Serum metabolomics signatures after an acute bout of combined traditional or high-intensity tactical training in young males and females

Zachary A. Graham[1,2,3,4*], Khyatiben V. Pathak[5,6*], Krystine Garcia-Mansfield[5,6], Kaleen M. Lavin[1,2,3], Anakaren R. Torres[5,6], Jeremy S. McAdam[1,2,3], Timothy Broderick[1], Patrick Pirrotte[5,6#], Marcas M. Bamman[1,2,3#]

[1]Healthspan, Resilience and Performance, Florida Institute for Human and Machine Cognition, Pensacola, FL

[2]Center for Exercise Medicine, The University of Alabama at Birmingham, Birmingham, AL

[3]Department of Cell, Developmental and Integrative Biology, The University of Alabama at Birmingham, Birmingham, AL

[4]Birmingham VA Health Care System, Birmingham, AL

[5]Cancer and Cell Biology Division, Translational Genomics Institute, Phoenix, AZ

[6]Integrated Mass Spectrometry Shared Resource, City of Hope Comprehensive Cancer Center, Duarte, CA

[7]Neurogenomics Division, Translational Genomics Institute, Phoenix, AZ

*Authors contributed equally

[#]Co-Senior Authors

Corresponding Author

Zachary A. Graham

Florida Institute for Human and Machine Cognition

41 S Alcaniz Street

Pensacola, FL

zgraham@ihmc.edu

Abstract (250 words)
Exercise is a multipotent stimulus that results in large-scale dynamic changes to the systemic molecular profile. Alternative exercise prescriptions and doses would be expected to result in distinct signatures due to differences in duration and intensity. We tested two novel combined endurance and resistance exercise regimens to better understand how differing prescriptions alter the acute metabolomics response at multiple timepoints up to 24h post-exercise. Serum metabolomics for n=37 untrained individuals was analyzed for participants completing traditional combined exercise [TRAD; n = 20 (11M/9F)] or high-intensity tactical training [HITT; n= 17 (9M/8F)] before exercise (pre), and immediately (h0), 3 and 24 h post-exercise (h3 and h24, respectively). We found minimal metabolites had a group by time interaction (2 with FDR < 0.10; 31 with nominal p < 0.05;), but both stimuli resulted in large-scale within-group changes to the circulating metabolome. TRAD consistently had greater numbers of differentially abundant metabolites (FDR < 0.10) as compared to HITT at h0 (431 vs. 333), h3 (435 vs. 331) and h24 (168 vs. 76). The major metabolite classes altered were related to key energy substrates for both groups at h0 (e.g., glucose, pyruvate) and energy replenishment for h3 and h24 (e.g., 12,13 diHOME, palmitylcarnitine, free fatty acids). In summary, our data are the first to describe the acute changes in the circulating metabolome following combined endurance and resistance exercise. Additionally, we show the two distinct doses of combined exercise led to generally similar patterns of responses, with the longer duration TRAD dose resulting in a higher magnitude of change.

Introduction

Exercise improves whole body health through a coordinated stress response that activates almost all tissue systems and cell types. Long term adaptation to consistent exercise training prevents or slows the onset of many disease states and greatly improves health and functional outcomes in those with existing pathologies (1–3). The diverse cues initiated by exercise (4) are required to meet the demands of the activity (e.g., timely delivery of energy substrates, removal of waste products) while additionally providing the molecular framework for long-term changes in cellular architecture (e.g., mitochondrial biogenesis, muscle hypertrophy) to improve overall functional capacity.

Acute exercise creates a unique energy demand that is not encountered during any other volitional activity. Relative maximal oxygen consumption can increase by ~25-fold during maximal exercise compared to resting levels in elite individuals (4, 5), largely driven by the metabolic demands of working cardiac tissue and skeletal muscle. Sustaining such an elevated level of work, even at submaximal levels, requires a well-coordinated inter-and intra-tissue molecular response. Increased accessibility and reductions in costs for next generation molecular profiling has greatly improved how we understand biological change at the molecular level (6). Metabolomics in particular is experiencing rapid advancements in sensitivity and analyte coverage. This enhancement in detection capacity allows better resolution to monitor crucial substrates, co-factors, signaling molecules, metabolic pathway intermediaries, and enzymatic reaction endpoints (8) as well as to discover novel and unique metabolite-specific signaling mechanisms. This was recently demonstrated with the proper identification and mechanistic characterization of Lac-Phe (7). Thus, metabolomics is becoming a key platform to understand the molecular interplay required to maintain exercise performance, with unique metabolomic signatures within the circulation depending on mode of exercise, training status, duration of the session, and timeframe after the end of the exercise bout (8, 9).

Continuous, moderate intensity exercise training has been widely established to improve fitness and health. Recently, high-intensity interval exercise programing (e.g., CrossFit, Tabata) has been adopted as a popular alternative to traditional steady-state exercise and has been widely adopted for military personnel (10). Metabolomics profiling has been performed across a range of acute and chronic exercise studies (11), though the vast majority of acute exercise studies have been completed on blood or urine following unimodal aerobic exercise (8). US public health guidelines include both aerobic and resistance training as integral to a healthy lifestyle(12). To our knowledge, there has not been a timecourse study investigating the acute metabolomic response to a combined aerobic and resistance exercise bout. Accordingly, no study has compared how the metabolome responds to different combined exercise prescriptions. This study aimed to address this knowledge gap by investigating the effect of an acute bout of traditional, long-duration combined moderate intensity exercise (TRAD) or a bout of combined exercise high-intensity tactical training (HITT) on serum metabolites over time.

Methods

Participants

Participants were recruited from the Birmingham, AL metro area as part of a larger parent trial (Department of Defense Office of Naval Research: N000141613159, ClinicalTrials.gov Identifier: NCT03380923). This cohort of acute bout-only participants (n=40 total; 23 males, 17 females) has also been reported in detail (13). In brief, a subset of young, sedentary females and males completed a single acute bout of TRAD (n=20; 11M/9F) or HITT (n=17; 9M/8F). All individuals provided written, informed consent to participate, and the protocol was approved by the University of Alabama-Birmingham Institutional Review Board (Protocol F160512012).

Study enrollment, exercise bout and blood collection

Full screening, randomization, familiarization and exercise bouts have been previously described (13). In brief, after enrollment into the study, all exercise bouts were carried out in the early morning with participants arriving having fasted overnight. Participants rested supine for 30 min with blood collected via venipuncture (pre); exercise started approximately 15 min post-blood draw. Participants randomized to HITT performed 10 rounds of a high-intensity circuit exercise program at maximal effort for 30 s with 30 s of rest. The exercises consisted of box jumps, burpees, split squat jumps, kettlebell swings, 2 cycling sprints, a rowing sprint, battle ropes, wall balls, and dips. Participants randomized to the TRAD group performed 30 min of treadmill running at 70% of heart rate reserve (HRR) as calculated from their VO_{2peak} test. Both HITT and TRAD groups then performed whole-body resistance exercise training with the following exercises: squat, knee extensions, heel raise, chest

press, overhead press, seated row, lateral pulldown, triceps pushdowns, biceps curl and abdominal crunches. The TRAD group completed 3 sets x 13 repetitions at an intensity of a 13-repetition max with ~60 s rest. The HITT group completed the same exercises in superset form at 3 sets of 9 repetitions at an intensity of a 9 repetition max with 30 s rest intervals between supersets. The total time for the acute bout was ~90 min for TRAD and ~45 min for HITT. Following the exercise bout, an immediate post-exercise blood draw was collected (h0). Participants were then provided the uniform meal of a protein shake (Ensure Plus: 350 kcal; 11 g fat, 16 g protein, 47 g carbohydrate), with another blood draw at 3 h post-exercise (h3) and a fasting blood draw 24 h post-exercise (h24). Blood serum was used for all components of this study. Whole blood was drawn into serum-separating tubes, allowed to clot at room temperature for 30 min, then centrifuged at 2000 x g for 10 min at 4ºC. Serum was placed in 500 µL aliquots and frozen at -80ºC until analysis. Sample sizes are slightly different between groups and along timepoints compared to our previous report (13) using these individuals because of serum availability. The resulting sample sizes across the groups and timepoints for TRAD were: n=20 at 'pre', n=17 at 'h0', n=16 at 'h3' and n=18 at 'h24'. For HITT, n=16 at 'pre', n=14 at 'h0', n=14 at 'h3' and n=16 at 'h24'.

Serum processing and mass spectrometry

Sera were sent overnight on dry ice to the Translational Genomics Research Institute for sample processing and data generation. Serum metabolites were extracted using a 1:3 plasma to organic solvent (3:1 v/v methanol:acetonitrile) ratio where organic solvent was spiked with four isotopically labelled internal standards (d8-valine, ^{13}C6-phenylalanine, ^{13}C6-adipic acid, d4-succinic acid). The samples were vortexed for 30 s and incubated at -20 °C for 10 min for protein precipitation. The metabolites in the supernatant were recovered by centrifuging samples at 15,000 rpm for 10 min at 4 °C and subjected to untargeted metabolomics profiling as described previously (14). 10 µL from each serum specimen were combined to create a pooled quality control (QC) sample and subjected to the same extraction protocol. The pooled QC sample was injected prior to sample batches for system conditioning, and every five samples to monitor instrument performance, extraction efficiency, correct retention time drift and perform batch normalization (15, 16). Raw data were subjected to Compound Discoverer 3.2 for metabolite annotation and relative quantification (15, 17).

Data processing and statistical analysis

Data were processed and analyzed using R (v3.6)(18), with the following packages used to generate figures: UpSet (19), pheatmap (20), mixOmics (21). Metabolite abundances were compared using the R *limma* pipeline with the 'pre' timepoint as the reference (22, 23) to allow for linear mixed-model approaches across time, similar to our previous report on these participants at the transcriptomic level (13). The statistical threshold for a differentially abundant metabolite was a Benjamini-Hochberg false discovery rate (FDR) of < 0.10. One participant was removed from data analyses based on our outlier detection (6 > median absolute deviations; TRAD Male) and one participant was removed from data analyses for not having a 'pre' timepoint (HITT Female), which is a requirement for the *limma* pipeline. The data matrix and metadata, along with complete statistical outcomes for each comparison, can be found in Supplemental File 1.

Results

Participant Characteristics

General descriptions of these participants were provided in prior work (13). Overall characteristics of HITT vs. TRAD were not different, such as age, height, weight, body composition, maximal aerobic (VO_{2peak}) and anaerobic (Wingate) power, and strength.

Metabolites

We detected 10,793 compounds across both reverse phase and hydrophilic interaction liquid chromatographies in both positive and negative modes (Fig. 1A). 5,215 were annotated, including 3,243 non-redundant compounds. 3,126 total compounds met thresholds for statistical differences in at least one comparison (FDR < 0.10), including 784 annotated endogenous metabolites belonging to major metabolite classes (Fig. 1B). PLS-DA plots show unique clustering across both TRAD and HITT groups at the h0 timepoint (Fig. 1C), while h3 also clusters from other time points, though not to the extent of h0. The h24 and 'pre-' clusters did not form distinct clusters.

To maximize our biological understanding, we performed the rest of the analyses on annotated metabolites only.

Between groups comparisons across time

Comparisons of TRAD vs. HITT at each time point resulted in 30 metabolites with nominal p values < 0.010 ('h0v pre': 9; 'h3vpre': 18; 'h24vpre': 4) and can be visualized via heatmap and UpSet plot (Fig. 2B). However, only 2 had an FDR < 0.100: the amino acid valine (FDR = 0.021) and the amino acid capryloylglycine (FDR = 0.022), which were both lower in the TRAD group compared to HITT at the 'h0' timepoint (Fig. 2C red squares). Fig. 2C also shows the boxplots for each of the metabolites for each group over time.

Response of combined exercise using within-group comparisons

The between group comparisons do not allow full understanding of the magnitude of acute exercise-induced responses, so we investigated this using within-group comparisons across time relative to 'pre' levels. Exercise led to large scale changes in circulating annotated metabolites across time in both groups, with TRAD showing a consistently greater number of differentially abundant metabolites. Almost all 784 detected annotated metabolites showed a significant change in abundance at one of the measured postexercise timepoints: 667 and 552 metabolites were differentially abundant (FDR < 0.10) post-exercise in TRAD and HITT, respectively (Fig. 3A). There were 431 metabolites (↑207, ↓224) differentially abundant in TRAD at 'h0 vs. pre' compared to 333 (↑174, ↓159) for HITT. At h3, 435 metabolites (↑328, ↓107) and 331 metabolites (↑273, ↓58) were altered in TRAD and HITT, respectively. The 'h24 vs. pre' comparison showed a smaller number of metabolites changed by exercise with 168 (↑77, ↓91) altered following TRAD and 76 (↑37, ↓39) changed following HITT.

The differentially abundant metabolite signatures across our comparisons are shown in an UpSet plot along with donut plots representing the proportion of membership to their biological class (Fig. 3B). Our focus was principally on the 6 intersections with > 25 metabolites. The 'h3 up' profile had the most shared metabolites, with 98 metabolites common to both TRAD and HITT. These were mostly related to lipid and fatty acid metabolism. The next most abundant was the 'h0 down', in which 57 metabolites were shared between exercise groups. There were three principal functional groups within this shared grouping: amino acids and biogenic amines, lipids, and peptides. The 'h0 up and h3 up' had 43 metabolites shared across both TRAD and HITT groups and was mostly composed of biogenic amines, acyl carnitines, fatty acids and derivatives. The next group of 40 metabolites was only decreased in TRAD at 'h0'. The majority of these metabolites were phospholipids, fatty acids and derivatives, and amino acids. TRAD and HITT shared 39 upregulated metabolites at 'h0' only, which were mostly amino acids and biogenic amines. Lastly, there was another unique set of 27 metabolites increased only in TRAD at 'h3' that were mostly annotated to phospholipids.

Differential abundance patterns across time

We used unbiased clustering to characterize the timecourse for differentially abundant metabolites compared to baseline levels setting cluster a membership threshold of ≥ 10 metabolites) for the annotated metabolites (Fig. 4A-B). Most patterns were shared between groups and are summarized in Table 1. These patterns show a wide range of responses, from immediate increase (TRAD Cluster 1, HITT Cluster 3;) or decrease (TRAD Cluster 5, HITT Cluster 7) with a return to baseline a h24, immediate and sustained decrease (TRAD Cluster 6, HITT Cluster 6), or a unique signature such as HITT Cluster 8 which had stable resting and h0 abundances, but gradually increasing levels at h3 and h24.

Discussion

Our data strongly support the established literature that exercise creates a substantial change in the circulating metabolome with conserved patterns of molecular abundance immediately to 24 h post-exercise. This ebb and flow in abundance of compounds is required for sustaining performance during an exercise session and we were able to detect and describe wide variety of metabolite classes and their cellular functions. Importantly, we also provide novel metabolomic findings related to a long duration traditional combined exercise session as well as a shorter high intensity alternative.

Moderate and continuous aerobic exercise has been the predominant focus of metabolomics-based acute exercise research (8). While others have demonstrated efficacy of subacute and chronic combined exercise training to change the resting metabolome as reviewed in (11), this is the first study to our knowledge that has investigated how an acute bout of combined exercise alters the circulating metabolome and, further, how different combinatorial exercise prescriptions differentially impact this metabolomic response. We identified 2 metabolites that met FDR criteria as being differentially abundant between TRAD and HITT: the branched-

chain amino acid (BCAA) valine and the amino acid metabolite capryloylglycine. These were both downregulated in the TRAD group at 'h0'. Valine is an important BCAA that can be used as an upstream substrate to generate the TCA intermediary succinyl-CoA (24). Serum valine levels are reduced following 80 min of sustained treadmill walking in males exposed to lab-based high-altitude equivalency (25) and following 80 min of sustained moderate cycling (~65% VO_2max) in males with adequate carbohydrate intake; though those with calorically-matched, low-carbohydrate intake saw elevated abundances of valine and other BCAAs (26). Our TRAD prescription was roughly 90 min and more sustained than HITT, and our data mostly supports that long-duration exercise reduces circulating valine. Capryloylglycine is a medium-chain fatty acid conjugate often used in cosmetic products. However, it has been described as a metabolite in mammalian system. In pigs, capryloylglycine is a principal metabolite produced by the pancreas (27) and in humans it has been observed to be elevated in skeletal muscle biopsies of older adults compared to younger adults (28). Genetically-induced mitochondrial β-oxidation deficiencies lead to elevated capryloylglycine levels that are excreted in urine (29); thus, it is suggestive of a potential link for capryloylglycine to be altered with sustained elevated mitochondrial activity during long duration exercise bouts. Why capryloylglycine was reduced immediately after TRAD is unknown as glycine levels were similar across both groups, suggesting equal availability. It is possible levels of caprylic acid were lower but it was not among our annotated metabolites. Another possibility is TRAD reduces activity of glycine N-acyltransferase, the enzyme that conjugates glycine to fatty acids. Future investigations are needed to resolve whether this change is biologically impactful and if so, to probe potential mechanisms.

TRAD had consistently more metabolites with differential abundance at each timepoint compared to HITT in our within-group analyses. This is in line with our previous transcriptomics work from this same cohort that reported generally elevated numbers of differentially expressed RNA species in TRAD vs. HITT (e.g., long non-coding RNA, protein coding RNA, microRNA) in serum-derived extracellular vesicles and skeletal muscle (13). This is likely due to a combination of factors such as the sustained metabolic demand from the continuous nature of the endurance portion of the exercise bout as well as the greater length of exercise duration for the TRAD group (~90 min) compared to HITT (~45 min). Despite the 'h0' timepoint being a unique cluster as identified by PLS-DA in both TRAD and HITT as shown in our PLS-DA plot, the 'h0' and 'h3' timepoints had roughly equivalent numbers of metabolites for each group. There was a differential pattern as 'h0' had roughly identical numbers of upregulated and downregulated metabolites while 'h3' was composed largely of upregulated metabolites. The upregulated metabolites in 'h3' were mostly annotated to fatty acids, conjugated fatty amines, and ketones. These represented classes fall in-line with systemic replenishment of energy stores that is supported by both TRAD and HITT having elevated levels of the adipose tissue-released exerkine 12,13 di-HOME, which increases fatty acid uptake in skeletal muscle (30), elevated levels of palmitoylcarnitine, which is derived from palmitoyl-CoA and shuttled across the inner membrane of the mitochondria (where it is subsequently converted back into palmitoyl-CoA for β-oxidation), and a host of other long chain fatty acids and their metabolites. The 'h0' timepoint was more associated with the active substrates of ATP generation (glucose, pyruvate, citrate, aconitate, fumarate) and the purine catabolism/salvage pathways (purine, guanine, hypoxanthine, uridine monophosphate), and is supported by unimodal studies using 45-60 min of moderate to moderate-vigorous steady state cycling or traditional resistance training at similar post-exercise timepoints (9, 31). Purine metabolism and ultimate salvage is more energy-efficient than de novo formation to maintain ATP levels, but the elevation in the blood levels of these metabolites would be expected to be from working skeletal muscle. Xanthine oxidase, the enzyme that converts hypoxanthine to uric acid, is not expressed in skeletal muscle so these metabolites are likely taken up by tissues such as the liver to be salvaged or further metabolized to uric acid (32).

There were also unique differences in metabolite signatures at 'h24' vs. 'pre' with TRAD having a more sustained change in the metabolomic signature. Of the 168 differentially abundant metabolites in TRAD at h24, 111 of them (68%) were also differentially abundant in our 'h3vpre' comparison. This was not the case with HITT, as only 43% (36/76) remained differentially abundant. Despite the differences in overall numbers and the proportion common at 'h3' and 'h24' between TRAD and HITT, amino acid metabolites, in particular the BCAAs leucine and isoleucine, were similarly elevated. This is contrasted by unimodal aerobic or resistance training acute bouts were reduced BCAAs, though their metabolites were elevated in the immediate and short timecourse after exercise (0-120 min)(9, 26), which we did observe. Similar to TRAD having a more robust response in the number of differentially abundant metabolites, this continual change in metabolite levels is likely related to the longer duration and continuous mode of exercise of the TRAD exercise dose.

Our combinatorial approach complicates direct comparisons against the literature as both TRAD and HITT were novel prescriptions. However, the magnitude of response with our annotated metabolites seen was similar to both acute resistance and aerobic bouts of exercise that saw peak changes immediately, 15 min, and out to 3 h post-exercise (9). In both of our groups, the aerobic portion was completed first immediately followed by the resistance exercise. Thus, there is potential that our metabolomic profiling would more mimic a resistance training prescription, however, the relative TRAD and HITT up and downregulated proportions at h0 and h3 timepoints do not align with resistance and endurance unimodal proportions at these same timepoints (9), suggesting our combinatorial intervention results in a unique metabolomic dynamic.

Our study has a few considerations. For consideration as sex as a biological variable, our randomization strategy included stratification by sex to ensure relative balance between HITT and TRAD, but we did not covary or normalize for sex as it is a non-random variable and it is likely the level of each metabolite is not directly related to the participant's sex, as was our approach for transcriptomics analyses from these same participants (13). Another important consideration is we supplied a supplemental protein drink after the 'h0' blood draw to mimic a real-world exercise scenario and cannot rule out the possibility some of the shared metabolites observed to be changed at 'h3', or less likely, 'h24' between both groups were from the supplement. However, this concern is dampened by evidence showing our general patterns of change in classes such as lipids and amino acids match those from both aerobic and resistance training acute response studies where participants remained fasted for serial blood analyses over 3h post-exercise (9). In summary, our novel combined exercise prescriptions resulted in robust metabolomic changes across multiple timepoints post-acute exercise that share major characteristics of unimodal exercise bouts, but also suggest distinct signatures related to exercise dose.

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Figure legends
Fig. 1. Serum metabolome and statistical outcomes following an acute bout of combined training of TRAD and HITT in young male and females. A) Summary of serum metabolic profiling including total compounds, annotated and unannotated compounds, and non-redundant metabolites. B) Pie chart representing distribution of biochemical classes for 784 non-redundant significant metabolites across the multiple group comparisons (h0vspre, h3vpre, h24vspre) between TRAD vs HITT. C) Partial least squares-discriminant analysis (PLS-DA) of differentially abundant metabolites (FDR < 0.10) across time for TRAD (lower left panel) and HITT (lower right panel), respectively.

Fig. 2. Differential circulating metabolomic responses for TRAD and HITT. A) Hierarchical clustering of differentially abundant metabolites by nominal p value ($p < 0.01$). B) Upset plot showing the patterns of time and direction response and C) box and whisker plots showing log-transformed values for each metabolite per group and per timepoint. Metabolites highlighted with a red border are metabolites with FDR < 0.10.

Fig. 3. Within group comparisons reflects transient, delayed or persistent effects of an acute bout of combined exercise modalities. A) Hierarchical clustering of differentially abundant metabolites (FDR < 0.10) post-exercise (h0, h3 and h24) relative to 'pre' within TRAD (Upper left panel) and HITT (Upper right panel). B) Upset plot representing shared or unique metabolite signatures post-exercise for both TRAD and HITT alongside donut charts representing the biochemical classes of differentially abundant metabolites common and unique in TRAD and HITT.

Fig. 4. Unbiased clustering of all differential abundant metabolites in post exercise compared to 'pre' for A) TRAD and B) HITT.

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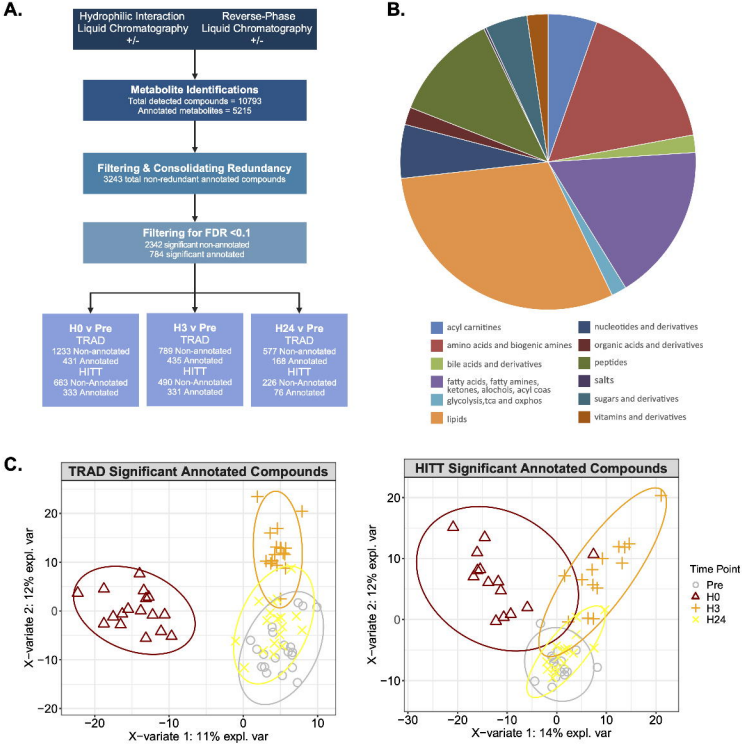
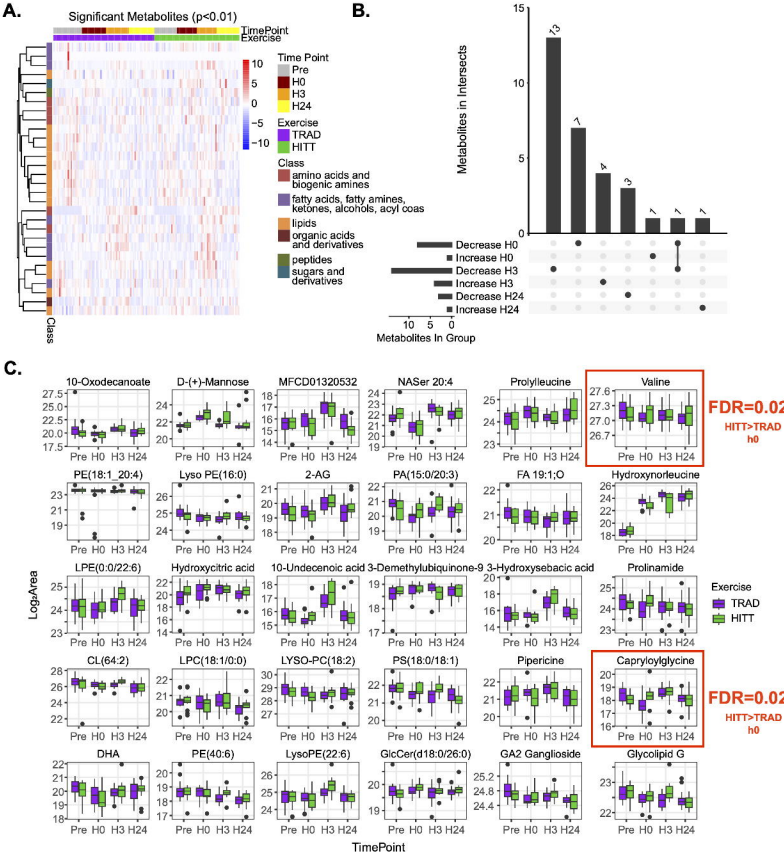
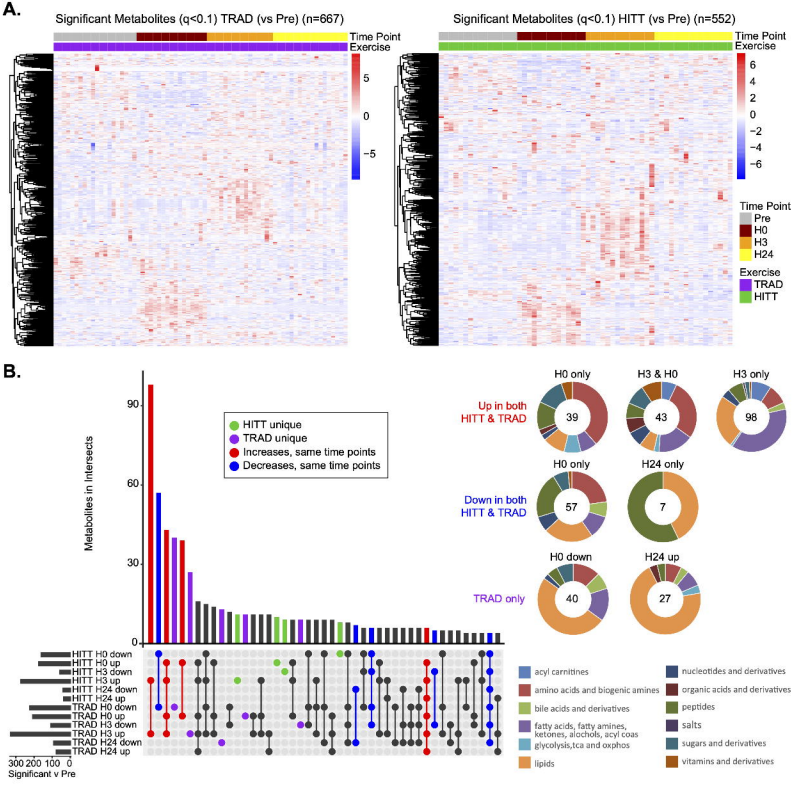
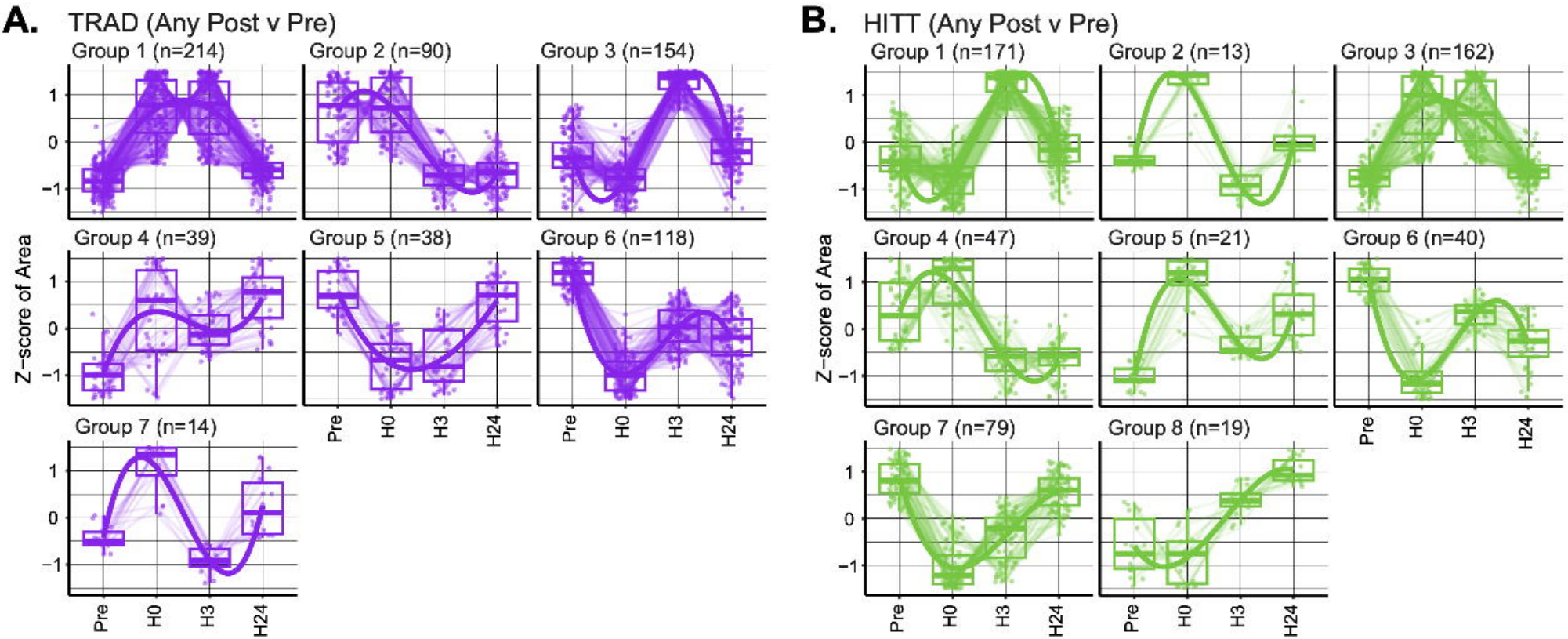
Table 1. Unbiased clustering of differentially abundant metabolites across time

Any timepoint vs. 'pre'					
Shared					
TRAD	HITT	Shared between Clusters	Shared % TRAD	Shared % HITT	Key metabolites
Cluster 1	Cluster 3	117	55%	72%	Glucose, aconitate, pyruvate
Cluster 2	Cluster 4	27	30%	57%	Cortisol, tryptophan, lactate
Cluster 3	Cluster 1	97	63%	58%	12,13 di-HOME, palmitoylcarnitine, oleic acid
Cluster 4	Cluster 5	7	18%	33%	Glutathione
Cluster 5	Cluster 7	18	47%	23%	Succinate, thymidine, asparagine
Cluster 6	Cluster 6	13	11%	33%	Phenylalanine, 1-hydroxy-L-tryptophan
Cluster 7	Cluster 2	0	0%	0%	N/A


A.
Hydrophilic Interaction Liquid Chromatography +/-
Reverse-Phase Liquid Chromatography +/-
Metabolite Identifications
Total detected compounds = 10793
Annotated metabolites = 5215
Filtering & Consolidating Redundancy
3243 total non-redundant annotated compounds
Filtering for FDR <0.1
2342 significant non-annotated
784 significant annotated
H0 v Pre
TRAD
1233 Non-annotated
431 Annotated
HITT
663 Non-Annotated
333 Annotated
H3 v Pre
TRAD
789 Non-annotated
435 Annotated
HITT
490 Non-Annotated
331 Annotated
H24 v Pre
TRAD
577 Non-annotated
168 Annotated
HITT
226 Non-Annotated
76 Annotated
B.
acyl carnitines
amino acids and biogenic amines
bile acids and derivatives
fatty acids, fatty amines, ketones, alochols, acyl coas glycolysis,tca and oxphos
lipids
nucleotides and derivatives
organic acids and derivatives
peptides
salts
sugars and derivatives
vitamins and derivatives
C.
TRAD Significant Annotated Compounds
X−variate 2: 12% expl. var
X−variate 1: 11% expl. var
HITT Significant Annotated Compounds
X−variate 2: 12% expl. var
X−variate 1: 14% expl. var
Time Point
Pre
H0
H3
H24


A.
Significant Metabolites (p<0.01)
TimePoint
Exercise
Time Point
Pre
H0
H3
H24
Exercise
TRAD
HITT
Class
amino acids and biogenic amines
fatty acids, fatty amines, ketones, alcohols, acyl coas
lipids
organic acids and derivatives
peptides
sugars and derivatives
Class
B.
Metabolites in Intersects
Decrease H0
Increase H0
Decrease H3
Increase H3
Decrease H24
Increase H24
Metabolites In Group
C.
10-Oxodecanoate
D-(+)-Mannose
MFCD01320532
NASer 20:4
Prolylleucine
Valine
FDR=0.021
HITT>TRAD
h0
PE(18:1_20:4)
Lyso PE(16:0)
2-AG
PA(15:0/20:3)
FA 19:1;O
Hydroxynorleucine
LPE(0:0/22:6)
Hydroxycitric acid
10-Undecenoic acid
3-Demethylubiquinone-9
3-Hydroxysebacic acid
Prolinamide
CL(64:2)
LPC(18:1/0:0)
LYSO-PC(18:2)
PS(18:0/18:1)
Pipericine
Caprylovlglycine
FDR=0.022
HITT>TRAD
h0
DHA
PE(40:6)
LysoPE(22:6)
GlcCer(d18:0/26:0)
GA2 Ganglioside
Glycolipid G
Log2Area
Pre H0 H3 H24
Exercise
TRAD
HITT
TimePoint


A.
Significant Metabolites (q<0.1) TRAD (vs Pre) (n=667)
Significant Metabolites (q<0.1) HITT (vs Pre) (n=552)
Time Point
Exercise
Pre
H0
H3
H24
TRAD
HITT
B.
Metabolites in Intersects
HITT unique
TRAD unique
Increases, same time points
Decreases, same time points
HITT H0 down
HITT H0 up
HITT H3 down
HITT H3 up
HITT H24 down
HITT H24 up
TRAD H0 down
TRAD H0 up
TRAD H3 down
TRAD H3 up
TRAD H24 down
TRAD H24 up
Significant v Pre
Up in both HITT & TRAD
Down in both HITT & TRAD
TRAD only
H0 only
H3 & H0
H3 only
H24 only
H24 up
acyl carnitines
amino acids and biogenic amines
bile acids and derivatives
fatty acids, fatty amines, ketones, alcohols, acyl coas
glycolysis,tca and oxphos
lipids
nucleotides and derivatives
organic acids and derivatives
peptides
salts
sugars and derivatives
vitamins and derivatives


A.
TRAD (Any Post v Pre)
Group 1 (n=214)
Group 2 (n=90)
Group 3 (n=154)
Group 4 (n=39)
Group 5 (n=38)
Group 6 (n=118)
Group 7 (n=14)
Z−score of Area
Pre
H0
H3
H24
B.
HITT (Any Post v Pre)
Group 1 (n=171)
Group 2 (n=13)
Group 3 (n=162)
Group 4 (n=47)
Group 5 (n=21)
Group 6 (n=40)
Group 7 (n=79)
Group 8 (n=19)