Baseline Urinary Metabolite Profiles of Primary Mitochondrial Myopathy Subjects in the MMPOWER Study Compared with Literature Healthy Controls

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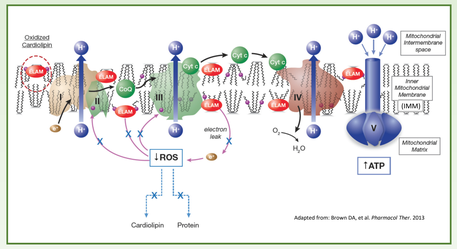
ABSTRACT

INTRODUCTION: MMPOWER was a multi-center, randomized, double-blind, three-dose-ascending, placebo-controlled study of elamipretide (ELAM) for 5 days in 36 subjects with genetically confirmed primary mitochondrial myopathy (PMM). The study demonstrated a dose-dependent (P=0.014) increase in six-minute-walk distance (6MWD) (Karaa et al., *Neurology,* 90: e1212-e1221, 2018). In the high-dose ELAM group, changes in urinary adipic acid and ornithine correlated with one another and with 6MWD improvement (*Muscle & Nerve*, 58 (S1): S13, 2018). To further define the metabolic profile of PMM subjects, we report here an analysis of baseline urinary concentrations of selected metabolites in *untreated* MMPOWER subjects (group PMM) relative to healthy literature controls (group N). **METHODS:** MMPOWER urine metabolites were quantified by LC-ESI-MS/MS in the UCSD Biochemical Genetics and Metabolomics Laboratory. Reference concentrations of 64 energy-linked metabolites for fasted healthy subjects, 3 years of age to adulthood, were compiled from the Human Metabolome Data Base (HMDB) and U.S. National Library of Medicine publications. HMDB listings were screened for referenced source, duplicates, and gender differences; multiple data values per publication were averaged. After interquartile range filtering, mean values for each metabolite in group N (average n=7 unique reports/metabolite) and in group PMM (n=33/metabolite) were compared using logarithmic transformation followed by univariate statistical analyses with correction for multiple comparisons at Q=1%. **RESULTS**: Coefficients of Variation (SD/mean) of 64 metabolites in groups N and PMM averaged 62% and 69%, respectively. Unless otherwise noted, all changes mentioned below were considered statistically significant when P<0.01. Compared to group N, PMM baseline urine concentrations for hexoses, lactate, citrate, aconitate and malate were low (14-48% of N), whereas isocitrate was elevated >2-fold. PMM succinate was also increased ~2-fold, but was variable (P>0.01 vs. N). PMM aspartate was ~8-fold elevated with ~2- to 3-fold rises in glycine, proline, serine, and threonine while ornithine was below normal (42% of N). PMM 3-hydroxybutyrate and adipate were normal, while acylcarnitines were normal or low, e.g., lauroylcarnitine and myristoylcarnitine were 4% and 18% of N, respectively. PMM urate was elevated 36% (P=0.02), while adenine, adenosine, cytidine, hypoxanthine and inosine were 4-26% of N. By contrast, uracil and orotic acid were ~8- and 10-fold above N, respectively. **CONCLUSIONS**: These data reveal a urinary metabolic baseline profile in PMM subjects suggesting that they exhibit marked energy thriftiness and substantial ATP expenditure to detoxify ammonia via synthesis and excretion of urea, pyrimidines and some NH_3-consuming amino acids. Excess flux of ammonia presumably results from enhanced proteolysis consequent to impaired citric acid cycle energy metabolism caused by increased intramitochondrial NADH/NAD+. In addition to robust glycolysis and gluconeogenesis, increased proteolysis and extra-mitochondrial fatty acid oxidation appear to support increased energy production via succinate metabolism through Complex II. These results underpin a previous MMPOWER analysis indicating that increased energy efficiency associated with ELAM treatment in PMM is manifest by a dose-dependent hierarchy of metabolic effects: 1) stabilization or reduction of proteolysis and ATP-requiring ammonia detoxification, 2) reduction of resting fuel consumption and/or increased 6MWD capability.

INTRODUCTION

- Elamipretide (ELAM) is a peptide that transiently localizes in mitochondria and improves bioenergetics. Data suggests that by associating with cardiolipin, ELAM stabilizes mitochondrial cristae and improves the efficiency of oxidative phosphorylation (1). ELAM was found also to reduce mitochondrial ROS emission without detectable *in vitro* scavenging (2).

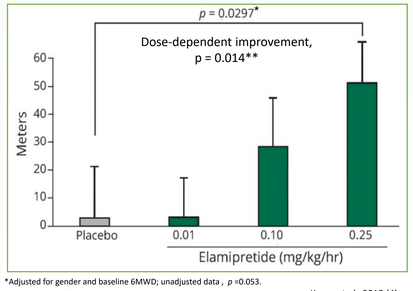


- Preclinical study with ELAM showed rapid improvement of ATP production in skeletal muscle of aged mice (3), and the MMPOWER clinical study of ELAM with primary mitochondrial myopathy (PMM) subjects found a dose-dependent improvement in 6-minute walk test distance (6MWD) (4).

MMPOWER Phase 1/2 Study

Efficacy Study in Primary Mitochondrial Myopathy

Change in Distance Walked at Day 5



*Adjusted for gender and baseline 6MWD; unadjusted data, p =0.053.
**Mixed model for repeated measures, day 5.

Karaa *et al.*, 2018 (4)

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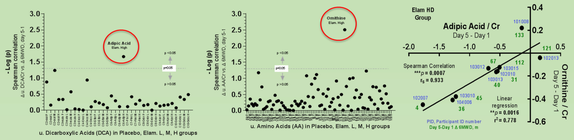
INTRODUCTION (cont'd)

MMPOWER CORRELATIONS: Δ 6MWD VS. Δ URINARY METABOLITES

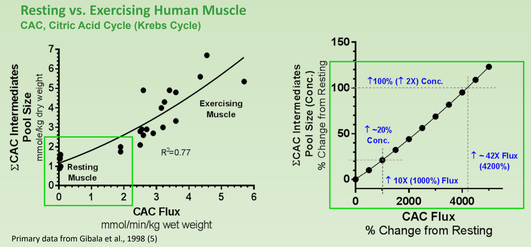


- Correlations between Δ 6MWD and Δ metabolites found in HD group only
- Increased Δ 6MWD correlated with Δ adipic acid (p = 0.0074, r^2 =0.67)
- Improved 6MWD also correlated with Δ ornithine (p = 0.0054, r^2 = 0.69)
- *Per HD ELAM subject*, Δ adipic & Δ Orn correlated (***p* = 0.0007**, r_s**=0.933**)
- Consistent w. ↑ ATP_{max} ELAM HD: ↑ 6MWD and ↓ "Resting" Fuel Demand

CHANGE IN (Δ) METABOLITE CONCENTRATION vs. Δ METABOLIC FLUX

Resting vs. Exercising Human Muscle

CAC, Citric Acid Cycle (Krebs Cycle)



Primary data from Gibala et al., 1998 (5)

- CAC metabolite concentration is directly proportional to CAC flux
- From resting, % Δ Flux relative to % Δ metabolite conc. ~ 40:1
- ***Small* changes in CAC metabolite *concs.*** linked to ***large* changes in *flux***

OBJECTIVE

- The objective of this exploratory urinary metabolomic analysis is to further define the metabolic profile of PMM subjects by estimating baseline urinary concentrations of selected metabolites in untreated MMPOWER subjects (group PMM) relative to healthy literature controls (group N).

MATERIALS and METHODS

CLINICAL STUDY DESIGN

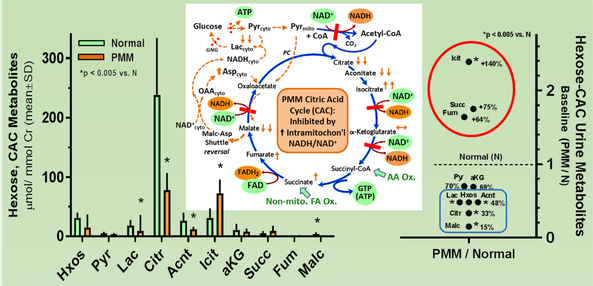
- MMPOWER (SPIMM-201) was a multi-center, randomized, double-blind, 3-dose-ascending, placebo (PCBO)-controlled study of **36 PMM participants with 4 treatment groups, n = 9/group**: PCBO, 0.01, 0.1, or 0.25 mg/kg, i.v., ELAM once-a-day for 2 hours/day X 5 days.
- Each subject was sampled for exploratory urine metabolomics (first morning void, FMV) three times: pre-drug Baseline (Day 1), pre-drug Day 5 (last dose), follow-up day 7 (washout).

URINARY METABOLOMICS

- PMM Group. 106 urine samples were analyzed for 294 metabolites by LC-ESI-MS/MS in Dr. Bruce A. Barshop's laboratory at UCSD. Data were normalized by stable-isotope dilution and urinary Creatinine (Cr).
- Normal Reference Values: Reference concentrations of 64 energy-linked metabolites for fasted healthy subjects, 3 years of age to adulthood, were compiled from the Human Metabolome Data Base (HMDB) and U.S. National Library of Medicine publications. HMDB listings were screened for referenced source, duplicates, and gender differences; multiple data values per publication were averaged. After interquartile range filtering, mean values for each metabolite in group N (average n = 7 unique reports/metabolite) and in group PMM (n = 33/metabolite) were compared using logarithmic transformation followed by univariate statistical analyses with correction for multiple comparisons at Q = 1%.

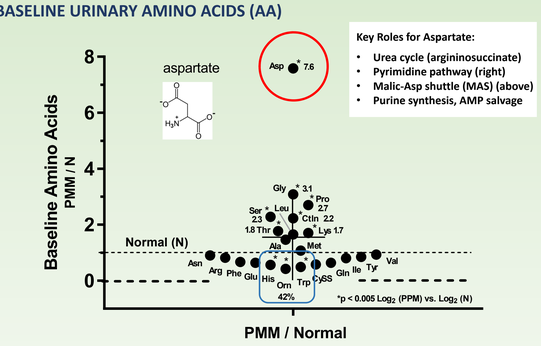
RESULTS and DISCUSSION

BASELINE URINARY HEXOSE AND CITRIC ACID CYCLE METABOLITES



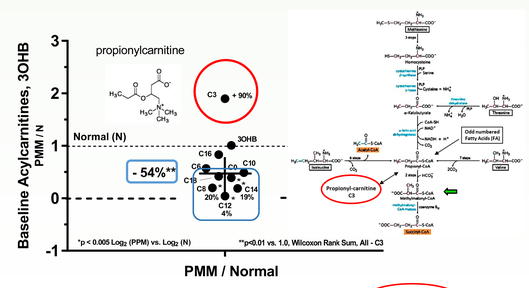
- PMM First Morning Void Creatinine baselines, 11.8±5.5 (34) mM, were not different from Normal, 10.1±2.2 (15) mM, *p*=0.67, NS (data not shown)
- PMM isocitrate (2.4X), succinate (75%), fumarate (64%) were high vs. N
- Hexose, lactate, citrate, aconitate and malate were low (14-48% of N)
- CAC and hexose metabolite profiles are consistent with
  - ↑ intramitochondrial NADH/NAD+ in PMM, e.g., consequent to Complex I dysfn.
  - ↑ Succinyl-CoA & succinate metabolism, e.g., bypassing Complex I deficiency
  - Malate-Aspartate Shuttle (MAS) "reversal": ↓ intramito'l NADH (see below also)

BASELINE URINARY AMINO ACIDS (AA)



- **PMM Aspartate** strikingly **up 7.6-fold** – consistent with multiple roles
  - ↑ Reverse Malate-Aspartate shuttle activity (see above): ↓ intramito. NADH
  - ↑ Pyrimidine biosynthesis for NH_3 detox and energy: ↑ $CoQH_2$ (see right)
  - ↑ Urea Cycle activity, arginosuccinate; ↑ NH_3 detox from ↑ proteolysis
  - ↑ Purine synthesis and AMP salvage from IMP: IMP + Asp > AMP + Fum
- PMM citrulline (Ctln) ↑ 2.2X, but ornithine (Orn) 42%: ↑ NH_3 ,↑ proteolysis
- PMM glycine, serine ↑ 2-3X; syntheses consume NH_3 from ↑ proteolysis

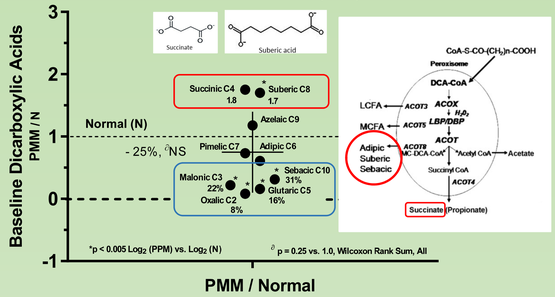
BASELINE URINARY ACYLCARNITINES, 3-HYDROXYBUTYRIC ACID



- PMM **C3,** precursor to succinyl-CoA from AA Oxidation, **↑ 90% ;** variable, NS
- Excepting C3, **PMM acylcarnitines 54% below N** (p<0.01): ↑ FA oxidation
- C12, C14 v. low: *non-mito'l* FA omega and peroxisomal oxidation to succinate

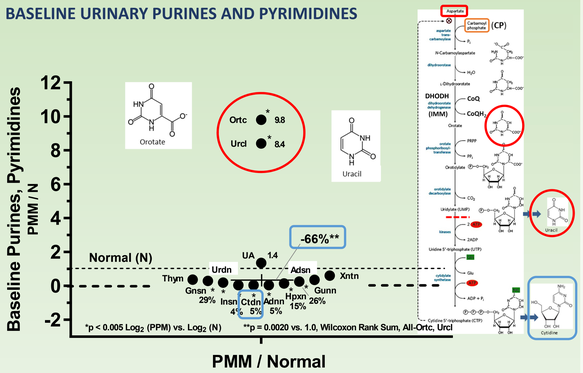
RESULTS and DISCUSSION (cont'd)

BASELINE URINARY DICARBOXYLIC ACIDS (DCA)



- PMM baseline suberic and succinic acids elevated ~2X vs. N
- Succinic and suberic are products of **peroxisomal** fatty acid oxidation
- Adipic, pimelic acids are ~ N; others, e.g., oxalic C2, are below N

BASELINE URINARY PURINES AND PYRIMIDINES



- Pyrimidines orotic acid (Ortc) and uracil (Urcl) ↑ 8-10X (like Aspartate)
  - CP, excess from Urea Cycle or made in cytoplasm, reacts w. Asp
  - Dihyroorotate dehydrogenase (DHODH) step provides $CoQH_2$ to IMM
- Remainder ↓ average of -66% of N, although uric acid (UA) ↑ 40% (p=0.03)
- ↓ adenosine, inosine, hypoxanthine with ↑ UA: ↑ AMP degradation, low ATP

CONCLUSIONS

- New alogrithm developed to reach "consensus" Normal metabolite values
- PMM/N: ↑ Icit, Succ, Fum suggests: ↑ mito NADH/NAD+, ↑ Succ metabolism
- PMM/N: ↑ Asp, MAS reversal, ↑ UC activity (AA oxid'n), Ortc, AMP synthesis
- PMM/N: Gly, Ser, Ctln ↑ ~2X, Orn 42%: suggests ↑ NH_3 detox, ↑ proteolysis
- 90% ↑ C_3-carnitine suggests ↑ AA oxidation > succinyl-CoA production
- ↓ C_{12}, C_{14} carnitines: suggest ↑ microsomal (ω-)/peroxisomal β-FA oxidation
- ↑ Ortc, Urcl: energy from DHODH; ↑ UA and low purines: ↑ AMP degradation
- Functionally important metabs (Orn, Adpc) not evident from baseline concs.
- Results provide 1) a new metabolite-based resting "PMM energy profile", 2) a metabolic framework for elucidation of ELAM's positive effects in PMM

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ACKNOWLEDGEMENTS

The authors wish to thank all members of the Stealth Biotherapeutics Development Team, members of the Phase 2 Clinical Team and all participants and their supporters who participated in the MMPOWER study. We also thank Julie M. Romano and James A. Shiffer, RPh, of Write On Time Medical Communications, LLC for their assistance acquiring relevant literature reports.