Plasma Metabolomic Effects of 8-Week Red Wine Consumption in CHD Patients

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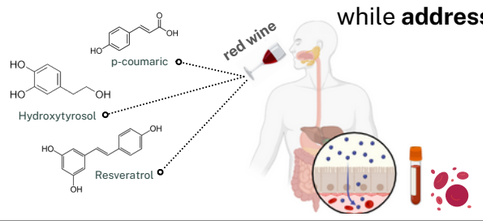
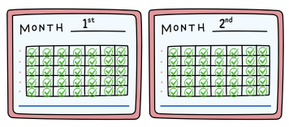
INTRODUCTION

Objective

To investigate the metabolic effects of **red wine consumption over 8 weeks**, focusing on the identification of key metabolites and their temporal changes, while **addressing inter-individual variability using multilevel analysis**.



8 weeks of daily wine consumption



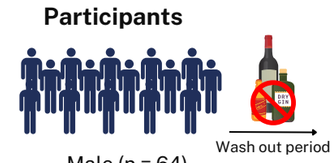
Moderate alcohol consumption, particularly **red wine**, has long been **associated with cardioprotective effects**, largely attributed to its polyphenolic content. However, the **long-term metabolic impact** of such consumption **in patients with coronary heart disease (CHD) remains poorly characterized**. To date, most studies have either focused on short-term interventions or observational associations without directly comparing ethanol versus polyphenol-rich alcoholic beverages. **This study** addresses this gap by conducting **the first 8-week randomized clinical trial using untargeted metabolomics.**

EXPERIMENTAL FLOW CHART

1. Clinical Trial

Randomized controlled 3-arm parallel intervention

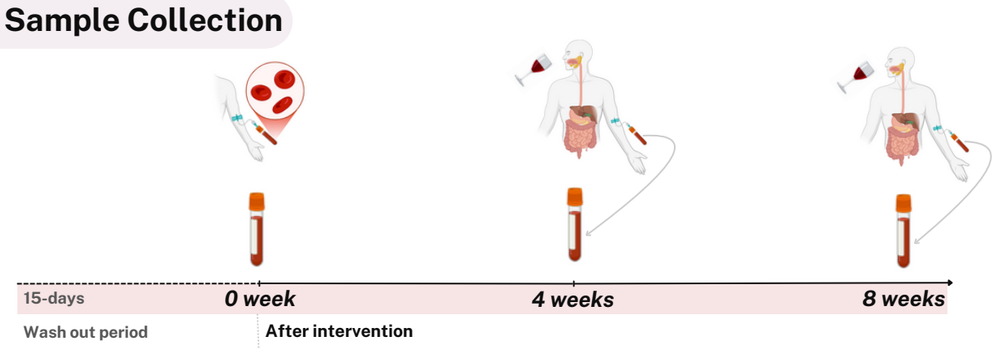
Participants



Male (n = 64)
CHD patients
Age range: **61-63 years**
stable medication

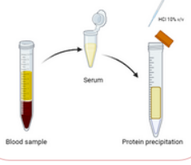
Trial CS	Trial T	Trial W
Male (n = 21)	Male (n = 16)	Male (n = 20)
red wine	alcoholic distillate	control group
Cabernet Sauvignon	**Tsipouro**	**Water**
27 g alcohol/ day	27 g alcohol/ day	

2. Sample Collection

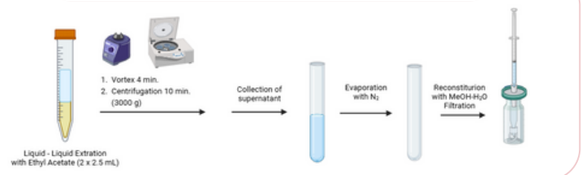


3. Sample Preparation

I. Protein Precipitation



II. Liquid-Liquid Extraction
III. Evaporation
IV. Reconstitution
V. Filtration



4. Analysis

Liquid Chromatography

Reverse Phase (RP) (+)
C18 column (2.1 m × 100 mm, 2.2 µm)
Mobile phase mixures:
(A) Mili-Q H_2O: MeOH (90:10)
(B) MeOH,
with both A and B containing 5mM ammonium formate

High Resolution Mass Spectrometry

Electrospay ionization (ESI)
Capillary voltage: 3500 V
m/z range: 50 - 1000
(with a scan rate of 2 Hz)
Collision energies: 4 eV and 25 eV
in **Data Independent Acquisition (DIA) mode**

Data Processing

Non-targeted metabolomics data were acquired in AIF mode and processed in **MS-DIAL**. The dataset was **log-transformed** and **Pareto-scaled** in **RStudio** using MetaboAnalystR. Features with **RSD > 30%** were removed using **QC samples**. Multilevel analysis was performed with **mixOmics** and **MetaboAnalystR** to account for intra-subject variability.

RESULTS

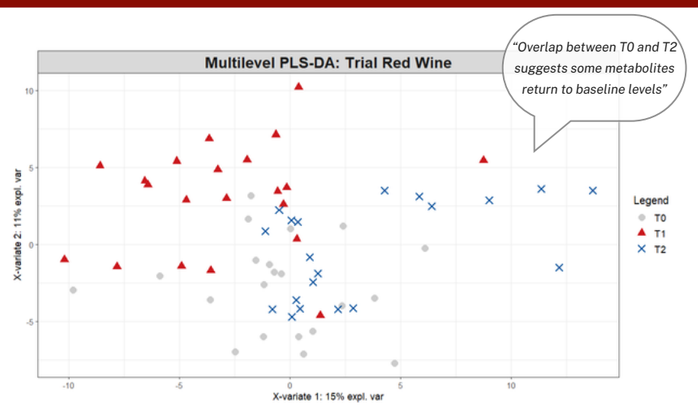


Figure A1. Partial least squares discriminant analysis (PLS-DA) of the red wine trial, with adjustment for inter-individual variability (multilevel).

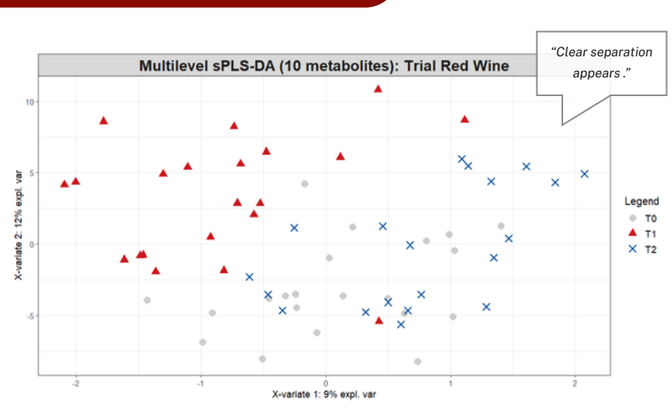


Figure A2. Multilevel sparse PLS-DA (sPLS-DA) of the red wine trial, with top 10 metabolites.

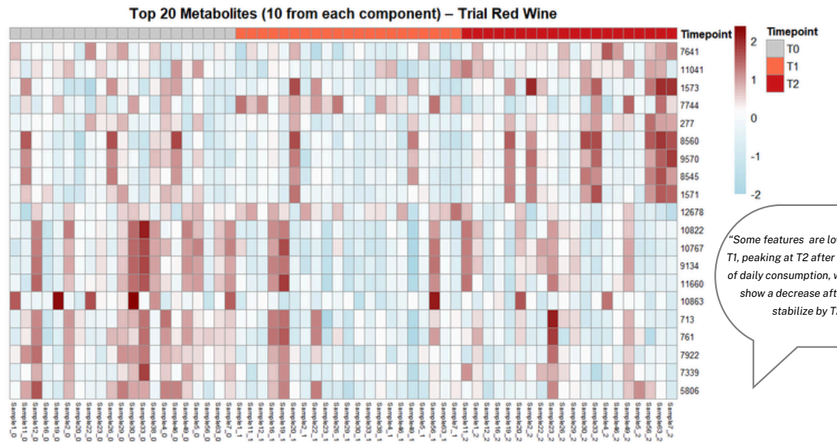


Figure B. Scaled heatmap of the top 20 discriminating metabolites identified by multilevel sPLS-DA (10 from each component), illustrating inter-individual and temporal variation across T0, T1, and T2.

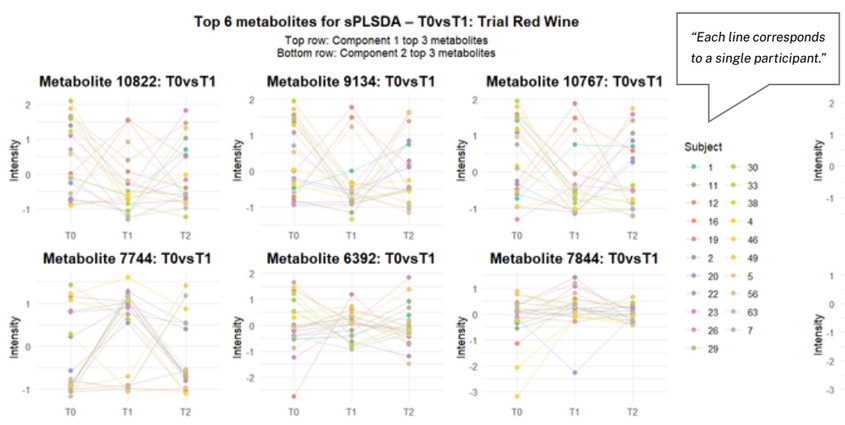


Figure C1. Longitudinal trajectories for the top 6 discriminating scaled features from the sPLS-DA comparison between T0 and T1.

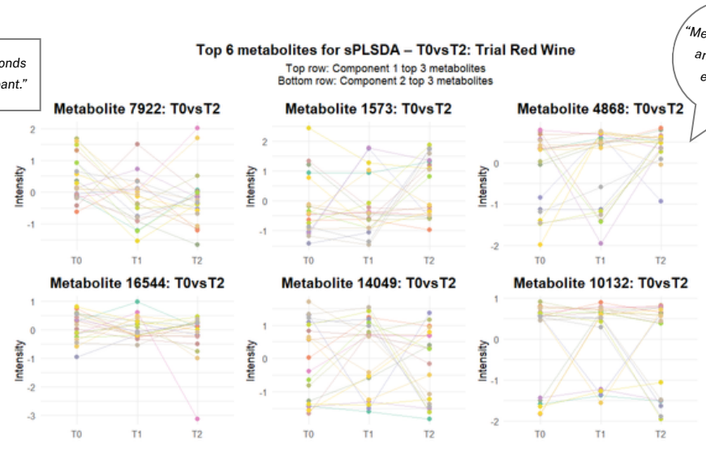


Figure C2. Temporal evolution of the top 6 discriminating features between T0 and T2 in the red wine group.

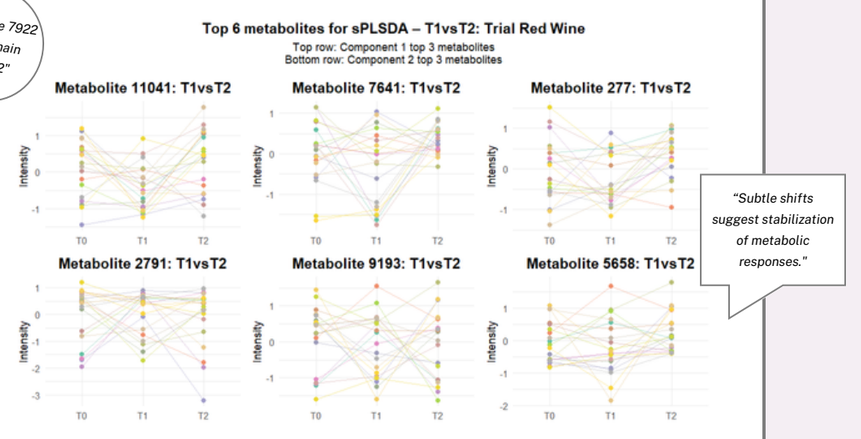


Figure C3. Inter-subject trajectories of the top 6 features distinguishing the 1st and 2nd month timepoints (T1 vs T2).

CONCLUSIONS / FUTURE PERSPECTIVES

1. **Metabolic Changes:** Red wine consumption induces significant metabolic shifts, with key metabolites increasing at T2 and others dicrease after T1.
2. **Inter-Individual Variability:** Substantial variability across subjects highlights the need for multilevel modeling to account for baseline differences.
3. **Metabolic Stabilization:** Some metabolites return to baseline (T0) by T2, indicating metabolic adaptation to red wine consumption.

*"**Future work** will focus on **annotating metabolites** and integrating them into pathway analyses to uncover underlying metabolic mechanisms."*

Related Literature

[1] J. M. **Guilford** and J. M. **Pezzuto**, "Wine and Health: A Review," **2011**
[2] M. **Lombardo**, A. Feraco, E. Camajani, M. Caprio, and A. Armani, "Health Effects of Red Wine Consumption: A Narrative Review of an Issue That Still Deserves Debate," **2023**
[4] E. **Fragopoulou**, M. Choleva, S. Antonopoulou, and C. A. Demopoulos, "Wine and its metabolic effects. A comprehensive review of clinical trials," **2018**
[5] P. **Lekka**, E. Fragopoulou, A. Terpou, and M. Dasenaki, "Exploring Human Metabolome after Wine Intake — A Review," **2023**

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