-EpDoPE										
19,20-EpDoPE										
19,20-DiHDoPE										
20-HDoHE										

Legend

Shading indicates differences (P<0.05):

Higher than control and all other groups

Higher than control and 2-3 other groups

Higher than control and/or 1 other group

Lower than control and all other groups

Lower than control and 2-3 other groups

Lower than control and/or 1 other group

RG n-6 Oxylipins										
Diet Sex	ALA		EPA		DHA		LA		LA+ALA	
	F	M	F	M	F	M	F	M	F	M
AA oxylipins										
PGE ₂										
6k-PGF _{1α}										
PGF _{2α}										
12-HHTrE										
5-HETE										
5-OxoETE										
8-HETE										
9-HETE										
11-HETE										
12-HETE										
12-OxoETE										
15-HETE										
15-OxoETE										
8,15-DiHETE										
5,6-EpETrE										
8,9-EpETrE										
8,9-DiHETrE										
11,12-EpETrE										
11,12-DiHETrE										
14,15-EpETrE										
14,15-DiHETrE										
16-HETE										
LA oxylipins										
9-HODE										
9-OxoODE										
13-HODE										
13-OxoODE										
9,10,13-TriHOME										
9,12,13-TriHOME										
9,10-EpOME										
9,10-DiHOME										
12,13-EpOME										
12,13-DiHOME										
DGLA oxylipins										
8-HETrE										
15-HETrE										

RG Precursor PL PUFA										
Diet Sex	ALA		EPA		DHA		LA		LA+ALA	
	F	M	F	M	F	M	F	M	F	M
n-3 PUFAs										
ALA										
EPA										
DPA										
DHA										
n-6 PUFAs										
AA										
LA										
DGLA										

WG n-3 Oxylipins										
Diet Sex	ALA		EPA		DHA		LA		LA+ALA	
	F	M	F	M	F	M	F	M	F	M
ALA oxylipins										
9-HOTrE										
9-OxoOTrE										
13-HOTrE										
9,10-EpODE*										
12,13-EpODE										
15,16-EpODE*										
EPA oxylipins										
5-HEPE										
8-HEPE										
9-HEPE										
11-HEPE										
12-HEPE										
15-HEPE										
14,15-EpETE										
14,15-DiHETE										
17,18-EpETE										
18-HEPE										
DPA oxylipins										
17k-DPA										
DHA oxylipins										
4-HDoHE										
7-HDoHE										
8-HDoHE										
10-HDoHE										
11-HDoHE										
13-HDoHE										
14-HDoHE										
16-HDoHE										
17-HDoHE										
16,17-EpDoPE										
19,20-EpDoPE										
20-HDoHE										

Legend

Shading indicates differences (P<0.05):

- Higher than control and all other groups
- Higher than control and 2-3 other groups
- Higher than control and/or 1 other group
- Lower than control and all other groups
- Lower than control and 2-3 other groups
- Lower than control and/or 1 other group

WG n-6 Oxylipins										
Diet Sex	ALA		EPA		DHA		LA		LA+ALA	
	F	M	F	M	F	M	F	M	F	M
AA oxylipins										
PGD ₂										
PGE ₂										
6k-PGF _{1α}										
PGF _{2α}										
12-HHTrE										
5-HETE										
5-OxoETE										
8-HETE										
11-HETE										
12-HETE										
15-HETE										
15-OxoETE										
8,15-DiHETE										
5,6-EpETrE										
8,9-DiHETrE										
11,12-EpETrE										
11,12-DiHETrE										
14,15-DiHETrE										
16-HETE										
LA oxylipins										
9-HODE										
9-OxoODE										
13-HODE										
13-OxoODE										
9,10,13-TriHOME										
9,12,13-TriHOME										
9,10-EpOME										
9,10-DiHOME										
12,13-EpOME										
12,13-DiHOME										
DGLA oxylipins										
15-HETrE										

WG Precursor PL PUFA										
Diet Sex	ALA		EPA		DHA		LA		LA+ALA	
	F	M	F	M	F	M	F	M	F	M
n-3 PUFAs										
ALA										
EPA										
DPA										
DHA										
n-6 PUFAs										
AA										
LA										
DGLA

Oxylipins

PUFA

Control

ALA

LA

SO

RG

WG