



# Changes in blood metabolic profile during repeated cardiopulmonary exercise testing (2xCPET) in ME/CFS patients.

Henrique Chapola<sup>1</sup>, Katarina Lien<sup>2,3</sup>, Sissel Dyrstad<sup>1</sup>, Per Ole Iversen<sup>2</sup>, Karl Johan Tronstad<sup>1</sup>

<sup>1</sup> Department of Biomedicine, University of Bergen, Bergen, Norway

<sup>2</sup> Department of Nutrition, Institute of Basic Medical Sciences, University of Oslo, 0317 Oslo, Norway.

<sup>3</sup> CFS/ME Center, Division of Medicine, Oslo University Hospital, Oslo, Norway.

## Abstract

ME/CFS is characterized by post-exertional malaise (PEM) and delayed recovery after physical activities, possibly indicating a bioenergetic imbalance. This study analyzed 70 biochemical markers in blood serum, including amino acid metabolites, during repeated cardiopulmonary cycling exercise tests over two consecutive days (2xCPET). Eighteen ME/CFS patients were compared with 15 healthy controls. Samples were taken at rest, peak exercise intensity, and 15 and 60 minutes post-exercise [1]. Multiple tests evaluated significant alterations in concentration and proportional changes. Metabolite enrichment pathway analysis assessed changes in three sets: concentration-related, change between groups, and temporal dynamic behavior. The study explored exercise-induced differences in nicotinamide, tryptophan, BCAA, one-carbon, and butanoate metabolism. Findings provide insights into ME/CFS physiological response to exercise and biochemical elements of exercise intolerance and PEM. Metabolites overlapping the three sets were further investigated for potential discriminatory value, and their correlation with blood gas parameters was assessed to understand mechanism shifts in ME patients.

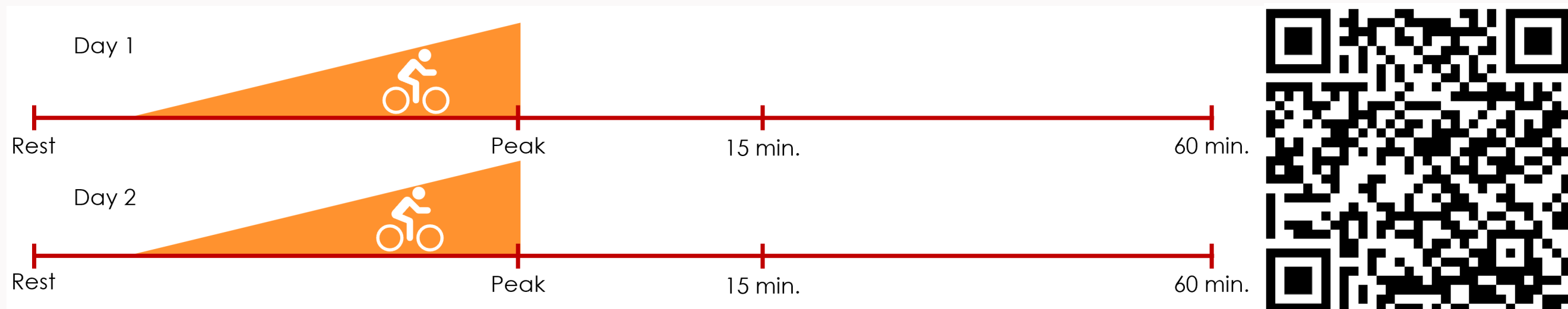


Fig 1: Experimental design and timepoints for sampling. For more information from the original study, scan the QR code.

## Results

- Concentration: difference in abundance.
- Change: differences relative to the first measurement.
- Behavior: differences in dynamic.
- 27 out of the 70 metabolites across the comparison methods had at least one significant finding (e.g.: Fig. 2).
- Those 27 metabolites were mostly related to BCAA, nicotinate, one carbon, and butanoate metabolism (Fig. 3).
- From 27 metabolites, 5 had significant findings in all comparisons, at least in one timepoint (Fig. 3).

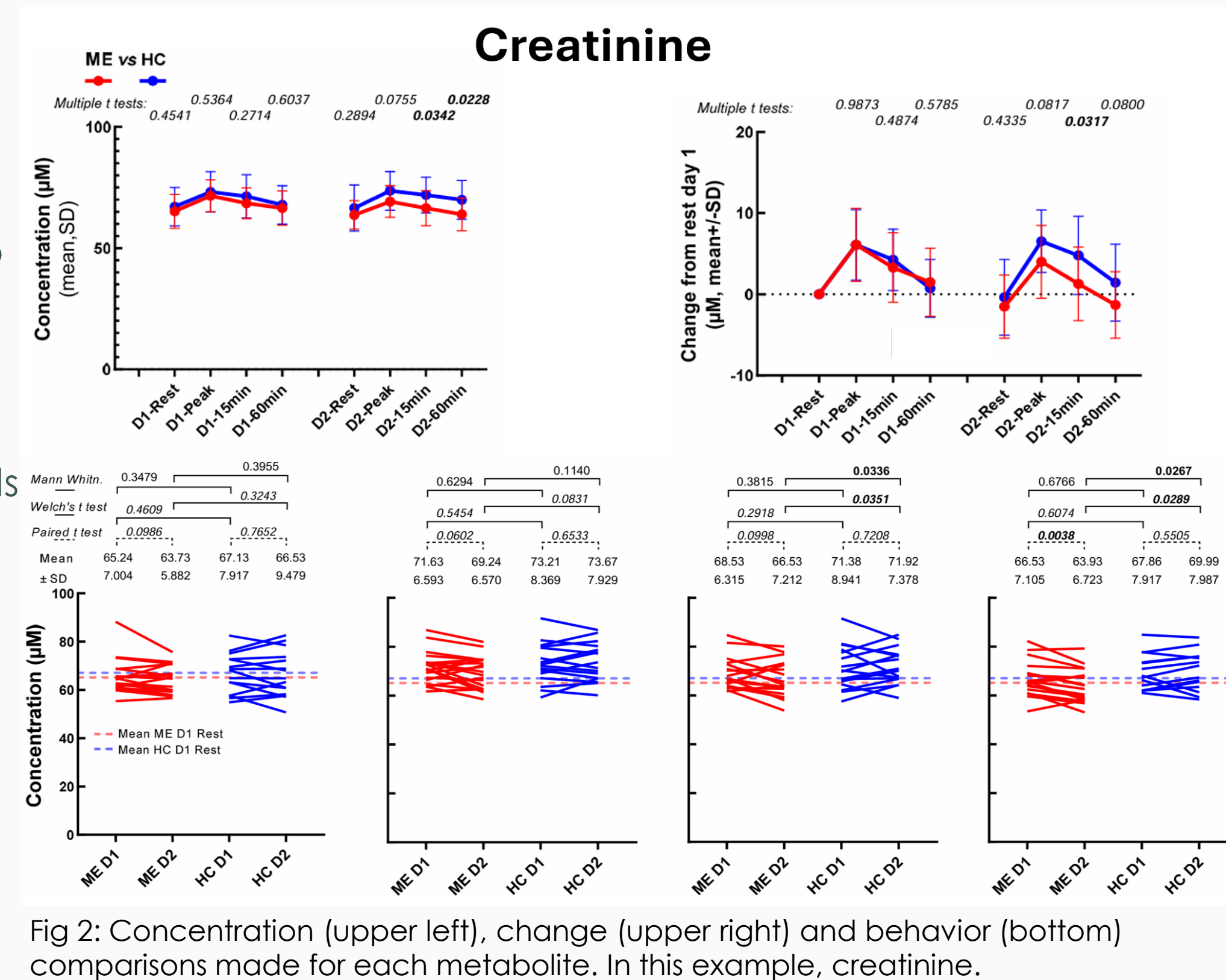


Fig 2: Concentration (upper left), change (upper right) and behavior (bottom) comparisons made for each metabolite. In this example, creatinine.

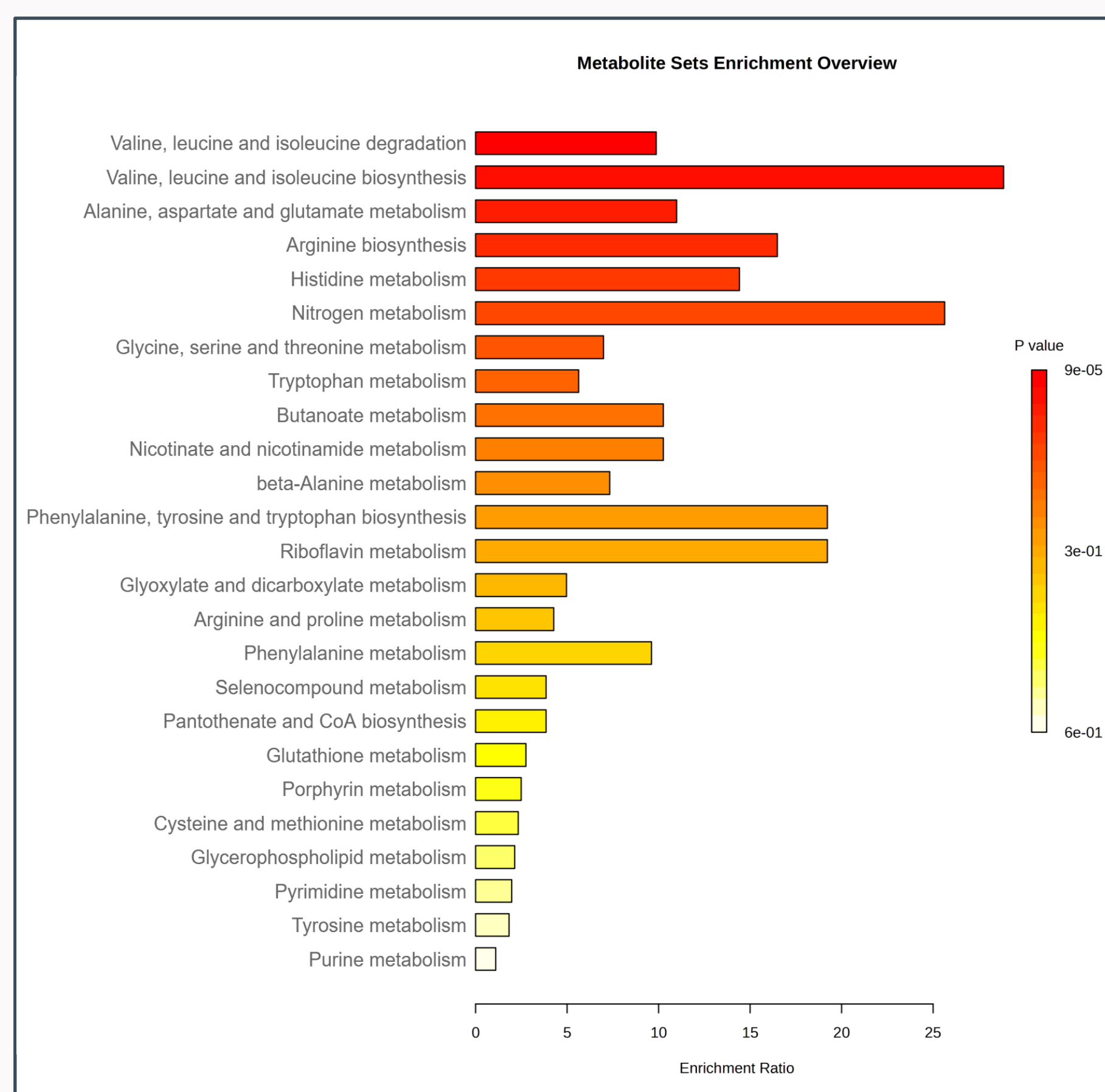


Fig 3: KEGG pathways enrichment results of 27 metabolites

	Concentration	Change	Behavior
Methionine	X		
Choline	X		
Methionine sulfoxide	X		
Trigonelline	X		
Alanine		X	
Glutamine		X	
Histidine		X	
Leucine		X	
Threonine		X	
2-Aminoadipic acid		X	
Phenylalanine			X
Creatine			X
3-Hydroxyanthranilic acid			X
Kynurenine			X
Picolinic acid			X
Riboflavin			X
Aspartic acid	X	X	
Isoleucine		X	X
Methylmalonic acid		X	X
Xanthurenic acid		X	X
Glutamic Acid	X	X	X
Tryptophan	X	X	X
Creatinine	X	X	X
3-Hydroxyisobutyrate	X	X	X
N1-methylnicotinamide	X	X	X
Acetoacetate	X	X	X

Table 1: Metabolite significant findings per type of comparison

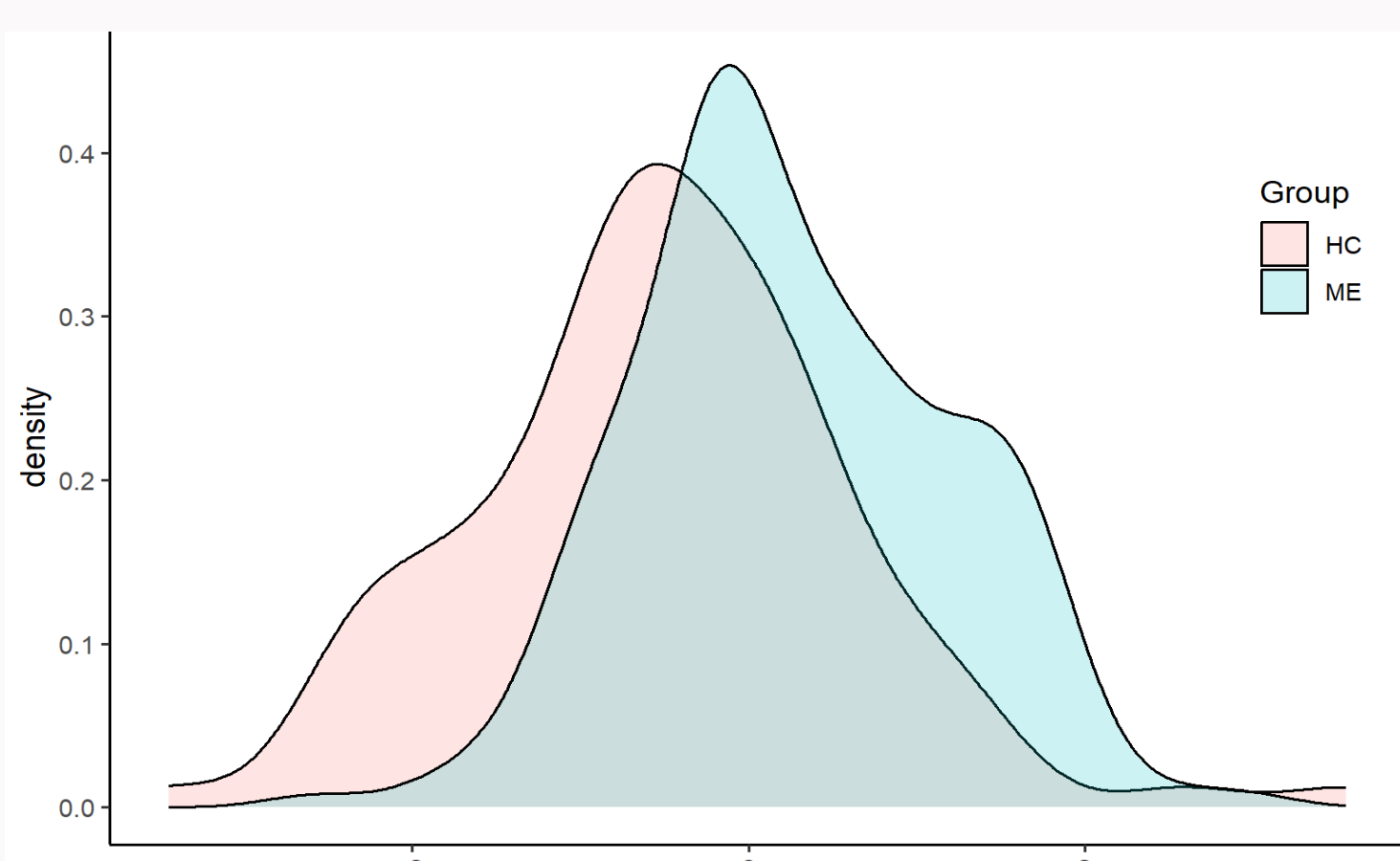


Fig 4: MECFS and HC predicted data distribution according to LDA

## References

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- N1-methylnicotinamide presented most consistent and greater changes throughout the experiment (Fig. 5).
- Creatinine most significant and relative changes are concentrated in the last two timepoints (Fig. 2 and 5).

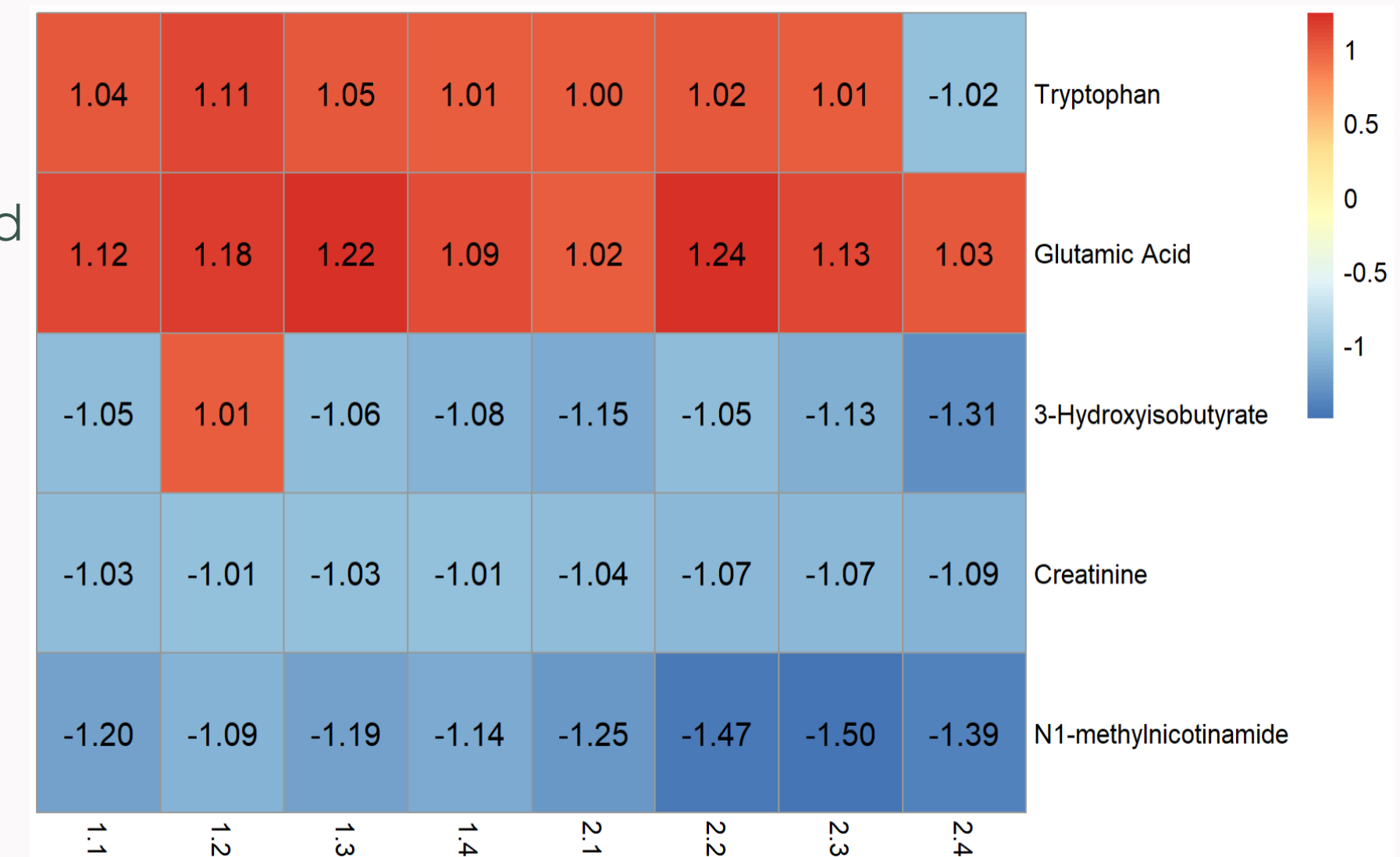


Fig 5: Heatmap with the mean fold change values of ME/CFS data compared to HC, for each timepoint

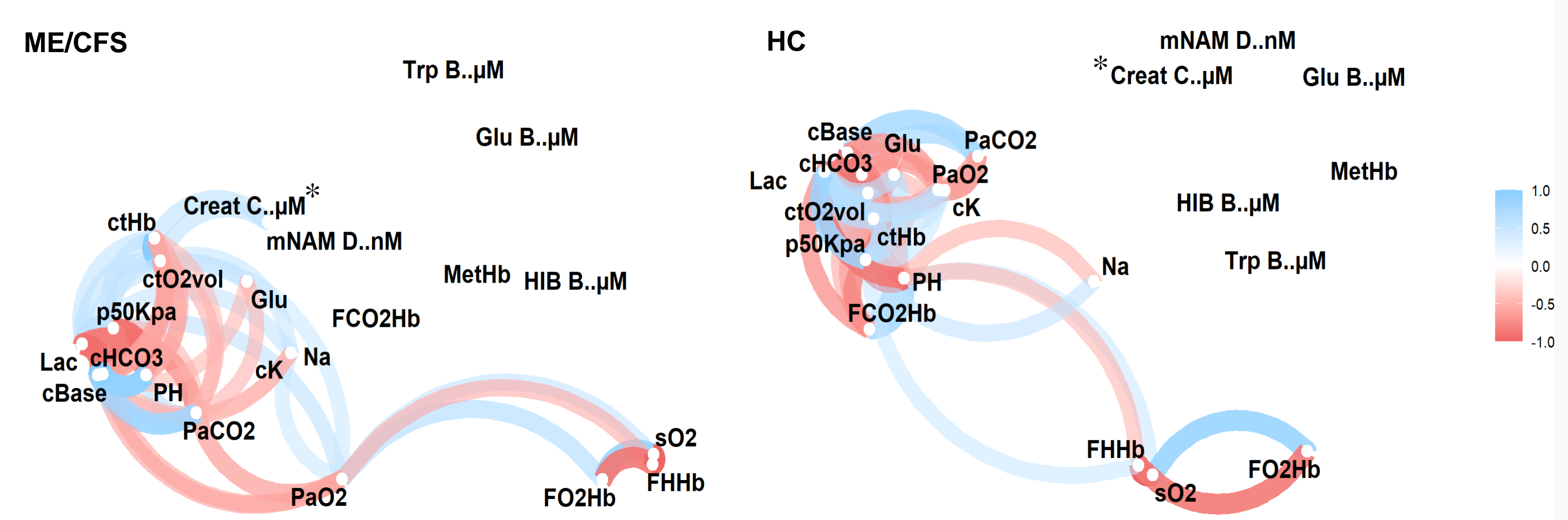


Fig 6: Correlation maps for ME/CFS (left) and HC (right) with metabolites and blood gas parameters, showing interactions with Spearman correlation coefficient above 0.5.

- Mapping blood gas parameters correlations greater than 0.5, we observed a notable association between creatinine (mapped as Creat C..µM) and hemoglobin (ctHb) as well as oxygen concentration (ctO2vol).
- The p-value between creatinine and hemoglobin in ME/CFS and HC groups was 0.0007, with a confidence interval ranging from 0.1331 to 0.5281. Similarly, the correlation coefficients difference between creatinine and oxygen volume had a p-value of 0.0023. Both changes in association were significantly different, indicating a meaningful shift in the correlation network.

## The findings

- Support the role of metabolic limitations in the pathomechanism, including exertion-triggered elements [2].
- Reveal plausible signatures of impaired metabolic recovery after exercise
- Indicate possible associations with oxygen transport and utilization.
- Expanded analyses will be performed to enlighten these aspects.

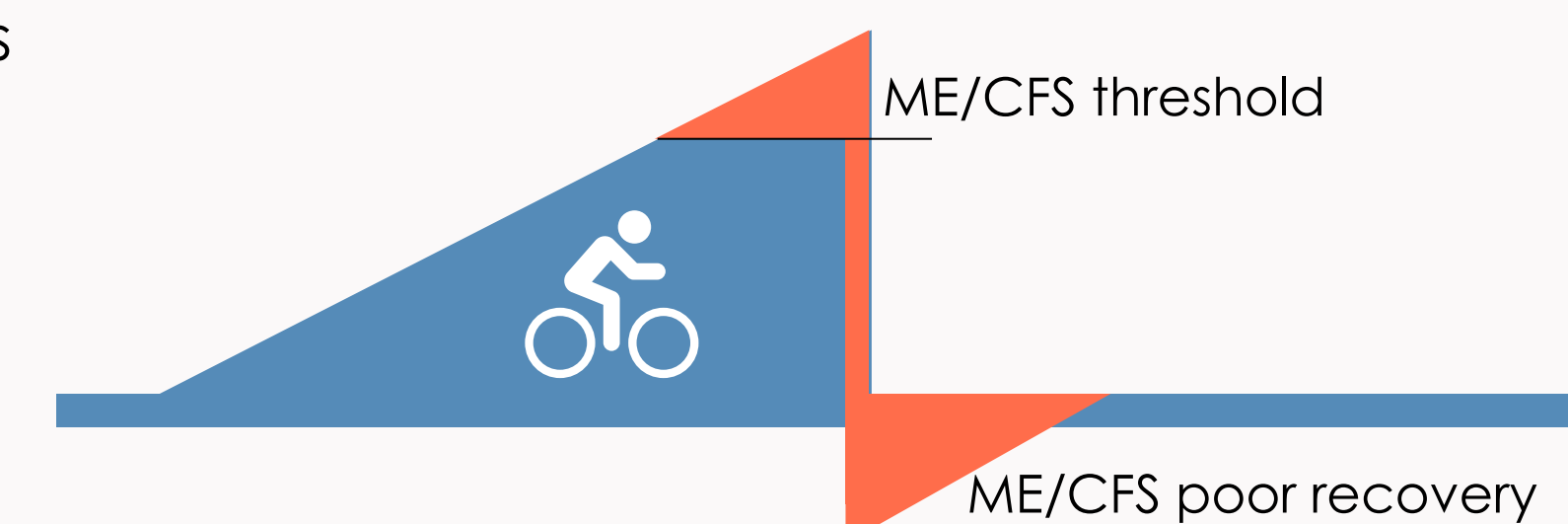


Fig 7: Schematics interpretation of the findings. Metabolic limitations may contribute to physical exhaustion and poor recovery.

## Conclusion

The study identified significant alterations in 27 out of 70 metabolites, with five showing consistent differences across all comparisons. However, using these five metabolites in linear discriminant analysis (LDA) failed to distinguish between groups due to considerable overlap in LD1 values, suggesting that LDA is insufficient for longitudinal data with high variability. Significant correlations were found between creatinine and hemoglobin, as well as creatinine and oxygen concentration, indicating a meaningful shift in the correlation network between ME/CFS and HC groups. Our next steps are conducting longitudinal discriminant analysis and investigate correlation networks further with other clinical parameters and network analysis to enhance our understanding of ME/CFS compensatory mechanisms.

## Methods

Participants (18 female ME/CFS patients and 15 healthy female controls) were pre-screened based on age, health status, medication, and physical activity level. Patients met the Canadian Consensus Criteria for ME/CFS. Participants performed 2xCPET 24 hours apart on a cycle ergometer, with gas exchange and ventilation measured breath-by-breath and blood samples were taken (Fig 1)[1]. Metabolites were analyzed through three strategies: comparisons for each time point between groups, changes relative to baseline on day 1, and pairwise. The first two sets focused on "concentration" and "change," while the third set, "behavior," required a change between patients and controls or a significant finding exclusive to one group. The enrichment analysis parameters included the Hypergeometric test, relative-betweenness centrality topology measure, and KEGG annotations for Homo sapiens [3]. To distinguish between the two groups, made a linear discriminant analysis, modeling the scaled measurements of the five selected metabolites from all the points, randomly sampling 70% of the dataset for training and 30% for testing [4]. Blood gas and metabolite correlation matrices and mapping were with Spearman's method and correlation coefficients threshold of 0.5. The significance of independent correlation coefficients was evaluated using Fisher's test [5, 6].

## Acknowledgments

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