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Profiling Redox and Energy Coenzymes in Tissue and Blood using NMR Spectroscopy

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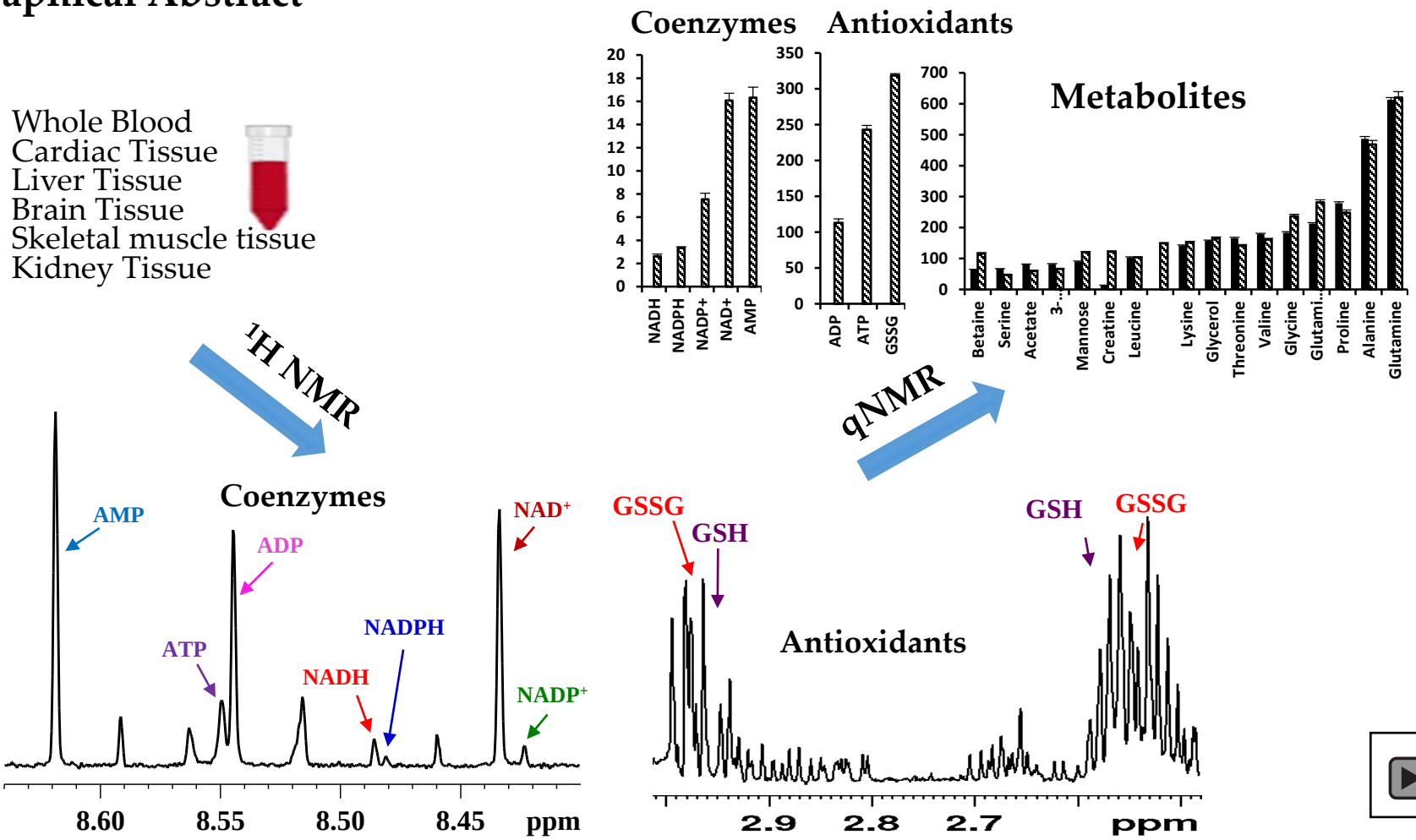
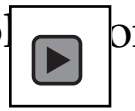
Profiling Redox and Energy Coenzymes in Tissue and Blood using NMR Spectroscopy

Graphical Abstract



Abstract: Coenzymes of redox reactions and cellular energy mediate biochemical reactions fundamental to the functioning of all living cells. To date, no simple method exists to profile these coenzymes in biological samples such as blood and different types of tissue in a single step. Conventional methods that measure them individually carry high risk of errors outweighing their biological variations. We show here that coenzymes including antioxidants and a vast pool of other metabolites can be profiled quantitatively using a simple ^{1}H NMR experiment, simultaneously, in tissue and whole blood. Coenzymes of redox reactions including oxidized/reduced nicotinamide adenine dinucleotide (NAD^{+} and NADH) and nicotinamide adenine dinucleotide phosphate ($NADP^{+}$ and NADPH); the coenzymes of energy including adenosine triphosphate (ATP), adenosine diphosphate (ADP) and adenosine monophosphate (AMP) and the antioxidants including the sum of oxidized and reduced glutathione (GSSG and GSH) can be measured with essentially no additional effort along with the nearly 70 metabolites that were shown to be quantitated in serum/plasma, recently. Considering that coenzymes and antioxidants represent a sensitive measure of cellular functions in health and numerous diseases, the simple NMR spectroscopy method described here for profiling tissue and blood potentially opens a new avenue for appl ons in the metabolomics field.



Keywords: Redox and energy coenzymes; antioxidants; ^{1}H NMR; quantitation

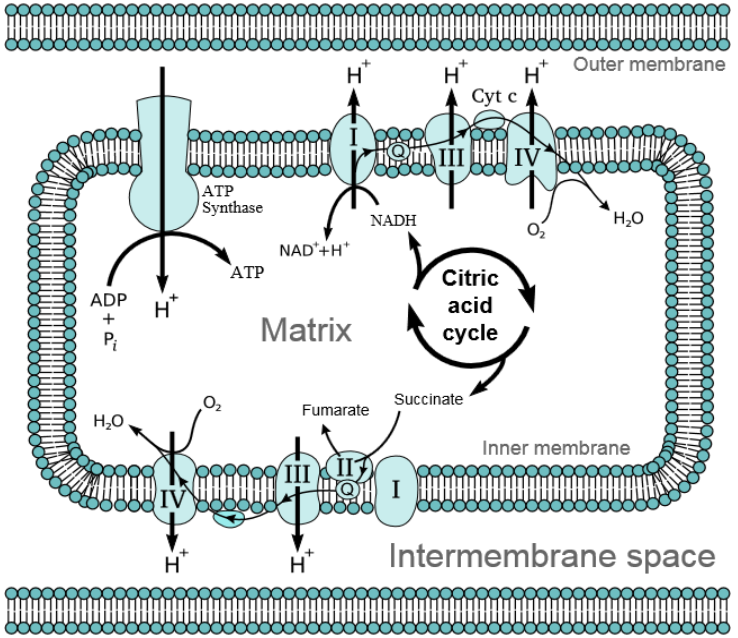
Introduction

- NAD^+, NADH, $NADP^+$ and NADPH mediate biochemical reactions fundamental to the functioning of living cells
- ATP along with ADP and AMP, represent the cellular energy currency.
- No simple method exists currently to measure all quantitatively and simultaneously

Conventional analysis method: Enzymatic Assay:

Challenges:

- Separate protocol required for each coenzyme.
- Interference from sample matrix
- Finite linear range



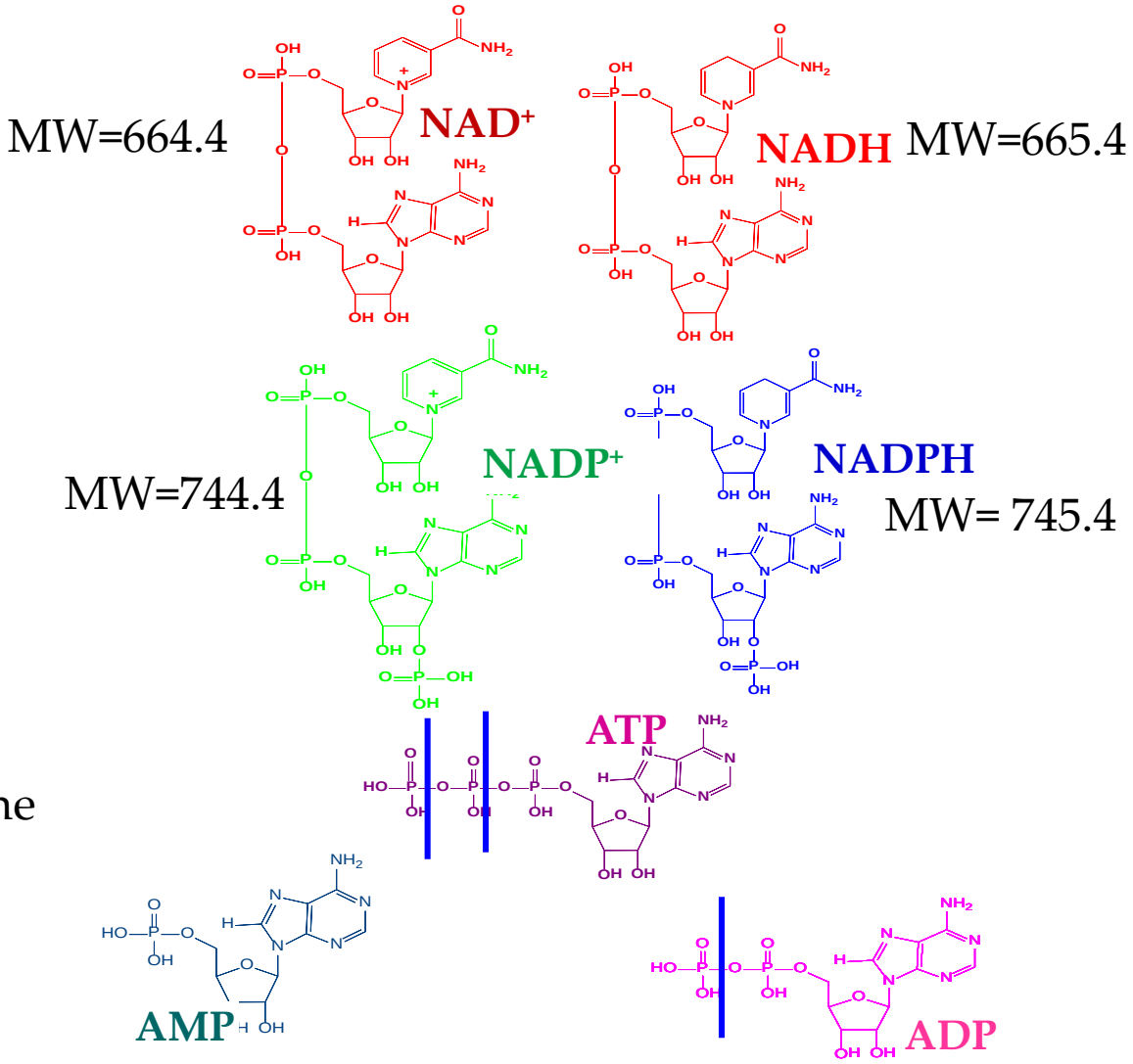
Introduction

Can you Analyze by Mass Spectrometry?

Yes, but…

Challenges

- Ion suppression
- Interference due to unit mass difference
- Interference due to similar chromatographic retention time
- In-source fragmentation

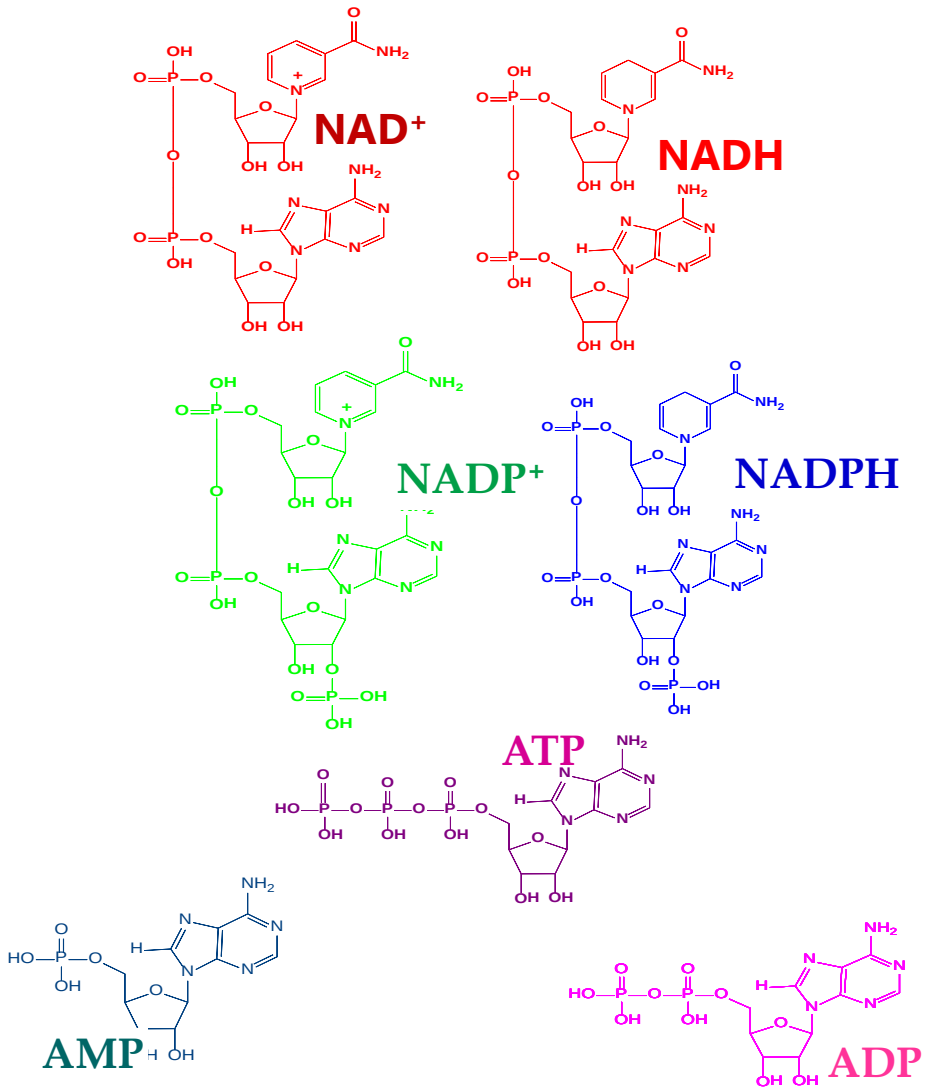


Introduction

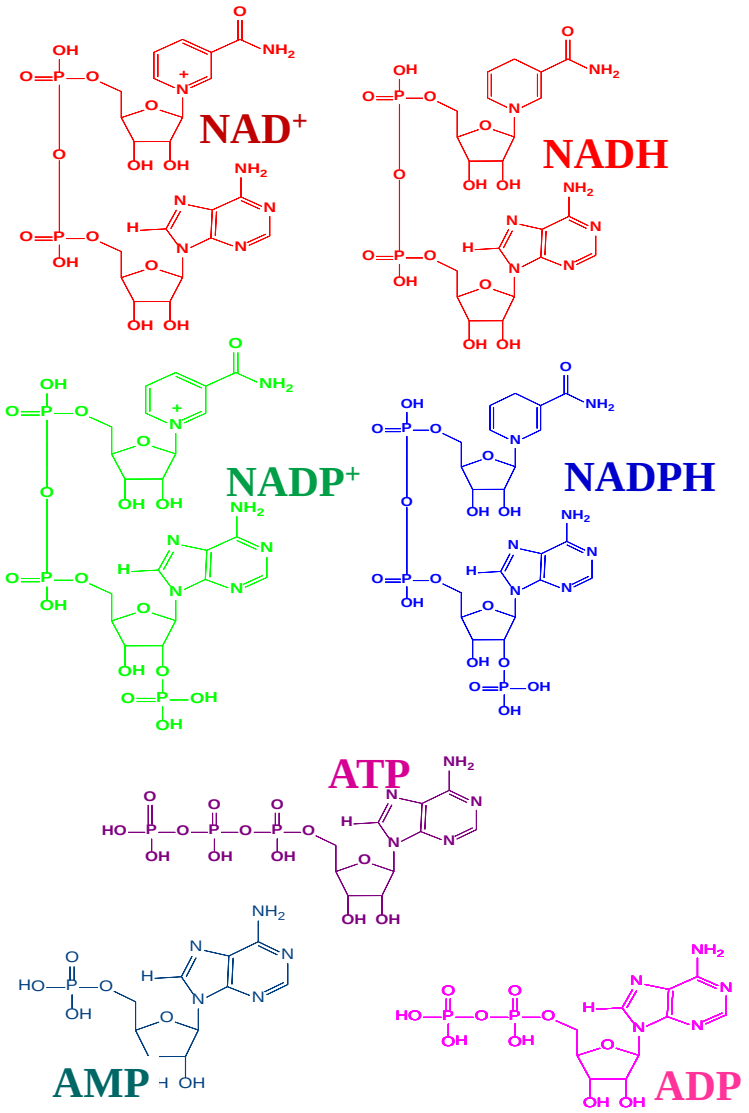
Can you Analyze by NMR Spectroscopy?

Challenges:

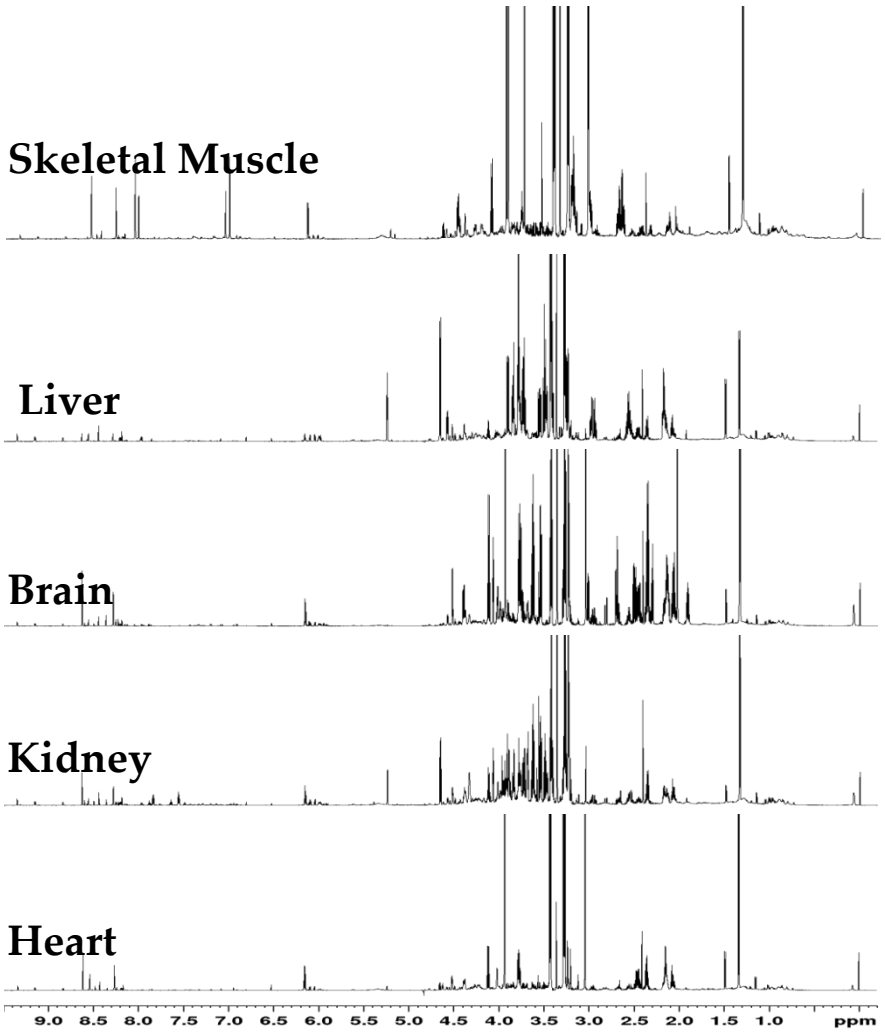
- Complexity
- Similar structures
- Low concentration
- Instability
  - Enzyme activity
  - Oxidation



Results and Discussion



Coenzymes in Tissue Extracts



Results and Discussion

Founding work for coenzymes analysis

analytical chemistry

Article

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Expanding the Limits of Human Blood Metabolite Quantitation Using NMR Spectroscopy

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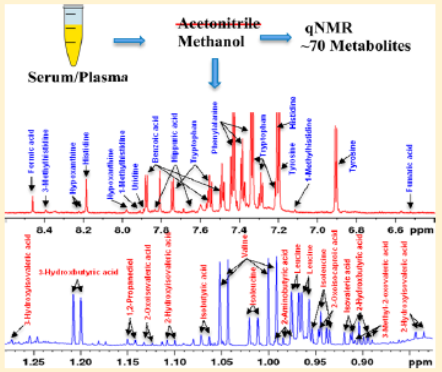
Supporting Information

ABSTRACT: A current challenge in metabolomics is the reliable quantitation of many metabolites. Limited resolution and sensitivity combined with the challenges associated with unknown metabolite identification have restricted both the number and the quantitative accuracy of blood metabolites. Focused on alleviating this bottleneck in NMR-based metabolomics, investigations of pooled human serum combining an array of 1D/2D NMR experiments at 800 MHz, database searches, and spiking with authentic compounds enabled the identification of 67 blood metabolites. Many of these (~1/3) are new compared with those reported previously as a part of the Human Serum Metabolome Database. In addition, considering both the high reproducibility and quantitative nature of NMR as well as the sensitivity of NMR chemical shifts to altered sample conditions, experimental protocols and comprehensive peak annotations are provided here as a guide for identification and quantitation of the new pool of blood metabolites for routine applications. Further,

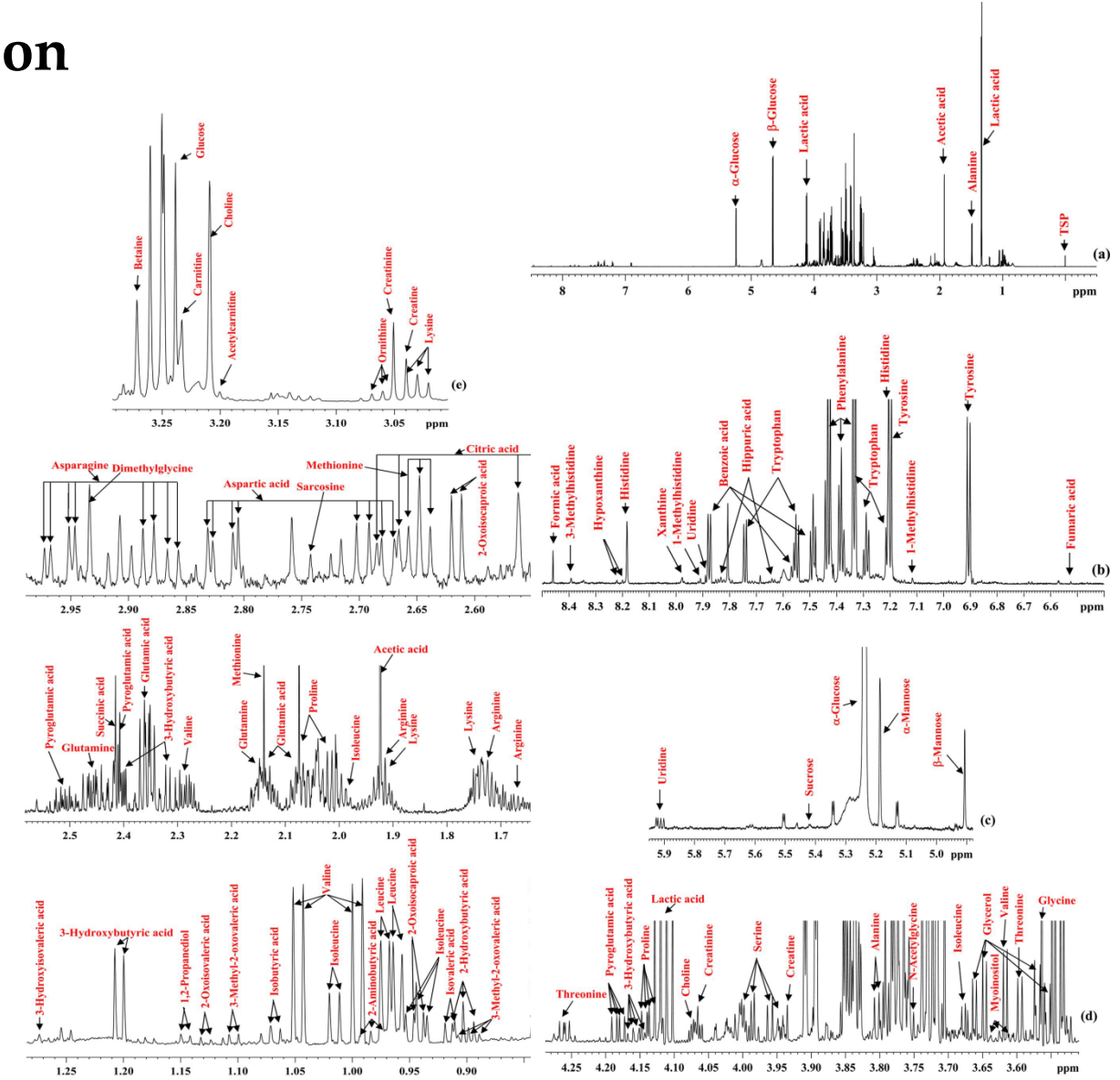


Anal Chem. 2015 Jan 6;87(1):706-15

Results and Discussion

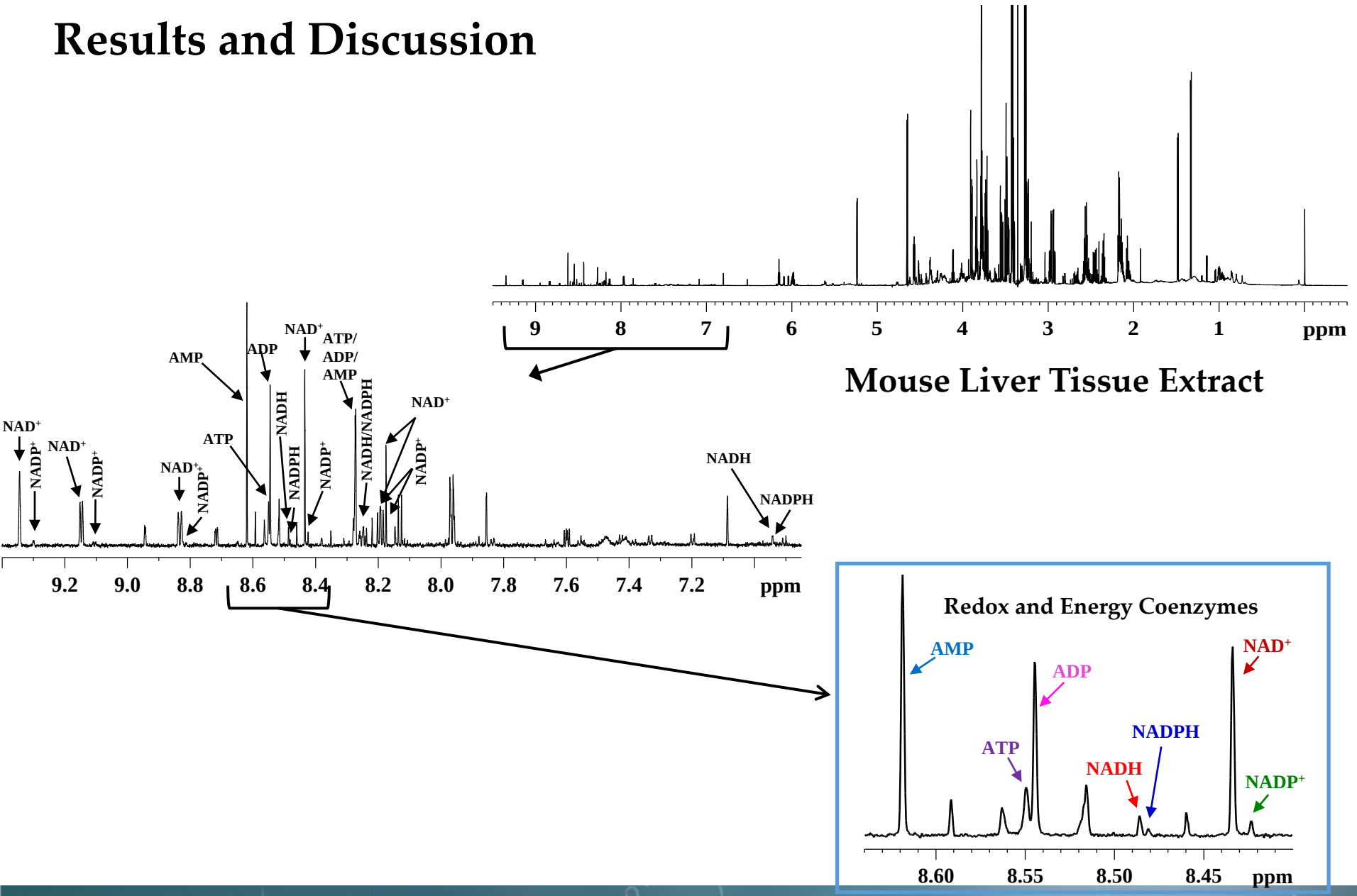
Founding work for coenzymes analysis

- Nearly 70 metabolites identified and quantitated in human blood
- ~35% were newly identified
- Unprecedented for 1D NMR.

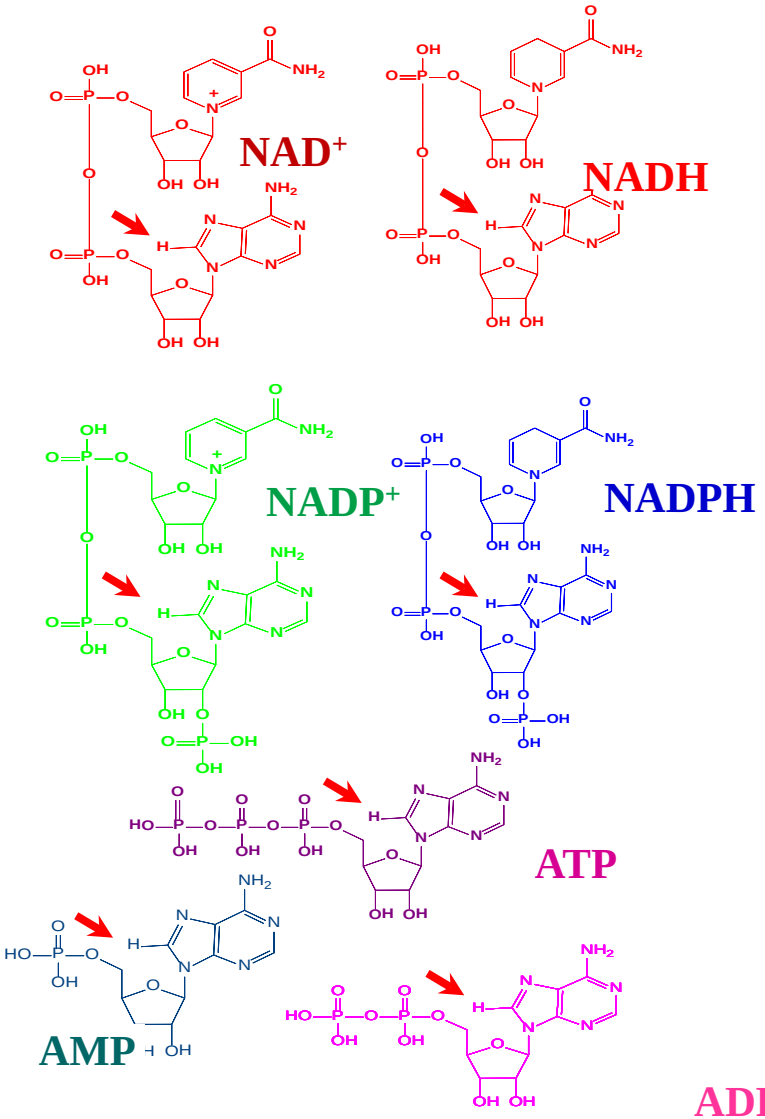


Anal Chem. 2015 Jan 6;87(1):706-15

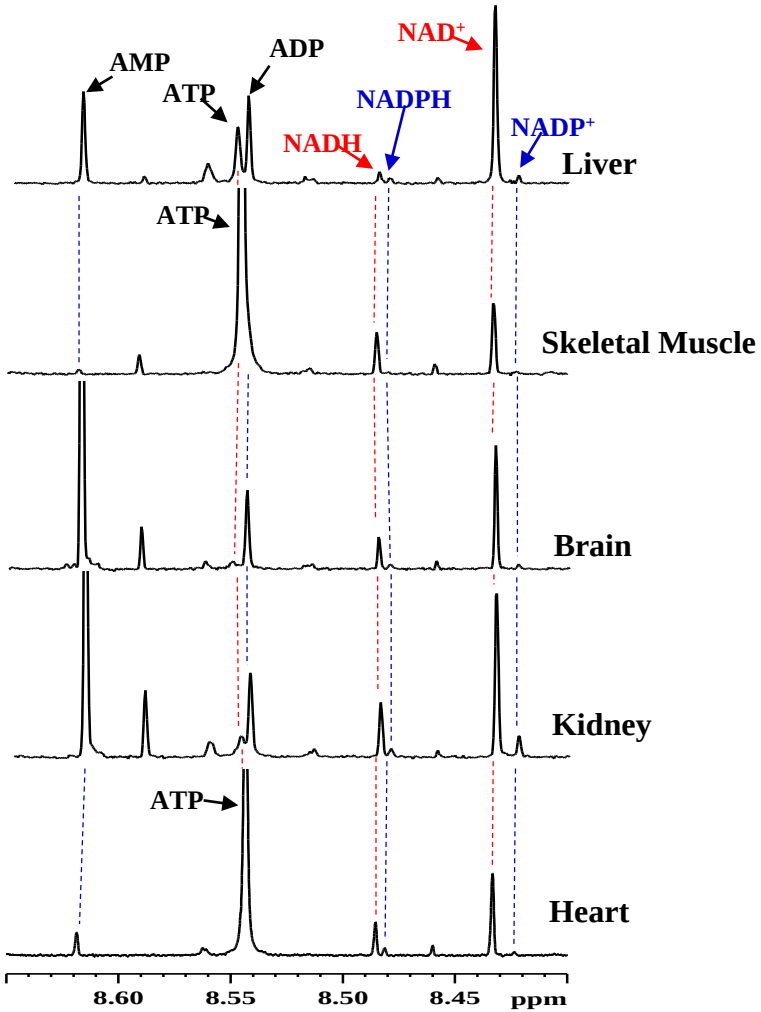
Results and Discussion



Results and Discussion:



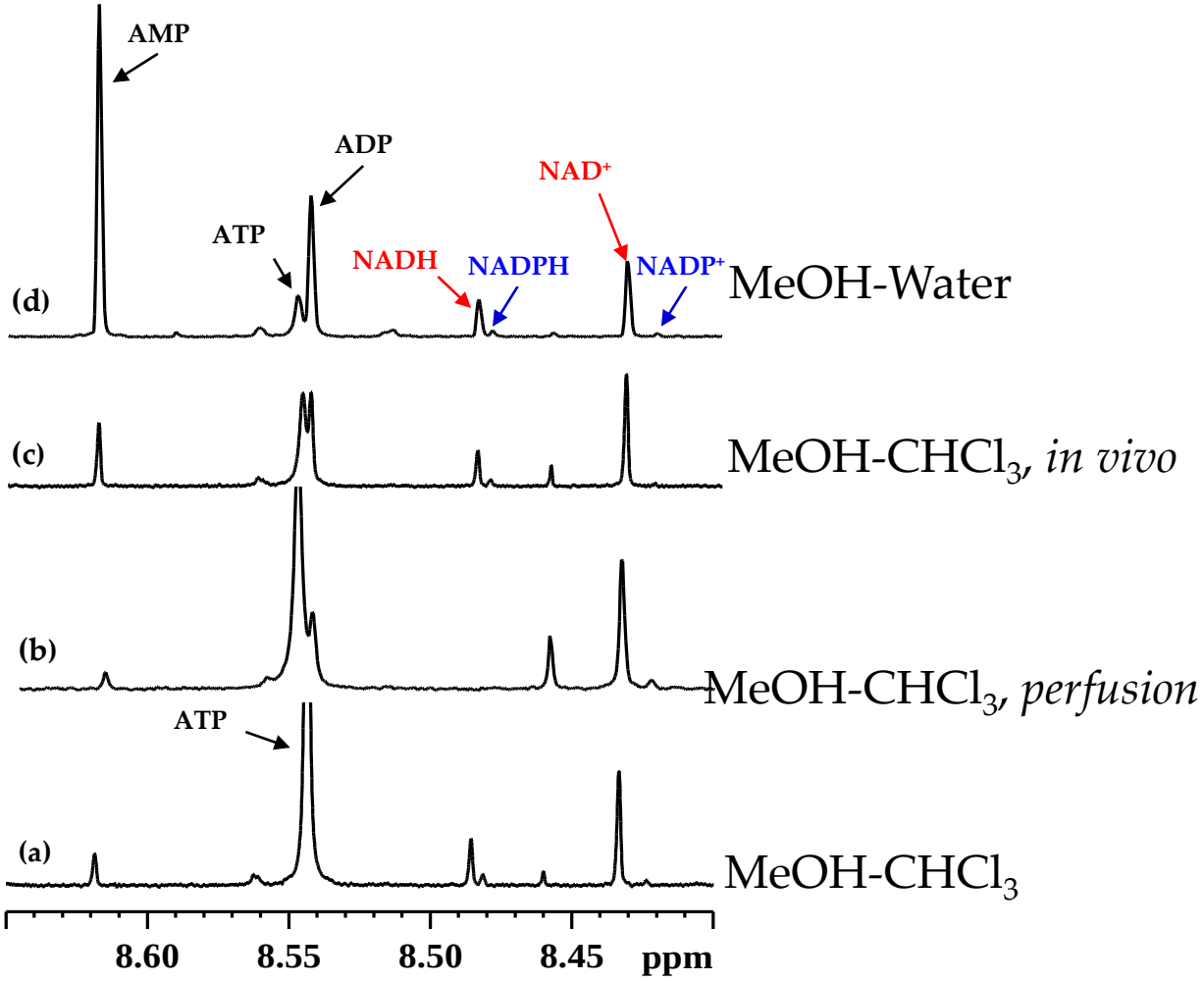
Coenzymes in Different Tissues



Results and Discussion

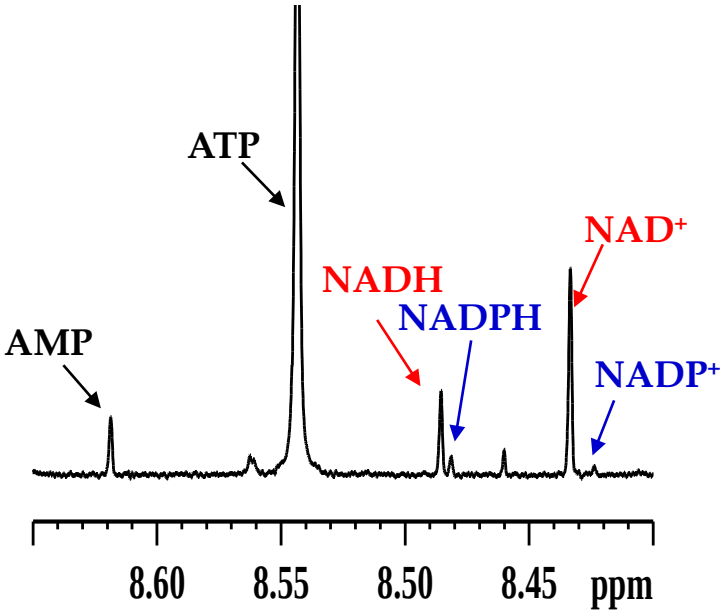
Sensitivity of coenzymes to tissue harvesting/extraction

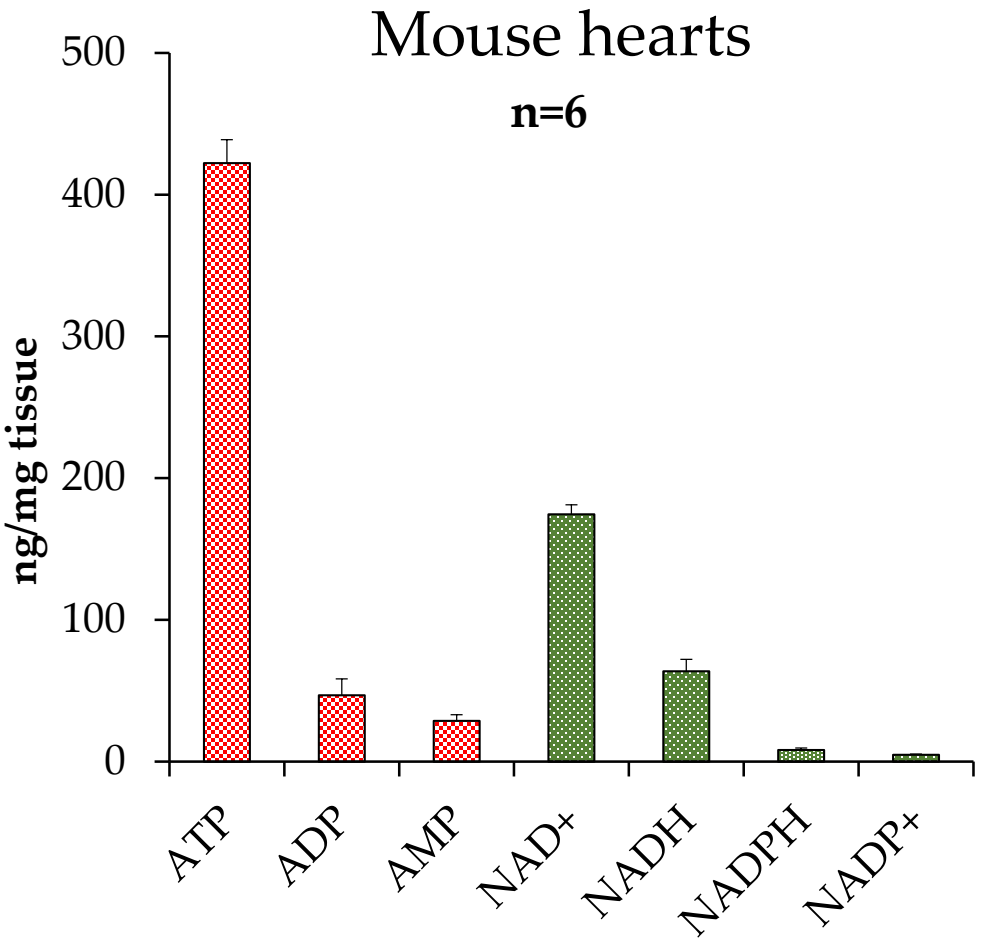
The same mouse heart tissue extracted under different conditions



Results and Discussion

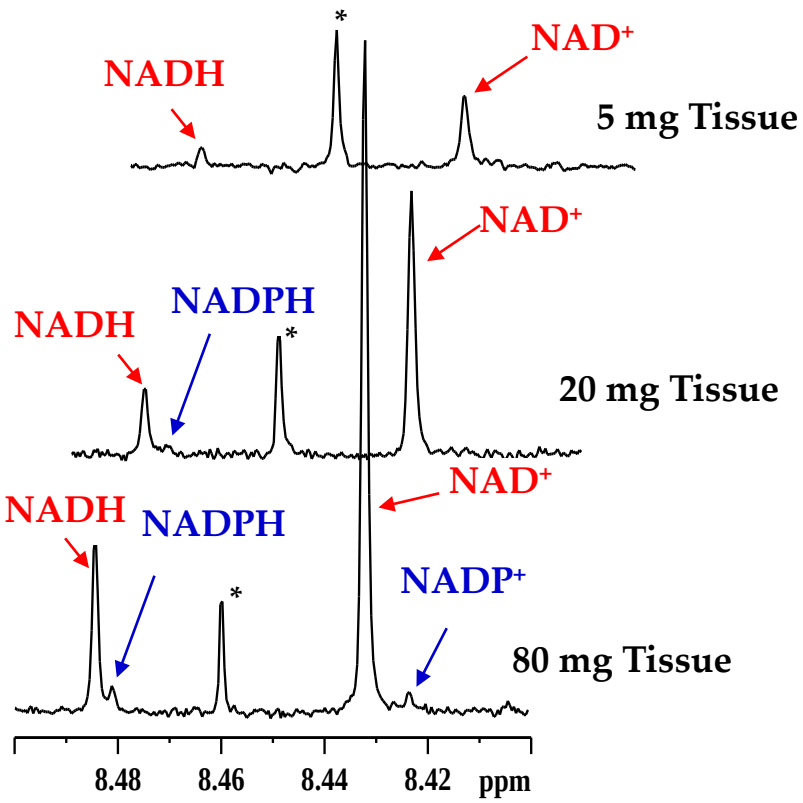
Simultaneous Analysis of Coenzymes

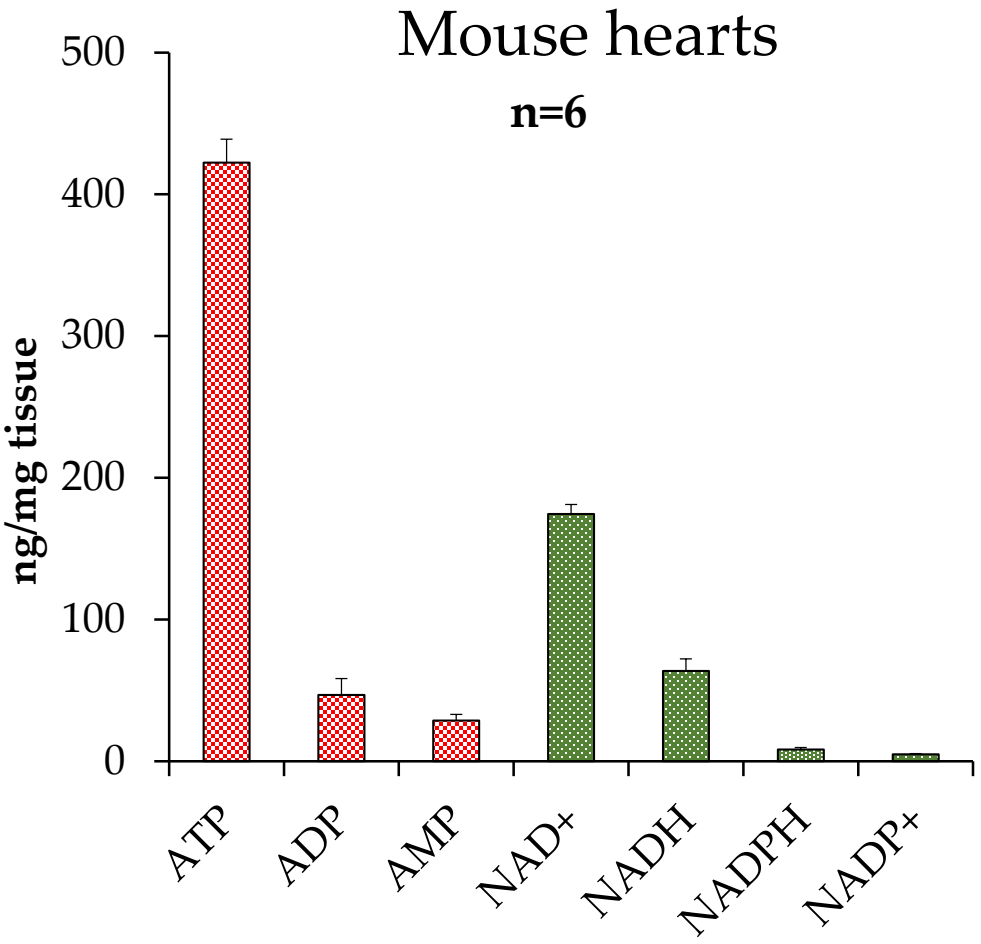




Results and Discussion

Coenzymes from ~5mg tissue





Results and Discussion

Coenzymes and Antioxidants in Whole Blood

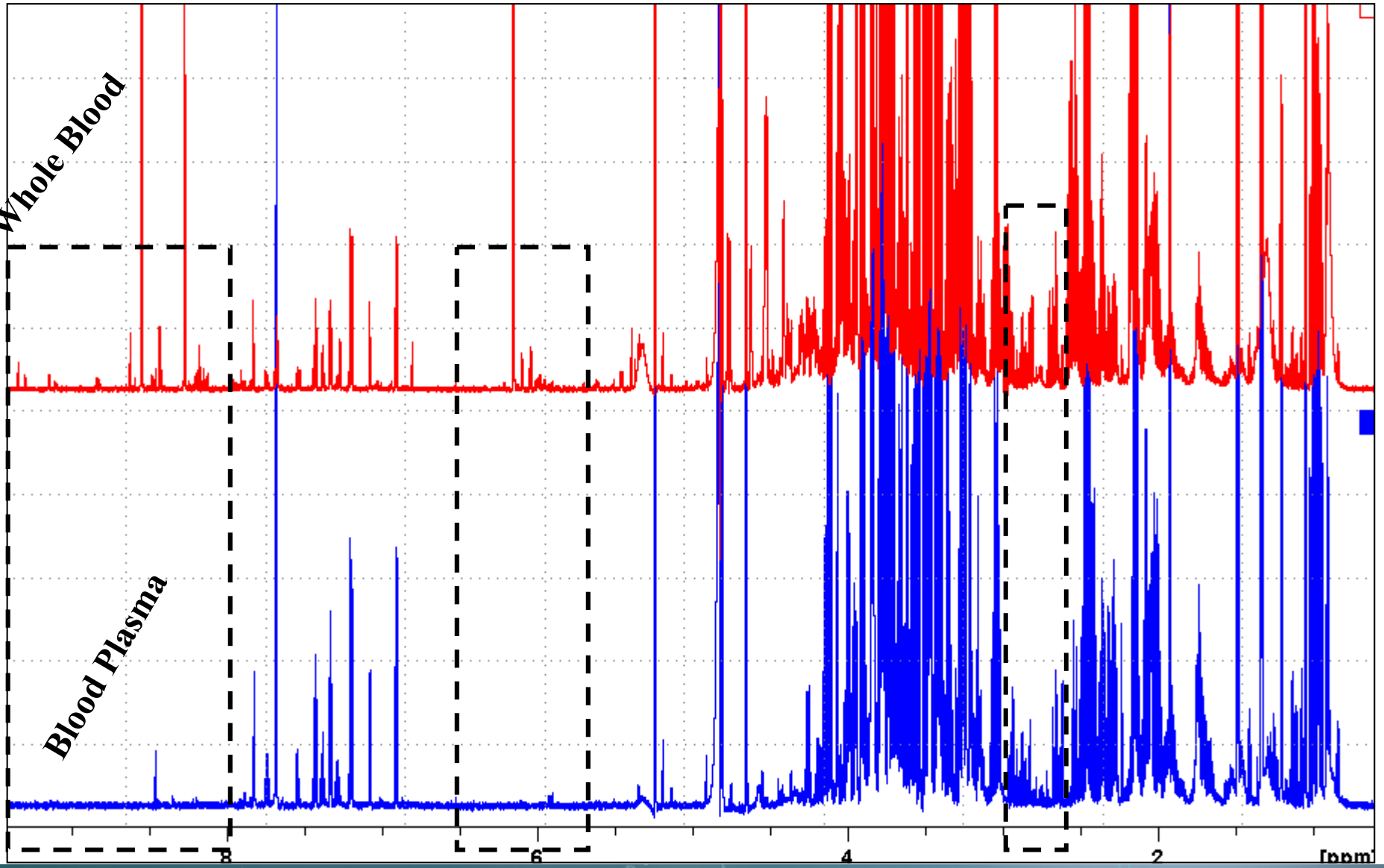
- Blood Metabolomics: Serum or Plasma
- Whole blood: serum/plasma + Cells

  -Nearly 50 % of blood volume is cells and 99% of cells are RBC.

- Whole Blood Metabolomics:
  -Provides access to wider pool of metabolites with no additional efforts.

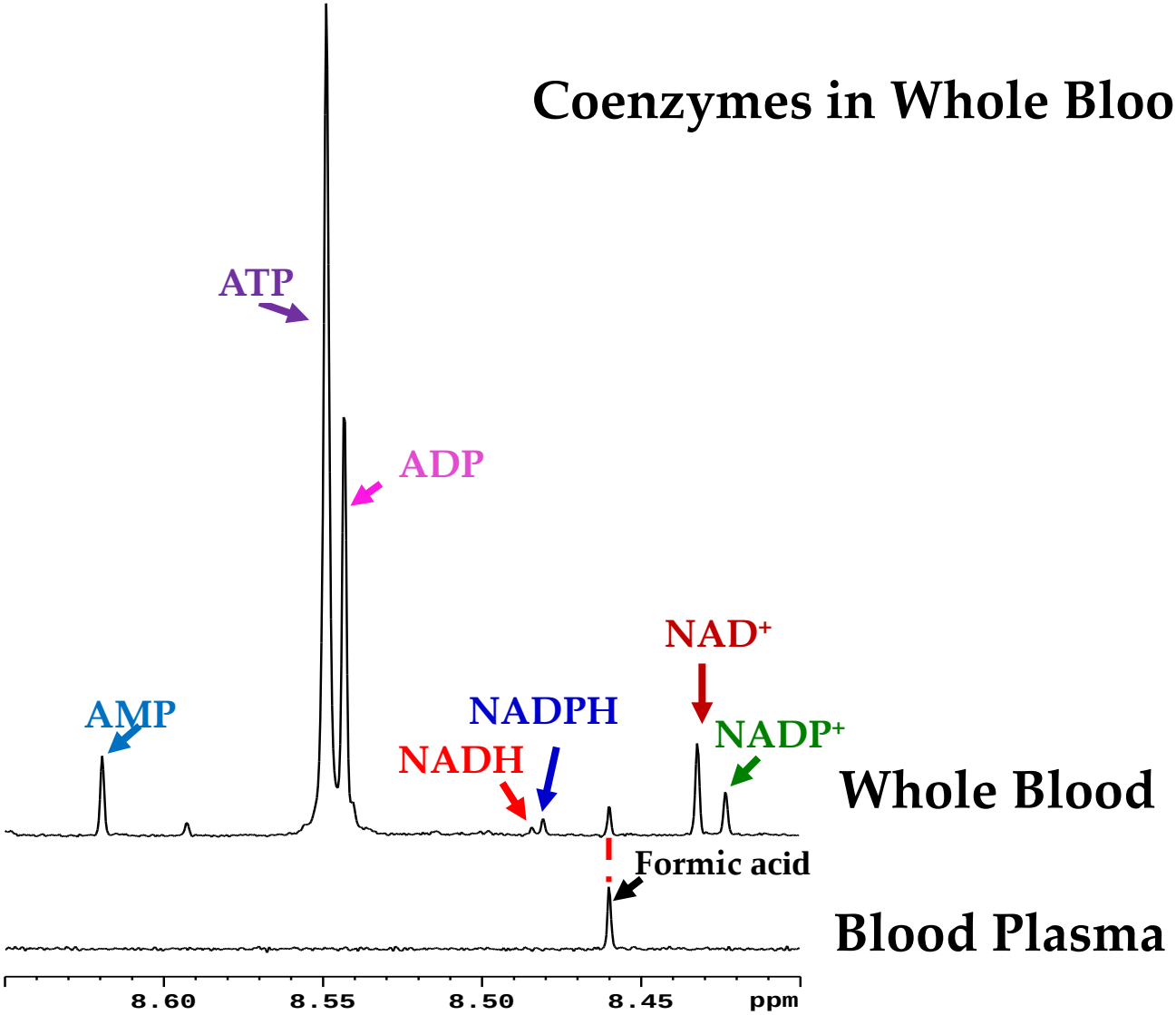
Results and Discussion

Coenzymes and Antioxidants in Blood



Results and Discussion

Coenzymes in Whole Blood extract

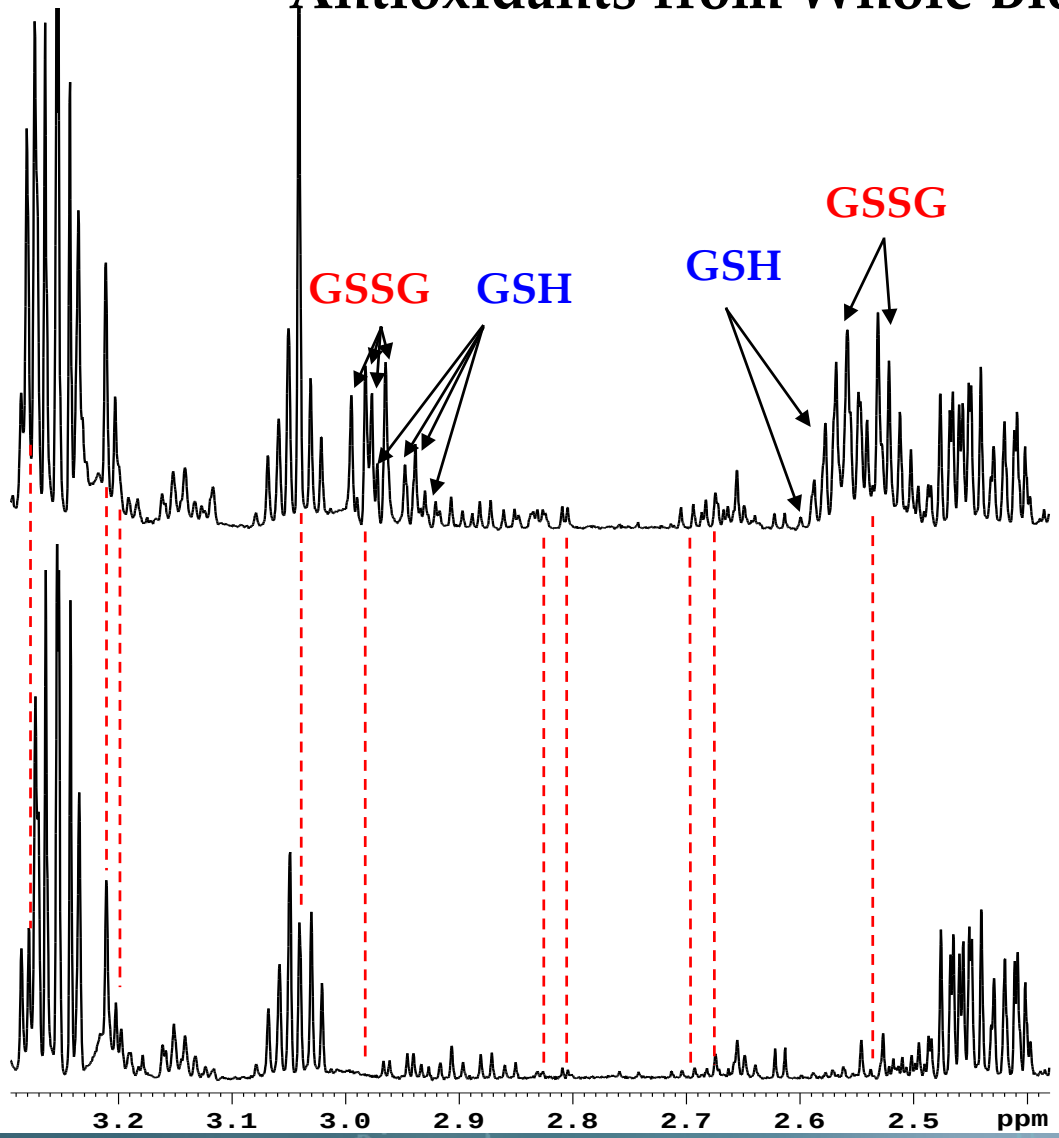


Results and Discussion

Antioxidants from Whole Blood

Whole Blood

Blood Plasma

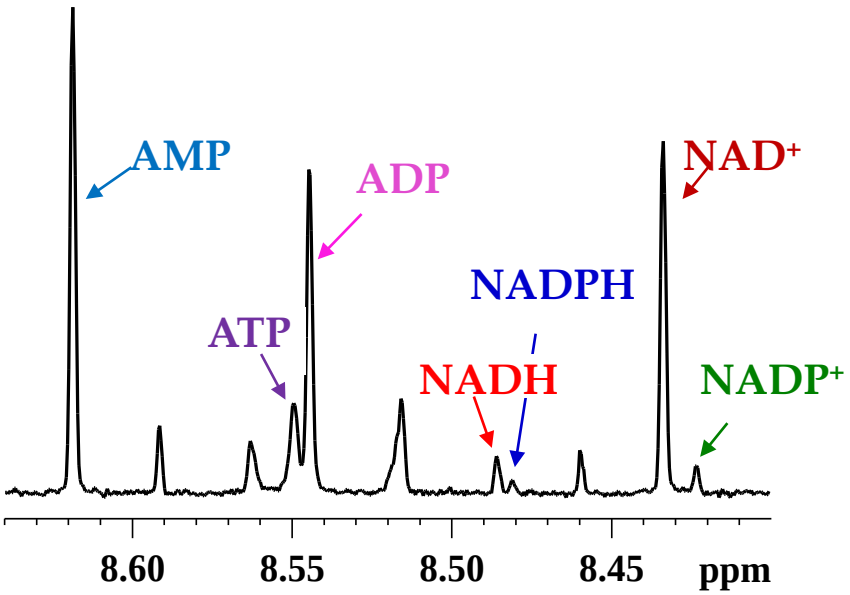


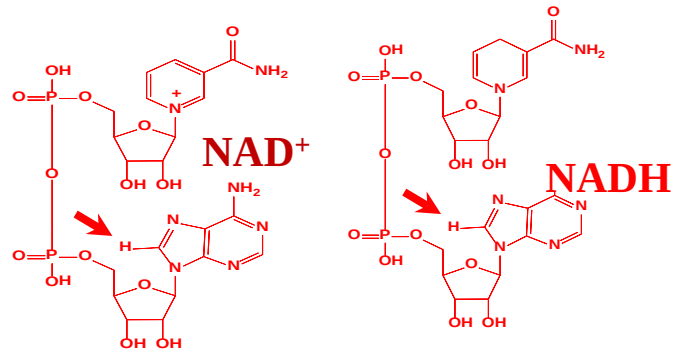
Results and Discussion

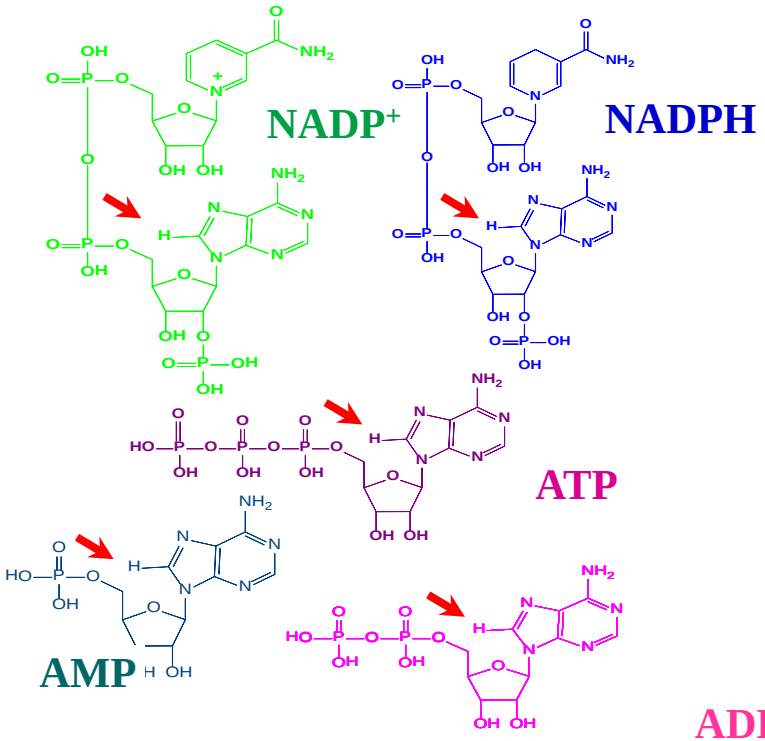
Peak Ratios are Same as Coenzyme Ratios

$NAD^+/NADH$

$NADP^+/NADPH$





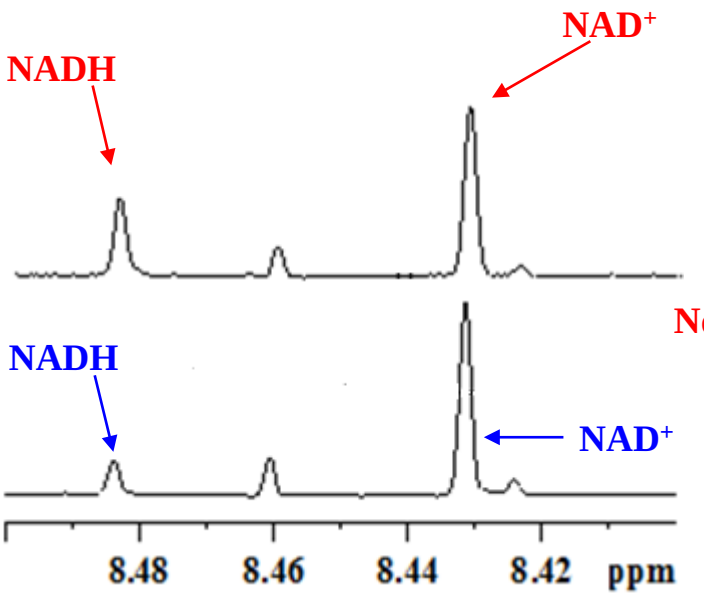


Results and Discussion

Biological validation

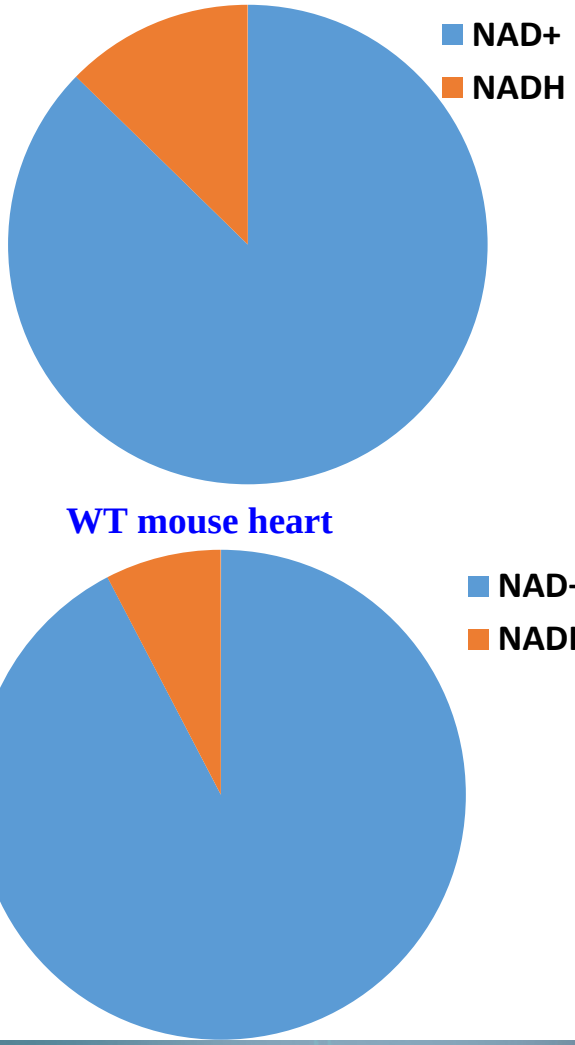
Mice with *Ndufs4* gene Knockout

- Ndufs4 protein critical for mitochondrial function and ATP synthesis.
- *Ndufs4-KO:* NADH/NAD$^+$ ratio ↑



Ndufs4-KO mouse heart

WT mouse heart

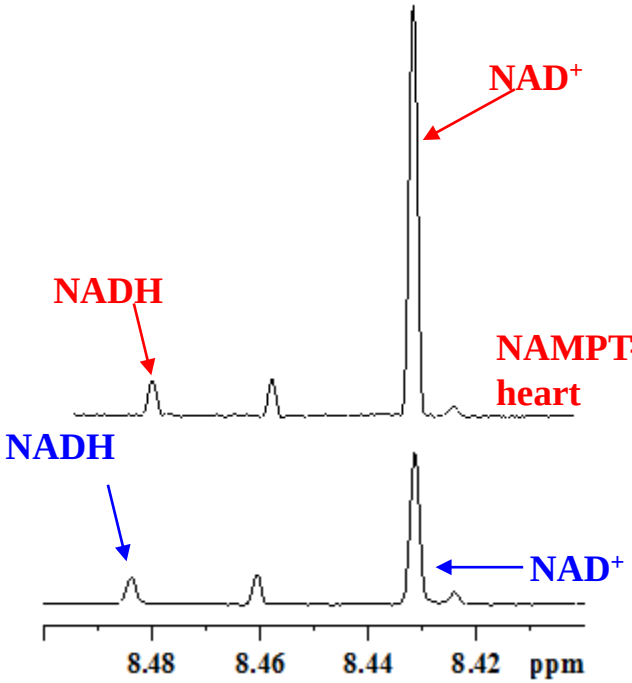


Results and Discussion

Overexpression of NAMPT Protein

(Nicotinamide phosphoribosyltransferase)

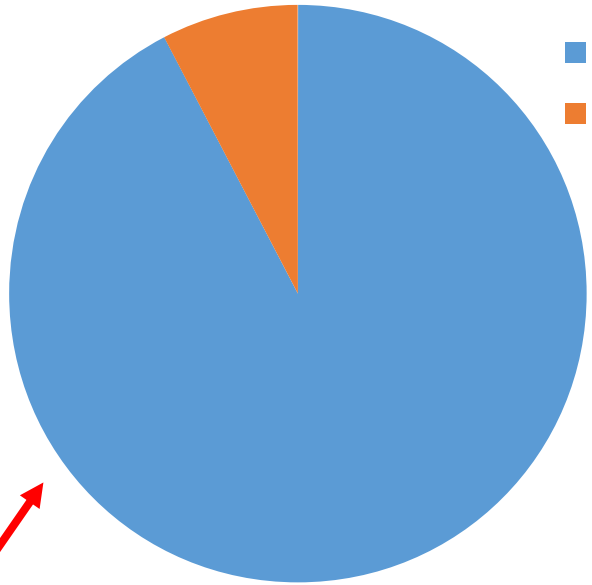
- Increased NAD^+ synthesis
- NAD^+/NADH pool ↑



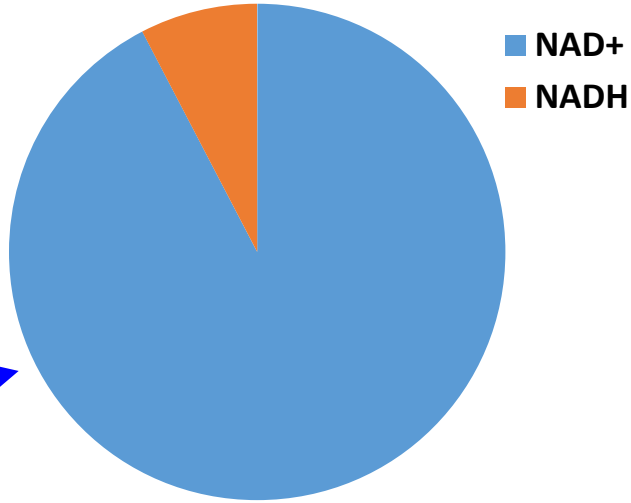
mouse

WT mouse heart

NAMPT-TG mouse heart



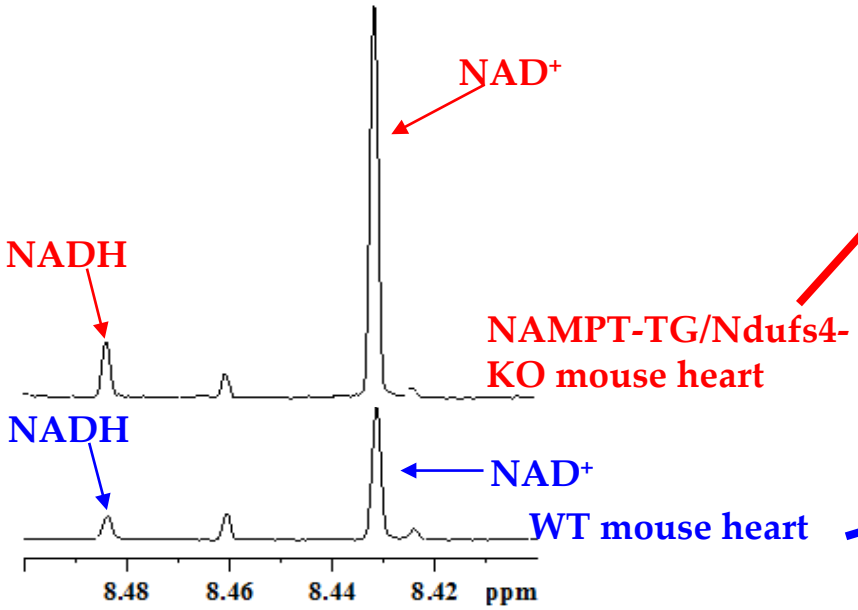
WT mouse heart

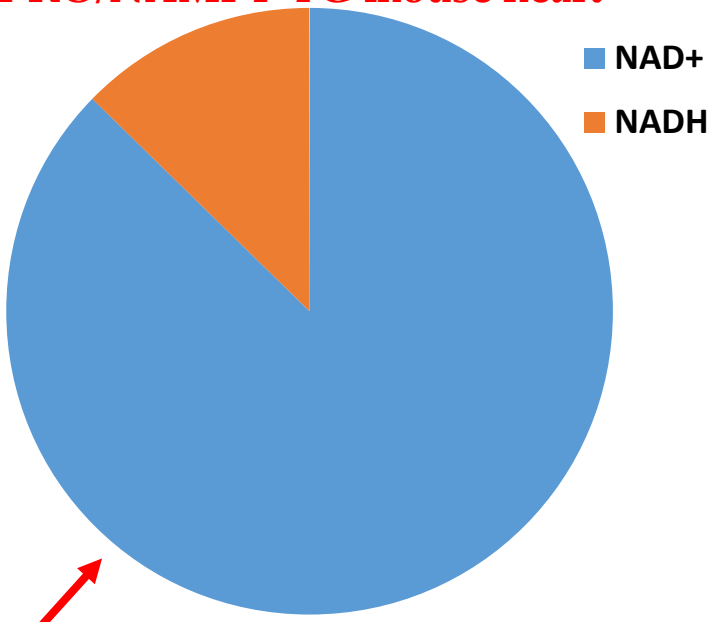


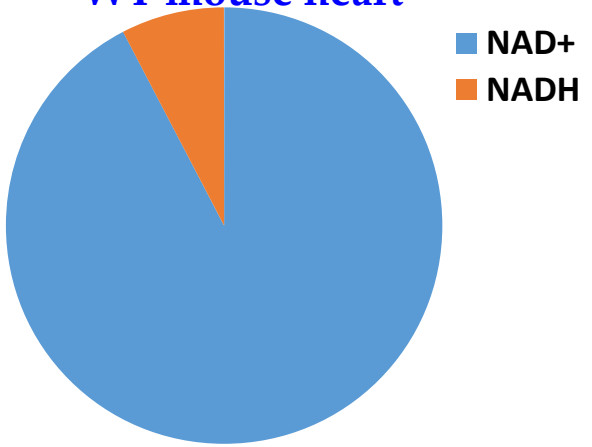
Results and Discussion

Both NAMPT-TG/Ndufs4-KO

- Ndufs4-KO: NADH ↑
- NAMPT-TG : NADH/NAD⁺ pool ↑

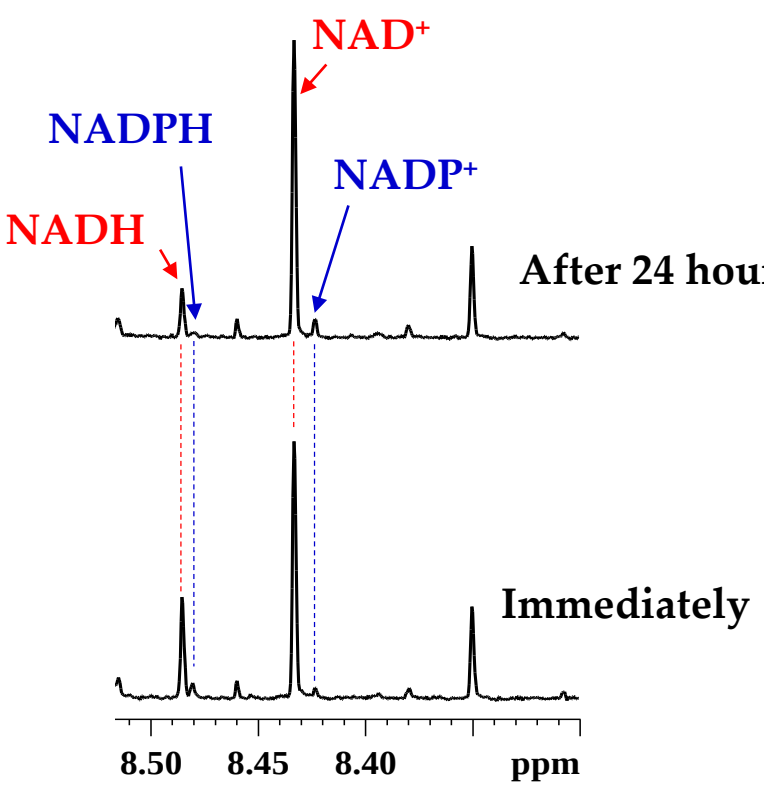




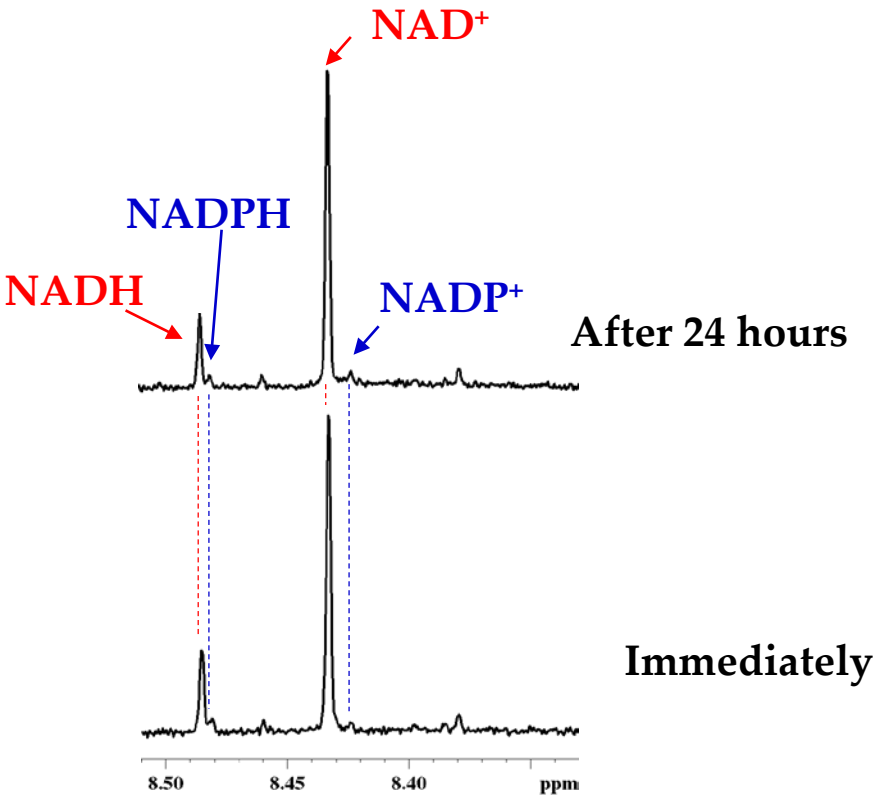


Results and Discussion

Degassing Halts NADH/NADPH Oxidation



No degassing

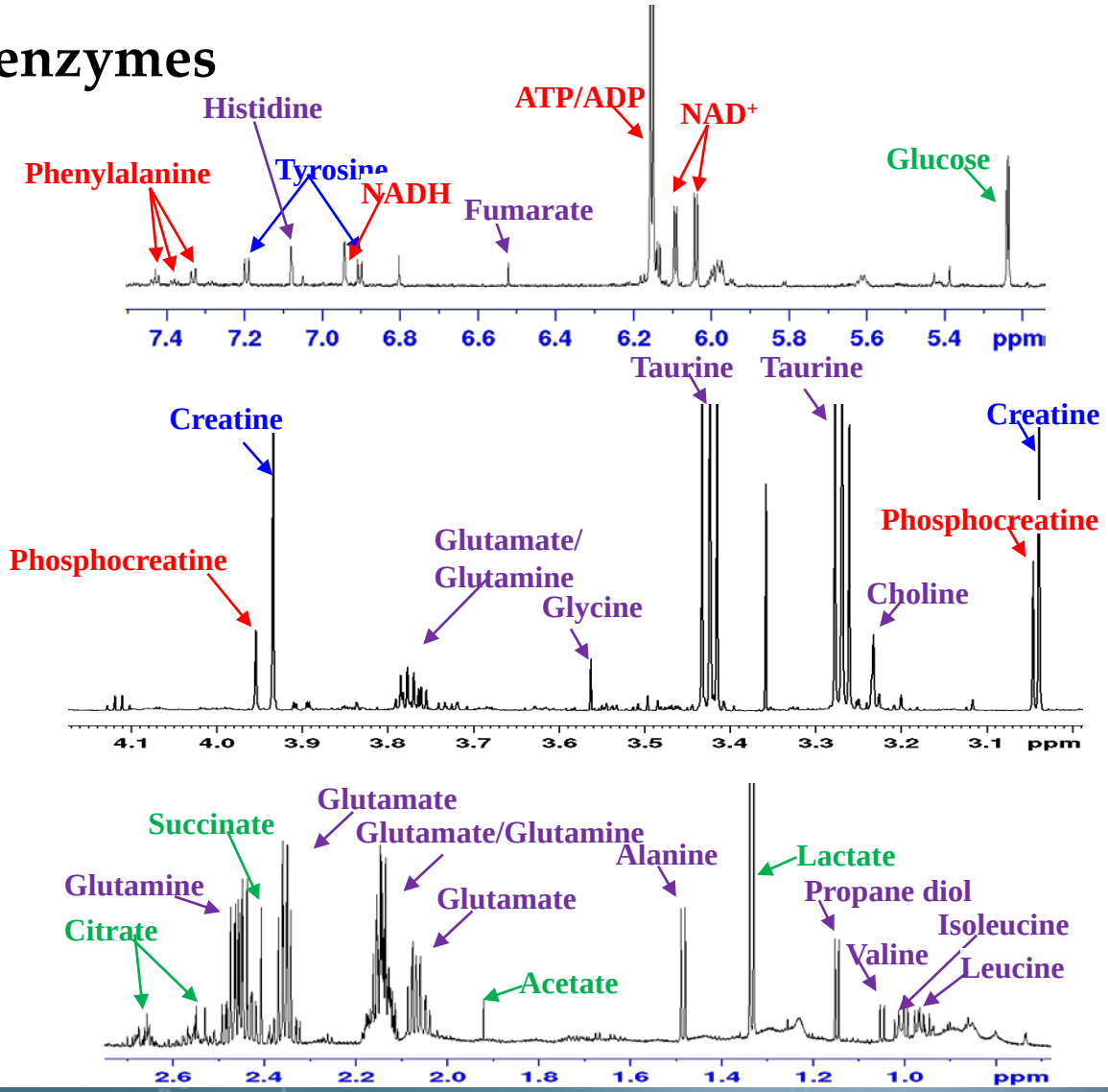


Degassing

Results and Discussion

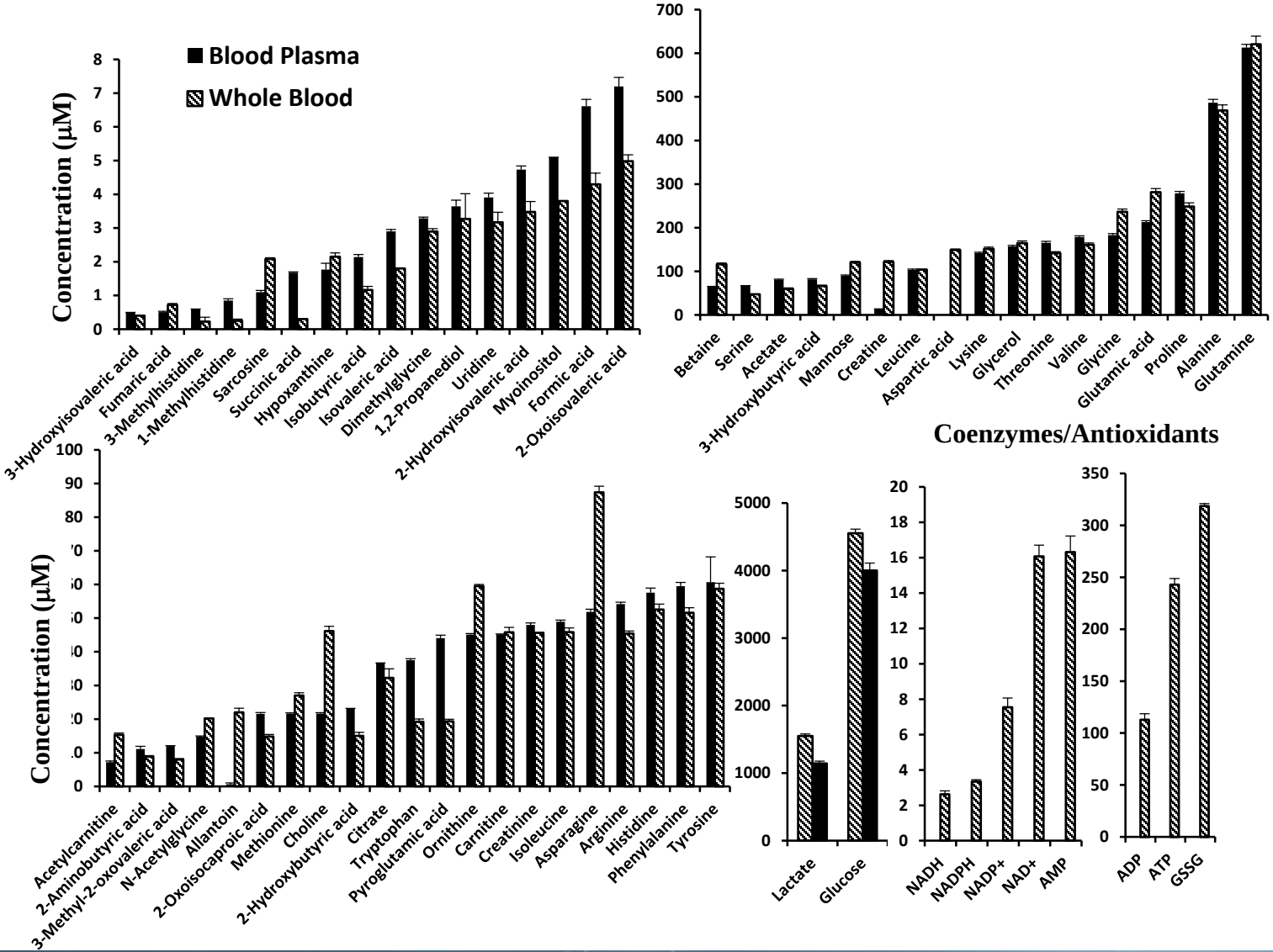
Much More Than the Coenzymes

- Antioxidants
- TCA Cycle
- Glycolysis
- Amino acids
- Carboxylic acids
- Creatine/Phosphocreatine



Results and Discussion

Concentrations of coenzymes, antioxidants and other metabolites in human blood



Conclusions

- We provide a simple NMR method for simultaneous quantification of coenzymes of cellular redox reactions and energy in tissue and blood.

- We demonstrate importance of harvesting/extraction methods for reliable analysis, as many coenzymes can evade detection wholly or partly depending on the method.

- We show that over a period of time NADH and NADPH levels diminish due to oxidation in solution; however, degassing can halt such a change.

- Most of the coenzymes can be measured using as little as 5 mg tissue and a further improvement in the detection limit can be achieved using sensitivity enhancement methods such as using micro-coil probes.

- The ability to measure coenzymes and antioxidants along with a vast pool of other metabolites using NMR potentially opens a new chapter in the metabolomics field.

Supplementary Materials

Links:

http://pubs.acs.org/doi/abs/10.1021/acs.analchem.7b00171

http://pubs.acs.org/doi/abs/10.1021/acs.analchem.6b00442

http://pubs.acs.org/doi/abs/10.1021/ac503651e

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UNIVERSITY of WASHINGTON

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NIH National Institute of Diabetes and Digestive and Kidney Diseases

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