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Intramuscular inflammatory and resolving lipid profile responses to an acute bout of resistance exercise in men.

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Running head: Effect of resistance exercise on muscle lipid mediators

Abstract:

Lipid mediators including classical arachidonic acid derived eicosanoids (e.g. prostaglandins and leukotrienes) and more recently identified specialized pro-resolving mediator metabolites of the omega-3 fatty acids play essential roles in initiation, self-limitation and active resolution of acute inflammatory responses. In the current study, we examined the bioactive lipid mediator profile of human skeletal muscle at rest and following acute resistance exercise. Twelve male subjects completed a single bout of maximal isokinetic unilateral knee extension exercise and muscle biopsies were taken from the *m.*vastus lateralis before and at 2, 4 and 24 h of recovery. Muscle tissue lipid mediator profile was analysed via liquid chromatography–mass spectrometry (LC-MS)-based targeted lipidomics. At 2 h post-exercise, there was an increased intramuscular abundance of cyclooxygenase (COX)-derived thromboxanes (TXB_2: 3.33 fold) and prostaglandins (PGE_2: 2.52 fold and $PGF_{2\alpha}$: 1.77 fold). Resistance exercise also transiently increased muscle concentrations of lipoxygenase (LOX) pathway derived leukotrienes (12-Oxo LTB_4: 1.49 fold and 20-COOH LTB_4: 2.91 fold), monohydroxy-eicosatetraenoic acids (5-HETE: 2.66 fold, 12-HETE: 2.83 fold, and 15-HETE: 1.69 fold) and monohydroxy-docosahexaenoic acids (4-HDoHE: 1.69 fold, 7-HDoHE: 1.58 fold and 14-HDoHE: 2.35 fold). Furthermore, the abundance of CYP pathway derived epoxy- and dihydroxy-eicosatrienoic acids was increased in 2 h post-exercise biopsies (5,6-EpETrE: 2.48 fold, 11,12-DiHETrE: 1.66 fold and 14,15-DiHETrE: 2.23 fold). These data reveal a range of bioactive lipid mediators as present within human skeletal muscle tissue and demonstrate that acute resistance exercise transiently stimulates the local production of both pro-inflammatory eicosanoids and pathway markers in specialized pro-resolving mediator biosynthesis circuits.

Keywords: Exercise recovery, inflammation, lipids, inflammatory resolution.

Introduction:

Skeletal muscle is a remarkably heterogeneous tissue with the capacity to adapt and respond to external stress. It is well established that intense resistance exercise can lead to improvements in muscle strength through changes in muscle fiber type, myofibrillar hypertrophy and neuromuscular mechanisms (9). However, unaccustomed exercise, especially when comprising a large eccentric component, can cause skeletal muscle injury and initiate an acute inflammatory response (1, 8, 52). Experimental models targeted at manipulating the post-exercise inflammatory response have identified that exercise-induced inflammation is a key regulatory feature in the normal process of tissue regeneration and adaptation following acute muscle damage (25, 32, 57). This suggests that molecular signaling events occurring early during acute inflammation play an active role in promoting the restoration of normal tissue function and promote skeletal muscle adaptation following an exercise stimulus.

The humoral and local muscular changes that occur during exercise-induced inflammation closely resemble that of an acute phase response to cellular stress. Exercise stimulus triggers the production of pro-inflammatory signaling molecules, establishing a chemotactic gradient and the diapedesis as well as potential infiltration of inflammatory leukocytes (30). These chemoattractants consist of lipid-derived mediators such as leukotrienes (LTs) and prostaglandins (PGs), as well as protein mediators, including cytokines and chemokines (40). The usual outcome of an acute inflammatory response is its successful resolution and repair of damaged tissue (47). Traditionally, the resolution of inflammation was thought to be a passive process involving the dilution and catabolism of pro-inflammatory mediators leading to the exodus of leukocytes from the site of muscle damage. However, with the discovery of novel classes of lipid-derived mediators, the resolution of acute inflammation is now seen as an active and finely controlled biochemical and metabolic process that may provide a critical link between cellular stress and tissue regeneration/adaptation (2, 17).

Lipid mediators are biosynthesized endogenously from essential omega-6 (n-6) and omega-3 (n-3) polyunsaturated fatty acids (PUFA) and are involved in a wide range of physiological and pathophysiological processes (2). The majority of research in post-exercise inflammation has focused on classical prostaglandins (synthesized via

cyclooxygenase (COX) enzymes, COX-1 and COX-2) and leukotrienes (synthesized via the 5-lipoxygenase (5-LOX) enzyme), which are derived from the n-6 PUFA arachidonic acid (AA). These lipid mediators play a diverse role in stimulating acute inflammation by controlling local blood flow, vascular permeability, cytokine production, leucocyte chemotaxis and sensation of pain (24). On the other hand, a second class of eicosanoids also generated from AA, termed the lipoxins (LX) (20, 35, 45), together with more recently identified eicosapentaenoic (EPA) (E-Series) and docosahexaenoic acid (DHA) (D-Series) derived resolvins (Rv), protectins (PD) (6, 12, 38, 41, 42, 44), and maresins (MaR) (Serhan et al. 2009 & 2012) have been shown to play pro-resolution functions following acute inflammation. These novel lipid mediators, collectively termed specialized pro-resolving mediators (SPMs), act to block acute inflammatory signals by inhibiting pro-inflammatory cytokine production and subsequent neutrophil chemotaxis (36, 46). They simultaneously promote the nonphlogistic infiltration of blood monocytes/macrophages and stimulate tissue macrophages to phagocytize and clear apoptotic neutrophils whilst promoting wound healing (10, 20).

SPMs are formed during inflammatory transcellular interactions, involving the sequential actions of two or more cell types expressing the required LOX and/or COX enzymes in a compartmentalized manner. During the time-course of inflammation cell-cell interactions between platelets, leucocytes, the vasculature and resident tissue cells facilitates the transcellular biosynthesis of unique SPMs (23). The temporal regulation of these lipids is therefore specific to the tissue type, inciting inflammatory stimulus (2, 17). For example, LX biosynthesis involves cellular interactions between 5-LOX expressing neutrophils with 12-lipoxygenase (12-LOX) expressing platelets or 15-lipoxygenase (15-LOX) expressing M2 monocytes (43). Recent findings from Markworth et al. (2013) demonstrated that SPMs, including lipoxins, resolvins and protectins, were elevated in human blood serum samples collected following an acute bout of resistance exercise (24). Peak induction of pro-inflammatory mediators including the prostaglandins and leukotrienes occurred during the early stages of post-exercise muscle recovery (1-2 h), while elevated concentrations of specific SPMs were detected during both early (0-3 h: LXA_4/LXB_4, RvE1 and RvD1) and later (24 h: PD1) stages of muscle recovery. In the present study, we used the same targeted lipidomics approach to characterize the time-course of changes in concentrations of

eicosanoid and docosanoid species locally within human skeletal muscle tissue following an acute bout of resistance exercise. We aimed to identify which species of bioactive lipid mediators are present within skeletal muscle tissue and hence may be locally generated and acting following an acute bout of resistance exercise. We hypothesized that there would be a rapid increase in pro-inflammatory prostaglandin and leukotriene biosynthesis, followed by the activation of SPM pathways at the onset of inflammatory resolution. Identification of the lipid mediator profile of skeletal muscle and ability of exercise stress to modulate intramuscular bioactive lipids will help to contribute to the understanding of a biologically active inflammatory resolution pathway that may be essential to muscle recovery and adaptation following an inflammatory event.

Materials and Methods:

Subjects:

As previously described (7), fourteen untrained but recreationally active men aged 18-25 years were recruited to participate in the acute exercise study. A subset of 12 male participants, for which sufficient muscle biopsy tissue remained, were included in the analysis performed here (Table 1). Exclusion criteria included participation in regular resistance exercise within one year prior to commencing the study, and/or the consumption of any nutritional or purported muscle building supplements. Each participant also completed a medical history questionnaire to identify any potential risk factors that would prevent the subjects from completing strenuous exercise.

Ethics approval:

Each participant was provided with a written and oral explanation of the nature of the study and potential risks of the experimental procedures before providing written consent to participate. All procedures involved in the study were formally approved by the Deakin University Human Research Ethics Committee (DUHREC 2004-017) and muscle biopsy procedures were performed in order with Helsinki declaration.

Familiarization:

At least seven days prior to the trial day, each subject completed a familiarization session on the Cybex NORM dynamometer (Cybex International Inc. UK). The session involved performing isokinetic maximal voluntary contractions (iMVC) during concentric and eccentric knee extension exercise. Maximal force production measured as peak torque (N.m) was determined at 60°/s over 12 maximal concentric and eccentric contractions. Subjects were provided with verbal encouragement throughout the test to ensure maximal effort.

Experimental design:

On the morning of the trial, subjects reported to the laboratory in an overnight fasted state having abstained from alcohol, caffeine and tobacco for the previous 24 h. Participants rested in a supine position for 30 min, following which a resting muscle biopsy sample was collected. Each participant then completed an acute bout of maximal concentric and eccentric isokinetic unilateral knee extension exercise on the Cybex NORM dynamometer. Subjects completed three sets of 12 maximal voluntary repetitions at a constant speed of 60°/s with 2 min of rest between each set. Subjects were instructed to contract maximally during each repetition and were provided with verbal encouragement throughout each set. Further muscle biopsy samples were obtained from the exercised leg at 2 and 4 h after completion of the exercise protocol. The following morning, subjects reported to the laboratory again in an overnight fasted state for a final follow up 24 h post-exercise muscle biopsy sample.

Muscle biopsy procedure:

Muscle biopsy samples were obtained from the *m.*vastus lateralis under local anaesthesia (Xylocaine 1%) using a percutaneous needle biopsy technique modified to include suction (3). A section of excised tissue was rapidly snap frozen in liquid nitrogen and stored at -80°C for further analysis. Repeat muscle biopsy samples were collected from the same leg through separate incisions separated by at least 2 cm from the previous biopsy site to minimize the risk of any localized inflammation arising from the biopsy procedure confounding exercise-induced inflammation.

Liquid chromatography-mass spectrometry (LC-MS):

Muscle biopsy samples were weighed and homogenized in 1 ml phosphate buffered saline (50 mM phosphate containing 0.9% sodium chloride, pH 7.4) using Zirconium beads on a high-frequency oscillator (Precellys homogenizer, Bertig Instruments). The homogenates were centrifuged at 6,000g for 10 min and the supernatant was collected for the extraction of fatty acyl lipid mediators using C18 solid phase extraction cartridges as described earlier (18, 19, 24). Fatty acyl lipid mediator extracts were subjected to LC-MS analysis essentially as described before (22, 24). Under the LC-MS conditions employed, the detection limit for most of the lipid mediators was 1 pg on the column and the quantitation limit was 5 pg on the column with a signal/noise ratio >3. Tissue weights from each sample (range: 14-70 mg, average: 43 mg, inter-quartile range: 32-56 mg) were used for normalization of the LC-MS data and the data are reported as ng per gram (ng/g) of tissue.

Statistics:

Statistical analysis was performed using SigmaPlot v12.3 (Systat Software Inc, Chicago, IL). Data were analyzed using a one-way repeated measures ANOVA. Following a statistically significant main ANOVA effect, Student-Newman-Keuls post hoc tests were used to determine the significance of pair-wise comparisons between individual time points. Data is presented as mean ± standard error of the mean (SEM). Statistical significance was set at $P < 0.05$.

Results:

Metabolipidomic profile of human skeletal muscle tissue:

Lipid mediator profiles of human skeletal muscle biopsies were generated via targeted LC-MS/MS based metabolipidomics. Of the total 125 multiple reaction monitoring (MRM) transitions, 84 unique lipid mediators were reliably detected in resting skeletal muscle tissue (signal/noise ratio >3 in ≥50% of samples) (Supplemental Table 1). Detected analytes included a range n-6 and n-3 PUFA metabolites enzymatically derived from the COX, LOX and CYP pathways (Supplemental Table 1). Metabolites of linoleic acid (LA, 18:2n-6) including hydroxy-octadecadienoic acid (9-, 13-HODEs) and epoxy-octadecadienoic acids (9(10)-, 12(13)-EpOMEs) were most abundant (50-100 ng/g), followed by major

enzymatic metabolites of the n-6 PUFA AA (20:4n-6). Numerous metabolites of the n-3 PUFAs EPA (20:5n-3) and DHA (22:6n-3) were also detected at relatively lower concentrations

Cyclooxygenase pathways:

Omega-6 derived: COX enzymes catalyse the first step in the conversion of AA to prostaglandins (PGE_2, $PGF_{2\alpha}$, PGD_2 and PGI_2) and thromboxane (TXA_2). TXA_2 is highly unstable and non-enzymatically decomposes to TXB_2 and 12(S)-HHTrE. Thus, both these metabolites serve as surrogate markers of TXA_2 biosynthesis. TXB_2 and 12(S) HHTrE were both detected within resting skeletal muscle tissue at concentrations of 1.12 ng/g and 7.68 ng/g respectively (Figure 1). At 2 h post-exercise, muscle TXB_2 increased to 3.73 ng/g ($P = 0.002$) (Figure 1A) and 12(S)-HHTrE increased to 13.50 ng/g ($P = 0.002$) (Figure 1B). The prostaglandins PGE_2 and $PGF_{2\alpha}$ were also detected in resting skeletal muscle tissue at concentrations of 1.13 ng/g and 0.68 ng/g respectively. At 2 h post-exercise, intramuscular PGE_2 increased to 2.84 ng/g ($P = 0.009$) (Figure 1C) and $PGF_{2\alpha}$ increased to 1.20 ng/g ($P = 0.013$) (Figure 1D). Intramuscular TXB_2, 12(S)HHTrE, PGE_2 and $PGF_{2\alpha}$ were no longer elevated above pre-exercise levels by 4 h and 24 h of recovery from the exercise bout. Other major AA derived prostaglandins including PGD_2 and PGI_2 (measured as the stable downstream non-enzymatic metabolite 6-keto-$PGF_{1\alpha}$) were below the limit of detection of the assay used here in human muscle biopsy samples collected both before and after the resistance exercise intervention (Supplemental Table 1).

Omega-3 derived: The majority of series three (EPA derived) prostaglandins were below the limit of detection of our assay in human skeletal muscle tissue (Supplemental Table 1). However, a downstream bioactive metabolite of the EPA-derived PGD_3, 15-Deoxy-$\Delta^{12,14}$-prostaglandin J_3, was detected at concentrations of 3.09 ng/g in resting muscle. Furthermore, 15-Deoxy-$\Delta^{12,14}$-prostaglandin J_3 increased 1.35 fold to concentrations of 4.45 ng/g at 2 h post-exercise ($P = 0.010$) (Figure 1E). 15-Deoxy-$\Delta^{12,14}$-prostaglandin J_3 returned to basal levels by 4 h ($P = 0.764$) and 24 h ($P = 0.344$).

Lipoxygenase pathways:

5-LOX: The 5-LOX pathway primarily converts AA substrate to 5-hydroperoxy-eicosatetranoic acid (5-HpETE), which can be reduced to 5-hydroxy-eicosatetranoic acid (5-HETE), or undergo further metabolism via 5-LOX to form the leukotrienes. 5-HETE was detected in resting muscle at a concentration of 3.38 ng/g (Figure 2A). Muscle 5-HETE levels increased at 2 h post-exercise to 8.99 ng/g (p=0.017) (Figure 2A). Leukotriene B_4 (LTB_4) was below the level of detection of our assay in the majority of resting muscle biopsies, but was consistently detected at concentrations of ~3 ng/g at 2 h of recovery from the bout of resistance exercise (Figure 2B). Furthermore, downstream degradation products of LTB_4, including 12-Oxo-LTB_4 and 20-COOH-LTB_4 and, were both detected in resting muscle and increased in abundance at 2 h post-exercise to concentrations of 2.29 ng/g ($P < 0.041$) (Figure 2C) and 5.33 ng/g ($P < 0.001$) (Figure 2D) respectively. Intramuscular 5-LOX products of AA including 5-HETE, 12-Oxo-LTB_4 and 20-COOH-LTB_4 were no longer increased above basal levels at 4 h or 24 h of recovery.

12-LOX: The 12-LOX enzyme is expressed primarily in human platelets and metabolizes AA to form 12-hydroxy-eicosatetraenoic acid (12-HETE), which is a key stimulator of leucocyte chemotaxis and platelet aggregation (4, 59, 60). 12-HETE was by far the most abundant monohydroxylated-FA product detected in resting muscle tissue, present at concentrations of 22.51 ng/g. Muscle 12-HETE further increased above resting levels at 2 h post-exercise to concentrations of 63.81 ng/g ($P = 0.01$) (Figure 3A). Tetranor 12-HETE, a downstream degradation product of 12-HETE, was also detected in resting muscle tissue at lower concentrations of 0.62 ng/g and increased markedly at 2 h post-exercise to reach intramuscular concentrations of 3.97 ng/g ($P = 0.006$) (Figure 3B). Furthermore, the 12-LOX metabolites of n-3 EPA, 12-hydroxy-eicosapentaenoic acid (12-HEPE) was present in resting muscle biopsies and increased at 2 h post-exercise ($P = 0.016$) (Figure 3C). By 4 h and 24 h of recovery, intramuscular 12-LOX products including 12-HETE, tetranor 12-HETE and 12-HEPE no longer differed from resting levels.

15-LOX: The 15-LOX pathway converts AA to 15-hydroxy-eicosatetranoic acid (15-HETE) (58). 15-HETE was detected in resting muscle at 3.84 ng/g. Muscle 15-HETE tended to increase at 2 h post-exercise to 6.50 ng/g, but this did not achieve statistical significance ($P = 0.10$) (Figure 4A). The 15-LOX metabolite of n-3 EPA,

15-hydroxy-eicosapentaenoic acid (15-HEPE) was also detected in resting muscle tissue, but was not influenced by the exercise intervention (main effect p=0.308) (Figure 4B).

Docosanoids: In addition to the 20-carbon PUFA AA and EPA, LOX pathway converts the 22-carbon n-3 PUFA DHA to docosanoid metabolites which most notably are key pathway markers and intermediates in the biosynthesis of the SPM family of bioactive lipid mediators.

The 5-LOX enzyme oxidizes n-3 DHA to form the monohydroxylated-DHA (HDoHE) products 4-hydroxy-docosahexanoic acid (4-HDoHE) and 7-hydroxy-docosahexanoic acid (7-HDoHE). Both 4- and 7-HDoHE were detected in resting muscle at concentrations of 2.23 ng/g (Figure 5A) and 0.98 ng/g (Figure 5B) respectively. 7-HDoHE increased at 2 h post-exercise to 1.54 ng/g ($P = 0.008$) (Figure 5B). Similarly, muscle 4-HDoHE tended to increase from pre-exercise levels at 2 h post-exercise ($P = 0.069$), and was statistically greater at 2 h compared than both 4 h ($P = 0.011$) and 24 h ($P = 0.016$) of recovery (Figure 5A).

The 12-LOX enzyme converts the 22-carbon DHA to 14-hydroperxy-docosahexanoic acid (14-HpDoHE) which can then be reduced to 14-HDoHE or metabolised to form the maresins (MaR 1 & 2) via the further action of 12-LOX. Similarly, the 15-LOX enzyme converts DHA to 17-hydroxy-docosahexanoic acid (17-HDoHE) which can be converted to the D-series resolvins via the subsequent action of 5-LOX. Therefore, 14-HDoHE and 17-HDoHE are pathway markers of increased MaR and RvD biosynthesis respectively. We detected 14-HDoHE in resting skeletal muscle at a concentration of 0.68 ng/g (Figure 5C). Muscle 14-HDoHE increased at 2 h post-exercise to concentrations of 1.60 ng/g ($P = 0.005$) (Figure 5C). In contrast, 17-HDoHE was not found to be present at detectable concentrations within human muscle biopsies at rest or at any time-point throughout exercise recovery (Supplemental Table 1).

Multiple reaction monitoring (MRM) transitions corresponding to mature SPMs including the lipoxins (LXA_4, LXB_4, LXA_5), E-series resolvins (RvE1 & RvE3), D-series resolvins (RvD1, RvD2, RvD5, RvD6), protectins (PD1 & 10S,17S-DiHDoHE) and maresins (MaR1) were additionally monitored by our LC-MS/MS assay. Resting muscle tissue was found to contain low but detectable concentrations

of RvD6 (1.04 ng/g), PD1 (0.64 ng/g) and MaR1 (0.75 ng/g). The greatest average intramuscular concentrations of RvD6 (1.81 ng/g), PD1 (2.58 ng/g) and MaR1 (1.33 ng/g) were observed at 2 h post-exercise. Despite this, the low and sporadic concentrations in certain subjects at particular time-points, combined with our repeated measures study design, precluded statistical analysis. Other mature SPMs including LXA_4, LXB_4, LXA_5, RvD1, RvE3, RvD1, RvD2, RvD5 and the protectin D1 isomer 10S,17S-DiHDoHE were not found to be present at detectable levels locally within human muscle tissue biopsies at rest or throughout 24 h of post-exercise recovery under the conditions used here (Supplemental Table 1).

Epoxygenase pathway: The cytochrome P-450 (CYP) enzymes metabolize n-6 PUFA AA to a family of epoxyeicosatrienoic acid (EpETrE) regioisomers. Once formed, these bioactive EpETrEs are rapidly metabolized by the soluble epoxide hydrolase (sEH) enzyme to form corresponding downstream dihydroxyeicosatrienoic acids (DiHETrEs) vicinal diols. AA epoxides including 5,6-, 8,9-, 11,12- and 13,14-EpETrE, were detected in resting muscle tissue at concentrations between 3-10 ng/g. Despite this, 5,6-EpETrE was the only primary enzymatic epoxy AA-metabolite that showed an effect for the exercise intervention, increasing 3-fold to 14.12 ng/g at 2 h post-exercise ($P < 0.003$) (Figure 6A). Downstream epoxide products of the sEH enzyme, 11,12-DiHETrE and 14,15-DiHETrE were present at much lower absolute concentrations in resting muscle (~1 ng/g), but increased in response to exercise with a peak response at 2 h of recovery (11,12-DiHETrE $P = 0.026$, 14,15-DiHETrE $P = 0.011$) (Figure 6B & C). The 5,6-DiHETrE regioisomer was also detected at low levels in resting muscle, but was unchanged during post-exercise recovery (Supplemental Table 1).

CYP epoxidase enzymes also have the capacity to metabolise the n-6 linoleic acid to bioactive lipid mediators including 9(10)-EpOME and 12(13)-EpOME and their downstream sEH products 9(10)-DiHOME and 12(13)-DiHOME. Both 9(10)- and 12(13)-EpOME were highly abundant in resting human muscle present at concentrations of 100.87 ng/g and 54.36 ng/g respectively, but were not influenced by the exercise intervention (Supplemental Table 1). In contrast, 9(10)- and 12(13)-DiHOME were present at lower concentrations in resting muscle, but increased significantly at 2 h post-exercise (p=0.034 and p=0.020 respectively) (Figure 7). CYP

pathway metabolites of both AA and LA no longer differed from pre-exercise levels by 4- and 24-h of post-exercise recovery.

Discussion:

The present study explored the intramuscular lipid mediator response following an acute bout of resistance exercise. We identified 84 unique lipid mediators as present within skeletal muscle tissue. The early post-exercise response was characterized by increased tissue concentrations of a range of bioactive lipid derivatives of the cyclooxygenase (COX-1 & 2), lipoxygenase (5-, 12- & 15-LOX), and epoxygenase (CYP) pathways. Unexpectedly, however, peak induction of both pro-inflammatory and SPM pathway intermediates occurred simultaneously at 2 h of recovery. The findings from this study identify a complex parallel adaptive lipid response to resistance exercise that may play an essential role in regulating the onset and resolution of acute exercise-induced skeletal muscle inflammation.

This is the first paper to use a targeted lipidomics approach to extensively characterize the lipid mediator profile of human skeletal muscle tissue at rest and in response to acute exercise. The majority of human research exploring the effect of exercise on lipid species has focused primarily on a select few prostaglandins, specifically PGE_2 and $PGF_{2\alpha}$, likely due to their complex role in regulating acute inflammation, perceptions of pain and purported roles in muscle cell growth/regeneration (13, 14, 53, 54). Although some studies have reported elevated circulating levels of PGE_2 following exercise-induced muscle injury (5, 24, 50, 56), these findings have not yet been replicated in skeletal muscle tissue (29, 54). $PGF_{2\alpha}$ increases locally within skeletal muscle tissue following both eccentric (54) and

isotonic resistance exercise protocols (53). It plays a significant role in *in-vitro* myofibre hypertrophy (21) and post-exercise muscle protein synthesis (55). We previously found a significant increase in serum levels of the circulating $PGF_{2\alpha}$ metabolite 15-keto-$PGF_{2\alpha}$ early (1 h) following resistance exercise in humans (24). In the current study, PGE_2 and $PGF_{2\alpha}$ were the most abundant AA derived PGs detected within muscle tissue and both transiently increased in abundance at 2 h following resistance exercise. Furthermore, intramuscular production of TXA_2 (measured by heightened local TXB_2 and 12(S)-HHTrE concentrations) increased at 2 h post-exercise. This finding within the exercised musculature is consistent with prior reports of transiently increased systemic TXB_2 concentrations following both acute maximal aerobic (16) and resistance exercise (24). COX enzymes are also able to metabolize linoleic acid to form hydroxyoctadecadienoic acids (HODEs), which function stimulate the maturation of monocytes to form macrophages. Previous research has identified an increase in plasma in 9- and 13-HODE following 75km of cycling (27), however the present study is the first to detect an increase in 9- and 13- HODEs in skeletal muscle tissue following acute resistance exercise.

Another major metabolic pathway leading to the formation of lipid species involved in the regulation of inflammation is the 5-lipoxygenase pathway. 5-LOX derived LTB_4 is increased in human blood serum following acute resistance exercise (24) and high speed running (11). LTB_4 is a potent neutrophil chemoattractant and a powerful stimulator of vasoconstriction and blood vessel permeability (26). Expression of 5-LOX is essentially limited to bone-marrow derived cells including inflammatory neutrophils and monocytes/macrophages (34). It is therefore not surprising that in the present study LTB_4 was very lowly expressed in skeletal muscle tissue prior to exercise. At 2 h post-exercise, LTB_4 was present at detectable concentrations in muscle biopsies from the majority of subjects, indicative of a potential increase from resting levels. Consistently, downstream derivatives of LTB_4, including 12-Oxo LTB_4 and 20-COOH LTB_4, increased above resting levels at 2 h post-exercise. A less well-described branch of the 5-LOX pathway involves the metabolism of 22-carbon n-3 PUFA DHA to form mono-hydroxylated fatty acids 4- and 7-HDoHE. Both of these fatty acids were detected in resting skeletal muscle tissue and increased above basal levels at 2 h post-exercise. On the other hand, we

observed no change in 4- or 7-HDoHE during recovery from resistance exercise in human blood serum samples previously (24).

In addition to 5-LOX, metabolites of the human platelet type 12-LOX enzyme 12-HETE and its downstream derivate tetranor 12-HETE were also elevated within muscle post-exercise. Both metabolites are pro-inflammatory in nature and act transcellularly to modify the responsiveness of neutrophils to other chemotactic factors (31). We previously observed a similar increase in 12-HETE and downstream tetranor 12-HETE in human blood serum samples during recovery from resistance exercise (24). Interestingly 12-LOX expressing platelets are implicated in the transcellular biosynthesis of pro-resolution LX mediators through interactions with 5-LOX expressing PMNs. This pathway involves leucocyte-platelet interactions during which the 5-LOX derived leukotriene intermediate LTA_4 is taken up by 12-LOX expressing platelets for subsequent conversion to LXA_4 (33, 48). Further, the 15-LOX pathway is implicated as a second endogenous route of LX biosynthesis species. 15-LOX is highly expressed in alternatively activated macrophages and epithelial cells. The secretion of the 15-LOX product, 15-HETE, can be taken up by 5-LOX expressing cells and converted to LXA_4 and LXB_4 (37). In the current study we observed a trend towards an increase in 15-HETE 2 h post-exercise, which supports our prior observation in human blood serum samples (24). The simultaneous induction of the primary products of both the 5-LOX/12-LOX and 15-LOX/5-LOX pathways within skeletal muscle during post-exercise recovery is presumably a permissive environment for local LX biosynthesis. We were however, unable to detect LXA_4 or LXB_4 themselves within the muscle biopsy homogenates analysed here. Nevertheless, these local changes within the exercised musculature suggests that skeletal muscle tissue may potentially contribute to the previously reported systemic lipoxin response to resistance exercise following their release from exercised myofibers (24).

The CYP pathway is a third and less well-characterized branch of the AA metabolic pathway. The increase in epoxyeicosatrienoic acid regioisomer 5-6-EpETrE and dihydroxyeicosatrienoic acids 11,12- and 14,15-DiHETrE in skeletal muscle supports observations made in serum samples when measured during the early stages of post-exercise inflammation (24). The physiological function of these derivatives remains unexplored in skeletal muscle tissue. However, in vascular smooth muscle

and endothelium cells they play anti-inflammatory roles through in the inhibition of prostaglandin and cytokine induced inflammatory responses (49). CYP enzymes also metabolise linoleic acid via epoxidation to form epoxy-octadecanoic acids (EpOMEs). EpOMEs are rapidly hydrolysed by the sEH enzyme to form corresponding dihydroxy-octadecanoic acids (DiHOMEs) (15). EpOMEs and DiHOMEs are leukotoxins that play a role in the suppression of neutrophil respiratory burst activity, vasodilation and cellular apoptosis (28, 51). A cycling based intervention comprising of a 75km time trial had no effect on 9-10,DiHOME 1.5 h and 21 h post-exercise in plasma samples of competitive road cyclists (28). Alternatively, a bout of acute resistance exercise triggered an increase in 9(10)-EpOME and 9-10,DiHOME in serum samples (24). Results from the present study showed an increase in 9,10-DiHOME and 12,13-DiHOME suggesting that discrepancies in the previous literature may be due to the differences in the type of exercise performed and the training status of the subjects.

Collectively, these findings demonstrate an increase in bioactive lipid derived mediators of the COX, LOX and CYP pathways during post-exercise muscle recovery. The concept of a biologically active inflammatory resolution programme governed by lipid derivatives was first proposed in a TNF-α-stimulated model of acute inflammation in the murine air pouch (17). Within this model, early formation of LTB_4 and PGE_2 at the onset of inflammation was succeeded by a class-switching of eicosanoids to LXA_4. During this process, interactions between inflammatory and host tissue cells enabled the biosynthesis of resolution mediators (17). Alternatively, in a model of zymosan-A stimulated murine peritonitis, the onset of inflammation was characterized by a concomitant increase in LTB_4 and LXA_4 followed by a late appearance of PGE_2 at the onset of resolution (2). These findings demonstrate that the temporal regulation of lipids and their role in inflammation is likely cell-type and stimulus specific. Recent work from our group profiled the human lipid response to acute resistance exercise in serum samples (24). This study showed an increase in key prostaglandin, leukotriene, lipoxin and resolvin species during the early stages of acute inflammation (1-3 h), followed by an increase in 15-LOX derivatives and some prostaglandin metabolites (6-keto-$PGF_{1\alpha}$ and 13,14dh-15kPGE_2) 24 h post- exercise (24). The present finding of increased intramuscular abundance of bioactive lipid mediators at 2 h post-exercise is overall consistent with findings from serum samples

(24), suggesting that muscle may be a major source of blood lipid mediators. Interestingly lipid species that require transcellular interactions, including lipoxins, resolvins and protectins, were either undetected, or very lowly expressed in skeletal muscle tissue, disproving the original hypothesis predicting a delayed increase in SPMs coincident with the resolution of acute inflammation. These lipids were detectable previously in human serum samples during post exercise recovery and play a vital role in the active resolution of acute inflammation (24).

Conclusion:

This is the first study to characterize the lipid mediator profile of human skeletal muscle tissue at rest and following acute resistance exercise. We identified an increase in lipids autocoids derived from the COX, LOX and CYP pathways. Peak induction of AA derived classical pro-inflammatory prostaglandins (TXB_2, PGE_2 and $PGF_{2\alpha}$) and leukotrienes (LTB_4, 12-Oxo LTB_4 and 20-COOH LTB_4) occurred at 2 h post-exercise. Further various derivatives of the 5-LOX (5-HETE, 4-HDoHE and 7-HDoHE), 12-LOX (12-HETE, tetranor 12-HETE and 14-HDoHE) and 15-LOX (15-HETE) pathways were identified in abundance in skeletal muscle tissue at 2 h post-exercise and may resemble transient cellular intermediates for the formation of pro-resolution lipoxin and resolvin species. In alternative models of acute inflammation, these lipids are involved in coordinating a biologically active inflammatory resolution programme that is mechanistically linked to tissue healing. The present study was limited in that the analysis was performed during the first 24 hours of post-exercise recovery. Later time points may provide insight in to an ongoing cascade of complex lipidomic alterations (35, 39, 40). This study represents a descriptive analysis of the skeletal muscle lipid response to acute resistance exercise. Further mechanistic research exploring the physiological significance and function of these lipids, both locally within skeletal muscle tissue and following their systemic release, will be useful in further characterizing the significance of the intramuscular inflammatory response in post-exercise muscle recovery.

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723 **Figure Legends:**

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725 **Figure 1:** Metabolites of the cyclooxygenase (COX) pathway derived from
726 arachidonic acid and eicosapentaenoic acid. Values depicted are mean values ± SEM.
727 * denotes statistical significance compared to pre-exercise values ($P < 0.05$).

728 **Figure 2:** Metabolites of the 5-lipoxygenase (5-LOX) pathway derived from
729 arachidonic acid. Values depicted are mean values ± SEM. * denotes statistical
730 significance compared to pre-exercise values ($P < 0.05$).

731 **Figure 3:** Metabolites of the 12-lipoxygenase (12-LOX) pathway derived from
732 arachidonic acid and eicosapentaenoic acid. Values depicted are mean values ± SEM.
733 * denotes statistical significance compared to pre-exercise values ($P < 0.05$).

734 **Figure 4:** Metabolites of the 15-lipoxygenase (15-LOX) pathway derived from
735 arachidonic acid and eicosapentaenoic acid. Values depicted are mean values ± SEM.
736 * denotes statistical significance compared to pre-exercise values ($P < 0.05$).

737 **Figure 5:** Metabolites of the lipoxygenase pathways derived from docosahexaenoic
738 acid. Values depicted are mean values ± SEM. * denotes statistical significance
739 compared to pre-exercise values ($P < 0.05$). ^ denotes statistical significance
740 compared to 4 and 24 h post-exercise values ($P < 0.05$).

741 **Figure 6:** Metabolites of the epoxygenase pathway derived from arachidonic acid.
742 Values depicted are mean values ± SEM. * denotes statistical significance compared
743 to pre-exercise values ($P < 0.05$).

744 **Figure 7:** Metabolites of the epoxygenase pathway derived from linoleic acid. Values
745 depicted are mean values ± SEM. * denotes statistical significance compared to pre-
746 exercise values ($P < 0.05$).