Human inflammatory and resolving lipid mediator responses
to resistance exercise and ibuprofen treatment

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Abstract

Classical pro-inflammatory eicosanoids, and more recently discovered lipid mediators with anti-inflammatory and pro-resolving bioactivity, exert a complex role in the initiation, control, and resolution of inflammation. Using a targeted lipidomics approach we investigated circulating lipid mediator responses to resistance exercise and treatment with the non-steroidal anti-inflammatory drug (NSAID) ibuprofen. Human subjects undertook a single bout of unaccustomed resistance exercise (80% 1RM) following oral ingestion of ibuprofen (400 mg) or placebo control. Venous blood was collected during early recovery (0-3 h and 24 h post-exercise) and serum lipid mediator composition analyzed by LC-MS based targeted lipidomics. Post-exercise recovery was characterized by elevated levels of cyclooxygenase (COX-1 and 2) derived prostanoids (TXB₂, PGE₂, PGD₂, PGF₂α, PGI₂), lipooxygenase (5-, 12-, and 15-LOX) derived hydroxyeicosatetraenoic acids (HETEs) and leukotrienes (e.g. LTB₄), and epoxygenase (CYP) derived epoxy/dihydroxyeicosatrienoic acids (EpETrEs/DiHETrEs). Additionally, we detected elevated levels of bioactive lipid mediators with anti-inflammatory and pro-resolving properties including arachidonic acid derived lipoxins (LXA₄ & LXB₄), and the EPA (E-series) and DHA (D-series) derived resolvins (RvD₁ & RvE₁), and protectins (PD₁ isomer 10S, 17S-diHDoHE). Ibuprofen treatment blocked exercise-induced increases in COX-1 and 2 derived prostanoids, but also resulted in off target reductions in leukotriene biosynthesis, and a diminished pro-resolving lipid mediator response. CYP pathway product metabolism was also altered by ibuprofen treatment, as indicated by elevated post-exercise serum 5,6- and 8,9- DiHETrE only in those receiving ibuprofen. These findings characterize the blood inflammatory lipid mediator response to unaccustomed resistance exercise in humans and show that acute pro-inflammatory signals are mechanistically linked to the induction of a biological active inflammatory resolution program, regulated by pro-resolving lipid mediators during post-exercise recovery.
Introduction

Lipid mediators are a diverse class of bioactive autocrine/paracrine signaling molecules which are synthesized endogenously from essential omega-6 and omega-3 fatty acids. Oxidation of free fatty acid substrates via cyclooxygenase (COX-1 and 2), lipoxygenase (5-, 12-, and 15-LOX), epoxygenase (CYP), and non-enzymatic pathways, produces a potential array of more than 100 distinct lipid mediators. Bioactive lipid mediators are involved in a wide range of physiological and pathological processes, one of the best characterized of which is their key role in the inflammatory response (reviewed in 91). Classical eicosanoids derived from omega-6 arachidonic acid (AA, ω-6 20:4) including prostaglandins (PGs; synthesized via COX-1 and 2) and leukotrienes (LTs, synthesized via 5-LOX), are well established pro-inflammatory signaling molecules which stimulate blood vessel vasodilation, enhance vascular permeability, promote neutrophil chemotaxis/activation, and sensitize neural afferents; stimulating the cardinal signs of inflammation; heat, redness, swelling and pain (reviewed in 57, 64). More recently, classes of lipid mediators with dual anti-inflammatory and pro-resolving of inflammation bioactivity were also identified (as reviewed in 81, 86), including the AA derived lipoxins (LXs) (54, 93, 94), and the eicosapentaenoic (EPA) (E-series)/docosahexaenoic acid (DHA) (D-series) derived resolvins (Rv) (83, 85) and protectins (P) (42, 84). In humans, these pro-resolving mediators are synthesized during inflammatory transcellular interactions involving the sequential action of two or more different cell types, which express distinct compartmentalized LOX enzyme isoforms in a tissue specific manner (e.g. neutrophil 5-LOX and platelet 12-LOX) (reviewed in 3). These non-classical lipid mediators act to antagonize PG/LT action, block pro-inflammatory cytokine expression and inhibit neutrophil recruitment/activation, whilst actively promoting recruitment of monocytes/macrophages which act to phagocytize apoptotic neutrophils and promote wound healing (77, 87). This has led to understanding that the initiation and subsequent resolution of the acute inflammatory response are not passive processes as once thought, but rather actively
regulated, ordered and intricately linked events (11, 82). Temporal changes in lipid mediator profile can lead to dynamic shifts in inflammatory cell type, cytokine expression, and cellular signaling from a pro-inflammatory state, to an environment that facilitates tissue adaptation and repair (82, 86).

Intense or unaccustomed exercise, especially involving a large eccentric component, can result in exercise-induced skeletal muscle injury (16). The humoral and local muscular changes during post-exercise recovery resemble acute-phase responses to tissue trauma/inflammation, including pro-inflammatory cytokine production, and the mobilization, migration, muscle tissue accumulation (and potentially infiltration by leukocytes) (69). Thus, exercise-induced muscle injury is an in-vivo model of acute inflammation applicable to human experimentation. Compared with other aspects of the immune response (e.g. leukocytes and cytokines (69)), the lipid mediator response during post-exercise recovery has received little attention. The limited studies available have focused on the potential role of select COX-1 and 2 pathway derived pro-inflammatory/hyperalgesic PGs (e.g. PGE$_2$) in underlying the symptoms of exercise-induced muscle injury which occur $\geq 24$ h into recovery. Whilst some studies have reported elevated circulating levels PGE$_2$, concurrent with the onset of delayed onset muscle soreness (DOMs) 24-72 h post-exercise (95, 102), others have found no such response (4, 9, 13, 23, 24, 41).

Additionally, a large body of research on the use of non-steroidal anti-inflammatory drugs (NSAIDs), the main therapeutic mechanism of action of which is thought to be blockade of the COX-1 and 2 pathways, to relieve symptoms of DOMs, has yielded conflicting and inconclusive results (reviewed in 1). Both COX-1 and 2 enzymatic activities are elevated in human skeletal muscle tissue 4 h post-resistance exercise (17), suggesting that PG biosynthesis may be heightened acutely and mediate inflammatory processes during early post-exercise recovery. However, the eicosanoid response during early post-exercise recovery and effect of exercise on the wider lipid mediator metabolome remains uncharacterized.
In addition to their role in inflammation, lipid mediators have recently been implicated to play an important role in soft tissue healing and regeneration (reviewed in 105). The use of NSAIDs is common amongst elite athletes (37, 101), in the clinical treatment soft tissue injury (67), and in efforts to relieve the muscular soreness that follows intense or unaccustomed physical activity (44). The efficacy of such interventions has however been brought into question and concerns have been raised regarding possible long-term negative implications on muscle growth and regeneration (78). For example, blockade of COX-1 and 2 mediated PG synthesis with NSAID treatment was found to blunt contraction induced protein synthesis (98, 100) and satellite cell proliferation (52, 59) responses in human skeletal muscle. Nevertheless, whereas oral ingestion of the non-selective NSAID ibuprofen blocked resistance exercise-induced skeletal muscle protein synthesis (100), local infusion of indomethacin (another distinct non-selective NSAID) failed to do so (61). Furthermore, in contrast to animal studies specifically implicating the COX-2 isoform in skeletal muscle growth/regeneration (7, 8, 65, 88, 89), COX-2 selective NSAIDs have been found to have no effect on contraction induced protein turnover or satellite cell activation in human skeletal muscle (14, 68). The primary mechanism of action of NSAIDs as potent COX-1 and 2 inhibitors is well established (74), but NSAIDs may also have other incompletely understood effects. These include shunting of mobilized free AA substrate via parallel enzymatic pathways (e.g. LOX) (15), COX-1 and 2 independent anti-inflammatory effects (96), and direct in vivo physiological responses to COX-1 and 2 pathway blockade not evident in in-vitro assays (5, 12). Interestingly, in animal models of acute self-limited inflammation, treatment with certain NSAIDs has been shown to delay or diminish normal resolution of the inflammatory response (80). Comprehensive lipidomic analysis of the effect of NSAID treatment on temporal inflammatory responses in humans in vivo are required in order to characterize the impact of anti-inflammatory treatment on inflammation-resolution, as well as muscle adaptation to loading.
In the present study we utilized a targeted lipidomics approach to characterize temporal changes in the human peripheral blood fatty acyl lipid mediator profile following a single bout of unaccustomed resistance exercise, with or without treatment with the NSAID ibuprofen. Early post-exercise recovery was characterized by elevated circulating levels of bioactive lipid mediators implicated in multiple aspects of inflammation and resolution, including thromboxanes, prostaglandins, HETEs, leukotrienes, lipoxins, resolvins, and protectins, amongst others. Ibuprofen treatment blocked exercise-induced increases in COX-1 and 2 derived prostanoids, and more surprisingly was also associated with deregulated CYP product metabolism, off target reductions in leukotriene biosynthesis, and a diminished anti-inflammatory/pro-resolving lipid mediator response during post-exercise recovery.
Materials and Methods

Participants

Sixteen healthy male subjects (characteristics shown in Table 1) volunteered and provided informed written consent to participate in the study; after the nature, purpose and risks of the study were explained. Exclusion criteria deemed that participants had not engaged in lower body resistance exercise training for a period $\geq 6$ months, and had no history of chronic anti-inflammatory drug use. Current use of medications, nutritional supplements or a previous history of a diagnosed condition or illness that would endanger the participants during strenuous exercise and/or anti-inflammatory drug treatment also excluded subjects from participation. All procedures involved in this study were formally approved by the Deakin University Human Research Ethics Committee.

Familiarization and strength testing

Participants underwent a familiarization and strength testing session one week prior to the exercise study trial day. Participants were instructed on correct exercise technique and underwent multiple repetition maximum strength testing to determine experimental exercise load (80% of 1 repetition maximum (1RM)). The maximal weight that subjects could lift for 3-6 repetitions (3-6 RM) on the barbell squat, leg press, and leg extension exercises was determined. Participants 1-RM was estimated from multiple RM testing results using the Brzycki equation (1-RM $= 100 \times \frac{\text{load rep}}{(102.78 - 2.78 \times \text{reps completed})}$), and 80% of subjects estimated 1 RM was determined. Participants were instructed to abstain from any vigorous physical activity in the following week prior to the experimental trial day.
Experimental protocol

The evening prior to the exercise trial day participants were provided with a standardized meal (CHO 57%, FAT 22%, PRO 21%), which they were instructed to consume before 10:00 pm. Participants then arrived the following morning (7:00 AM day 1) in a fasted state. During 30 minutes of supine rest a cannula was inserted in the anticubital vein to allow venous blood sampling. Participant’s rested supine following collection of resting blood samples (see blood sampling below) for approximately 10-15 min at which time the exercise protocol commenced. Following a 10 min warm up consisting of light cycling on bicycle ergometer and 1 low resistance warm up set on each exercise, participants performed 3 sets each of 8-10 repetitions of bilateral barbell smith rack squat, 45 degree leg press and seated knee extension, at 80% of their pre-determined 1RM. Exercises were performed sequentially in a circuit manner with participants resting for 1 minute between each exercise and 3 minutes between each set. This same exercise protocol has been previously used by our research group to induce local pro-inflammatory cytokine expression and associated NF-κB signaling in human skeletal muscle (106). Following completion of the exercise bout, subjects rested supine throughout a 3 h recovery period during which post-exercise blood samples were obtained. Participants were then provided with a pre-prepared standardized meal to be consumed at the laboratory (CHO 71%, FAT 13%, PRO 16%). Additional snacks and a standardized evening meal (CHO 64%, FAT 27%, PRO 18%) were provided for subjects to consume throughout the remainder of the trial day. Participants were instructed not to consume any additional food or drink (besides water) than that provided. Participants were instructed to consume the food provided prior to 10:00 pm and returned to the laboratory the following morning at ~9 am (24 h post-exercise) for follow up testing. A schematic of the experimental protocol is shown in Figure 1.
NSAID administration

Participants were randomly allocated to either the NSAID (1200 mg/day orally administered ibuprofen) (N=8) or the placebo group (gelatin capsules identical in appearance containing powdered sugar in place of ibuprofen) (N=8). Participants in the NSAID group ingested the maximal recommended 1200 mg over-the-counter daily dose of ibuprofen throughout the trial day, in three 400 mg doses. The first ibuprofen dose (400 mg) was administered upon arriving to the laboratory on the morning of day 1, immediately prior to baseline blood/muscle sampling (~30 min pre-exercise). Two additional 400 mg ibuprofen doses were subsequently provided which subjects were instructed to ingest at 2:00pm and 8:00pm on the evening of the trial day. The ibuprofen used was a commercially available over-the-counter brand consisting of a racemic mixture of the S+ ibuprofen and R- ibuprofen optical isomers. Based on the pharmacokinetics of orally administered ibuprofen (26), we expected this dosing protocol to elevate circulating ibuprofen to biologically active concentrations during the early post-exercise period, and aimed to maintain elevated levels as best as possible throughout the study day. A similar ibuprofen dosing protocol was used by Trappe et al. who reported that it was sufficient to blunt acute resistance exercise-induced elevation of intramuscular PGF$_2$α and muscle protein synthesis in young human subjects (99, 100).

Sample collection

Venous blood samples were drawn at rest, immediately post-exercise, at 30 min intervals throughout 3 h of recovery, and at 24 h post-exercise. All blood samples were drawn through an indwelling cannula within the antecubital vein into VACUETTE serum separator tubes (Greiner Bio-One). Whole blood was allowed to clot at room temperature for 10 min and was then centrifuged at $1000 \times g$ for 10 min. Serum was collected immediately on ice and stored at -80° C until further analysis. Skeletal muscle biopsy samples were additionally collected from all participants for further analysis not included in the present manuscript. Muscle biopsies were
taken from the *vastus lateralis* musculature under local anaesthesia (Xylocaine 1%) by percutaneous needle biopsy technique modified to include suction. Biopsy time points were as follows: Pre-exercise, immediately post-exercise, 3 h post-exercise, and 24 h post-exercise.

Sample preparation and LC-MS analysis of lipid mediators

Serum samples (0.5 ml) were spiked with 10 ng (in 10 µl) each of 15(S)-HETE-d8, 11dh-TXB2-d4, and TXB2-d4 as internal standards for analyte recovery and quantitation and mixed thoroughly. The samples were then extracted for fatty acyl lipid metabolites using C18 extraction columns as described earlier (53). Briefly, the internal standard spiked samples were applied to conditioned C18 cartridges, washed with water followed by hexane and dried under vacuum. The cartridges were eluted with 0.5 ml methanol. The eluate was dried under a gentle stream of nitrogen. The residue was redissolved in 50 µl methanol-25 mM aqueous ammonium acetate (1:1) and subjected to LC-MS analysis.

HPLC was performed on a Prominence XR system (Shimadzu) using Luna C18 (3µ, 2.1x150 mm) column. The mobile phase consisted of a gradient between A: methanol-water-acetonitrile (10:85:5 v/v) and B: methanol-water-acetonitrile (90:5:5 v/v), both containing 0.1% ammonium acetate. The gradient program with respect to the composition of B was as follows: 0-1 min, 50%; 1-8 min, 50-80%; 8-15 min, 80-95%; and 15-17 min, 95%. The flow rate was 0.2 ml/min. The HPLC eluate was directly introduced to ESI source of QTRAP5500 mass analyzer (ABSCIEX) in the negative ion mode with following conditions: Curtain gas: 35 psi, GS1: 35 psi, GS2: 65 psi, Temperature: 600 ºC, Ion Spray Voltage: -1500 V, Collision gas: low, Declustering Potential: -60 V, and Entrance Potential: -7 V. The eluate was monitored by Multiple Reaction Monitoring (MRM) method to detect unique molecular ion – daughter ion combinations for each of 125 transitions (to monitor a total of 144 lipid mediators and 3 internal standards) with 5 msec dwell time for each transition. Optimized Collisional Energies (18 – 35 eV) and Collision Cell Exit Potentials (7 – 10 V) were used for each MRM transition. The data was collected using Analyst 1.5.2 software and the MRM transition chromatograms were quantitated by MultiQuant.
software (both from ABSCIEX). The internal standard (15(S)-HETE-d8, 11dh-TXB2-d4, or TXB2-d4) signal in each chromatogram was used for normalization for recovery as well as relative quantitation of each detected analyte as per Supplementary Table 1.

**LC-MS analysis of ibuprofen and arachidonic acid in serum**

Arachidonic acid and deuterated arachidonic acid standards for LC-MS analysis were purchased from Cayman Chemicals. Ibuprofen and butylated hydroxytoluene (BHT) were purchased from Sigma Aldrich and ammonium acetate was obtained from Univar. Hexane, citric acid, ethyl acetate and methanol were purchased from Merck and acetonitrile was obtained from Honeywell. Arachidonic acid and ibuprofen were extracted by adding 0.75 ml of methanol to serum samples (0.5 ml) followed by the addition of 0.75 ml of acetonitrile containing 100 ng/mL of deuterated arachidonic acid (as an internal standard) and 10% of BHT. Mixtures were vortexed for 30 seconds and centrifuged at 10000 rpm for 10 min at 0°C (Beckman Coulter 22R centrifuge). Supernatant was transferred to fresh glass tubes, evaporated under a stream of nitrogen at room temperature and reconstituted in 75 µL of methanol containing 10 mM ammonium acetate. Chromatography was performed using an Agilent binary HPLC system consisting of an Agilent 1200 LC pump, an Agilent 1200 well plate auto-sampler and Luna phenyl hexyl (3 µm, 2.0 mm internal diameter and 150 mm length) column (Phenomenex) equipped with a Phenomenex Security guard column of the same packing material; both maintained at a column temperature of 50°C. The mobile phase A and B consist of water/10 mM ammonium acetate and acetonitrile/10 mM ammonium acetate, respectively at a flow rate of 200 µL/min. Samples (10 µL) were injected onto the column and eluted using the following gradient profile; initial composition was 21% B which was increased to 50% B in 10 minutes, held at 50% B for 1.5 min, returned to 21% B over 0.5 min and the column re-equilibrated for 3 min.
Liquid chromatography mass spectrometry analysis for arachidonic acid and ibuprofen was performed using Agilent Jet Stream Triple Quad 6460 in the negative mode electrospray ionization with following conditions: capillary voltage: 4000 V, collision gas temperature: 250°C, sheath gas temperature: 220°C. Nebulizer pressure was 30 psi, nozzle voltage was 500, nebulizer gas flow and sheath gas flow was maintained at 7 L/min. Ibuprofen, arachidonic acid and the deuterated arachidonic acid internal standard were quantified using MRM with 20 msec dwell time for each transition. MRM transitions of m/z 205 to 160.8, m/z 303 to 259 and m/z 310 to 266 were monitored for the ibuprofen, arachidonic acid and deuterated arachidonic acid, respectively. Optimum fragmentor voltage of 70 and 173 V and collision energy of 10 and 9V were used for the ibuprofen and arachidonic acid MRM transition. LC-MS data were processed by Agilent MassHunter software version 3. The deuterated arachidonic acid signal was used for normalization of each sample.

Data analysis

Subject characteristics were compared with independent sample t-tests. In addition to quantitative analysis, the entire lipidomic data generated were expressed as a heat map (using the R gplots package (heatmap.2 function)) to identify patterns of relative changes in families of related serum lipid mediators over time. Quantitative statistical analysis was then performed on a subpopulation of total lipid mediators detected. Serum lipid mediator concentrations were compared across time-points and between the placebo and ibuprofen groups using a 2-way ANOVA with repeated measured for time (between subject factor = group (drug), within subject factor = time) (SigmaPlot v12.0). Following statistically significant main or interaction effects, Student-Newman-Keuls post-hoc tests were used to determine the significance of pair-wise comparisons between individual time-points and groups with p≤0.05 considered statistically significant. All data is presented as the mean ± standard error of mean (SEM).
Results

**NSAID kinetics**

Ibuprofen was not present in any serum samples obtained from any subjects at baseline and was detectable during post-exercise recovery only in those subjects assigned to NSAID treatment group (Figure 2A). The average time which elapsed between initial pre-exercise NSAID dose ingestion and immediate post-exercise blood sampling was 48.69 ± 3.35 min. Serum ibuprofen concentrations in the NSAID group during recovery ranged from 16.85 ± 0.46 μg/mL immediately post-exercise to 22.52 ± 4.03 μg/mL at 2h of recovery (p<0.05 vs. immediately post-exercise) (Figure 2A). The peak circulating concentration (C_{max}) of ibuprofen achieved in subjects in the NSAID group was 25.65 ± 3.78 μg/mL, which was reached at a time to peak concentration (T_{max}) of 101.25 ± 17.87 min post-exercise (or 149.94 ± 21.22 min post-NSAID ingestion). Serum ibuprofen concentrations had returned to basal levels by 24 h post-exercise, approximately 12 h following administration of the third and final 400 mg ibuprofen dose.

**Serum arachidonic acid**

During animal models of experimental inflammation, AA is the predominant substrate for elevated lipid mediator biosynthesis, as well as itself being elevated in systemic circulation as free circulating substrate (2, 5, 32). We found that circulating free AA increased significantly (p<0.05) above baseline levels in human serum in both the placebo and ibuprofen groups at 90 min post-exercise (~1.3 fold) (Figure 2B). Serum AA peaked between 2.5 h (IBU ~1.7 fold, p<0.001) and 3 h (PLA ~1.8 fold p<0.001) into post-exercise recovery, with comparable increases above baseline levels in both groups observed (Figure 2B). Serum AA was no longer significantly elevated at 24 h post-exercise for either group. These results show that circulating
free AA substrate exhibits a delayed elevation during the early hours of post-exercise recovery, and that this response is not influenced by ibuprofen treatment.

**Lipidomic analysis of human serum lipid mediator profile**

Lipid mediator profiles of human serum were generated by LC-MS based targeted lipidomic analysis. Mass spectrometric conditions for each analyte parent-daughter transition was optimized under the HPLC flow conditions and the detection limit for most of the analytes was <100 pg on the column. Identical MRM transitions were resolved by HPLC separation to accurately quantify individual analytes. A total of 125 MRM transitions were used to monitor 147 individual analytes within the 17 min chromatography (Supplementary Table 1).

In total, 87 unique lipid mediators (and the 3 spiked internal standards) were reliably detected in human serum, including metabolites derived from COX (COX-1 and 2), LOX (5-, 12-, and 15-LOX), CYP, and non-enzymatic/degradative pathways (see Supplemental Table 1 for full analyte list and Supplemental Table 2 for full chemical names of detected lipid mediators). Significant temporal changes were identified in a diverse range of both pro-inflammatory and anti-inflammatory/pro-resolving lipid mediators and their downstream metabolites during post-exercise recovery (Figure 3). Peak elevation in the majority of detected lipid analytes was observed between 1-3 h of post-exercise recovery; although products of different enzymatic origin exhibited distinct temporal peak responses ranging immediately post-exercise (e.g. TXB₂ and 12(S)-HHTrE) to 24 h post-exercise (e.g. 6-keto-PGF₁α, 15-HETE, and 10(S),17(S)-DiHDoHE). Ibuprofen treated individuals exhibited a markedly altered post-exercise lipidomic metabolic profile, which notably did not appear to be exclusively limited to reductions in products of the COX-1 and 2 pathways (the major purported mechanistic target of NSAIDs including ibuprofen). Selected metabolites from each of the major classes of bioactive lipid
mediators are described herein, and their proposed contribution to the inflammation-resolution and physiological responses to resistance exercise is suggested. The complete quantitative data set has also been made available (Supplemental Table 3).

**COX-1 and 2 pathways**

Prostanoids including thromboxane (TXA\(_2\)) and the four primary biologically active prostaglandins: PGE\(_2\), PGD\(_2\), PGF\(_{2\alpha}\) and PGI\(_2\); are the major metabolic products of the COX-1 and 2 pathways which induce/regulate inflammatory responses (75), and blockade of their synthesis is a well characterized mechanism of action of NSAIDs (74). TXA\(_2\) is a transient platelet COX-1 product, which is a potent arterial vasoconstrictor and platelet aggregator involved in blood coagulation before undergoing rapid non-enzymatic degradation to the inactive metabolites TXB\(_2\) or 12(S)-HHTrE (63). TXB\(_2\) and 12(S)-HHTrE were increased in human serum obtained from the placebo group, but not the ibuprofen group, immediately (TXB\(_2\): p<0.001, 12(S)-HHTrE: p<0.01), 30 min (both p<0.05), 1 h (both p<0.05), 2 h (TXB\(_2\): p<0.05, 12(S)-HHTrE: p<0.001), 3 h (TXB\(_2\): p<0.01, 12(S)-HHTrE: p<0.001), and 24 h (only 12(S)-HHTrE: p<0.05) (Figure 4). Detected COX-1 and 2 derived prostaglandins including PGD\(_2\) and PGE\(_2\), also increased in the placebo group at 2 h post-exercise (PGD\(_2\): p<0.001, PGE\(_2\): p<0.01). Circulating prostaglandins are rapidly cleared by enzymatic oxidation to form the less biologically active primary 15-keto and secondary 13,14-dihydro-15-keto metabolites. 15k-PGF\(_{2\alpha}\) and 15k-PGE\(_2\), downstream markers of PGF\(_{2\alpha}\) and PGE\(_2\) biosynthesis respectively, increased in the placebo group at 1 h (15k-PGF\(_{2\alpha}\): p<0.05) and 2 h (15k-PGE\(_2\): p<0.05) post-exercise (Figure 4). Additionally, 13,14-dihydro-15-k PGE\(_2\), a tertiary PGE\(_2\) metabolite, was unaltered during early recovery, but significantly elevated above baseline later at 24 h post-exercise in the placebo group (p<0.05). Finally, 6k-PGF\(_{1\alpha}\), the stable circulating non-enzymatic product of the highly transient
COX product PGI₂ (prostacyclin) increased significantly at 3h (p<0.01) and then peaked at 24 h (p<0.001) post-exercise in the placebo group. Ibuprofen treatment blocked the exercise induced prostanoid response, with no significant elevation from pre-exercise levels in any detected circulating prostanoids or their downstream metabolic products observed in the ibuprofen group throughout 0-24 h of post-exercise recovery (Figure 4).

Lipoxygenase pathways

Lipoxygenase isoform expression has been characterized in human neutrophils (5-LOX), human platelets (platelet type 12-LOX) and human monocytes (reticulocyte-type 15-LOX) (29, 38). 5-LOX catalyzes the oxygenation of AA to form the epoxide intermediate leukotriene A₄ (LTA₄), which is a precursor to the two major classes of biologically active leukotrienes; LTB₄, one of the most potent neutrophil chemoattractants known to date, and the cysteinyl-leukotrienes, LTC₄, LTD₄, and LTE₄, powerful stimulators of bronchial/vascular smooth muscle contraction and blood vessel permeability (57). Additionally, secreted LTA₄ is one potential substrate for the subsequent biosynthesis of the anti-inflammatory/pro-resolving arachidonic acid derived lipoxins (LXA₄ & LXB₄) via the subsequent enzymatic action of platelet 12-LOX (77). Circulating LTB₄ and the non-enzymatic hydrolysis product of LTA₄ 5,12-DiHETE (6-trans-12-epi Leukotriene B₄), increased significantly from baseline levels at 1h (LTB₄: p<0.05) and 2 h (both p<0.001) post-exercise, returning to basal levels by 3 h of recovery (Figure 5A). On the other hand, no significant changes from baseline occurred in response to resistance exercise in the ibuprofen group at any time point. These findings show that circulating LTs are elevated during early post-exercise recovery (~1-2 h) and that surprisingly ibuprofen treatment appears to greatly suppress the LT response to resistance exercise.
The platelet type 12-LOX pathway metabolizes AA to the primary biologically active product 12-HETE, which possesses pro-inflammatory, pro-aggregatory and leukocyte chemotactic properties (29, 110). Additionally, as mentioned above, 12-LOX expressing cells (e.g. human platelets) can participate in anti-inflammatory/pro-resolving lipoxin biosynthesis from LTA₄ substrate during transcellular interactions with 5-LOX expressing cells (e.g. neutrophils) (77). We found human serum 12-HETE to exhibit a transient and significant increase immediately post-exercise (p<0.01 vs. pre, pooled group data), followed by a second more gradual significant elevation 3 h into recovery (p<0.01 vs. pre, pooled group data) (Figure 5B). Similarly, tetranor 12-HETE, a downstream metabolic byproduct of 12-HETE, increased during recovery, peaking 3 h post-exercise in both groups (PLA p<0.001, IBU p<0.001). The 12-LOX pathway lipid mediator response to resistance exercise was not influenced by ibuprofen treatment, with a comparable significant elevation in 12-HETE and tetranor 12-HETE observed in both groups.

15-LOX oxidizes AA to form the metabolic product 15-HETE, which via undergoing further enzymatic degradation can form the breakdown product 15-OxoETE. 15-HETE itself exhibits direct anti-inflammatory activity by blocking LTB₄ actions including superoxide production and neutrophil migration (103). Additionally, 15-HETE is a second potential precursor for anti-inflammatory/pro-resolving lipoxin biosynthesis by transcellular release, uptake, and sequential metabolism by 5-LOX expressing cells (77). Consistently 15-LOX expression is induced in peripheral blood monocytes during alternative (M2 type)-activation in response to the anti-inflammatory cytokines IL-4/IL-13, and is highly expressed in resolution phase, but not inflammatory phase macrophages (103). We found circulating 15-HETE was increased transiently in human serum immediately post-exercise in both the placebo and ibuprofen groups (PLA p<0.05, IBU p<0.05), followed by a second delayed and sustained elevation 24 h into recovery (PLA p<0.05, IBU p<0.05) (Figure 5C). Similarly, circulating 15-OxoETE was also significantly elevated above basal levels at 24 h post-exercise in both groups.
These findings show that oxidation of AA via the 15-LOX pathway is heightened both transiently in response to acute exercise, and then subsequently during the latter stages of recovery at 24 h post-exercise. This response however, was not influenced by ibuprofen administration.

**LOX/LOX interaction products: Pro-resolving lipid mediators.**

Lipoxins are omega-6 AA derived lipid mediators, which unlike the classical pro-inflammatory AA derived eicosanoids, possess anti-inflammatory and pro-resolving actions including antagonizing LT/PG signaling and inhibiting neutrophil chemotaxis, whilst acting as potent chemoattractants for alternatively activated macrophages (77). Since lipoxin synthesis in humans requires the sequential action of either 5- and 12-LOX, or 15- and 5-LOX, lipoxin production is thought to require sequential transcellular metabolism of locally secreted intermediates (e.g. LTA₄ or 15-HETE) during inflammatory cell to cell interactions between distinct cell types (77). We found lipoxin A₄ (LXA₄) to be significantly elevated above baseline levels in human serum at 1 h post-exercise in the placebo group (p<0.05) (Figure 5D). Additionally, lipoxin B₄ (LXB₄) was significantly increased from immediately post-exercise (p<0.01) through until 2 h post-exercise (p<0.05) in the placebo group (Figure 5D). In contrast, in the ibuprofen group, LXA₄ and LXB₄ failed to achieve significant changes from baseline at any time point during post-exercise recovery. These findings show that circulating lipoxins are elevated in human serum during early post-exercise recovery (~0-2 h) and that this response appears to be diminished in those receiving the ibuprofen treatment.

Resolvins and protectins are novel omega-3 fatty acid (EPA: E-series, and DHA: D-series) derived LOX enzyme interaction products which were recently discovered in self-resolving inflammatory exudates (87). Like the lipoxins, these lipid mediators have been...
characterized as possessing anti-inflammatory and pro-resolving properties (87). We found endogenous basal levels of the DHA derived RvD1 to be low in human serum and numerous sample signals were below the limit of detection, precluding within subject analysis over time. Analysis of available data by between subjects 2-way ANOVA did however show overall significantly higher circulating RvD1 in the placebo group when compared to the ibuprofen group (main effect of drug; RvD1 p<0.001) (Figure 6). RvE1 was detectable in basal human serum, and increased in the placebo group, but not the ibuprofen group, immediately post-exercise (p<0.05). RvE1 was however not reliably detectable in 2 h, 3 h and 24 h post-exercise samples in either group (possibly due to instability of this highly labile analyte throughout the latter stages of the LC-MS protocol). The protectin D1 isomer, 10(S),17(S)-DiHDoHE, was detectable in basal human serum and increased significantly immediately following exercise, as well as at 2 h and 24 h of post-exercise recovery in the placebo group, with no significant change observed in the ibuprofen group (Figure 6).

Epoxygenase pathway

The cytochrome p450 (CYP) or epoxygenase pathway is a third, less well known, branch of the AA cascade, which has more recently been established to play an important physiological role in health and disease (48). CYP epoxygenase enzymes convert arachidonic acid to a family of four epoxyeicosatrienoic acid regioisomers (5,6-, 8,9-, 11,12-, and 14,15- EpETrE). These EpETrEs are bioactive, simulating blood vessel vasodilatation, promoting angiogenesis, and possessing anti-inflammatory and anti-thrombotic properties (48). Once formed, they are rapidly metabolized by the soluble epoxide hydrolase (sEH) enzyme to corresponding downstream dihydroxyeicosatrienoic acids (DiHETrEs). 5,6- and 8,9- EpETrE specifically (but not 11,12 and 13,14-EpETrE) are also potential substrates for COX-1 and 2, and certain physiological effects including 5,6 and 8,9- EpETrE stimulated vasodilatation are mediated secondary to the
downstream synthesis of the COX products 5,6-epoxyprostaglandin E₁ and 11-hydroxy-8,9-EpETrE (90, 97). We found that circulating 11,12- and 14,15- DiHETrE, but not 5,6- and 8,9- DiHETrE, increased in human serum 3 h post-exercise in the placebo group (11,12-DHET p<0.001 vs. baseline & 14,15- DiHETrE p<0.001 vs. 30 min post) (Figure 7). In those receiving ibuprofen treatment, however, a significant increase during post-exercise recovery was observed for all four DiHETrE regioisomers (5,6- DiHETrE, 8, 9- DiHETrE, 11,12- DiHETrE and 14,15- DiHETrE) (Figure 7). These data suggest that EpETrE synthesis is elevated during acute post-exercise recovery, and that under normal physiological circumstances (in the absence of ibuprofen) sEH is not a major degradative pathway for 5,6- and 8,9- EpETrE regioisomers. Elevated 5,6- and 8,9 DiHETrE exclusively in the presence of ibuprofen treatment may be indicative of diversion of the 5,6- and 8,9 EpETrE regioisomers away from normal physiological COX dependent metabolic pathways during post-exercise recovery.

Linoleic acid metabolites

Linoleic acid can undergo enzymatic oxidation to form HODEs (from the 5-, 12- or 15-LOX pathways) and EpOMEs (from the CYP pathway). Additionally, HODEs and EpOMEs are also commonly used as indicators of free radical mediated lipid peroxidation. In particular, activated neutrophils are a major source of 9,10- and 12,13-EpOME, with excessive formation being associated with neutrophil oxidative burst. We observed that all detected linoleic acid derived eicosanoids including HODEs and EpOMEs, as well as their downstream OxoODE and DiHOME metabolic products, exhibited a distinct temporal response characterized by an early transient decline 0-30 min post-exercise, followed by subsequent significant elevation 2-3 h into recovery (Figure 8). This linoleic acid derived lipid mediator response to resistance exercise was highly similar in those receiving placebo or ibuprofen treatment, and thus was not influenced by NSAID administration.
Discussion

The present study utilized a targeted lipidomics approach to characterize in vivo temporal changes in human peripheral blood inflammatory lipid mediator profile following unaccustomed resistance exercise and treatment with the NSAID ibuprofen. The lipid mediator response during post-exercise recovery was characterized by elevated circulating levels of COX-1 and 2 derived prostaglandins, as well as numerous 5-, 12-, 15-LOX, and CYP pathway products implicated in multiple aspects of inflammation and resolution. Ibuprofen treatment blocked exercise-induced increases in COX-1 and 2 derived prostanoids, and notably was also associated with off target reductions in the biosynthesis of the inflammatory leukotrienes (e.g. LTB₄), as well as pro-resolving lipid mediators including lipoxins (LXA₄ and LXB₄), resolvins (RvD₁ and RvE₁), and protectins (protectin D1 isomer 10S, 17S-diHDHA). Furthermore, CYP pathway product (EpETrE) metabolism was found to be altered by ibuprofen treatment. These findings identify a complex bioactive lipid mediator response during post-exercise recovery that may play an important physiological role in inflammatory/adaptive responses to resistance exercise and show that NSAID treatment can have previously unappreciated effects on in vivo lipid mediator dynamics in a human model of acute inflammation.

Despite the well-established role of individual bioactive lipid mediators in eliciting aspects of inflammation-resolution (91), and recent reports of lipid mediator profiling of human biological fluids (34, 36, 71), few studies have investigated in vivo human lipid mediator dynamics during physiological models of inflammation. By the use of LC-MS targeted lipidomic approach employed in the present study, the majority of the known inflammatory mediator lipidome can be profiled in a single human biological sample. Recent animal studies have used a similar targeted lipidomics approach to characterize temporal lipid mediator responses during inflammatory models including experimental arthritis (5, 20, 32), acute administration of the hyperalgesic/pro-inflammatory agents carrageenan (12, 31) or lipopolysaccharides (LPS) (2, 27),
and maternal hypercholesterolemia (72). The majority of previous human studies have focused on a select few inflammatory eicosanoids, resulting in a fragmented view of the \textit{in vivo} lipid mediator dynamics. The most investigated lipid mediator in human models of exercise-induced muscle injury has been PGE$_2$, likely due to its pro-inflammatory and hyperalgesic properties and their hypothesized association with symptomatology of DOMs. Whilst some studies have reported elevated circulating PGE$_2$ concurrent with the onset of DOMs 24-72 h following exercise-induced muscle injury (95, 102), others have found no such response (4, 9, 13, 23, 24, 41). The reasons for this are unclear, but may be related to differences in the intensity of skeletal muscle loading (for example downhill running (13) vs. resistance exercise (95, 102)) and/or the volume of active muscle mass (unilateral/isolation (4, 9, 23, 24, 41) vs. bilateral/compound exercises (95, 102). In the present study, the vast majority of inflammatory lipid mediators, including PGE$_2$, were no longer significantly elevated in human serum at 24 h post-exercise. Whilst no measures of the extent of muscle injury were included in the present study, the traditional resistance exercise protocol used is a realistic muscle hypertrophy training stimulus which would be expected to inflict only ‘minor exercise-induced muscle injury’ on the basis of previous studies implementing similar exercise protocols (69). More extreme eccentric exercise protocols can result in longer lasting impairment of muscle function, myofiber necrosis, and a robust inflammatory cell response, which may last many days to weeks (69). Thus, it is possible that a more extreme exercise stimulus may induce a longer lasting pro-inflammatory lipid mediator response.

Several studies have also shown that PG biosynthesis is elevated with modest intensity aerobic exercise, which would presumably not be eliciting muscle damage or injury. Thus lipid mediators may play an important role in adaptive responses to exercise that are evoked not only with classic injury or damaging stimuli. Heightened circulating COX-1 and 2 derived PGE$_2$ (28, 50, 66, 70, 107), PGF$_{2\alpha}$ (28, 30), and 6-keto-PGF$_{1\alpha}$ (18, 28, 33, 50, 58, 62, 76, 108) have been observed during continuous sub-maximal exercise. These transient changes appear to play an important role in the regulation of circulatory system responses during exercise, as NSAID
administration can block exercise induced muscle hyperemia and the cardiovascular pressor reflex (22, 25, 79). Similarly, increases in 5-LOX derived plasma LTB$_4$ have been observed in humans immediately following high intensity running in some (40), but not all studies (70). In the present study, circulating PGs and LTs (with the exception of TXB$_2$) were unchanged immediately post-resistance exercise, but exhibited a delayed peak elevation between ~1-2 h (PGD$_2$, PGE$_2$, PGF$_{2\alpha}$, and LTB$_4$) and 24 h (6-keto-PGF$_{1\alpha}$) into recovery. TXA$_2$ is the major product of platelet COX-1 and elevated TXB$_2$ immediately post-exercise likely reflects an acute pro-aggregatory cardiovascular response as has been observed during maximal, but not sub-maximal, aerobic exercise (33, 50, 58). PGs and LTs are classical pro-inflammatory lipid mediators, the physiological effects of which include vascular vasodilation, increase blood vessel permeability, and neutrophil chemotaxis/activation (57, 75). Although infiltrated neutrophils have been scarcely detected within human muscle biopsies following the kind of resistance exercise employed in the present study (69), both elevated circulating numbers of neutrophils and local accumulation of neutrophils in the region of the exercised musculature are physiological responses commonly observed in the early hours of recovery (69). PGs and LTs may play an important role in regulating mobilization, activation, and migration of neutrophils during the early hours of post-exercise recovery. Additionally, skeletal muscle cells themselves express PG and LT receptors and have been shown to respond to exogenous treatment with lipid mediators LTB$_4$ (92), PGF$_{2\alpha}$ (43, 46, 56), PGI$_2$ (6), or free AA substrate (55) with enhanced \textit{in-vitro} growth. Therefore, eicosanoids may be important autocrine/paracrine growth signaling molecules within skeletal muscle tissue during post-exercise recovery, independent of their classical inflammatory roles.

Ibuprofen is a non-selective NSAID, the main therapeutic mechanism of action of which thought to be non-selective inhibition of both COX-1 and 2 activities (73). As expected the PG and TX response to resistance exercise was blocked by ibuprofen treatment, indicating that the dosing regimen used was effective to inhibit post-exercise COX-1 and 2 enzymatic activities.
More surprisingly however, we found that ibuprofen administration also greatly blunted the LT response to resistance exercise. This unanticipated finding may have important implications for concerning the effect of NSAIDs on physiological responses to exercise previously reported. Whereas oral ibuprofen ingestion impaired the skeletal muscle protein synthesis response to resistance exercise (100), COX-2 specific drugs (14) or intramuscular infusion of a different non-selective COX-1 and 2 inhibitor (indomethacin) (60) have failed to do so. It is possible that ibuprofen may be unique amongst NSAIDs in that it can exert COX-1 and 2 independent anti-inflammatory effects by direct inhibition of 5-LOX enzyme activity (45, 49, 104). Alternatively, in vivo loss of COX-1 and 2 activity itself may simply have direct effects on LT biosynthesis not appreciated from in vitro enzymatic or biological assays. Interestingly, using a similar targeted lipidomics approach as employed in the present study, COX-2 knockout mice were recently found to exhibit blunted LTB₄ biosynthesis during induction of experimental arthritis (5). Indeed, these findings appear indicative of a direct mechanistic link between COX-2 activity and LT biosynthesis via the 5-LOX pathway during in vivo inflammation. To our knowledge the present study is the first report of a similar effect of NSAID treatment on 5-LOX products in human subjects in vivo.

In addition to 5-LOX, humans express distinct lipoxygenase isoforms in a tissue specific manner, including platelet type 12-LOX, reticulocyte type 15-LOX, and epidermal type 15-LOX. Comparatively very little is known about the effect of exercise on the products of these pathways. We observed transient elevation of circulating levels of both the 12-LOX product 12-HETE and 15-LOX product 15-HETE immediately post-exercise. This transient response was followed by a second delayed elevation in 12-HETE (as well as its downstream metabolite tetranor 12-HETE) at 3 h, and then 15-HETE (as well as downstream 15-OxoETE) at 24 h of recovery. Unlike PGs and LTs, which are synthesized exclusively from mobilized free fatty acid substrate, free 12-HETE and 15-HETE can also be incorporated into membrane phospholipids and undergo subsequent direct release by phospholipase action (10). It is possible that the bi-modal 12-HETE
and 15-HETE responses observed may thus reflect early release of intracellular stores immediately post-exercise, followed by subsequent enzymatic biosynthesis during the latter stages of recovery. Notably 15-LOX expression is low or undetectable in most cell type, but is inducibly expressed in alternatively activated macrophages (19, 21, 39). Delayed elevation in 15-LOX products only at 24 h post-exercise is consistent with macrophages being the predominant inflammatory cell type present during the latter stages of inflammation, including ≥24 h following exercise-induced muscle injury (69).

Biosynthesis of pro-resolving mediators in humans including the lipoxins, resolvins, and protectins involves the sequential transcellular metabolism by distinct compartmentalized LOX enzymes which are expressed in a cell type specific manner. For example, LXA₄ and LXB₄ can be synthesized from neutrophil 5-LOX generated LTA₄ by sequential release, uptake, and oxidation by platelet 12-LOX. Alternatively, 15-LOX expressing cells (e.g. human monocytes) can convert free AA to 15-HETE which can subsequently be taken up and converted by 5-LOX expressing neutrophils to form LXA₄ and LXB₄. To our knowledge the present study is the first to measure these pro-resolving lipid mediators in human blood during post-exercise recovery. It was demonstrated that lipoxins (LXA₄ & LXB₄), resolvins (RvE₁ & RvD₁), and the protectin D₁ isomer 10S,17S-diHDHA increased in human serum during the early hours of post-exercise recovery. Our findings with the lipoxins are consistent with those reported earlier by Gangemi et al. (2003) where LXA₄ in human urine were found to increase with treadmill exercise (35). Interestingly, we found that treatment with the NSAID ibuprofen, in addition to blocking pro-inflammatory PG and LT biosynthesis, also resulted in a diminished pro-resolving lipid mediator response to resistance exercise Although NSAIDs such as ibuprofen have not been found to inhibit 12-LOX or 15-LOX activity directly, biosynthesis of resolution phase mediators including LXs, Rvs and protectins is dependent on the induction of early inflammatory events (81). For example, LX and Rv biosynthesis requires cell to cell interactions which occur during inflammation for transcellular biosynthesis (e.g. neutrophils with platelets, or monocytes with
neutrophils). Additionally, elevated PGE$_2$ and PGD$_2$ levels have been shown to directly trigger the transcription of the 15-LOX enzyme in neutrophils, promoting a shift from LT to LX biosynthesis, a phenomenon termed lipid class switching (51). Given that ibuprofen administration seemingly did not influence post-exercise elevation in levels of 12-HETE or 15-HETE in the present study; impaired pro-resolving mediator synthesis during post-exercise-recovery may be predominantly related to blunted 5-LOX activity as was indicated by the diminished LT response. Whilst the precise underlying mechanisms involved remain to be determined, our findings are consistent with previous animal studies showing that NSAIDs can prevent or delay inflammatory resolution, and suggest that acute pro-inflammatory signals during post-exercise recovery are important in the sequential induction of pro-resolving lipid mediator biosynthesis in humans.

An important consideration is the likely cellular source(s) of the circulating components measured in the present study. Muscle tissue is a potential source of certain lipid mediators, especially PGs, which are known to be synthesized locally by muscle cells (55). Numerous potential non-muscle sources of lipid mediators also exist however, including neutrophils (PGs and LTs), platelets (TXB$_2$ and 12-HETE)), monocytes/macrophages (15-HETE), vascular endothelial cells (PGI$_2$) and transcellular interactions (lipoxins & resolvins). Previous reports of elevated post-exercise leukocyte COX-2 and 5-LOX expression (40, 47), COX-2 mRNA expression within human post-exercise muscle biopsies (60, 109), and 5-LOX expression within resting skeletal muscle tissue (111) suggests that leukocytes and exercised skeletal muscle tissue itself are potential sites of elevated LT and PG biosynthesis. Whether skeletal muscle can participate in the biosynthesis of other lipid mediator species measured including pro-resolving mediators, either directly, or via transcellular cross talk with locally accumulating inflammatory cells is not known. To date the majority of these lipid mediators have most commonly been measured in peripheral circulation as in the present study. It is important to note however, that local production of pro-inflammatory and pro-resolving mediators within exercised muscle may
be locally inactivated or metabolized before reaching the blood compartment. Future studies investigating intramuscular lipid mediator profile will be useful further charactering the role of inflammatory and resolving lipid mediators during post-exercise recovery.

**Perspectives and significance**

The data presented here characterize the acute inflammatory and pro-resolving lipid mediator response in human blood in response to unaccustomed resistance exercise. Peak induction of pro-inflammatory prostaglandin and leukotriene biosynthesis occurred in the early hours (1-2 h) of post-exercise recovery. In addition, elevated levels of potent pro-resolving mediators including lipoxins, resolvins, and protectins were observed during both the early (0-3 h post-exercise) (LXA4, LXB4, RvE1, RvD1) and later (24 h post-exercise) (15-HETE and protectin D1 isomer 10S, 17S-diHDHA) stages of recovery. Treatment with ibuprofen, a well-known over-the-counter COX-1 and 2 inhibitor, blocked the prostaglandin response to exercise, but more surprisingly also abolished leukotriene biosynthesis and lead to a diminished pro-resolving lipid mediator response during post-exercise recovery. We propose that the identified lipid mediators may play a previously unappreciated role in immunological and adaptive muscle signaling responses during the early post-exercise recovery period. Moreover, our data suggests that acute pro-inflammatory signals are mechanistically linked to the induction of a biological active inflammatory resolution program, regulated by pro-resolving lipid mediators during post-exercise recovery. Practically these findings suggest that the use of anti-inflammatory interventions in an effort to enhance post-exercise recovery may be at the cost of delayed and/or diminished natural resolution of the inflammatory response to exercise. Whilst the precise physiological implications remain to be determined, the effect of ibuprofen treatment human lipid mediator profile observed in the present study provides one potential underlying mechanism for
previous reports of deleterious effects of NSAIDs on skeletal muscle tissue responses to injury, mechanical overload, and exercise.
Table 1: Subject characteristics. Values are means ± SEM, n=8/group. NS denotes no significant difference between groups.

Figure 1: Schematic of experimental study design.

Figure 2: Ibuprofen and arachidonic acid response to resistance exercise ± ibuprofen treatment. A: Pharmacokinetics of ibuprofen in human serum during the post-exercise period following ibuprofen or placebo administration. Mean peak serum concentration = Cmax. Mean time to peak serum concentration = Tmax. B: Fold change in circulating human serum arachidonic acid (AA) during post-exercise recovery. Values are means ± SEM. ***p<0.001, **p<0.01, *p<0.05 vs. pre-exercise.

Figure 3: Lipidomic profiling of temporal changes in major eicosanoid biosynthetic pathways. Heat map representing average percentage change in the levels of lipid mediators detected in human serum during 24 h post-exercise recovery. Analytes are expressed relative to respective baseline (pre-exercise) levels (% change) for ibuprofen or placebo treated subjects. Increases in metabolite levels are indicated by red, decreases by green, and detectable but unchanged levels by white. Analytes with an increase ≥ 100% are displayed as a maximum of +100%.

Figure 4: Prostanoid response to acute resistance exercise ± ibuprofen treatment. Values are means ± SEM of IS normalized arbitrary units. ***p<0.001, **p<0.01, *p<0.05 vs. respective pre-exercise levels. ###p<0.001, ##p<0.01, #p<0.05 vs. placebo group at same time point.
Figure 5: Lipoxygenase pathway responses to resistance exercise ± ibuprofen treatment. Values are means ± SEM of IS normalized arbitrary units. ***p<0.001, **p<0.01, *p<0.05 vs. respective pre-exercise levels. #p<0.05 vs. placebo group at same time point.

Figure 6: Omega-3 fatty acid derived pro-resolving lipid mediator response to resistance exercise ± ibuprofen treatment. Values are means ± SEM of IS normalized arbitrary units. ***p<0.001, **p<0.01, *p<0.05 vs. pre-exercise. #p<0.05 vs. placebo group at same time point.

Figure 7: Cytochrome p450 metabolic response to resistance exercise ± ibuprofen treatment. Values are means ± SEM of IS normalized arbitrary units. ***p<0.001, **p<0.01, *p<0.05 vs. pre-exercise. ##p<0.01 vs. placebo group at same time point.

Figure 8: Linoleic acid metabolic response to resistance exercise ± ibuprofen treatment. Values are means ± SEM of IS normalized arbitrary units. ^^^p<0.001, ^^p<0.01, ^p<0.05 vs. 30 min post-exercise time-point.


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83. Serhan CN, Clish CB, Brannon J, Colgan SP, Chiang N, and Gronert K. Novel functional sets of lipid-derived mediators with antiinflammatory actions generated


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NS: Not Significant
A. Serum ibuprofen (µg/mL)

C_{max} = 25.65 ± 3.78 µg/mL
T_{max} = 101.26 ± 17.87 min

B. Serum Arachidonic Acid (Fold change)

Placebo
- Ibuprofen

Pre 0 0.5 1 1.5 2 2.5 3 24
Pro-resolving mediators

A

Drug p<0.001

B

Ibuprofen
Placebo

C

Protectin D1 isomer
10S,17S-DHDoHE

Time post-exercise (h)