

# Emerging roles of pro-resolving lipid mediators in immunological and adaptive responses to exercise-induced muscle injury

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## ABSTRACT

*Lipid mediators are bioactive metabolites of the essential polyunsaturated fatty acids (PUFA) that play diverse roles in the initiation, self-limitation, and active resolution of inflammation. Prostaglandins, classical pro-inflammatory lipid metabolites of arachidonic acid, have long been implicated in immunological and adaptive muscle responses to acute injury and exercise-induced stress. More recently, PUFA metabolites have been discovered during the resolution phase of inflammation which collectively function as endogenous 'stop signals' to control inflammation whilst actively promoting the return to a non-inflamed state. The apparent self-resolving nature of inflammatory responses holds important implications for contexts of musculoskeletal injury, exercise recovery, and chronic inflammatory diseases originating in or impacting upon muscle. 'Anti-inflammatory' interventions that strive to control inflammation via antagonism of pro-inflammatory signals are currently commonplace in efforts to hasten muscle recovery from damaging or exhaustive exercise, as well as to relieve the pain associated with musculoskeletal injury. However, the scientific literature does not clearly support a benefit of this anti-inflammatory approach. Additionally, recent evidence suggests that strategies to block pro-inflammatory lipid mediator pathways (e.g. NSAIDs) may be counterintuitive and inadvertently derange or impair timely resolution of inflammation; with potentially deleterious implications on skeletal muscle remodelling. The current review will provide an overview of the current understanding of diverse roles of bioactive lipid mediators in the initiation, control, and active resolution of acute inflammation. The established and putative roles of lipid mediators in mediating immunological and adaptive skeletal muscle responses to acute muscle injury and exercise-induced muscle load/stress will be discussed.*

**Key words:** Inflammation, resolution, PUFA, docosanoids, eicosanoids.

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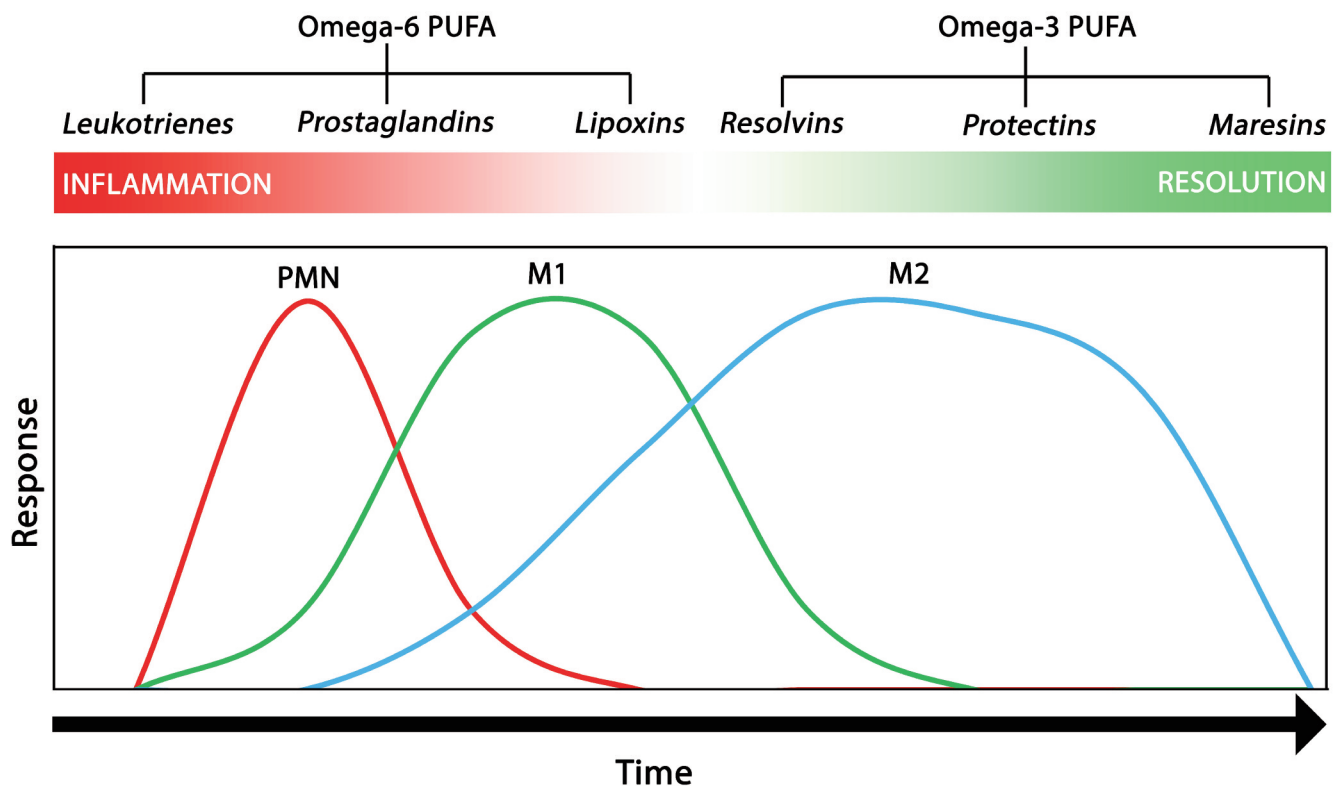
## 1. INTRODUCTION

Inflammation is an essential protective response to injury or infection to eliminate the harmful agent and promote tissue repair. Overwhelming and persistent inflammation, however, can result in secondary cellular damage, promote maladaptive tissue remodelling and lead to the onset of degenerative chronic inflammatory disease. In a typical self-limited acute inflammatory response, clearance of cellular debris and pathogen removal by phagocytic immune cells is followed by successful resolution of inflammatory infiltrates, involving clearance of invading leukocytes, cellular repair, and restoration of tissue homeostasis. The resolution phase of the inflammatory response, although once thought to be a passive

process, has only more recently been recognised to be an active and biologically controlled event (1-3). The nature and extent of resolution of inflammation is an area of critical importance in the context of muscle and exercise physiology, both to understand the inherent self-resolving nature of skeletal muscle injury/alterd use and gain insight into how this knowledge may be applied to contexts of exercise recovery, musculoskeletal injury management, and prevention/treatment of chronic inflammatory disease.

Skeletal muscle injury elicits an acute inflammatory response characterised by the secretion of soluble mediators within damaged tissue that promote influx of fluid and plasma proteins followed by the infiltration and local accumulation of

locally into tissue macrophages (MΦs) (6, 7, 12, 13) (Figure 1). Successful resolution of inflammation requires apoptosis of PMNs within the infiltrate, followed by their elimination by nonphlogistic MΦ phagocytosis. Systemic depletion of monocytes has been conclusively shown to delay resolution of muscle inflammation, leading to impaired myofiber regenerative/adaptive responses to both degenerative injury (13-17) and overload induced muscle hypertrophy (18). Early, following injury, circulating classically activated (M1) pro-inflammatory monocytes infiltrate muscle and phagocytose myofiber debris and apoptotic neutrophils, a process which triggers a switch to an alternatively activated anti-inflammatory (M2) phenotype (19). During the latter stages of inflammation these M2 populations are predominant and play an impor-



**Figure 1: Time-course of leukocyte populations and bioactive lipid mediators in the inflammatory response to muscle injury/overload.** Following tissue injury, neutrophils (PMNs) are rapidly mobilised, increase in circulation, and migrate from the blood stream into damaged tissue. Pro-inflammatory lipid mediators, the leukotrienes (LTs) and prostaglandins (PG) facilitate PMN trafficking via cellular effects on blood flow (vasodilation), vascular permeability, and PMN chemotaxis. Later in the inflammatory response there is a shift to biosynthesis of lipid mediators with anti-inflammatory and pro-resolving properties including n-6 PUFA derived lipoxins and n-3 PUFA derived resolvins, protectins and maresins. These signals function to limit and stop PMN trafficking, whilst promoting recruitment of blood monocytes/MΦ which phagocytose and clear apoptotic PMNs. Classically-activated M1 monocyte populations initially are predominant but are later replaced by an alternatively activated M2 phenotype which play important roles in facilitating muscle growth and regeneration.

blood leukocytes (4, 5) (Figure 1). Muscle inflammation occurs in response not only to traumatic injury, but also to a diverse range of loading stimuli including eccentric exercise and reloading following muscle disuse. Polymorphonuclear neutrophils (PMNs) constitute the first wave of host immune cells, infiltrating damaged muscle within minutes to hours (6-9) (Figure 1). PMNs are phagocytic granulocytes that ingest and eliminate damaged tissue, whilst releasing reactive molecules through degranulation which can potentially induce secondary muscle damage and exacerbate muscle injury (8, 10, 11). The early wave of PMNs is followed later by recruitment of blood monocytes which accumulate within muscle in the hours to days following mechanical insult and differentiate

tant active role in promoting muscle growth and regeneration (20-23) (Figure 1). Eosinophils, another key innate immune cell population active within the resolution phase of inflammation, were also recently shown to be indispensable in the muscle regenerative response to injury (24). Collectively these data show that resolution phase of inflammation plays an important role in skeletal muscle adaptation to injury.

A complex signalling network exists between infiltrating immune cells and resident populations within the musculature including muscle cells (myofibers), myogenic stem cells (satellite cells), endothelial cells, and fibroblasts. A key, yet poorly understood, aspect of this regulation which is frequent-

**Table 1:**  
**Inflammatory and muscle roles of arachidonic acid metabolites of the COX-1 & 2 pathways**

	Receptors	Major inflammatory role	Muscle role <i>Effect</i>	<i>Mechanisms</i>	References
<i>PGD<sub>2</sub></i>	DP1	Anti-inflammatory/pro-resolving	↑ Myoblast proliferation	↑ 15Δ-PGJ <sub>2</sub>	(150)
	DP2		↓ Myoblast differentiation	↓ MyoD, ↓ myogenin	(97)
<i>PGE<sub>2</sub></i>	EP1	Pro-inflammatory	↑ Myoblast proliferation	↑ ROS, ↑ cyclin E, ↓ myostatin	(98, 151)
	EP2	↑ <i>Vascular permeability</i>	↑ Myoblast differentiation	↑ MyoD & myogenin (Low dose)	(300)
	EP3	↑ <i>Leukocyte chemotaxis</i>	↓ Myoblast fusion	↓ Calcium (high dose)	(293)
	EP4	↑ <i>Vasodilation</i> ↑ <i>Algesia</i>	↑ Protein degradation	↑ MuRF/expression ↑ IL-6 expression	(139, 301) (301)
<i>PGF<sub>2</sub>α</i>	FP	Pro-inflammatory	↑ Myoblast number	↓ Apoptosis, ↑ BRUCE	(152, 153)
		↑ <i>leukocyte chemotaxis</i>	↑ Myoblast fusion	↑ Survival, ↑ NFATC2 signalling	(153, 154)
			↑ Protein synthesis		(139)
			↑ Myotube hypertrophy	↑ mTOR/ERK signalling	(149)
<i>PGI<sub>2</sub></i>	IP	Pro-inflammatory/anti-aggregatory			
		↑ <i>vascular permeability</i>	↓ Myoblast migration	↓ Cell motility	(101)
		↓ <i>platelet aggregation</i>	↑ Myoblast fusion		(101)
		↑ <i>vasodilation</i> ↑ <i>algesia</i>			
<i>TXA<sub>2</sub></i>	TP	Pro-inflammatory/pro-Aggregatory	?	?	
		↑ <i>platelet aggregation</i>			
		↑ <i>vasoconstriction</i>			
		↑ <i>leukocyte chemotaxis</i>			

ly overlooked in the context of muscle and exercise is the role played by bioactive lipids. Lipid mediators are autocrine/paracrine signalling molecules derived from cellular polyunsaturated acids (PUFA). Under basal conditions, the majority of PUFA substrate remains esterified within cell membrane phospholipids, but a proportion is rapidly released by the action of phospholipase A2 (PLA2) in response to injurious or inflammatory stimuli. Lipid mediator biosynthesis involves oxidation of mobilised PUFA substrate by three major enzymatic pathways: (1) cyclooxygenase (COX), (2) lipoxygenase (LOX) and (3) epoxygenase catalysed by cytochrome P450 (CYP). Hundreds of distinct lipid mediator species can be synthesised via these pathways from numerous fatty acid (FA) precursors including major omega-6 (n-6) arachidonic acid (AA) and long-chain (LC) omega-3s (n-3) eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA). During the early stages of tissue injury, classical pro-inflammatory lipid mediators are locally produced (e.g. prostaglandins and leukotrienes) which drive inflammation. Later in the inflammatory response, a shift in the profile of these bioactive lipids results in the predominant generation of mediators with anti-inflammatory and pro-resolving bioactivity which function to actively bring about the resolution phase of inflammation (e.g. lipoxins, resolvins, protectins and maresins) (25, 26) (Figure 1). The development of mass spectrometry (MS)-based lipidomic profiling methods has recently allowed for the simultaneous identification and quantification of large numbers of inflammatory and resolving lipid mediator metabolites in biological fluids. This approach sometimes referred in the literature to as

“mediator lipidomics” or “metabololipidomics” enables the profiling of the “mediator lipidome” of biological samples encompassing the bioactive metabolites of the PUFAs (27–29). A large number of studies have focused on the systemic and intramuscular leukocyte and inflammatory cytokine immunological responses to exercise and these topics have been covered in several recent comprehensive reviews (e.g 30, 31). In contrast, the potential diverse role of bioactive lipid mediators in exercise immunology is yet to undergo systematic investigation and the topic has not been comprehensively reviewed previously.

The present review aims to provide an overview of the diverse role of bioactive lipid mediators in driving acute inflammation and bringing about its timely resolution, whilst discussing the established and putative roles of lipid mediators within muscle tissue. The current understanding of the role of lipid mediators in the adaptive responses to exercise is reviewed whilst highlighting the many gaps in our current knowledge and significant areas for future investigation.

## 2. CYCLOOXYGENASE PATHWAYS

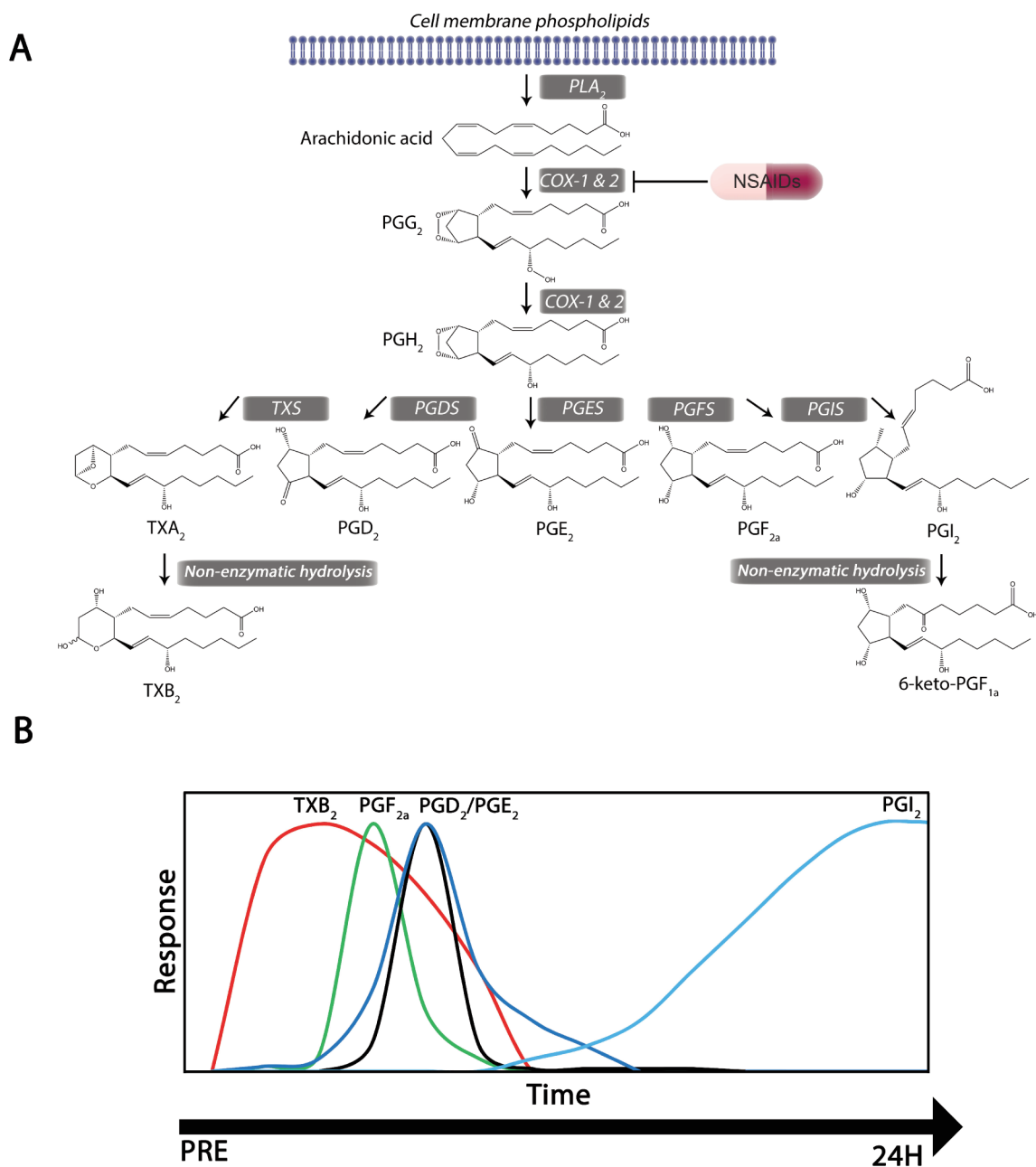
The role of bioactive lipids in inflammation has classically focused on PUFA metabolites of the cyclooxygenase (COX) pathway. The COX pathway converts mobilised free intracellular AA to prostaglandin G<sub>2</sub> (PGG<sub>2</sub>), and catalyses the subsequent reduction of PGG<sub>2</sub> to form prostaglandin H<sub>2</sub> (PGH<sub>2</sub>) (Figure 2A). Specific synthase enzymes convert PGH<sub>2</sub> to the

five primary prostanoids; thromboxane A<sub>2</sub> (TXA<sub>2</sub>), PGD<sub>2</sub>, PGE<sub>2</sub>, PGF<sub>2α</sub> and PGI<sub>2</sub> (Figure 2A). Collectively these eicosanoid metabolites elicit the cardinal signs of inflammation; rubor (redness), calor (heat), tumor (swelling) and dolor (pain), via their cellular actions in vasodilation (32), vascular permeability (32-36), hyperalgesia (37-41), and PMN chemotaxis (42-45) (Table 1). Two COX isoforms are expressed in mammalian tissue. COX-1 is a constitutively expressed enzyme that allows for rapid PG synthesis following AA release which regulates a variety of homeostatic functions, whilst, COX-2 is transcribed by an inducible gene, which is up-regulated in the hours following exposure to inflammatory stimuli. The COX 1 & 2 pathways are well known as the molecular target of the non-steroidal anti-inflammatory class

of drugs (NSAIDs), which chiefly function to prevent PG biosynthesis via inhibition of the cyclooxygenase activity of the COX-1 & 2 enzymes (46-48) (Figure 2A).

## 2.1 Prostanoid response to exercise:

The PG response to muscle loading has been studied for many years (49-51); however our understanding of the temporal changes in local and systemic PG concentrations in exercising humans *in-vivo* and their role in physiological exercise responses remains incomplete. Early human studies using experimental models of sub-maximal exercise consistently found heightened levels of PGs including PGE<sub>2</sub> (52-56), PGF<sub>2α</sub> (52, 57), and PGI<sub>2</sub> (measured as 6-keto-PGF<sub>1α</sub>, a non-enzymatic hydrolysis product of PGI<sub>2</sub>) (52, 53, 58-63) in



**Figure 2: Cyclooxygenase (COX-1 & 2) pathways of lipid mediator biosynthesis during exercise recovery.** A: COX-1 & 2 pathways of prostanoid biosynthesis. Free intracellular arachidonic acid (AA) substrate, mobilized from cell membrane phospholipids is converted to PGH<sub>2</sub> by the action of the COX-1 & 2 enzymes. PGH<sub>2</sub> is the precursor to the major bioactive PGs including TXA<sub>2</sub>, PGD<sub>2</sub>, PGE<sub>2</sub>, PGF<sub>2α</sub> and PGI<sub>2</sub> by action of specific PG synthases. B: Time-course of peripheral blood COX/PG responses during recovery from unaccustomed resistance exercise as determined by mediator lipidomic profiling (102).



human plasma/serum during exercise. Excretion of 2,3-dinor-6-keto-PGF<sub>1α</sub> (64-68), and tetranor-PGE<sub>2</sub> (69), urinary metabolites of PGI<sub>2</sub> and PGE<sub>2</sub> respectively, were also found to be elevated during exercise recovery. The effect of exercise on TXA<sub>2</sub> (measured as circulating TXB<sub>2</sub> a non-enzymatic hydrolysis product of TXA<sub>2</sub> or urinary 2,3-dinor-TXB<sub>2</sub> metabolites) is less clear. Low intensity exercise appears to predominantly increase the vasodilator/anti-platelet aggregator PGI<sub>2</sub>, with no (61, 64, 66, 68), or minimal (65), changes in the vasoconstrictor/platelet aggregator TXA<sub>2</sub>. In contrast, maximal (53, 59, 60, 70) or high intensity (71) exercise has been reported to elevate TXB<sub>2</sub> in most, but not all (63) studies.

Fewer studies have investigated the PG response to potentially damaging muscular contractions with resistance or eccentric exercise, but available data suggests a relatively delayed or prolonged response. A bout of intense stretch-shortening cycle exercise was reported to elevate circulating PGE<sub>2</sub>, peaking 2 h into recovery (72). Other studies have reported increased plasma/serum PG concentrations for 1-3 days following a bout of resistance exercise consisting of bench press (50-90% 1RM) (PGE<sub>2</sub>) (73), barbell squat (70% 1RM) (13,14-dihydro-15-keto-PGF<sub>2α</sub>) (74) and step-up (body weight) (PGE<sub>2</sub>) (75). In contrast, several studies failed to detect any changes in circulating PGE<sub>2</sub> following isolated eccentric contractions of the knee extensors (76, 77) or elbow flexors (78-80). Similarly, downhill running had no effect (81), or no greater effect than level surface high intensity running (55), on circulating PGE<sub>2</sub>. The reasons for such conflicting findings across different exercise protocols is unclear, but may relate to the intensity of muscle load (downhill running (55, 81) vs. resistance exercise (73, 75)) or the volume of active muscle mass (unilateral isolation (76-80) vs. bilateral compound exercises (73, 75, 82, 83). Additionally, the sensitivity and specificity of immunological assays (e.g. ELISA) used by the majority of these studies is a likely contributing factor (84, 85).

In addition to studies measuring PGs in peripheral circulation, a limited number of studies have attempted to detect PGs within exercised muscle tissue (86-92). At 24 h of recovery from eccentric resistance exercise, PGF<sub>2α</sub> was found to be increased in human muscle biopsy sample homogenates (90). A more traditional bout of resistance exercise, also increased PGF<sub>2α</sub> in muscle microdialysates at 5-6 and 8-9 h post-exercise, but returned to basal levels by 24 h (86). Enzymatic activity of both COX-1 and 2 are elevated in muscle biopsies collected during exercise recovery (93), a response which appears to be driven by delayed elevation of the inducible COX-2 (but not COX-1) at both the mRNA (94) and protein (93) level. The expression of COX-2 also increases in human peripheral blood mononuclear cells during exercise recovery, suggesting that infiltrating leukocytes are likely a key source of PGs in exercised muscle (95). Interestingly, however, muscle cells themselves express both COX-1 and 2 (96) and are capable of synthesising and secreting a range of COX metabolites including PGD<sub>2</sub> (97), PGE<sub>2</sub>/PGF<sub>2α</sub> (14, 49, 98-100) and PGI<sub>2</sub> (101). Therefore PGs are likely to be important autocrine/paracrine signalling molecules produced locally within muscle tissue, and contracting muscle presumably

contributes to elevated PGs and/or their metabolic degradation products in peripheral blood during post-exercise recovery.

The development of mediator lipidomic profiling methods has recently allowed for more comprehensive analysis of circulating COX metabolites during exercise recovery than previously available. By employing a human model of unaccustomed resistance exercise, we analysed the temporal changes in peripheral blood lipid mediators during post-exercise recovery using an unbiased metabolipidomic profiling approach (102). Immediately post-exercise we observed marked elevation in serum TXB<sub>2</sub>, which preceded the other COX metabolites. This early response was followed by delayed elevation of, PGD<sub>2</sub>, PGE<sub>2</sub>, PGF<sub>2α</sub> (and/or their circulating stable metabolic degradation products) between 1-2 h of recovery. Finally, peak elevation of PGI<sub>2</sub> (measured as the stable circulating product 6-keto-PGF<sub>1α</sub>) was not observed until 24 h of recovery. These results appear to show clear temporal differences in the systemic response to individual PG species throughout the time-course of post-exercise recovery (summarised in Figure 2B). Further studies using a metabolipidomic approach are needed to comprehensively address the local changes in prostanoids within exercise musculature throughout the time course of exercise recovery.

## 2.2 Role of the COX pathway in immunological and adaptive responses to exercise:

Classical NSAIDs (e.g. ibuprofen and indomethacin) administered at typically recommended doses have been shown to effectively block the exercise-induced elevation of PGs in both human muscle tissue (89-91, 103) and peripheral blood (74, 102). Acetaminophen (paracetamol), although not classically considered a NSAID and does not inhibit COX *in-vitro*, also appears to interfere with COX activity in muscle during exercise recovery (90). Therefore NSAID treatment can be effectively used to investigate the role of PGs in physiological responses to exercise.

The literature on the role of PGs in exercise-induced muscle injury and the purported benefit of NSAIDs to improve recovery or reduce symptoms of delayed onset muscle soreness (DOMs) is highly controversial. Many studies have reported positive short terms effects of NSAID treatment on muscle recovery including reduced swelling (104), DOMs (104-117), circulating creatine kinase (CK) (111, 113, 114, 116-118), and improved strength (104, 105, 108, 110, 115, 119). Conversely, numerous other studies have failed to find any significant influence of NSAIDs on muscle swelling (112, 120), DOMs (77, 82, 90, 118-132), circulating CK (77, 82, 107-110, 112, 119, 129, 130), or strength loss (77, 106, 112, 113, 117, 118, 123, 128, 131). Given their purported primary therapeutic role as anti-inflammatory, it also may be considered surprising that human studies to date have failed to find an effect of NSAIDs on systemic leukocytosis (117, 118, 133) or intramuscular leukocyte infiltration (112, 119, 134) responses to exercise stress. In fact, recent studies have found that acute NSAID treatment appears to augment exercise-induced increases in skeletal muscle inflammatory cytokine (e.g. IL-6 & MCP-1) (129, 135-137) and inducible COX-2 gene expression (136, 138). Although the underlying mechanisms remain unclear,

this response may be a compensatory response elicited secondary to reduced PG concentrations.

In addition to their immunoregulatory properties, a role of PGs in the control of muscle protein turnover has been proposed over three decades ago (139). Twenty years later, the first human study to investigate the effect of NSAID treatment on muscle protein turnover, showed that oral ingestion of the non-selective NSAID ibuprofen appeared to block the normal increase in the rate of muscle protein synthesis 24 h following a bout of maximal eccentric resistance exercise (132). Further key studies showed that a different non-selective NSAID (indomethacin) interferes with the muscle satellite cell proliferative response which occurred later (7 days) during exercise recovery (103, 128, 140, 141). However, subsequent follow up studies testing the effect of COX-2 selective NSAIDs on exercise recovery failed to show an effect on exercise-induced muscle protein synthesis (138) or muscle satellite cell responses (112). These findings suggest that despite the well-established indispensable role of COX-2 activity in rodent muscle reparative response to injury (14, 99, 142-146), COX-1 rather than COX-2, may be the primary isoform involved in human muscle adaptive responses to exercise. More recent studies have focused on the effect of NSAIDs on the molecular response to exercise stress have provided further mechanistic insight (136, 147, 148). Oral ibuprofen treatment was reported to blunt activation of anabolic signalling kinases early (3 h) during recovery from resistance exercise, showing that the early elevation in PG biosynthesis during post-exercise recovery (102) appear to be important for the anabolic signalling response in human muscle (148). On the other hand, impaired satellite cell proliferation in the presence of indomethacin treatment (103), was not found to be associated with any effect on the expression of growth factors and extracellular matrix-related genes (136), nor changes in the heat shock protein (HSP) response (147). Thus, the molecular mechanisms by which NSAIDs may impair satellite cell myogenesis in human muscle during post-exercise recovery remains to be elucidated.

A number of cell culture studies have also investigated the direct regulatory roles of specific PGs in various stages of muscle cell growth and development (summarised in Table 1). These studies suggest that individual PGs may play distinct and specific temporal roles in muscle tissue throughout exercise recovery. In the early hours post-exercise, myofiber protein turnover is rapidly increased and there is an expansion and migration of the myogenic stem cell (satellite cell) pool.

This timeframe corresponds to elevated PGs with roles in stimulating muscle protein turnover (139, 149) and enhancing myoblast proliferation (98, 150-152)/survival (153, 154), whilst limiting myogenic differentiation (97) (Figure 2A, Table 1). During the latter stages of recovery, myoblasts must cease to migrate and divide, allowed to undergo myogenic differentiation and stimulated to fuse in order to regenerate damaged tissue and support cellular hypertrophy. This latter phase is associated with an increase in PGI<sub>2</sub> which has been shown to act a “brake” on myoblast migration to facilitate cell-cell contact and promote myogenic fusion events (101). These speculative roles of individual PG species in specific stages of the skeletal muscle growth and regeneration require further demonstration *in-vivo*.

### 3. LIPOXYGENASE PATHWAYS

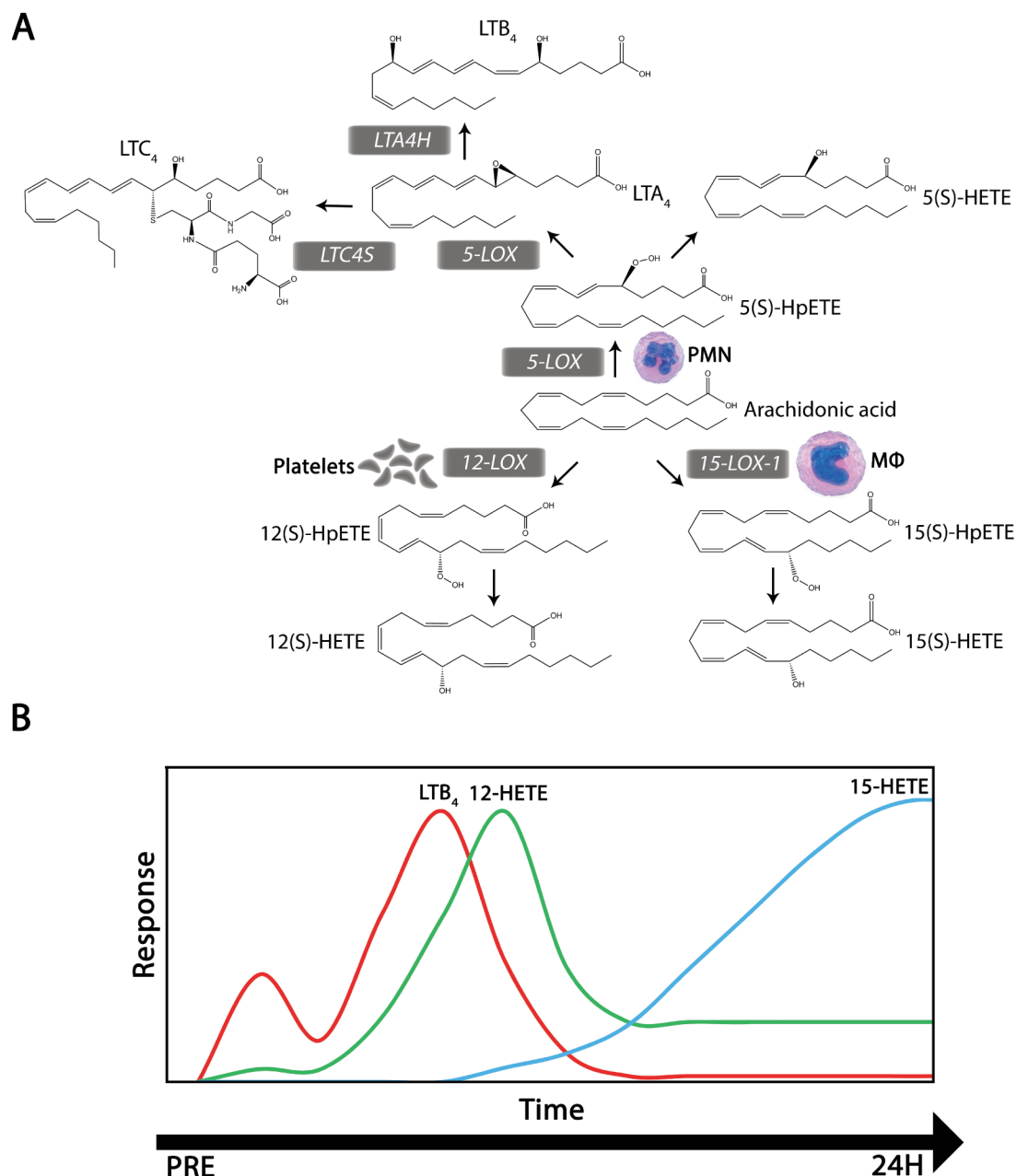
The lipoxygenase (LOX) pathways oxygenate PUFA substrate to form hydroperoxy products (e.g. hydroperoxy-eicosatetraenoic acid (HpETE) from AA), which are rapidly reduced to corresponding monohydroxy fatty acid metabolites (e.g. hydroxy-eicosatetraenoic acid (HETE) from HETE) (Figure 3A). The naming of LOX enzymes was originally based on their specificity with respect to 20-carbon AA. For example, 12-LOX oxygenates AA at carbon-12 to form 12-hydroxy-eicosatetraenoic acid (12-HETE) (Figure 3A). In animal tissues, enzymes exist with specificity for three major oxidation sites on AA at C-5, C-12, and C-15 and the enzymes are named 5-LOX, 12-LOX, and 15-LOX, respectively (Figure 3A). However, certain LOX enzymes possess dual specificities and interspecies differences exist (155). The human genome includes six functional LOX genes (*ALOX5*, *ALOX15*, *ALOX15B*, *ALOX12*, *ALOX12B*, *ALOXE3*) each encoding a distinct enzyme, often with cell-type specific expression (155) (summarised in Table 2). Signalling lipids produced by LOX pathways are involved in a range of physiological and pathological processes, one of the best characterised of which is their intricate role in the inflammatory response.

#### 3.1 Human LOX enzymes:

**3.1.1 5-LOX pathway:** A single 5-LOX enzyme is encoded by the *ALOX5* gene which is highly expressed in various leukocyte populations, especially PMNs (156, 157) (Table 2). The leukotrienes (LTs) are classical pro-inflammatory AA metabolites generated by the 5-LOX pathway with key roles in both the innate and adaptive immune system (158-160).

**Table 2:**  
**Arachidonate lipoxygenase (ALOX) genes and isoforms**

Gene	Enzyme	Aliases	Activity		Expression pattern in humans
			Human	Mouse	
<i>ALOX5</i>	5-LOX	5-LO	5(S)-HETE	5(S)-HETE	Leukocytes
<i>ALOX12</i>	12-LOX	12-LO, 12S-LOX, Platelet-Type 12-LOX	12(S)-HETE	12(S)-HETE	Platelets, macrophages, dendritic cells
<i>ALOX12B</i>	12-LOXB	12-LO, 12R-LOX, Epidermis-Type 12-LOX	12(R)-HETE	12(R)-HETE	Skin
<i>ALOX15</i>	15-LOX-1	15-LO-1, Leukocyte type 12-LOX, 12/15-LOX	15(S)-HETE	12(S)-HETE	Eosinophils, epithelium, M2 macrophages
<i>ALOX15B</i>	15-LOX-2	15-LO-2, 15-LOX-B, 15S-LOX, Mouse 8-LOX	15(S)-HETE	8(S)-HETE	Epithelium, macrophages
<i>ALOXE3</i>	ELOX-3	Epidermal LOX-3, E-LOX			Skin



**Figure 3: Human lipoxygenase (5-, 12-, and 15-LOX) pathways of lipid mediator biosynthesis during exercise recovery.** A: The 5-, 12-, and 15-LOX pathways of lipid mediator biosynthesis. Mobilised free intracellular arachidonic acid (AA) substrate derived from cell membrane phospholipids is converted to specific positional monohydroxy-AA isomers by the action of LOX enzymes expressed in a cell-type/tissue specific manner that oxygenate 20-carbon PUFA specifically at C-5 (5-LOX), C-12 (12-LOX), and C15 (15-LOX). B: Time-course of peripheral blood LOX metabolite responses during recovery from unaccustomed resistance exercise as determined by mediator lipidomic profiling (102).

Oxygenation of AA by 5-LOX forms the primary metabolite 5-HpETE, which can be reduced to 5-HETE (161), or further metabolised by the 5-LOX enzyme to generate leukotriene A<sub>4</sub> (LTA<sub>4</sub>) (159, 162, 163) (Figure 3A). LTA<sub>4</sub> is the transient intermediate precursor to the major bioactive leukotrienes including LTB<sub>4</sub> (159, 164) and the cysteinyl LTs; LTC<sub>4</sub>, LTD<sub>4</sub>, LTE<sub>4</sub> (160). Most notably, LTB<sub>4</sub> is a powerful chemotactic lipid for PMNs (165-168), acting through BLT1 and BLT2 receptors to promote PMN chemotaxis and migration into tissues (169-171).

**3.1.2 12-LOX pathway:** Humans express two 12-LOX isoforms in a cell type specific manner; platelet-type 12-LOX (encoded by the *ALOX12* gene) (172, 173) and epidermis-type

12-LOX (encoded by the *ALOX12B* gene) (174, 175) (Table 2). Expression of the platelet type 12-LOX was also recently reported in human MΦ and dendritic cell populations at levels considerably higher than that present in their monocyte precursors (176). *ALOX12* metabolises AA to form 12-HETE carrying its hydroxyl group predominantly in the S-configuration (Figure 2A). In contrast, *ALOX12B*, which is expressed in the skin, primarily produces the 12(R)-HETE isomer. Although first discovered in human blood platelets four decades ago (173), the precise role of 12-LOX in platelet physiology has remained somewhat unclear (177). In addition to its purported role in blood thrombosis, 12(S)-HETE is a potent pro-inflammatory lipid mediator which stimulates PMN chemotaxis (178-183), monocyte adhesion (184), and inflammatory



cytokine expression (e.g. IL-6, TNF $\alpha$ , MCP1) in both macrophages (185, 186) and adipocytes (187, 188). Similarly, 12(R)-HETE plays a similar key pro-inflammatory role in the skin (189-194).

**3.1.3 15-LOX pathway:** Humans express two 15-LOX isoforms, 15-LOX-1 and 15-LOX-2, encoded by the *ALOX15* and *ALOX15B* genes respectively (Table 2). 15-LOX-1 is highly expressed in eosinophils and epithelial cell populations. Whereas expression of 15-LOX-1 is typically low in blood monocytes, it is markedly induced by the anti-inflammatory cytokines IL-4 (195, 196) and IL-13 (197-200). Therefore, 15-LOX-1 is highly expressed in alternatively activated M2, but not classically activated M1 monocytes/ M $\Phi$  (201-205). The 15-LOX-1 pathway results in the formation of both 12(S)-HETE and 15(S)-HETE from AA substrate, with the ratio varying from one species to another. Human 15-LOX-1 primarily synthesises 15(S)-HETE, together with smaller amounts of 12(S)-HETE (206) (Figure 2A). In contrast, the murine *ALOX15* ortholog (often termed 12/15-LOX or leukocyte type 12-LOX), generates 12(S)-HETE as its primary product (207). Interestingly, 15(S)-HETE possesses primarily anti-inflammatory activity and counteracts the actions of the 5-LOX pathway by inhibiting PMN release of LTB $_4$  (208, 209) and dampening PMN chemotaxis to a LTB $_4$  gradient (209-212). In parallel, 15(S)-HETE has direct stimulatory effects on the migration of monocytes, indicative of a direct role in resolution phase of inflammation (213). A second 15-LOX isoform (15-LOX-2), encoded by the *ALOX15B* gene is expressed in human epidermis which also acts to convert AA almost exclusively to 15(S)-HETE (214). In contrast, the *ALOX15B* equivalent expressed in mouse skin primarily generates 8(S)-HETE from AA, and is thus often termed 8-LOX in the murine literature (215). In addition to its well-known role in the skin, 15-LOX-2 (*ALOX15B*) was recently reported to be a major 12/15-LOX isoform expressed during the differentiation of human monocytes to M $\Phi$ s, a response which was further induced in response to M2 polarisation (204). Thus both 15-LOX-1 and 15-LOX-2 appear to play a key role in recruitment and polarisation of monocyte/ M $\Phi$  populations required for successful resolution of the inflammatory response.

### 3.2 LOX pathway responses to exercise:

Despite the well-established role of LOX pathway PUFA metabolites in the inflammatory milieu, few studies exist regarding their potential roles in the immunological and adaptive responses to exercise stress. Whole blood mRNA expressions of both 5-LOX and 5-LOX activating protein (FLAP) have been reported to increase during recovery from running exercise (216). Consistently, elevated plasma levels of the major 5-LOX product LTB $_4$  has been reported following high intensity running in some (216), but not all studies (55). 5-LOX and FLAP in human muscle appears to predominantly co-localise with immune cells (217), although 5-LOX immunoreactivity has also been reported within skeletal myofibers themselves (218). To our knowledge no study to date has investigated the effect of exercise on expression of LT biosynthetic machinery (e.g. 5-LOX or FLAP) within muscle tissue. Nevertheless, a bout of cycling exercise was recently reported to increase intramuscular concentrations of

LTB $_4$  (a major 5-LOX product) in human myopathy patients (217), suggesting a possible local role of LTB $_4$  within the exercising musculature.

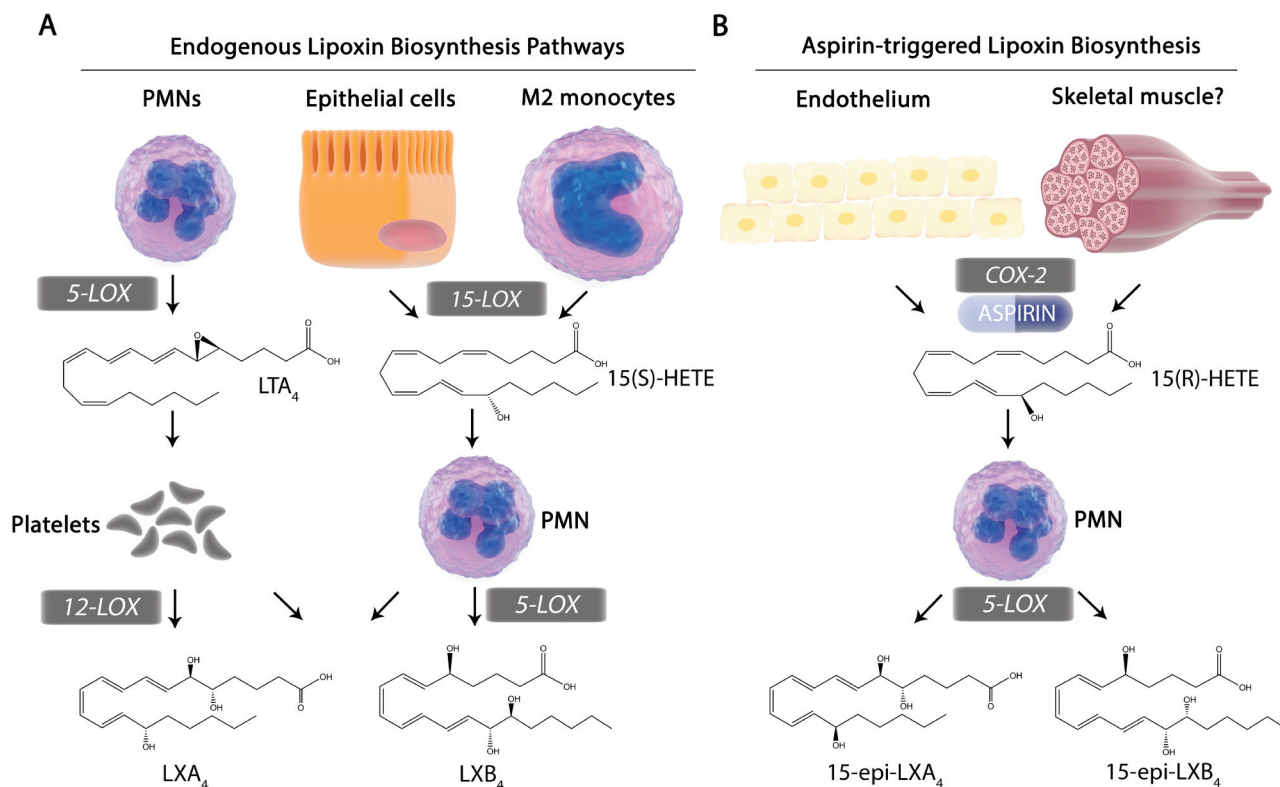
Metabolipidomic profiling has more recently provided a more detailed analysis of the response of LOX pathway products in human peripheral blood. We found that serum levels of the pro-inflammatory 5-LOX product LTB $_4$  were markedly elevated early (1-2 h) following an acute bout of unaccustomed resistance exercise (summarised in Figure 3B), a response which coincided with increased serum concentrations of inflammatory PGs (e.g. PGE $_2$ ) (102). This early transient response was followed by increases in 12-LOX pathway metabolites 12-HETE and its  $\beta$  oxidation product (termed tetranor 12-HETE), peaking later on at 3 h post-exercise (102). Finally, the 15-LOX pathway products 15-HETE and 15-Oxo-EETE were found to be elevated only during the latter stages of exercise recovery, achieving peak elevation 24 h post-exercise (102). This analysis suggests that specific PUFA metabolites of the LOX pathways as predominant at various stages of post-exercise recovery ranging from induction of pro-inflammatory metabolites early to a later switch to anti-inflammatory/pro-resolving lipids. It is important to note that this is the only study published to date to use a metabolipidomic approach to simultaneously profile PUFA metabolites of the LOX pathways in response to exercise stress. It is likely that exercise mode, duration, and intensity could lead to widely varying LOX pathway PUFA metabolite responses. Thus, future studies employing a metabolipidomic approach are needed to further characterise the PUFA LOX metabolic responses to exercise.

### 3.3 Role of LOX metabolites in immunological and adaptive responses to exercise:

Given their key roles in platelet and leukocyte biology, LOX metabolites may be hypothesised to play important and under-appreciated roles in mediating the immunological and vascular responses to exercise stress. Elevated circulating levels pro-inflammatory lipid mediators LTB $_4$  and 12-HETE in the early hours of recovery likely promote PMN mobilisation from bone marrow and migration to skeletal muscle via effects to stimulate chemotaxis and enhance vascular permeability (169-171). Subsequent elevation of 15-HETE during the latter stages of recovery would potentially inhibit release of LTB $_4$  by PMNs (208, 209) and dampen PMN chemotaxis/migration (209-212) whilst promoting subsequent migration of monocytes required for timely resolution of inflammation (213).

In addition to their key role in mediating the local and systemic inflammatory response, LOX metabolites may potentially act as important signalling molecules locally within muscle tissue. The LT receptors BLT1 and BLT2 have been shown to be expressed by cultured muscle satellite cells (219) and the L6 myoblast cell line (169). Additionally, exogenous LTB $_4$  treatment was found to promote L6 cell myogenesis *in vitro*, an action which is mediated by the BLT1 receptor (219). On this basis, increased LTB $_4$  biosynthesis following exercise stress (102, 216) may be potentially hypothesised to play a role in driving proliferation and differentiation of satellite cells involved in muscle growth/regeneration (219). However,





**Figure 4: Lipoxin (LX) biosynthetic pathways in generating n-6 AA derived specialised pro-resolving lipid mediators.** A: Endogenous pathways of LX biosynthesis involving cell-cell interactions of 5-LOX expressing cells (e.g. PMNs) with 12-LOX expressing cells (e.g. platelets), or 15-LOX expressing cells (epithelium or M2 monocytes/M $\phi$ ) with 5-LOX expressing cells (PMNs). B Aspirin-triggered (AT) pathway in which aspirin acetylated COX-2 generates 15(R)-HETE which participates in transcellular LX biosynthesis via the subsequent action of PMN 5-LOX.

to our knowledge no study to date has directly investigated the role of the 5-LOX pathway in muscle growth and regeneration.

On the other hand evidence also exists to suggest that chronic activation of the 5-LOX pathway can have deleterious effects on muscle tissue. For example, LTB<sub>4</sub> has been found to be chronically elevated in muscle microdialysates from polymyositis or dermatomyositis patients and appears related to the extent of muscle weakness in this context (217). Recently, LTB<sub>4</sub> was also reported to be elevated in muscle, adipose, and liver tissue of obese mice (169). In this model, inhibition of the LTB<sub>4</sub> receptor BLT-1, via genetic or pharmacologic means, protected against high fat diet induced insulin resistance, suggesting a deleterious effect of chronically elevated muscle LTB<sub>4</sub> (169, 220). Interestingly, this appeared at least partially attributable to a direct effect of LTB<sub>4</sub> to induce inflammatory signalling cascades in muscle cells themselves via the BLT1 receptor, leading to impaired muscle insulin sensitivity (169). These data show that chronically elevated pro-inflammatory 5-LOX metabolites can have apparent deleterious effects on muscle physiology under certain circumstances

Some limited evidence suggests that 12/15-LOX metabolites might also have direct regulatory effects on muscle. For example, treatment of C2C12 myoblasts *in-vitro* with exogenous 12-HETE has been reported to result in dose dependent activation of peroxisome proliferator-activated receptor gamma (PPAR $\gamma$ ) (221). Whilst platelets are a well-established

source of 12-HETE, C2C12 skeletal myoblast cultures apparently synthesise and secrete 12-HETE, an action which can be modulated by treatment with the n-3 PUFA EPA (222). Additionally, 12-LOX immunoreactivity has also been reported within skeletal myofibres *in-vivo* in one study, further suggesting that muscle tissue may possess 12-lipoxygenase activity (223). In a recent study, *ALOX15* (12/15-LOX) knockout mice were reported to be protected from denervation induced skeletal muscle atrophy, suggesting a negative regulatory role of 12/15-HETEs in the maintenance of muscle mass in this model (224). This finding may be complicated by the fact that the murine enzyme encoded by the *ALOX15* gene generates 12-HETE, as its primary product, in contrast to the 15-lipoxygenase activity of the human enzyme encoded by the *ALOX15* gene (see section 3.1). Nevertheless, exogenous treatment with 15-HETE was also been found to increase rates of protein degradation in C2C12 myoblasts/myotubes *in-vitro* in a series of studies (222, 225, 226). Together these findings appear to be consistent with the potential negative regulatory role of 15-LOX metabolites on skeletal muscle mass.

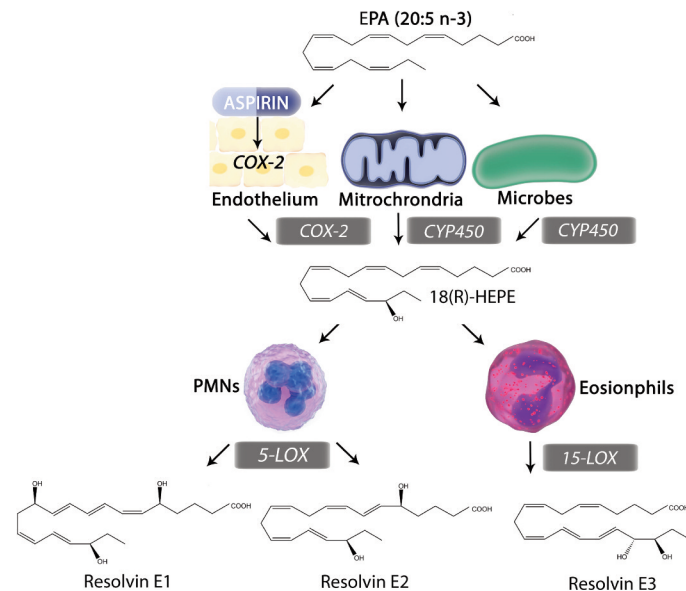
#### 4. TRANSCELLULAR LIPID MEDIATOR BIOSYNTHESIS: THE SPECIALISED PRO-RESOLVING MEDIATORS

In addition to generating their primary enzymatic products, the COX-2 and LOX pathways participate in transcellular biosynthetic pathways between two or more cell types which

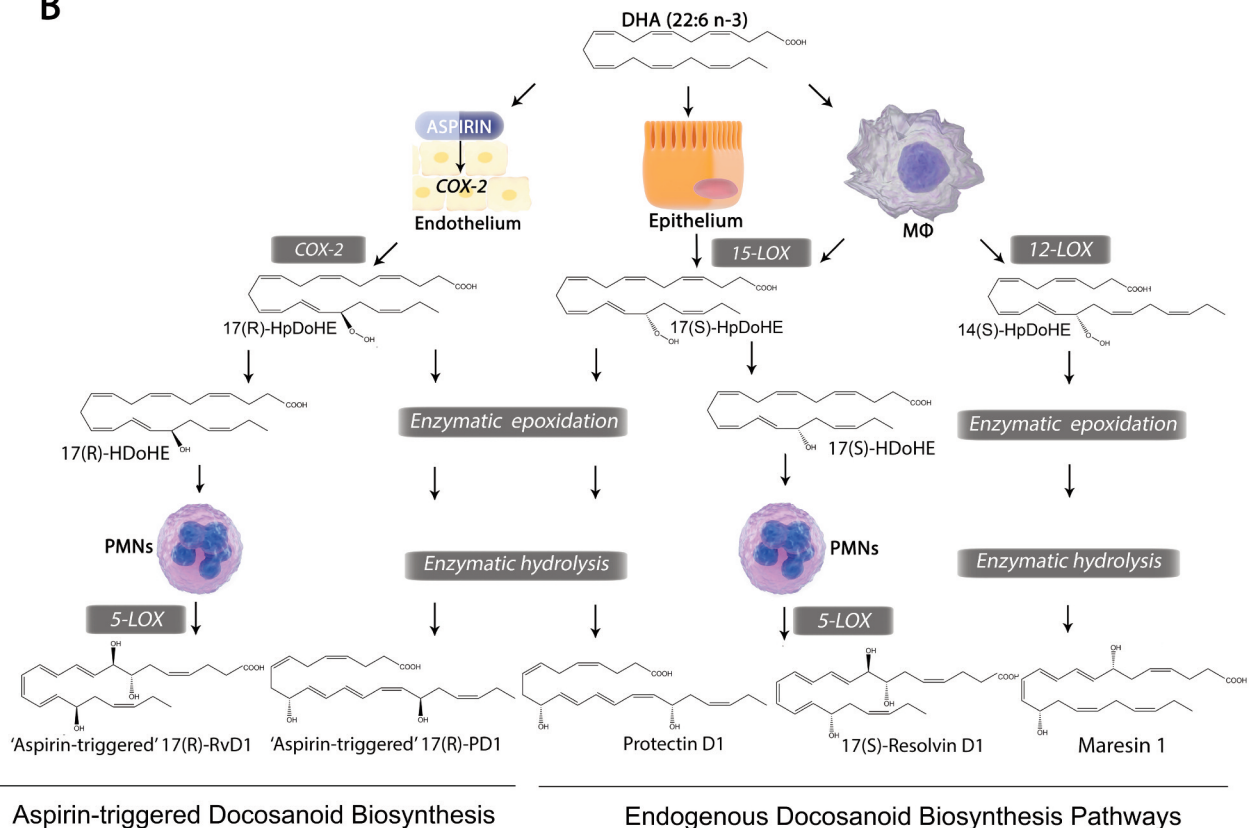
express the required enzymatic machinery in a compartmentalised manner (227). During inflammation, cell-cell interactions between platelets, leukocytes, the vasculature and resident tissue cells facilitates local transcellular biosynthesis of unique lipid mediator species. Interestingly, whilst the actions of the COX and LOX pathways expressed by cells in isolation

often generate products with pro-inflammatory properties, lipid mediators synthesised through transcellular routes are not only generally anti-inflammatory, but also actively function to promote resolution back to the non-inflamed state, i.e. 'pro-resolving'.

**A**



**B**



**Figure 5: n-3 PUFA derived specialized pro-resolving lipid mediator pathways.** A: Endogenous and aspirin-triggered routes of E-series resolvins (RvE) biosynthesis. EPA is converted to 18(R)-HEPE by aspirin acetylated COX-2 or alternatively CYP450 enzymes in host cells or resident microbes. 18(R)-HEPE is the primary intermediate in the biosynthesis of RvE1 and RvE2 by PMN 5-LOX, or RvE3 by eosinophil 15-LOX. B: Endogenous and aspirin triggered routes of DHA derived (D-series) resolvins (RvD), protectin (PD), and maresin (MaR) biosynthesis. DHA is converted to 17(R)-HpDoHE by aspirin acetylated COX-2 or 17(S)-HpDoHE by the endogenous 15-LOX pathway, precursors to the aspirin triggered and native PDs respectively. The corresponding 17-HDoHEs resulting from peroxidase catalysed reduction of the hydroperoxides serve as substrates for the RvDs by the action of the 5-LOX pathway. In an alternate pathway, DHA is converted by MΦ 12-LOX to 14(S)-HpDoHE, which is the primary precursor to the MaR family of SPM.

#### 4.1 Overview of the pro-resolving lipid mediators:

**4.1.1 Lipoxins:** The lipoxins (LXs or *lipoxygenase* interaction products) are bioactive metabolites of the n-6 PUFA AA originally isolated from human leukocytes (228), formed via transcellular biosynthetic routes (229) (Figure 4). In contrast to the classical AA derived eicosanoids (PGs and LTs), LXs possess potent anti-inflammatory actions by inhibiting the chemotaxis (230-233) and degranulation of PMNs (234). Furthermore, LXs also have direct pro-resolving bioactivity as they actively stimulate the migration/adherence of blood monocytes (233, 235-237) and eosinophils (238), whilst promoting MΦ nonphlogistic phagocytosis/clearance of apoptotic PMNs (239). Therefore LXs were the first lipid mediators identified to function as endogenous “braking signals” during the time course of inflammation to limit PMN infiltration and actively promoting their clearance from inflammatory infiltrates. Furthermore, LXs have direct analgesic properties which oppose that of the classical eicosanoids in the regulation of pain (240).

Two endogenous routes of LX biosynthesis exist in humans (Figure 4A). The first pathway involves leukocyte-platelet interactions during which the leukotriene biosynthesis intermediate  $LTA_4$ , secreted from 5-LOX expressing PMNs, is taken up by adherent platelets for conversion to lipoxin  $A_4$  ( $LXA_4$ ; 5S,6R,15S-trihydroxy-7E,9E,11Z,13E-eicosatetraenoic acid) or lipoxin  $B_4$  ( $LXB_4$ ; 5S,14R,15S-trihydroxy-6E,8Z,10E,12E-eicosatetraenoic acid) by the action of platelet-type 12-LOX (241-245). A second major route involves initial secretion of the 15-LOX product 15(S)-HETE by epithelial cells, eosinophils or M2 monocytes/MΦs, which is then taken up by 5-LOX expressing cells (e.g. PMNs) and converted to  $LXA_4$  and  $LXB_4$  (246-248). A third unorthodox pathway of LX biosynthesis operates only in the presence of aspirin (acetylsalicylic acid) (Figure 4B). Aspirin is unique amongst the NSAIDS in that it achieves inhibition of PG biosynthesis via irreversible acetylation of the COX-1 & 2 enzymes (249). Acetylation renders COX-1 entirely inactive, however, acetylated COX-2 obtains 15-lipoxygenase activity, generating 15(R)-HETE (rather than  $PGG_2$ ) as the primary product of AA (250-253). Secreted 15(R)-HETE participates in transcellular LX biosynthetic pathways, forming metabolites termed 15-epi-LXs (15(R)-LXs), or aspirin-triggered LXs (ATL) (254) which carry their C-15 hydroxyl group in a R-configuration, rather than the S-configuration characteristic of the native LX (254) (Figure 4B). The ATL and native LXs share their anti-inflammatory/pro-resolving (231, 233, 236, 254) and analgesic properties (240, 255, 256). On this basis, ATLs have in recent years been purported to account for a major mechanism underlying certain unique therapeutic effects of aspirin amongst the NSAID class.

**4.1.2 Resolvins:** The first pro-resolving metabolites of n-3 PUFAs identified were found to be synthesised from EPA (257), and later termed the E-series resolvins (Rv or *resolving* phase interaction products) (258). In the presence of aspirin, EPA is converted to 18(R)-hydroxy-EPA (18(R)-HEPE) by acetylated COX-2, in a similar manner to conversion of AA to 15(R)-HETE during AT-LX biosynthesis (257, 259) (Figure 5). In a second intriguing endogenous route, resident microbes expressing cytochrome p450 (CYP450) can generate and pro-

vide 18(R)-HEPE to their human host (260). Secreted 18(R)-HEPE obtained from endogenous or AT sources is taken up into PMNs and converted to resolvin E1 (RvE1; 5S,12R,18R-trihydroxy-6Z,8E,10E,14Z,16E-eicosapentaenoic acid) (257, 259, 260) and resolvin E2 (RvE2; 5S,18R-dihydroxy-6E,8Z,11Z,14Z,16E-eicosapentaenoic acid) (261-263) by the action of the 5-LOX pathway (Figure 5). RvE1 and RvE2 both possess potent multilevel anti-inflammatory and pro-resolving actions including inhibiting PMN migration and enhancing MΦ phagocytosis of apoptotic PMNs (257, 259, 264). The originally identified RvEs carry their C-18 hydroxyl group in the R-confirmation, although equivalent 18(S)-RvEs (e.g. 18(S)-RvE1; 5S,12R,18S-trihydroxy-6Z,8E,10E,14Z,16E-eicosapentaenoic acid) were later identified (265). In a distinct pathway, involving the 15-LOX pathway in human eosinophils, 18(S/R)-HEPE are converted to resolvin E3 (RvE3; 17R,18R-dihydroxy-5Z,8Z,11Z,13E,15E-eicosapentaenoic acid and 18(S)-RvE3; 17R,18S-dihydroxy-5Z,8Z,11Z,13E,15E-eicosapentaenoic acid) (266-268) (Figure 5A). Both 18S- and 18R-RvE3 exhibit direct inhibitory activity on PMN chemotaxis (266, 267).

Parallel biosynthetic pathways metabolise the 22-carbon DHA to generate resolvins of the D-series (docosanoids) (Figure 6). In aspirin triggered routes, during which the D-series resolvins were originally discovered, aspirin acetylated COX-2 generates 17(R)-hydroxy-DHA (17(R)-HDoHE) from DHA substrate (258). In an alternate endogenous route not requiring aspirin, DHA is metabolised by the 15-LOX pathway to 17-HDoHE carrying the C-17 hydroxyl group in an S-confirmation (17(S)-HDoHE) (269). Human PMNs take up and transform secreted 17(S/R)-HDoHE to the native 17(S)-D-series resolvins (RvD1-RvD6) or their aspirin-triggered 17(R)-counterparts (AT-RvD1-RvD6) by action of the 5-LOX pathway (258, 269-271) (Figure 5B). The DHA derived resolvins are potent anti-inflammatory and pro-resolving lipid mediators which counteract inflammation and actively promote restoration of a non-inflamed state by suppressing PMN chemotaxis and promoting MΦ recruitment and phagocytosis (272-275).

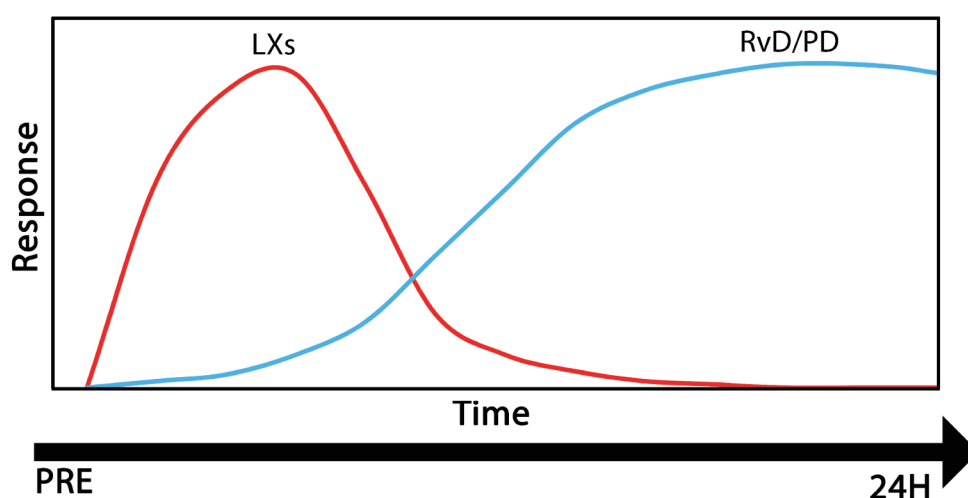
**4.1.3 Protectins:** In addition to contributing to RvD biosynthesis, the primary DHA metabolite of 15-LOX, 17(S)-HpDoHE, is also a precursor to a distinct group of 10,17-dihydroxy docosanoids (with conjugated trienes) termed the protectins (PD) or neuroprotectins (NPD) (269, 270, 276) (Figure 5B). The major bioactive PD synthesised endogenously by human cells has been shown to be 10R,17S-dihydroxy-4Z,7Z,11E,13E,15Z,19Z-docosahexaenoic acid, termed protectin D1 (PD1) (277). Additionally, in the presence of aspirin, an aspirin-triggered PD1 isomer carrying its 17-hydroxyl group in the R-confirmation is formed termed AT-PD1 (278) or AT-NPD1 (279) (10R,17R-dihydroxy-4Z,7Z,11E,13E,15Z,19Z-docosahexaenoic acid). Native PD1/NPD1 and AT-PD1/NPD1 are both potent anti-inflammatory pro-resolving molecules that inhibit PMN chemotaxis/migration and enhance MΦ efferocytosis of apoptotic PMNs.

**4.1.4 Maresins:** In a third and most recently identified alternate pathway of DHA metabolism, a new class of SPMs were

identified as products produced by human MΦ and named 'macrophage mediators in resolving inflammation' or Maresins (MaR) (280) (Figure 5B). Maresin biosynthesis involves the initial 14-lipoxygenation of DHA by the human MΦ 12-LOX pathway to form 14(S)-HpDoHE. Subsequent epoxidation of 14(S)-HpDoHE by the same enzyme within MΦ forms an 13S,14S-epoxy-DHA intermediate (recently termed 13(S),14(S)-epoxy-maresin or 13,14-eMaR). 13(S),14(S)-epoxy-DHA possesses its own bioactivity, as well functioning as a key intermediate in downstream MaR biosynthesis (281). Further enzymatic hydrolysis of 13(S),14(S)-epoxy-DHA by an as yet to be determined pathway yields Maresin 1 (MaR1; 7R,14S-dihydroxy-4Z,8E,10E,12E,16Z,19Z-docosahexaenoic acid) (281). Alter-

excretion of LXA<sub>4</sub> post-exercise (283). Additionally, a second more modest elevation in urinary LXA<sub>4</sub> was observed at 24 h or recovery (283). This led the authors to speculate that increased lipoxin biosynthesis may be reflective of cell-cell immunological interactions occurring during exercise and represent an endogenous anti-inflammatory and pro-resolving defence mechanism against exercise-induced stress (283).

In order to more comprehensively characterise the role of pro-resolving lipid mediators in exercise responses, we undertook unbiased metabololipidomic profiling of human peripheral blood samples collected throughout 24 h recovery from a single bout of unaccustomed intense resistance exercise (102). We found that pro-resolving AA metabolites, the lipoxins,



**Figure 6: Specialised pro-resolving lipid mediator response to exercise.** A: Time-course of human peripheral blood anti-inflammatory/pro-resolving lipid mediator responses during recovery from unaccustomed resistance exercise as determined by mediator lipidomic profiling (102).

natively, 13,14-eMaR can be converted by the action of soluble epoxide hydrolase (sEH) enzyme to the recently identified Maresin 2 (MaR2; 13R,14S-dihydroxy-4Z,7Z,9E,11E,16Z,19Z-hexaenoic acid) (176). MaR1 and MaR2 both possess potent anti-inflammatory, pro-resolving, tissue regenerative and anti-nociceptive actions (176, 280-282).

#### 4.2 Human pro-resolving lipid mediator responses to exercise

Appreciation of pro-resolving lipid mediator circuits in human acute inflammation has generally been limited to *in-vitro* assays utilizing blood immune cell population obtained from human volunteers. However, a small number of recent studies have suggested that exercise stress may be a human *in-vivo* model of self-limited inflammation during which resolving lipid mediator circuits play important physiological role and this process can be actively studied.

In the first study to address whether exercise might not only transiently increase concentrations of the pro-inflammatory eicosanoids, but also modulate bioactive lipids with anti-inflammatory and pro-resolving actions, maximal physical exertion was found to result in a rapid increase in the urinary

were increased early following exercise, with LXA<sub>4</sub> peaking immediately post-exercise and LXB<sub>4</sub> at 1 h of recovery. Similarly, the EPA derived pro-resolving lipid mediator, RvE1, was transiently increased in human serum 0-1 h post-exercise, rapidly decaying thereafter (102) (although we cannot discount that instability of highly labile RvE1 may have contributed to the latter response). Finally, docosanoids including resolvins (RvD1) and protectins (measured as the PD1 isomer (10S,17S-DiHDoHE) were increased in human peripheral blood later during recovery (2-3 h post) and remained elevated in circulation following 24 h of recovery (102). These results show that pro-resolving lipid mediator biosynthesis is increased in humans in response to a bout of unaccustomed resistance exercise and distinct classes of the resolving lipid mediators appear to exhibit specific temporal responses throughout the time-course of post-exercise recovery (Figure 6).

The utility of exercise as a model of self-resolving inflammation was further demonstrated in a recent study in which an exhaustive human exercise model was used alongside murine and cell-based experiments to demonstrate *in-vivo* detection and modulation of a new class of pro-resolving lipid mediators, termed the T-series resolvins (RvTs) in human peripheral



blood (284). This novel family of anti-inflammatory/pro-resolving lipid mediators synthesised from n-3 DPA (an intermediary PUFA between EPA and DHA) via the endogenous COX-2 pathway, were increased in human peripheral blood early ( $\leq 15$  min) post-exercise (284). Furthermore, this study confirmed previously reported findings of elevated DHA derived docosanoids in human peripheral blood in response to acute exercise stress (102, 284).

The cellular source and local changes in pro-resolving lipid mediator concentrations during post-exercise recovery is of interest. Lipid mediators typically act in an autocrine/paracrine action within tissue micro-environments and further studies are needed to profile the local changes in inflammatory and resolving lipid mediators within the exercised muscle tissue by utilising the skeletal muscle biopsy or microdialysis techniques. On the basis of apparent capacity of exercise to elevate circulating pro-resolving lipid mediators throughout recovery, an interesting question that remains unanswered is whether exercised skeletal muscle contributes to pro-resolving lipid mediator biosynthesis, either directly or via cell-cell interactions with the vasculature or infiltrating immune cell populations. In one early study, cultured skeletal myoblasts have been reported to secrete 5-HETE, 12-HETE and 15-HETE, suggesting they may possess various lipoxygenating activities (222). Future studies should address the possible role of skeletal muscle in transcellular lipid mediator biosynthetic pathways by interactions with infiltrating leukocytes.

#### 4.3 Lipid mediator class switching during post-exercise recovery

The time course of induction of inflammatory and resolving bioactive lipid mediators in peripheral blood of exercising humans appears to match well with common murine experimental models of self-limited inflammation (e.g. peritonitis and dorsal air pouch). In these spontaneously resolving murine models of inflammation, AA derived LTs increase rapidly after induction, followed by elevated PGs and LXs (within hours), and subsequently more delayed elevation of the docosanoids (e.g. RvD and PD1)  $\sim 12$ -24 h later (273, 285, 286). Interestingly, recent work using these models has suggested that the biosynthesis of specific resolving mediators such as resolvin D3 (RvD3) are uniquely positioned within the inflammatory response, appearing late (24-72 h) in the resolution time-frame (273). The time course of exercise recovery is known to be dependent on the extent of muscle injury inflicted, and in more extreme cases the inflammatory responses can persist for days to weeks (30). Thus, future studies that explore the latter time-frame of post-exercise recovery employ more diverse exercise models should provide valuable insight into the inflammatory and resolving lipid mediator response to exercise stress in humans.

Lipid mediator class-switching is a process first demonstrated by Levy et al. 2001 (286) in which increased concentrations of inflammatory COX-derived PGs early following exposure to an inflammatory stimuli actively triggers the subsequent induction on the enzymatic machinery of the pro-resolving lipid biosynthetic pathways, thereby bringing about resolution to a non-inflamed state (286-288). By employing a group of

human volunteers that received treatment with the NSAID ibuprofen, we were able to demonstrate that active lipid mediator class switching occurs during human exercise recovery. Subjects ingesting ibuprofen pre-exercise showed no early elevation in COX derived prostanoids during post-exercise recovery, but also displayed a complete lack of the subsequent elevation in pro-resolving lipid mediators (LXs, RvEs/RvDs & PDs) that was observed in subjects receiving placebo control (102). Therefore, blockade of COX-1 and 2 activities led to deleterious secondary downstream effects on the induction of resolution phase lipid mediator pathways. This data shows that lipid mediator class-switching pathways appear to be operational *in-vivo* in humans throughout the time course of post-exercise recovery. Furthermore, the use of NSAIDs (a common treatment of muscle injury and soreness), can have deleterious effects on induction of pro-resolving lipid mediator circuits.

#### 4.4 Putative roles of pro-resolving lipid mediators in the response to exercise

Several lines of evidence suggest that pro-resolving lipid mediators may play an important physiological role in the successful muscle regenerative and adaptive response following injury. The ability of PMNs to inflict secondary myofibre injury is well established (8, 10, 11). Thus, the activity of SPMs to limit PMN infiltration and actively promote their clearance by nonphlogistic M $\Phi$ s phagocytosis is likely to play an important role in injured muscle. Additionally, a hallmark of the SPMs is their ability to actively promote monocyte recruitment whilst stimulating to M $\Phi$  function and M2 polarization. Monocyte/M $\Phi$  recruitment (13-18) and in particular induction of a M2 phenotype (18, 20-23) are processes which are indispensable for successful muscle growth and regeneration. On this basis, we hypothesise that pro-resolving lipid mediators may play an important role in exercise recovery as well as in muscle healing/regeneration following injury. Nevertheless, to date no studies have been undertaken to test this hypothesis.

In addition to the potential effects secondary to their effect on immune cell population, SPMs may play more direct roles in muscle cell growth and development. For example, the lipoxin receptor (ALX), (also known as the formyl peptide receptor 2 (FPR2)) is expressed by the C2C12 skeletal myoblast cell line (289). The peptide annexin A1, another established ALX ligand, was shown to promote migration of muscle satellite cells and consequently enhances skeletal muscle cell differentiation (289, 290). RvD1 and AT-RvD1 also act via the ALX receptor (291). Whether LXs and RvDs can influence muscle satellite cell migration and differentiation via the muscle ALX remains to be determined, but appears a likely scenario. The RvE1 receptor, chemokine-like receptor 1 (chemR23 or CMKLR) (264) is also expressed in muscle, where it has been shown to play an important positive role in myogenesis (292). Despite these associations, to date no studies have directly investigated the effect of pro-resolving lipid mediators on muscle cell growth and development or the role of pro-resolving lipid mediator circuits in the context of skeletal muscle injury and adaptation. Therefore these are key potential areas for further research.

Whereas PGs/LTs play stimulatory roles on muscle cell growth and development, chronic activation of the COX and 5-LOX pathways can have deleterious effects on muscle (169, 217, 220, 293, 294). An intriguing hypothesis is whether failed pro-resolving lipid mediator circuits may underlie chronic non-resolving muscle inflammation and if targeting pro-resolving lipid mediator pathways could be of therapeutic benefit in conditions of rampant muscle inflammation. In support of this hypothesis, recently the DHA derived pro-resolving mediator PD1 was found to be lacking in muscle tissue of high fat-fed mice in association with chronic non-resolving inflammation and insulin resistance (295). In this model, administration of the PD1 isomer 10S,17S-dihydroxy-DHA (also known as protectin DX (PDX)) was found to exert unanticipated glucoregulatory activity by directly stimulating the release of the myokine interleukin-6 (IL-6) from skeletal muscle tissue (296). In this context, PDX acted to upregulate a myokine-liver signalling axis leading to an improved whole body insulin sensitivity (296). These results show for the first time that pro-resolving lipid mediators can exert direct regulatory effects on skeletal muscle tissue. Further studies are needed investigating the role of pro-resolving lipid mediators in the context of muscle responses to injury and mechanical overload, as well as the potential therapeutic value of resolving lipid mediators in settings of chronic muscle inflammation.

#### 4.5 Anti-inflammatory vs. pro-resolving approaches to acute muscle injury and exercise recovery?

The specialized pro-resolving lipid mediators possess bioactivity that simultaneously limits PMN trafficking (i.e. anti-inflammatory) and actively promotes monocyte/M $\Phi$  recruitment and function (i.e. pro-resolving). However, these actions are not one and the same, and it is important to distinguish between them. For example, common mainstay approaches to exercise recovery as well as the clinical treatment of musculoskeletal injury (e.g. NSAIDs) are anti-inflammatory in nature in that they strive to dampen inflammation via antagonism of pro-inflammatory mediators (reviewed in 297). In this context, however, classical anti-inflammatory agents such as the NSAIDs appear to prevent or delay resolution of inflammation by interfering with endogenous lipid mediator class switching circuits and can thus also be considered to be anti-resolving, or “resolution toxic” (286, 288, 298). The use of anti-inflammatory drugs (and potentially other interventions that are anti-inflammatory in nature e.g. cold exposure (299)) in an effort to provide analgesia and limit the cardinal signs of inflammation following muscle injury, may inadvertently derange or impair timely or complete resolution of muscle inflammation. Interestingly, this mechanism may help to explain the apparently deleterious effects of NSAIDs reported in numerous studies in rodent models of muscle growth and regeneration (14, 99, 142-146), and human adaptive responses to exercise (90, 128, 132, 140, 141, 148). An ideal strategy to enhance exercise recovery and manage musculoskeletal injury would perhaps be one that is analgesic and anti-inflammatory (in terms of effects on PMNs), yet simultaneously pro-resolving (by being conducive or even stimulatory in terms of effects on monocyte/M $\Phi$  recruitment and function). Interestingly, aspirin is distinct from other non-selective and COX-2 selective NSAIDs, in that whilst it inhibits PG biosynthesis

(potentially a deleterious outcome for endogenous SPM circuits); it simultaneously compensates by producing aspirin triggered pro-resolving lipid mediators (e.g. AT-LXs, AT-RvDs, & AT-PDs) from PUFA substrate by acetylated COX-2. Thus, future studies investigating the effect of aspirin on myogenesis and skeletal muscle growth/regeneration should help to delineate the roles of pro-inflammatory (e.g. PG and LT) vs. pro-resolving lipid mediators in successful muscle adaptation and whether certain interventions can be employed or developed which may potentially relieve pain and improve function whilst simultaneously ensuring endogenous regenerative mechanisms remain intact.

## 5. PERSPECTIVES AND FUTURE DIRECTIONS

Bioactive lipid mediators play diverse role in mediating the initiation, limitation, and active resolution of inflammation. Vigorous or unaccustomed exercise elicits a self-limited inflammatory response, involving cytokines/chemokines, blood leukocyte populations, and muscle-immune cell interactions. Classical pro-inflammatory lipid mediators (e.g. PGs and LTs) have long been implicated as playing a potential role in immunological and adaptive responses elicited following muscle loading. More recently, exercise has been found to be coupled to induction of a biologically active resolution of inflammation program involving the production of lipid mediators via cell-cell interactions which possess anti-inflammatory and pro-resolving bioactivity. These lipid mediators likely play important roles in the limitation and clearance of potentially myofiber damaging PMNs and the recruitment and polarisation of monocytes/ $\Phi$ s which restore tissue homeostasis and facilitate muscle growth and regeneration. Additionally, emerging data suggest that resolution phase lipid mediators may have direct effects on resident muscle cells including myofibers and satellite cells. Anti-inflammatory strategies targeted at relieving symptoms of exercise-induced muscle injury may perturb the resolution phase of inflammation by blocking key signals of resolution early in the inflammatory response (e.g. PGs), leading to deleterious effects on muscle remodelling. A paradigm shift away from current focus on anti-inflammatory approaches, towards interventions which are conducive or even facilitative to natural resolution of inflammation may lead to the development of novel approaches and therapeutics in order to enhance exercise recovery and more effectively manage and treat acute musculoskeletal injuries.

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