IL-6 Linkage to Exercise-Induced Shifts In Lipid-Related Metabolites: A Metabolomics-Based Analysis

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Abstract

Metabolomics profiling and bioinformatics technologies were Substantial increase In used to determine the relationship between exercise-induced increases in IL-6 and lipid-related metabolites 6 and lipid-related metabolites. Twenty-four male runners (age 36.5 +/- 1.8 y) ran on treadmills to exhaustion (2.26 +/- 0.01 h, 24.9 +/- 1.3 km, 69.7 +/- 1.9% Vastus lateralis muscle biopsy and blood samples were collected before and immediately after running and showed a 33.7 +/- 4.2% decrease in muscle glycogen, 39.0 +/- 8.8-, 2.4 +/- 0.3-, and 1.4 +/- 0.1-fold increases in plasma IL-6, IL-8, and MCP-1, respectively, and 95.0 +/- 18.9 and 158 20.6% increases in cortisol and epinephrine, respectively (all, $P < 0.001$). The metabolomics analysis revealed changes in 209 metabolites, especially long- and medium-chain fatty acids, fatty acid oxidation products (dicarboxylate and monohydroxy fatty acids, acylcarnitines), and ketone bodies. OPLS-DA modeling supported a strong separation in pre- and post-exercise samples (R2Y = 0.964, Q2Y = 0.902). OPLSR analysis failed to produce a viable model for the relationship between IL-6 and all lipid-related metabolites (R2Y = 0.76, Q2Y = -0.0748). Multiple structure equation models were evaluated based on IL-6, with the best-fit pathway model showing a linkage of exercise time to IL-6, then camitine, and 13-methylmyristic acid (a marker for adipose tissue lipolysis) and sebacate. These metabolomics-based data indicate that the increase in plasma IL-6 after long endurance running has a minor relationship to increases in lipid related metabolites.

Nieman, D. 2017. IL-6 Linkage to Exercise-Induced Shifts in Lipid-Related Metabolites: A Metabolomics-Based Analysis. Journal of Proteome Research. V 16.2. Pg 970-977. DOI: 10.1021/acs.jproteome.6b00892.

IL-6 Linkage to Exercise-Induced Shifts in Lipid-Related Metabolites: A Metabolomics-Based Analysis

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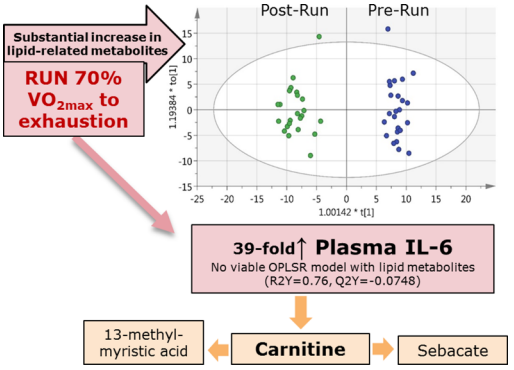
ABSTRACT: Metabolomics profiling and bioinformatics technologies were used to determine the relationship between exercise-induced increases in IL-6 and lipid-related metabolites. Twenty-four male runners (age 36.5 ± 1.8 y) ran on treadmills to exhaustion (2.26 ± 0.01 h, 24.9 ± 1.3 km, 69.7 ± 1.9% VO_{2max}). Vastus lateralis muscle biopsy and blood samples were collected before and immediately after running and showed a 33.7 ± 4.2% decrease in muscle glycogen, 39.0 ± 8.8-, 2.4 ± 0.3-, and 1.4 ± 0.1-fold increases in plasma IL-6, IL-8, and MCP-1, respectively, and 95.0 ± 18.9 and 158 ± 20.6% increases in cortisol and epinephrine, respectively (all, $P < 0.001$). The metabolomics analysis revealed changes in 209 metabolites, especially long- and medium-chain fatty acids, fatty acid oxidation products (dicarboxylate and monohydroxy fatty acids, acylcarnitines), and ketone bodies. OPLS-DA modeling supported a strong separation in pre- and post-exercise samples (R2Y = 0.964, Q2Y = 0.902). OPLSR analysis failed to produce a viable model for the relationship between IL-6 and all lipid-related metabolites (R2Y = 0.76, Q2Y = −0.0748). Multiple structure equation models were evaluated based on IL-6, with the best-fit pathway model showing a linkage of exercise time to IL-6, then carnitine, and 13-methylmyristic acid (a marker for adipose tissue lipolysis) and sebacate. These metabolomics-based data indicate that the increase in plasma IL-6 after long endurance running has a minor relationship to increases in lipid-related metabolites.



KEYWORDS: *running, interleukin-6, metabolomics, cortisol, epinephrine*

INTRODUCTION

Metabolomics is the study of small molecular weight molecules (metabolites) present in a biological system and when combined with bioinformatics can reveal new insights regarding complex metabolic processes following lifestyle and pharmaceutical interventions.[1] A nontargeted metabolomics approach provides a system-wide view of the metabolic response to exercise by simultaneously measuring and identifying shifts in hundreds of metabolites. Although the number of studies is limited, metabolomics-based investigations indicate that prolonged and intensive exercise is associated with extensive lipid mobilization and oxidation, marked by increases in metabolites related to carnitine metabolism and long-chain, dicarboxylate, and essential fatty acid metabolism.[2–7]

IL-6 is produced by a wide variety of cell types and has multiple, context-dependent immuno-modulating and immuno-metabolic roles.[8,9] During exercise, IL-6 serves as a complex communication factor between skeletal muscle, liver, pancreas, and other metabolic tissues.[9] Muscle- and adipose-derived IL-6 has been identified as a hormone-like signaling agent to adipose tissue to increase lipolysis and muscle fat oxidation, especially when muscle glycogen levels become depleted.[10–12]

In cell culture experiments, IL-6 markedly increases both lipolysis and free fatty acid (FFA) oxidation, but IL-6 concentration levels typically far exceed the increases in plasma IL-6 measured after prolonged and intensive exercise bouts.[11,13,14] Infusion of recombinant IL-6 (rIL-6) for several hours into resting humans increases whole-body lipolysis and FFA oxidation, but plasma IL-6 levels, as with cell culture studies, often exceed what is attained post-exercise. Additionally, interpretation of data from rIL-6 infusion studies can be confounded by concomitant increases in lipolytic hormones such as cortisol and epinephrine and the absence of the multitude of other hormones, metabolites, and proteins generated during intensive exercise.[11,15]

Exercise-induced increases in plasma IL-6 and lipolysis may be linked, but this has not yet been tested using metabolomics-

based procedures that allow the simultaneous evaluation of a high number of metabolites from the lipid super pathway. The primary purpose of this study was to use metabolomics profiling and bioinformatics technologies to evaluate the relationship of exercise-induced increases in plasma IL-6 and lipid-related metabolites in 24 athletes who ran on treadmills to exhaustion at high intensity.

MATERIALS AND METHODS

Subjects

Subject recruitment was conducted via direct messaging to runners and running clubs in the Charlotte, NC, metropolitan area. Subjects included 24 male runners (ages 22−55 years) who regularly competed in long-distance road races and were capable of running at ∼70% on treadmills to exhaustion. Subjects signed informed consent forms, and study procedures were approved by the Institutional Review Board at Appalachian State University (ASU).

Baseline Testing

Two weeks prior to the running trial, subjects were tested for VO_{2max} during a graded, treadmill test with the Cosmed Fitmate metabolic system (Cosmed, Rome, Italy). Body composition was measured with the Bod Pod body composition analyzer (Life Measurement, Concord, CA). Demographic and training histories were acquired with questionnaires.

Running Trial

During the 3-day period prior to the running trial, subjects were asked to taper their exercise training as if preparing for a long-distance race and to follow a moderate-carbohydrate dietary regimen (∼55% kcal as carbohydrates) by choosing foods from a list provided by the investigative team (to ensure a similar fat and carbohydrate intake before the run to exhaustion). A standardized meal consisting of Boost Plus (Néstle Nutrition, Slorham Park, NJ) was ingested at 12:00 pm, with energy intake adjusted to 5 kcal/kg body weight. Boost Plus is a nutritionally complete, high-energy oral supplement with an energy density of 6.4 kJ/mL (1.52 kcal/mL) and 15% of energy as protein, 35% as fat, and 50% as carbohydrate and 24 vitamins and minerals. Subjects reported to the lab at 2:15 pm and provided blood and muscle biopsy samples. At 3:00 pm, subjects ran on laboratory treadmills with the speed set at 70% of VO_{2max} and drank water ad libitum, without ingestion of any other beverages or food. Subjects ran as long as possible until exhaustion, defined as the inability of the subject to continue running at 70% VO_{2max} despite verbal urging from the laboratory staff. Metabolic measures from the Cosmed Fitmate metabolic system and the rating of perceived exertion (RPE) were taken at 15 min, then every 60 min during the running bout to verify that the appropriate intensity was maintained. Blood and muscle samples were taken again immediately following exercise.

Cytokine Analysis

Total plasma concentrations of three cytokines [monocyte chemoattractant protein-1 (MCP-1), IL-6, and IL-8] were determined using an electrochemiluminescence-based solid-phase sandwich immunoassay (Meso Scale Discovery, Gaithersburg, MD). All samples and provided standards were analyzed in duplicate, and the intra-assay CV ranged from 1.7 to 7.5% and the interassay CV ranged from 2.4 to 9.6% for all cytokines measured. Pre- and post-exercise samples for the cytokines were analyzed on the same assay plate to decrease interkit assay variability.

Stress Hormone Analysis

Serum cortisol was measured with an electrochemiluminescence immunoassay (ECLIA) and plasma epinephrine with high-pressure liquid chromatography (HPLC) and electrochemical (EC) detection through a commercial lab (LabCorp, Burlington, NC).

Muscle Biopsy Procedures and Glycogen Analysis

Pre- and post-exercise muscle biopsy samples were acquired from the vastus lateralis on the same leg approximately 2 cm apart using procedures previously described.[16] Local anesthesia (1% xylocaine, Hospira, Lake Forest, IL) was injected subcutaneously. After a small incision, a muscle biopsy sample was obtained using the suction-modified percutaneous needle biopsy procedure.[16] Muscle was trimmed of connective tissue and fat and immediately frozen in liquid nitrogen. Samples were stored at −80 °C until subsequent analysis (within 3 months of study completion). A glycogen assay kit (Catalog no. MAK016, Sigma-Aldrich, St. Louis, MO) was used to determine the concentration of glycogen in vastus lateralis muscle homogenates. In this coupled enzyme assay, glucoamylase hydrolyzed glycogen to glucose; then. the glucose was oxidized to yield a product that reacted with a probe to generate a color detectable with a microplate reader (Synergy H1 Hybrid Reader, BioTek Instruments, Winooski, VT) at 570 nm.

Metabolomics

The nontargeted metabolic profiling instrumentation employed for this analysis combined three independent platforms: ultrahigh performance liquid chromatography−tandem mass spectrometry (UHPLC−MS/MS) optimized for basic species, UHPLC−MS/MS optimized for acidic species, and gas chromatography−mass spectrometry (GC−MS).[17,18] Blood samples were collected in ethylenediaminetetraacetic acid (EDTA) tubes and centrifuged at 3000 rpm for 10 min at 4 °C, with the plasma aliquoted, snap frozen in liquid nitrogen, and then stored at −80 °C until analysis (within 3 months of study completion). For each plasma sample, 100 μL was used for analyses. Using an automated liquid handler (Hamilton LabStar, Salt Lake City, UT), protein was precipitated from the plasma with methanol that contained four standards to report on extraction efficiency. The resulting supernatant was split into equal aliquots for analysis on the three platforms. Aliquots, dried under nitrogen and vacuum-desiccated, were subsequently reconstituted in either 50 μL of 0.1% formic acid in water (acidic conditions) or 50 μL of 6.5 mM ammonium bicarbonate in water, pH 8 (basic conditions) for the two UHPLC−MS/MS analyses or derivatized to a final volume of 50 μL for GC−MS analysis using equal parts bistrimethylsilyltrifluoroacetamide and solvent mixture acetonitrile/dichloromethane/cyclohexane (5:4:1) with 5% triethylamine at 60 °C for 1 h. In addition, three types of controls were analyzed in concert with the experimental samples: aliquots of a well-characterized human plasma pool served as technical replicates throughout the data set, extracted water samples served as process blanks, and a cocktail of standards spiked into every analyzed sample allowed instrument performance monitoring. Standards to monitor extraction were d_6-cholesterol, fluorophenylglycine, and tridecanoic acid. A standard to monitor GC−MS derivatization was 2-*tert*-butyl-6-methylphenol (BHT). GC−MS standards to monitor GC and MS perform-

ance were C5−C18 alkylbenzenes. Pre- and post-exercise samples were randomized across platform run days.

For UHLC−MS/MS analysis, aliquots were separated using a Waters Acquity UPLC (Waters, Millford, MA) instrument with separate acid/base-dedicated 2.1 mm × 100 mm Waters BEH C18 1.7 μm particle columns heated to 40 °C and analyzed using an LTQ mass spectrometer (Thermo Fisher Scientific, Waltham, MA) that consisted of an electrospray ionization (ESI) source and linear ion-trap (LIT) mass analyzer.[17] Extracts reconstituted in formic acid were gradient eluted at 350 μL/min using (A) 0.1% formic acid in water and (B) 0.1% formic acid in methanol (0% B to 70% B in 4 min, 70−98% B in 0.5 min, 98% B for 0.9 min), whereas extracts reconstituted in ammonium bicarbonate used (A) 6.5 mM ammonium bicarbonate in water, pH 8, and (B) 6.5 mM ammonium bicarbonate in 95/5 methanol/water (same gradient profile as above) at 350 μL/min. The MS instrument scanned 99−1000 m/z and alternated between MS and MS2 scans using dynamic exclusion with approximately six scans per second. Derivatized samples for GC−MS were separated on a 5% diphenyl/95% dimethyl polysiloxane-fused silica column with helium as the carrier gas and a temperature ramp from 60 to 340 °C and then analyzed on a Thermo-Finnigan Trace DSQ MS (Thermo Fisher Scientific) operated at unit mass resolving power with electron impact ionization and a 50−750 atomic mass unit scan range. Metabolites were identified by automated comparison of the ion features in the experimental samples to a reference library of chemical standard entries that included retention time, molecular weight (m/z), preferred adducts, and in-source fragments as well as associated MS spectra and were curated by visual inspection for quality control using software developed at Metabolon (Durham, NC).[18] Common and biologically abundant isomers of unsaturated fatty acids containing the n3, n6, and n9 configuration are contained in Metabolon's chemical standard library. LC−MS standards to monitor LC and MS performance were d_3-leucine, chloro- and bromo-phenylalanine, d_2-maleic acid, amitriptyline, and d_{10}-benzophenone. Biochemical identifications were based on three criteria: retention index within a narrow window of the proposed identification, match to the library within 0.4 m/z, and the MS/MS forward and reverse scores between the experimental data and authentic standards. The MS/MS scores were based on a comparison of the ions present in the experimental spectrum to the ions present in the library spectrum.

Statistical Analysis

Data are expressed as mean ± SE. Cytokine, stress hormone, and muscle glycogen data were analyzed for pre-to-post exercise changes using paired t tests. For the metabolomics statistical analyses, any missing values were assumed to be below the limits of detection, and these values were imputed with the compound minimum (minimum value imputation). Each metabolite was median-scaled. Paired t-test analysis on log-transformed median-scaled metabolomics data was performed to identify metabolites that were significantly different between pre- and post-exercise samples. An estimate of the Benjamini-Hochberg false discovery rate (FDR) was calculated to take into account the multiple comparisons that normally occur in metabolomics-based studies, with FDR < 0.05 used as the cutoff for significance. Pre-to-post exercise fold changes were calculated using median-scaled intensity values. Metabolites with FDR < 0.05 and fold change >10% were considered as exercise responding metabolites. The correlations between the fold changes of exercise responding metabolites and the fold changes of plasma IL-6, serum cortisol, and plasma epinephrine were determined by Pearson correlation coefficient analysis. Orthogonal partial least-squares discriminant analysis (OPLS-DA) was also used to identify metabolites that distinguished post-exercise and pre-exercise samples. Variable influence on projection (VIP) scores greater than 1.75 were used as the cutoff to select the most important metabolites responding to exercise. Exercise responding metabolites from the lipid super pathway identified by paired t tests ($P < 0.05$ and fold change >10%) were further analyzed by orthogonal partial least-squares regression (OPLSR) in SIMCA (Version 14, MKS Data Analytics Solutions, Umeå, Sweden), which identified lipid-related metabolites whose changes were most associated with the change of plasma IL6, serum cortisol, plasma epinephrine, and muscle glycogen depletion. Two validation methods, including a 7-round cross validation and a permutation-based validation, were used to prevent overfitting. An OPLS model was considered valid if its prediction ability was better than 95% of the models built using the 999 permuted data sets. Structure equation models were used to evaluate potential pathways that explained the relationship between time to exhaustion and metabolic responses. The validity of each model was determined by several fit indices, including model chi-square, root-mean-square error of approximation (RMSEA), goodness of fit index (GFI), Bentler-Bonett normed-fit index (NFI), and Bentler comparative fit index (CFI).[19−22] Other than OPLS analysis using SIMCA, all other analyses were performed using SAS (SAS institute, Cary, NC)

RESULTS

Study participants included 24 trained male runners (ages 22 to 55 years). Table 1 summarizes demographic and performance data for these male endurance athletes. The runners were able

Table 1. Subject Characteristics and Run-to-Exhaustion Performance Measures (N = 24)[a]

variable	mean ± SE
age (years)	36.5 ± 1.8
height (m)	1.78 ± 0.01
weight (kg)	77.3 ± 1.9
body fat (%)	15.0 ± 1.0
VO_{2max} (mL kg $^{-1}$ min^{-1})	60.0 ± 1.5
HR_{max} (beats/min)	185 ± 2.3
Run Trial Performance	
time to exhaustion (h)	2.26 ± 0.01
distance (km)	24.9 ± 1.3
VO_2 (mL·kg^{-1} min^{-1})	41.5 ± 1.0
VO_2 (%VO_{2max})	69.7 ± 1.9
HR (beats/min)	157 ± 2.4
%HR_{max}	85.1 ± 0.9
ventilation (L/min)	77.6 ± 1.9
RPE	
average	13.7 ± 0.2
ending	17.7 ± 0.2

[a]VO_2, volume of oxygen consumed; HR, heart rate; RPE, rating of perceived exertion.

to maintain an average intensity of 69.7 ± 1.9% VO_{2max} for 2.26 ± 0.01 h and 24.9 ± 1.3 km until exhausted, with an ending RPE of 17.7 ± 0.2. Age had no influence on run time to exhaustion in these athletes (r = −0.119, P = 0.580).

Pre- and postrun plasma cytokine, stress hormone, and muscle glycogen data are summarized in Table 2. Plasma IL-6, IL-8, and MCP-1 increased 39.0 ± 8.8-, 2.4 ± 0.3-, and 1.4 ± 0.1-fold, respectively (all, P < 0.001). Serum cortisol and plasma epinephrine increased 95.0 ± 18.9 and 158 ± 20.6% post-exercise, respectively. Skeletal muscle glycogen sampled from the vastus lateralis decreased 33.7 ± 4.2% (P < 0.001).

Table 2. Run-to-Exhaustion Effects on Plasma Cytokines, Stress Hormones, and Muscle Glycogen in N = 24 Runners[a]

variable	pre-exercise	post-exercise
plasma IL-6 (pg/mL)	0.61 ± 0.08	16.4 ± 2.0[b]
plasma IL-8 (pg/mL)	4.23 ± 0.29	13.8 ± 1.1[b]
plasma MCP-1 (pg/mL)	105 ± 3.5	253 ± 14.0[b]
serum cortisol (pg/mL)	10.1 ± 0.6	18.4 ± 1.4[b]
plasma epinephrine (pg/mL)	66.0 ± 5.6	159 ± 15.2[b]
muscle glycogen (mmol/kg)	70.6 ± 3.4	47.0 ± 3.7[b]

[a]IL = interleukin; MCP-1 = monocyte chemoattractant protein-1. [b]P < 0.001 versus pre-exercise.

The metabolomics analysis revealed 380 detectable compounds of known identity with paired t tests indicating post-exercise increases and decreases for 166 and 43 metabolites, respectively (FDR < 0.05 and fold change >10%). OPLS-DA modeling supported a strong separation in post-exercise (green) and pre-exercise (blue) samples (R2Y = 0.964 and Q2Y = 0.902) (Figure 1). The predictive ability of the model was found to be better than any models generated from the 999 permutated data sets and therefore passed permutation-based validation. Table 3 lists 45 metabolites with VIP scores of 1.75 and higher and fold changes of 2.0 and higher (FDR < 0.01). All but 3 of these 45 metabolites were in the lipid super pathway, with a predominance of post-exercise increases in plasma long- and medium-chain fatty acids, fatty acid oxidation products (dicarboxylate and monohydroxy fatty acids, acylcarnitines), and ketone bodies. Supplemental Table S1 contains the full list of metabolites detected, with ranking by VIP score.

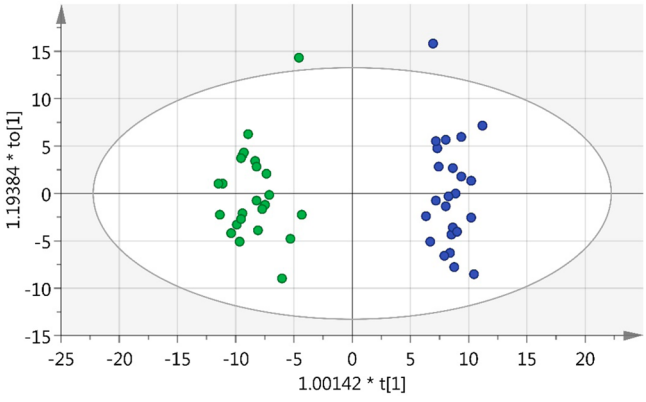


Figure 1. Post-exercise (green) and pre-exercise (blue) samples were well-separated in this OPLS-DA model (R2Y = 0.964 and Q2Y = 0.902 indicate very good model fitting and predictability). This model passed permutation-based validation, and the predictability of this model was found to be better than models built using any of the 999 permutated data sets. The metabolites that contributed the most to the separation are shown in Table 3.

Pearson correlation coefficients for relationships between pre-to-post exercise increases in plasma IL-6, cortisol, and epinephrine and changes in plasma metabolites from the lipid super pathway and other outcome measures (Table 2) are summarized in Table 4. The post-exercise increase in plasma IL-6 was most closely linked to exercise time to exhaustion, increases in IL-8 and MCP-1, the androgen hormone metabolite pregnen-diol disulfate, carnitine, and sebacate. The post-exercise increase in cortisol was related to exercise time to exhaustion and increases in 11 lipid-related metabolites including seven metabolites involved with lipid mobilization and oxidation. The increase in epinephrine was related to increases in three metabolites from the lipid super pathway, including two involved with bile acid metabolism.

OPLSR analysis was used to evaluate the relationship between exercise-induced changes in plasma IL-6 and all lipid-related metabolites listed in Supplemental Table S1 (minus any metabolite not from the lipid super pathway). No viable model was produced from this analysis (R2Y = 0.76, Q2Y = −0.0748). OPLSR analysis did reveal a relationship between changes in serum cortisol and all lipid-related metabolites (R2Y = 1, Q2Y = 0.434). Permutation-based validation found the predictive ability of this model was better than 94.1% of models generated from the permutated data sets (P = 0.059). (See Figure S1 for the scatter loading plot.) Similar OPLSR analyses for changes in plasma epinephrine and muscle glycogen with lipid-related metabolites failed to produce viable models.

Multiple structure equation models were evaluated to depict potential pathways that explained the relationship between exercise time to exhaustion, changes in plasma IL-6, and lipid metabolism. Of the various models evaluated, Figure 2 had the strongest fit when tested with five different model fit indices [P value (Chi-Square) = 0.8360 (P ranges between 0 and 1, with P > 0.05 indicating a good fit);[19] GFI = 0.9515 (GFI ranges between 0 and 1, with value >0.95 indicating a good fit);[20] Bentler-Bonett NFI = 0.9433 (NFI ranges between 0 and 1, with value >0.90 indicating a good fit);[21] RMSEA = 0 (RMSEA < 0.06 indicates a good fit);[22] and Bentler CFI = 1 (CFI ranges between 0 and 1, with value >0.95 indicating a good fit).[21] This model suggests that increased running time to exhaustion is linked to higher levels of plasma IL-6 that are then linked through carnitine to 13-methylmyristic acid (a marker of lipid metabolism) and sebacate (a dicarboxylic fatty acid).

DISCUSSION

This metabolomics-based analysis showed that the substantial increase in lipid metabolites in 24 male athletes after prolonged and intensive running was related more to changes in serum cortisol than increases in plasma IL-6, epinephrine, or muscle glycogen depletion. This finding was strengthened through use of bioinformatics procedures involving OPLSR analyses. A structure equation model supported one potential pathway for the relationship between run time to exhaustion and increases in plasma IL-6, carnitine, and two lipid-related metabolites (13-methylmyristic acid and sebacate).

As in previous global metabolomics-based laboratory studies using a prolonged exercise stress design, plasma IL-6 increased ~39-fold, and post-exercise metabolite shifts were predominately from the lipid super pathway.[2−4] Endurance running

Table 3. Rank Order of Metabolites (N = 45) with VIP Scores >1.75 and Fold Changes >2.0 in N = 24 Runners Following a Treadmill Run to Exhaustion at 70% VO_{2max}[a]

biochemical	super pathway	sub pathway	VIP score	fold change
glycerol	lipid	glycerolipid metabolism	2.15	7.59
myristate (14:0)	lipid	long-chain fatty acid	2.13	10.3
oleate (18:1n9)	lipid	long-chain fatty acid	2.12	7.61
margarate (17:0)	lipid	long-chain fatty acid	2.10	5.47
16-hydroxypalmitate	lipid	fatty acid, monohydroxy	2.09	4.83
10-heptadecenoate (17:1n7)	lipid	long-chain fatty acid	2.09	11.6
myristoleate (14:1n5)	lipid	long-chain fatty acid	2.09	13.3
palmitoleate (16:1n7)	lipid	long-chain fatty acid	2.08	15.3
palmitate (16:0)	lipid	long-chain fatty acid	2.08	3.62
dihomolinoleate (20:2n6)	lipid	long-chain fatty acid	2.08	6.78
eicosenoate (20:1n9 or 1n11)	lipid	long-chain fatty acid	2.07	8.23
laurate (12:0)	lipid	medium-chain fatty acid	2.07	8.47
10-nonadecenoate (19:1n9)	lipid	long-chain fatty acid	2.07	8.58
linoleate (18:2n6)	lipid	essential fatty acid	2.06	4.30
linolenate (18:3n3 or 3n6)	lipid	essential fatty acid	2.06	7.26
hexadecanedioate (C16)	lipid	fatty acid, dicarboxylate	2.06	10.2
stearate (18:0)	lipid	long-chain fatty acid	2.06	2.71
docosadienoate (22:2n6)	lipid	long-chain fatty acid	2.05	5.74
nonadecanoate (19:0)	lipid	long-chain fatty acid	2.02	3.43
5-dodecenoate (12:1n7)	lipid	medium-chain fatty acid	2.00	10.2
stearidonate (18:4n3)	lipid	long-chain fatty acid	1.99	11.8
arachidate (20:0)	lipid	long-chain fatty acid	1.98	3.01
pentadecanoate (15:0)	lipid	long-chain fatty acid	1.98	4.46
3-hydroxybutyrate (BHBA)	lipid	ketone bodies	1.97	7.24
caprate (10:0)	lipid	medium-chain fatty acid	1.97	3.51
decanoylcarnitine (C10)	lipid	carnitine metabolism	1.96	7.12
methionylalanine	peptide	dipeptide	1.95	4.70
tetradecanedioate (C14)	lipid	fatty acid, dicarboxylate	1.93	5.45
succinate	energy	krebs cycle	1.92	2.90
octanoylcarnitine (C8)	lipid	carnitine metabolism	1.92	6.78
acetoacetate	lipid	ketone bodies	1.92	5.13
3-hydroxydecanoate	lipid	fatty acid, monohydroxy	1.92	5.07
docosapentaenoate (DPA; 22:5n3)	lipid	essential fatty acid	1.89	3.63
13-HODE + 9-HODE	lipid	fatty acid, monohydroxy	1.89	4.33
17-methylstearate	lipid	fatty acid, branched	1.88	3.69
laurylcarnitine (C12)	lipid	carnitine metabolism	1.88	15.0
cis-4-decenoyl carnitine	lipid	carnitine metabolism	1.85	6.45
adrenate (22:4n6)	lipid	long-chain fatty acid	1.85	2.25
octadecanedioate (C18)	lipid	fatty acid, dicarboxylate	1.82	3.24
erucate (22:1n9)	lipid	long-chain fatty acid	1.77	3.86
10-undecenoate (11:1n1)	lipid	medium-chain fatty acid	1.77	3.53
methylpalmitate (15 or 2)	lipid	fatty acid, branched	1.76	4.34
3-hydroxyoctanoate	lipid	fatty acid, monohydroxy	1.75	3.41
malate	energy	Krebs cycle	1.75	2.34
dodecanedioate (C12)	lipid	fatty acid, dicarboxylate	1.75	3.83

[a]VIP scores were generated by OPLS-DA between pre- and post-exercise samples (Figure 1). Pre-to-post exercise fold changes were calculated using median scaled intensity values. These metabolites were also found to have FDR < 0.01 by paired t-test analysis between pre- and post-exercise samples.

was associated with substantial increases in long- and medium-chain fatty acids, fatty acid oxidation products, acylcarnitines, and ketone bodies. During prolonged and intensive exercise, IL-6 is produced by multiple cell types both within and outside muscle and adipose tissue, serving as a hormone-like signaling agent between metabolically active tissues. Cell culture, rodent-based, and recombinant IL-6 infusion studies indicate that one function for this pleiotropic cytokine is to increase lipolysis and FFA oxidation.[8−15,23] However, direct application of these data to what occurs in vivo during prolonged exercise bouts is problematic for several reasons. The IL-6 concentration levels used in vitro and attained during recombinant IL-6 infusion studies are substantially higher than what is typically measured in laboratory-based human exercise trials.[11,13−15] Increases in cortisol and other lipolytic hormones often accompany elevated IL-6 following rIL-6 infusion in resting adults,[11,15] but plasma glycerol and fatty acid levels are well below what is attained post-exercise in endurance athletes. Sympathetic nervous system (SNS) stimulation is a major inducer of adipose tissue lipolysis during exercise and may account for these disparities.[24]

Table 4. Pearson Correlation Coefficients for Pre-to-Post-Exercise Increases in IL-6, Cortisol, and Epinephrine with Changes in Plasma Metabolites from the Lipid Super Pathway and All Outcomes Listed in Table 2 and Exercise Time to Exhaustion[a]

variable	exercise fold change	lipid metabolite sub pathway	*R*	*P* value
IL-6 Correlations				
IL-8	2.40		0.756	<0.001
exercise time to exhaustion			0.739	<0.001
MCP-1	1.41		0.635	0.001
pregnen-diol disulfate	1.14	sterol/steroid	0.533	0.007
carnitine	1.14	carnitine metabolism	0.493	0.014
sebacate (decanedioate)	3.33	fatty acid, dicarboxylate	0.424	0.039
Cortisol Correlations				
pregnenolone sulfate	1.68	sterol/steroid	0.648	0.001
pregn steroid monosulfate	1.33	sterol/steroid	0.622	0.001
taurocholenate sulfate	1.83	bile acid metabolism	0.563	0.004
linolenate (18:3n3 or 3n6)	7.26	essential fatty acid	0.527	0.008
3-hydroxydecanoate	5.07	fatty acid, monohydroxy	0.454	0.026
myristoleate (14:1n5)	13.3	long-chain fatty acid	0.450	0.028
glycocholenate sulfate	1.38	bile acid metabolism	0.450	0.028
13-methylmyristic acid	1.78	fatty acid, branched	0.442	0.030
eicosapentaenoate (EPA; 20:5n3)	2.38	essential fatty acid	0.426	0.038
1-stearoylglycerol (18:0)	1.64	monoacylglycerol	0.416	0.043
exercise time to exhaustion			0.414	0.044
10-undecenoate (11:1n1)	3.53	medium-chain fatty acid	0.406	0.049
Epinephrine Correlations				
glycodeoxycholate (−)	0.30	bile acid metabolism	−0.558	0.005
glycocholenate sulfate	1.38	bile acid metabolism	0.525	0.008
pregnenolone sulfate	1.68	sterol/steroid	0.481	0.017

[a]Only significant correlations are shown.

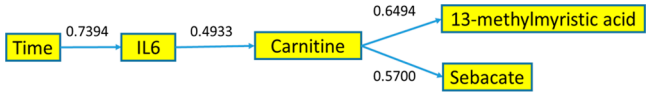


Figure 2. Best-fit pathway model for variables listed in Table 4. Numbers represent the standardized effect (beta weight) of the factor on the left to the one on the right. Time refers to time to exhaustion in 24 male runners who ran on treadmills at ~70% VO_{2max}. This analysis was performed using the structural equation model.

In our exercise-based study, plasma IL-6 increased to only 16 pg/mL but was accompanied by a 7.59-fold increase in plasma glycerol and 2- to 15-fold increases in a wide variety of lipid-related metabolites. In contrast, van Hall et al.[15] infused rIL-6 (or saline) for 3 h into healthy males at two doses resulting in plasma IL-6 levels of 140 and 320 pg/mL with arterial cortisol concentrations three times higher than saline controls and glycerol levels that never rose higher than 50% above controls. In another study using a more modest dose, eight healthy males were infused with rIL-6 for 4 h, with plasma IL-6 levels rising to 40 pg/mL.[23] Fatty acid oxidation increased modestly in muscle but not adipose tissue, and blood levels of palmitate increased <50%, far below the 3.62-fold increase measured in the current study.

The use of a systems biology approach such as metabolomics combined with cytokine, stress hormone, and muscle glycogen measurements provides a more global, in vivo, comprehensive understanding of the linkage between IL-6, lipolysis, and fat oxidation within an exercise context. OPLSR analysis was unable to produce a viable model when evaluating the relationship between exercise-induced changes in plasma IL-6 and all lipid-related metabolites listed in Supplement Table S1. The modest post-exercise increase in plasma IL-6 was most closely linked to exercise time to exhaustion and increases in IL-8 and MCP-1. We have previously reported that variance in plasma IL-6 increases in male cyclists in response to 2 h of endurance exercise was best explained by the level of self-selected exercise intensity.[25] The increase in IL-6 post-exercise in the current study was correlated to carnitine and sebacate, and our pathway model suggests that IL-6 may be linked to sebacate (a 10-carbon aliphatic dicarboxylic acid) and 13-methylmyristic (i.e., 13-methyltetradecanoic acid or 13-MTD) acid through carnitine. The branched fatty acid 13-MTD seems likely to be derived from the consumption of animal fats before becoming incorporated into complex lipids (including triglycerides). During lipolysis, 13-MTD can be used as a marker of mobility of fatty acyl chains in adipose tissue.[26] Sebacate is a probable product of omega-oxidation of fatty acids, and this water-soluble lipid substrate does not require hydrolysis and is immediately available for cellular utilization.[27] Limited data suggest that IL-6 may enhance long-chain fatty acid update into muscle.[28] In cultured primary human trophoblast cells, IL-6 but not TNF-alpha, promotes fatty acid accumulation.[29]

Taken together, the metabolomics-based data from the current study with 24 male runners do not support a strong relationship between the modest increase in IL-6 and the strong increase in numerous lipid-related metabolites following prolonged and intensive running. This finding is consistent with the finding that IL-6 had no effect on adipose tissue lipolysis in IL-6-deficient mice that exercised for 90 min.[30] In that study, IL-6 was involved in regulating the induction of glyceroneogenic enzymes that re-esterify fatty acids back into triglycerides in fat cells. The viewpoint that muscle-derived IL-6 stimulates adipose tissue lipolysis during exercise has not been consistently supported, as underscored by the data from the current study and others.[8,23,30]

OPLSR analysis did reveal a relationship between changes in serum cortisol and all lipid-related metabolites, confirming the well-established role of cortisol as a lipolytic hormone.[31] No viable models were produced with OPLSR analysis, however, for increases in epinephrine or muscle glycogen depletion and increases in lipid-related metabolites. Epinephrine is a regulator of intramuscular lipolysis, especially in oxidative muscle.[13] In one study, epinephrine infusion in healthy men increased plasma epinephrine levels to 221 pg/mL (39% above levels in the current study) and was associated with a ~70% increase in plasma glycerol levels (an index of lipolysis), far below the 7.59-fold increase measured in the current study.[32] rIL-6 infusion for 3 h in 12 healthy males increased plasma IL-6 to 152 pg/mL and was associated with a strong increase in plasma cortisol but no change in plasma epinephrine.[33] Epinephrine does have a role in glucoregulation, especially during intense exercise.[34] In the current study, change in plasma epinephrine was most strongly correlated with change in plasma pyruvate (R = 0.633, P = 0.001), with a modest relationship to increase in lactate (R = 0.487, P = 0.016) (data not shown). Norepinephrine was not measured in the current study, but adipose tissue displays a high sensitivity to this hormone, leading to high lipolysis rates.[35]

CONCLUSIONS

This is the first study to test the relationship between exercise-induced increases in plasma IL-6 and a high number of lipid-related metabolites using metabolomics-based procedures. These data indicate that the increase in plasma IL-6 after long endurance running has at best a minor relationship to increases in lipid-related metabolites. This study did not include the acquisition of a series of exercise-recovery blood samples, but this approach may not produce useful data due to the substantial decrease in plasma IL-6 and lipid metabolite concentrations during the first 1.5 h following vigorous exercise.[4] The measurement of other metabolic hormones such as glucagon and insulin may have added additional insights regarding the IL-6 and lipolysis relationship. The pathway model suggests some involvement of IL-6 with two lipid-related metabolites through carnitine, an amino acid derivative that transports long-chain acyl groups into the mitochondrial matrix for β-oxidation to acetyl-CoA. No prior publications have shown this linkage between post-exercise increases in plasma IL-6 and carnitine, and additional research is needed to investigate this relationship. Although many potential combinations for the pathway model were tested using the outcome measures summarized in Tables 1−3, we cannot rule out that IL-6 may operate by activating other intermediate factors.

ASSOCIATED CONTENT

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jproteome.6b00892.

Supplement Figure S1: Scatter loading plot for the relationship between exercise-induced changes in serum cortisol and lipid-related metabolites. (PDF)

Supplement Table S1: Full VIP listing of metabolites (pre-to-post change) related to exercise time to exhaustion in 24 male runners. (XLSX)

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We gratefully acknowledge Mary Pat Meaney and Zack Shue for their technical assistance in this research project. We acknowledge Reoxcyn Discoveries Group, Salt Lake City, Utah.

ABBREVIATIONS

9-HODE + 13-HODE, 9- and 13-hydroxyoctadecadienoic acid; ANOVA, analysis of variance; BHBA, 3-hydroxybutyrate; CBC, complete blood count; CFI, comparative fit index; EC, electrochemical; ECLIA, electrochemiluminescence immunoassay; EDTA, ethylenediaminetetraacetic acid; ESI, electrospray ionization; FDR, false discovery rate; GFI, goodness of fit index; HPLC, high-pressure liquid chromatography; LC−MS, liquid chromatography/mass spectrometry; MCP-1, monocyte chemoattractant protein-1; NFI, normed-fit index; OPLS-DA, orthogonal partial least-squares discriminant analysis; OPLSR, orthogonal partial least-squares regression; RER, respiratory exchange ratio; RMSEA, root-mean-square error of approximation; RPE, rating of perceived exertion; SE, standard error of the mean; UHPLC/MS/MS, ultrahigh performance liquid chromatography/tandem mass spectrometry; VIP, variable influence on projection; VO_{2max}, maximal volume of oxygen consumption

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