Acute Mountain Sickness:
Validating Risk Level Biomarkers and Investigating High Altitude Induced Alternative Energy Recruitment Mechanisms

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Non-Disclosure

Camilla Mauzy Ph.D. has no conflicts to report.

High Altitude Exposure

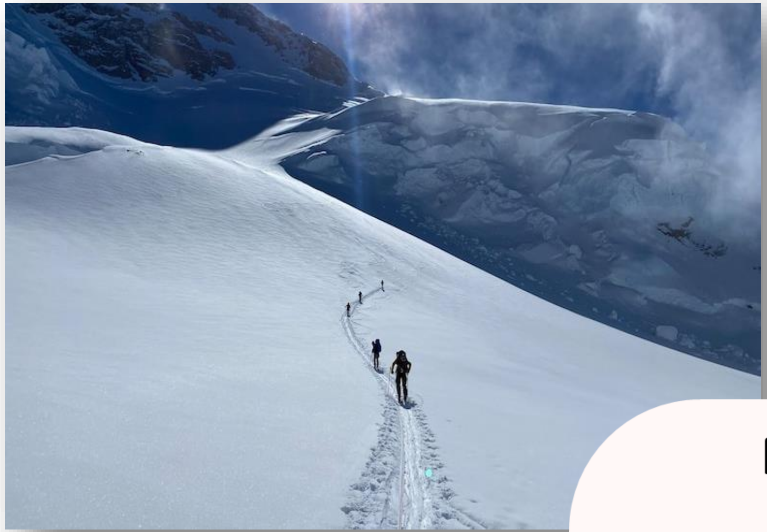


IMAGE: https://www.af.mil/News/Article-Display/Article/2696021/highest-risk-biggest-reward-planning-for-denali/
Disturbed eating at high altitude: influence of food preferences, acute mountain sickness and satiation hormones - PubMed (nih.gov)
https://pubmed.ncbi.nlm.nih.gov/10817145/

High altitude-induced hypobaric hypoxia triggers physiological responses which are highly variable in humans.

Acute Mountain Sickness (AMS) susceptible individuals develop symptoms after ascent to high altitudes of ≥ 2500 m (8200 ft).

Mild AMS (mAMS)

- Dizziness
- Insomnia
- Headache
- Rapid heartbeat
- Muscle aches
- Nausea and vomiting
- Irritability
- Loss of appetite
- Swelling of the hands, feet, and face
- Shortness of breath with physical exertion

Severe AMS (sAMS)

Additional symptoms, including

- Coughing
- Chest congestion
- Pale complexion and skin discoloration
- Inability to walk or lack of balance
- Social withdrawal

Metabolomic Analysis Pipeline

Human Cohort Testing

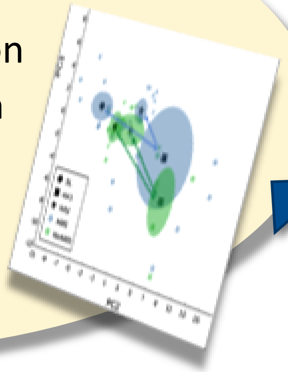
- **High Altitude Exposure**
- **Cohort of fit personnel (men or women, age 18-39)**
- **At both SL and HA**:
  - Physiological monitoring (SpO_2)
  - Environmental Symptoms Questionnaire (ESQ) to measure severity of AMS.
  - Sleep assessments, cognitive/mood evaluations, GI symptoms questionnaire, Physiologic status monitoring, Metabolic evaluation, electrolyte evaluation, Gut barrier eval, inflammation eval, hypoxic gene responses, and Hematologic Responses
  - Activity monitoring
  - Collect urine samples

Metabolomics Analysis

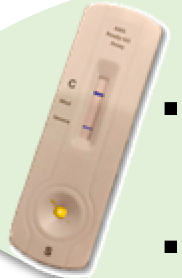
- **NMR-based metabolomics (850 MHz or 1.2 GHz)**
- **Acquire spectra from urine samples from sea level and at altitude**
- **AMS Symptom quantification**
  - **Lake Louise LLS**
  - **Environmental Symptom ESQ Scoring**
    - 0.7 No AMS
    - >0.7 to < 1.53 mAMS
    - > 1.53 sAMS
- **Conduct multivariate data analyses on binned and scaled spectral data Principal component analysis (PCA), both supervised and unsupervised**

Biomarker Validation

- Secondary Method Validation
- Additional cohort evaluation
- Responsivity
- Detection



Assay Development



- Development of biorecognition elements to target metabolites
- Lateral Flow Assay

Separation of AMS vs. NoAMS groups seen *at Sea Level*

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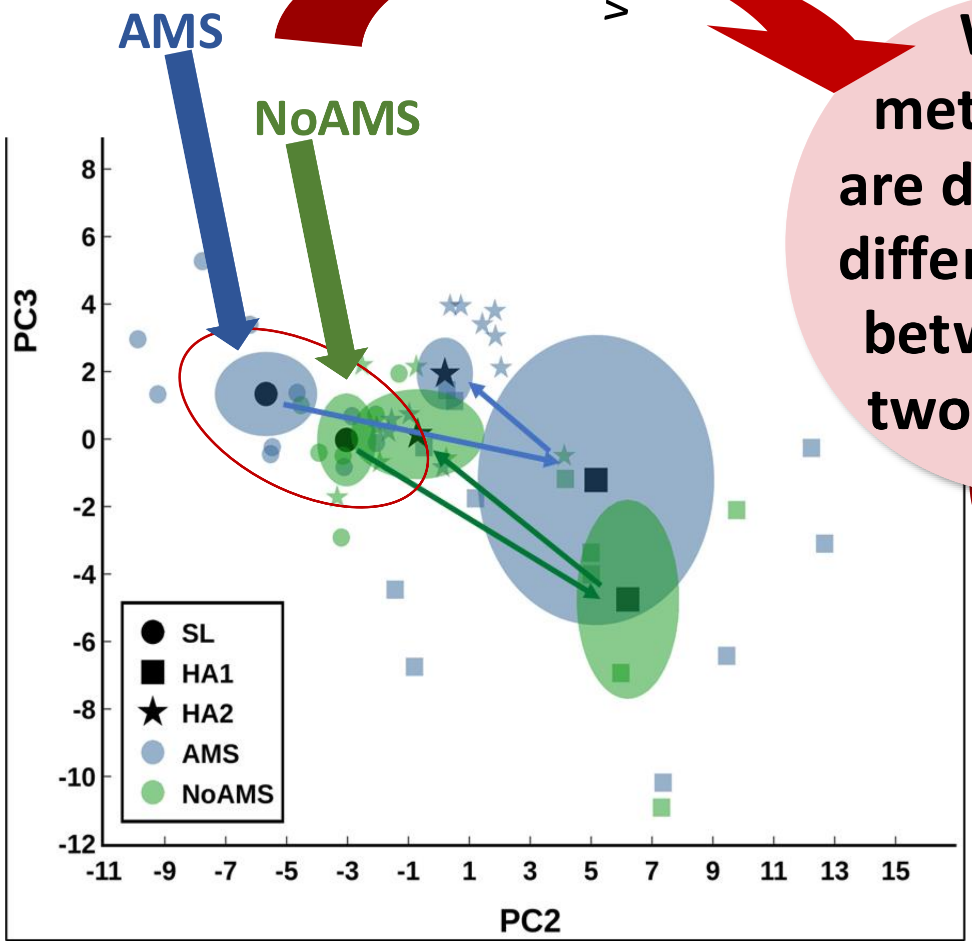
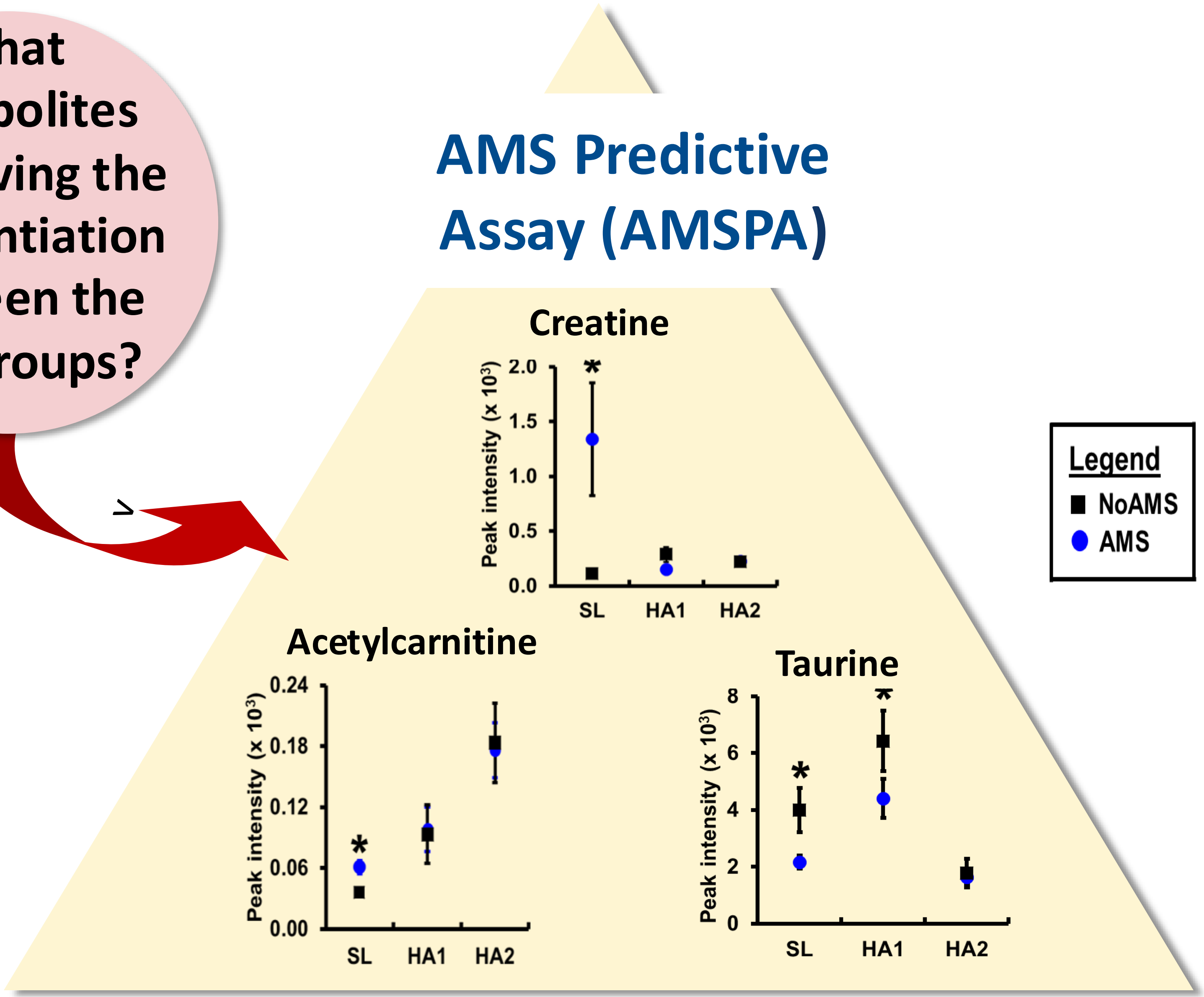


PCA scores plot modeling the urine data for AMS group at sea level (SL) and high altitude at Day 1 (HA1).

What metabolites are driving the differentiation between the two groups?



Biomarker Validation

Validation Studies need data, data, data, data… and many collaborations!

High Altitude study 1 : USAREIM Beidleman/Karl (17 subjects): Published

High Altitude study 2: USARIEM Beidleman/Karl (41 subjects): Recently Published

High Altitude study 3: USARIEM Beidleman/Karl (38 subjects)

High Altitude study 4: USARIEM Beidleman/Karl (32 subjects)

High Altitude study 5: USARIEM Seeley (16 subjects): Altitude Chamber Study

High Altitude study 6: Univ de Atacama Garotte (58 subjects): Chilean Study

High Altitude study 7: NHRC-San Diego Kelly *Initiated:* delayed due to Furlough

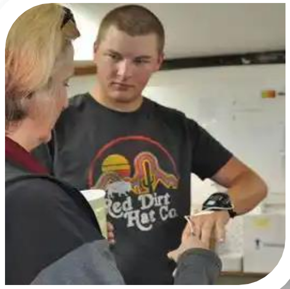
High Altitude study 8: USARIEM Beidleman *in progress*

High Altitude study 9: NAMRU-D Robinson *Initiated:* Altitude Chamber Study

High Altitude study 10: Nepal, NAST. *Planning*

High Altitude study 11: Univ de Atacama. *Planning*

DOD and OCONUS Collaborations



US Army Research institute of Environmental Medicine

Beth Beidleman Ph.D.
J. Phillip Karl Ph.D.
Afton Seeley Ph.D.

Human Altitude Studies for Biomarker Validation

SOARD
Universidad de Atacama

Copiapó, Chile

Claudio Allard Garotte Ph.D.

Human Altitude Studies – Possible Cofounders

- Gender differences in Metabolite Profiling
- Diet Interventions

Naval Medical Research Unit - Dayton

F. Eric Robinson Ph.D.

Human Hypoxia Chamber Studies – individual metabolic signatures

Navy Health Research Center San Diego

Karen Kelly Ph.D.

AMSPA Validation, Targeted intervention

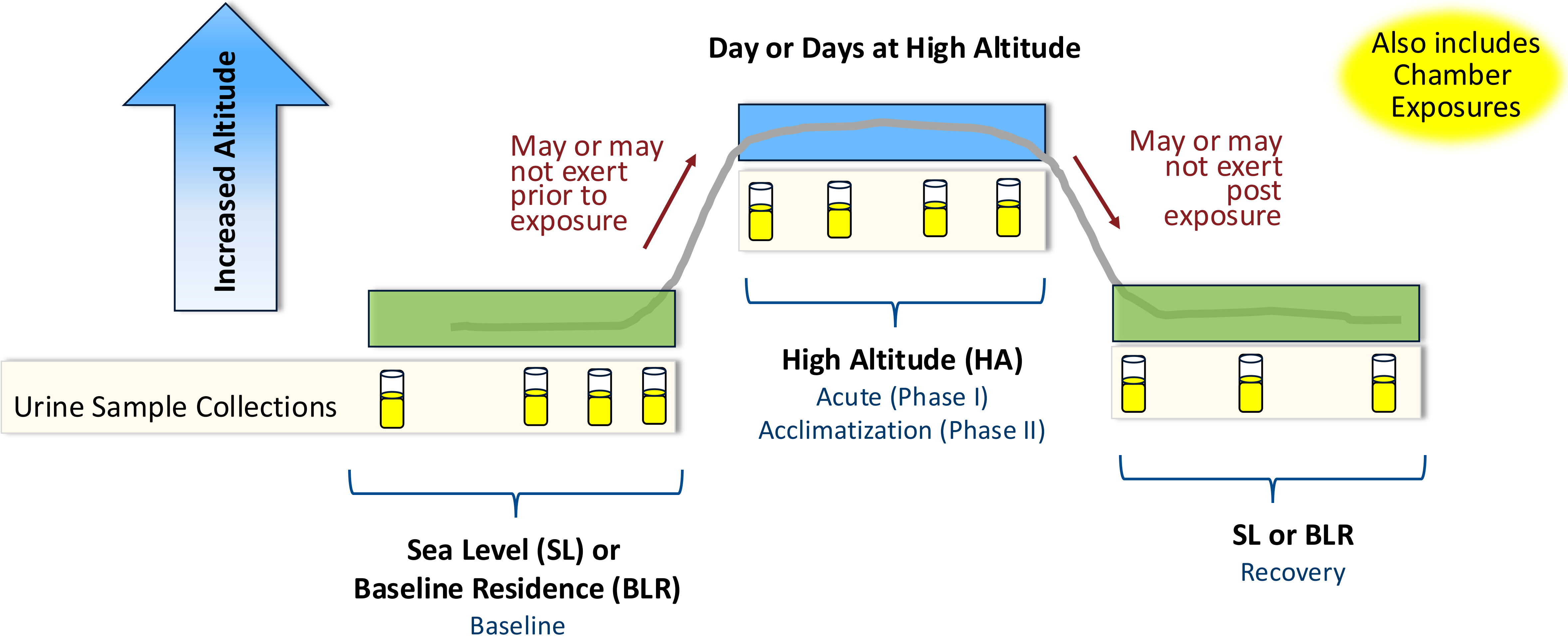
PLANNED AOARD
Nepal Academy of Science and Technology

Lalitpur, Nepal

Sajan Shyaula Ph.D.

Human Altitude Studies – Altitude exposure, Acclimatization

General High Altitude Human Study Design



Clarifying the Hypoxic Metabolomic Response Signature

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Normoxia
Sea Level

What are the urine metabolite signatures of AMS high-risk individuals in females?

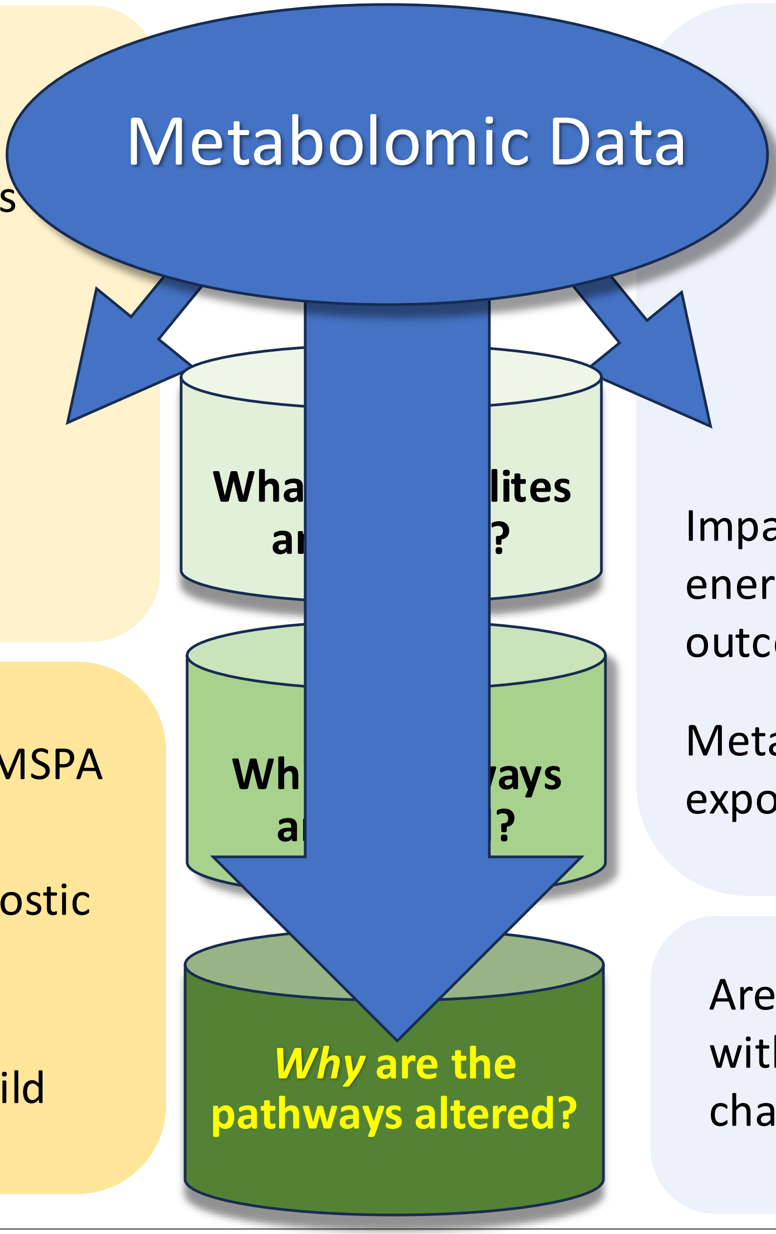
Why is the metabolite profile altered in subjects with higher risk of AMS?

Can AMSPA biomarkers be used to monitor dietary/prophylaxis outcome?

How far out (days prior to altitude exposure) does AMSPA demonstrate accuracy?

Does gender impact diagnostic efficiency AMS Predictive Signature?

Establish biomarker test range(s) for mild and severe AMS risk.



Hypobaric Hypoxia
High Altitude

Do we see an Acute response and an Acclimatization response?

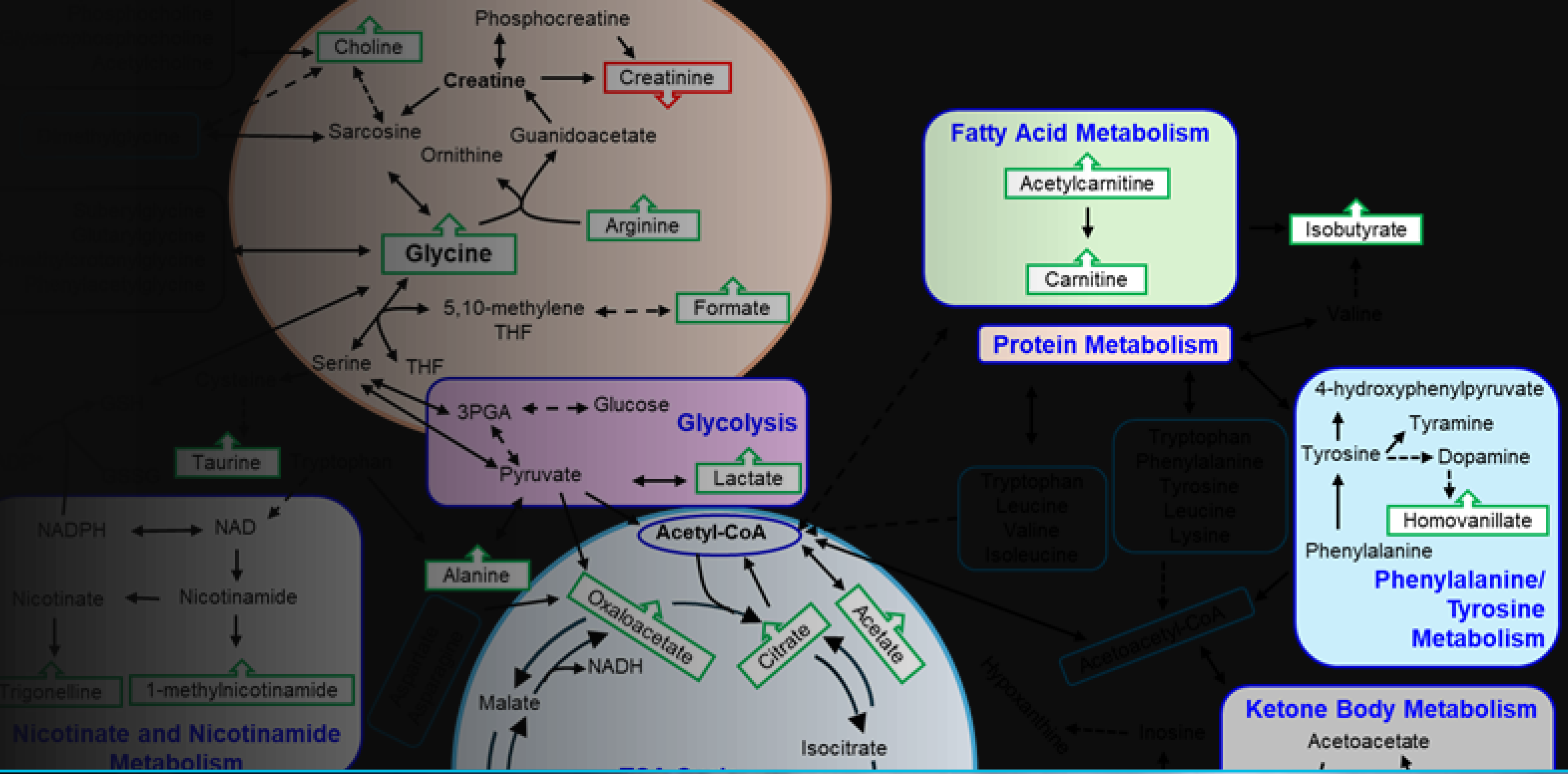
Are females more/less susceptible to hypoxic-induced outcomes?

Impact of energy deficient or additional energy expenditure on Acute Exposure outcome and Acclimatization?

Metabolite Response at different masl altitude exposures? Dose Response or other?

Are AMSPA metabolite signatures similar without hypobaric conditions, (altitude chambers)?

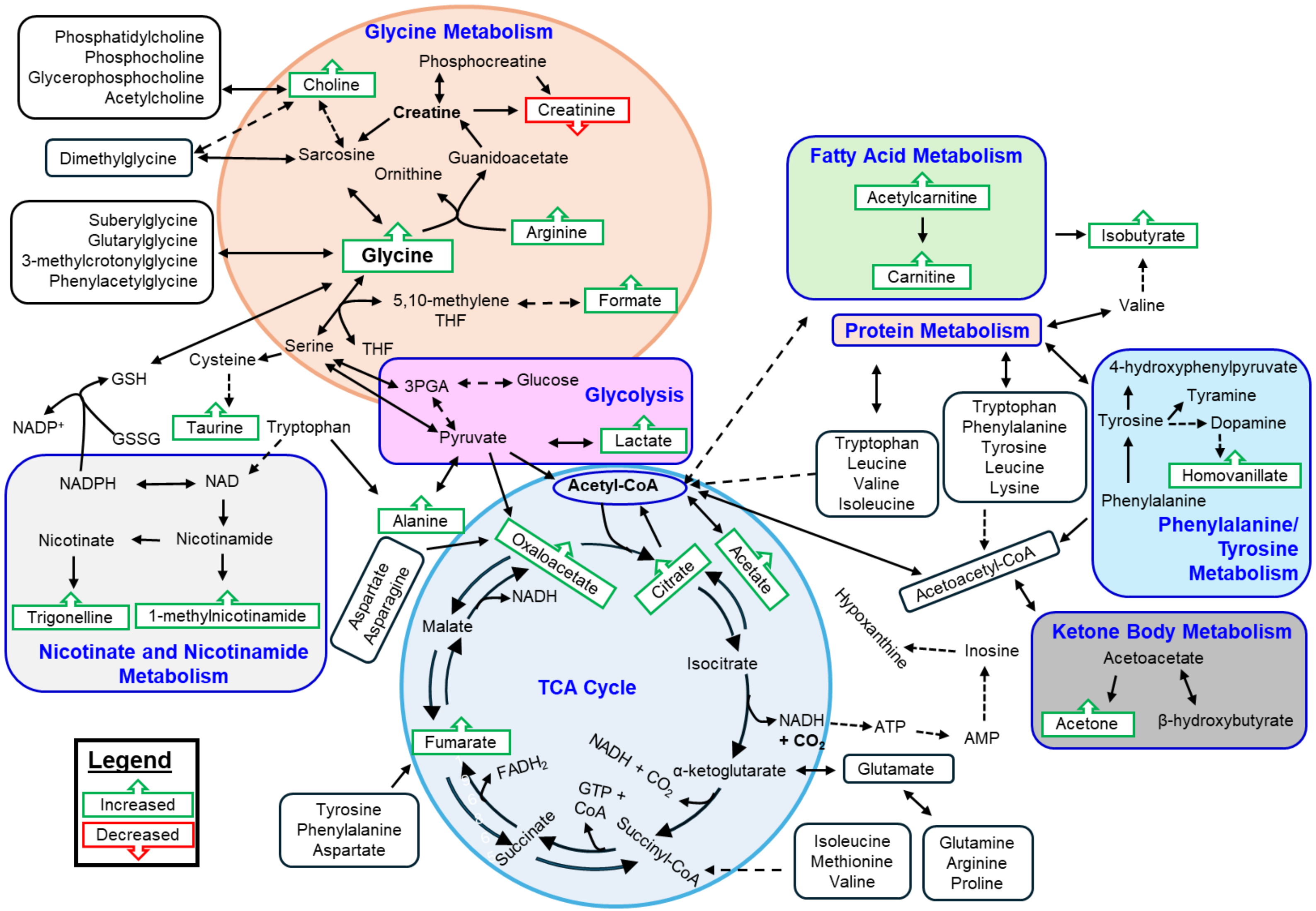
Shifts in Alternative Energy Recruitment



Shifts in Alternative Energy Recruitment

Current Hypotheses in Pathway Shifts in Energy Resourcing

- **Passive Ascenders**
- Metabolite shifts at HA1 (Acute Phase)
- Shifts due to High Altitude ONLY
- Normal response to hypobaric hypoxia includes increased urinary clearance of glycine, arginine, formate, and decreased creatinine excretion.

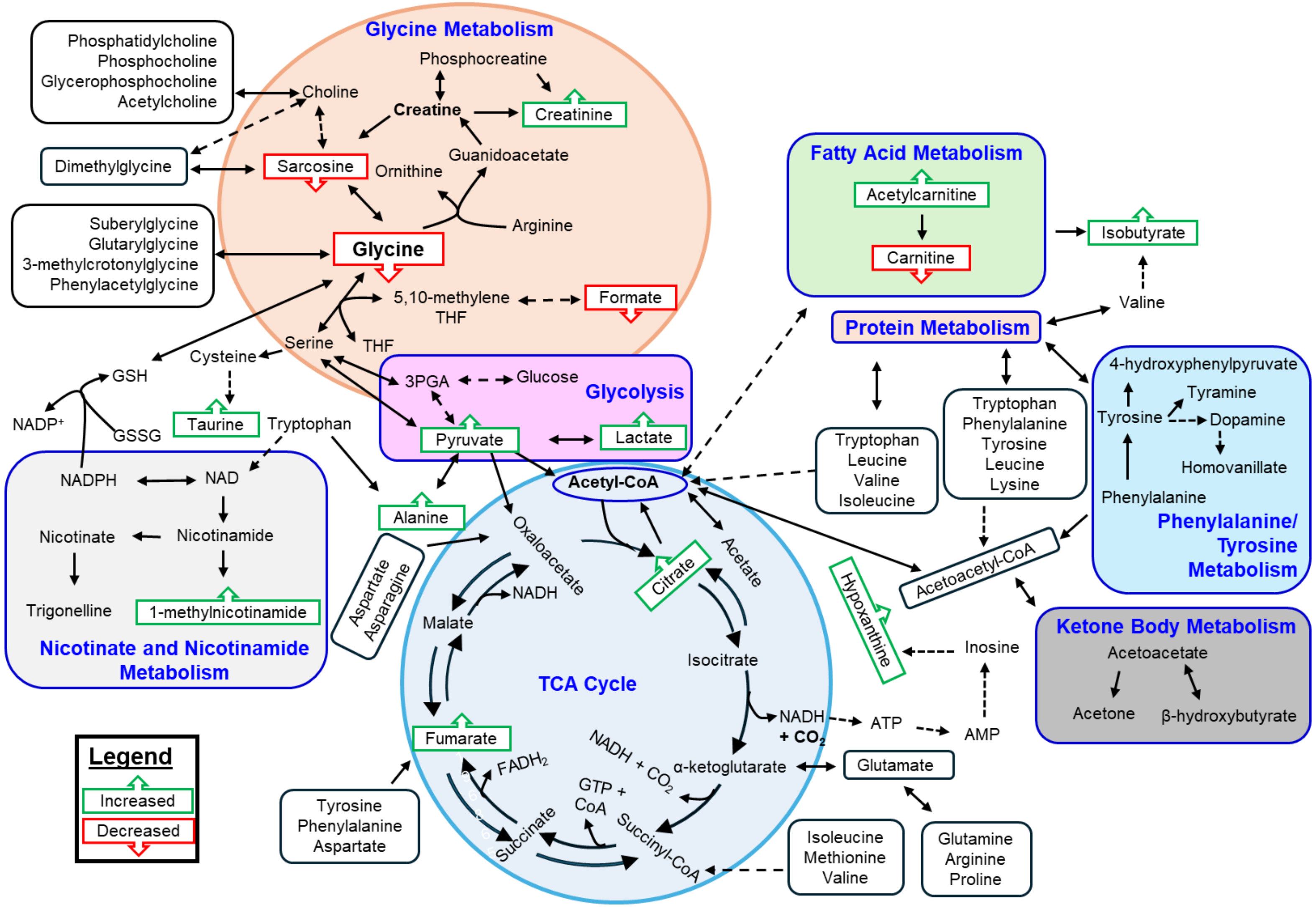


Meta Analysis Reveals Alterations in Glycine Metabolism with Increased Energy Demand Under Hypobaric Hypoxia. Submitted. Sibomana et al.

Shifts in Alternative Energy Recruitment

Current Hypotheses in Pathway Shifts in Energy Resourcing

- **Active Ascenders**
- Metabolite shifts at HA1 (Acute Phase)
- Metabolite shifts due to High Altitude <u>and</u> Hiking
- Physical exertion during ascent altered normal hypoxia responses:
  - ➢ Limited clearance of glycine and its by-products (arginine, formate).
  - ➢ Decreased excretion of creatine and sarcosine.
  - ➢ Changes in clearance of energy substrates (pyruvate, lactate, citrate, fumarate, oxaloacetate, carnitine).
  - ➢ Altered clearance of NAD and ATP degradation by-products (e.g., 1-methylnicotinamide, hypoxanthine).

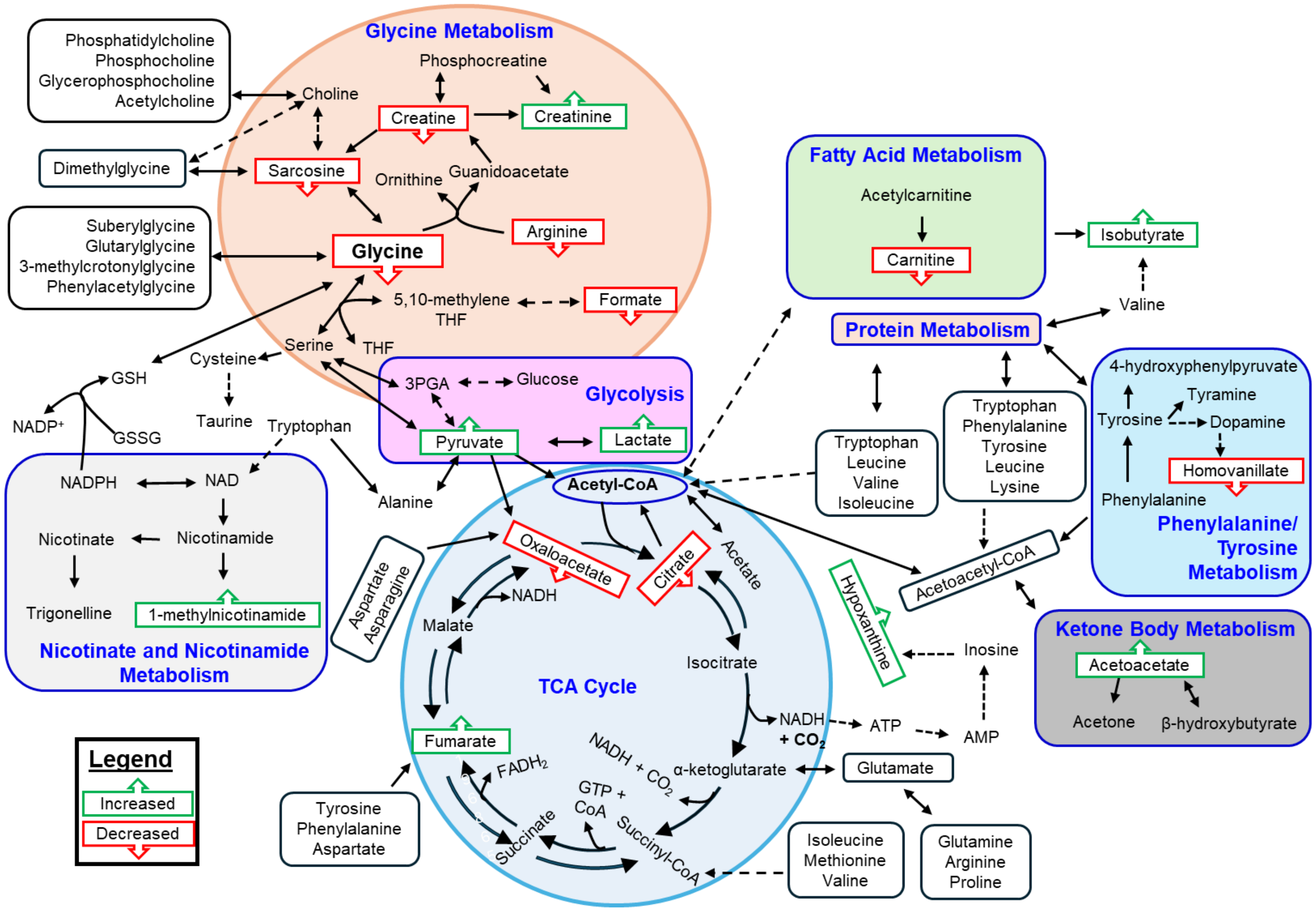


Meta Analysis Reveals Alterations in Glycine Metabolism with Increased Energy Demand Under Hypobaric Hypoxia. Submitted. Sibomana et al.

Shifts in Alternative Energy Recruitment

Current Hypotheses in Pathway Shifts in Energy Resourcing

- **Active vs. Passive Ascender Metabolite Profile Comparison**
- Metabolite shifts at HA1 (Acute Phase)
- Glycine by-products actively utilized for creatinine synthesis under hypobaric hypoxia.
- Physical activity just prior or during hypoxia increases energy demands, leading to altered glycine metabolism and cellular responses.
- Physical exertion impacts metabolic pathways, creating a shift in energy production processes and affecting cellular homeostasis of key substrates for energy.



Meta Analysis Reveals Alterations in Glycine Metabolism with Increased Energy Demand Under Hypobaric Hypoxia. Submitted. Sibomana et al.

Hypotheses and Thoughts

- **Data indicate that a lag in alternative energy resourcing may be one aspect associated with AMS risk.**

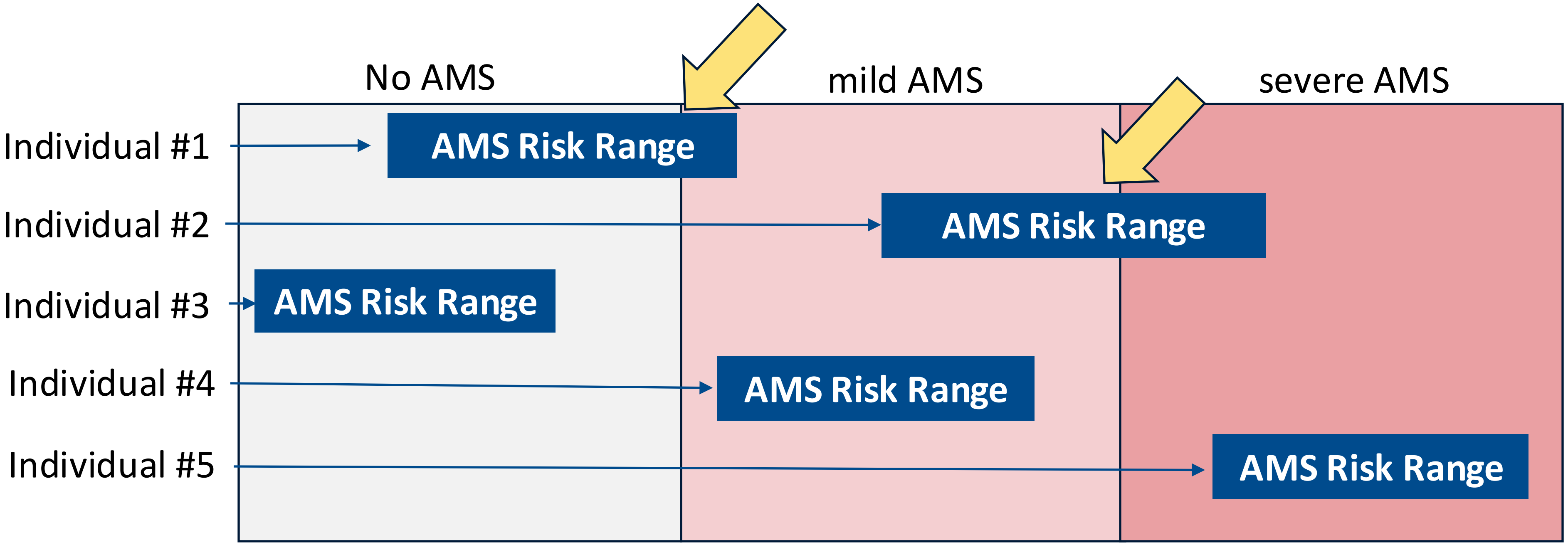
  But not the only one!

- **Is AMS risk temporal or permanent?**

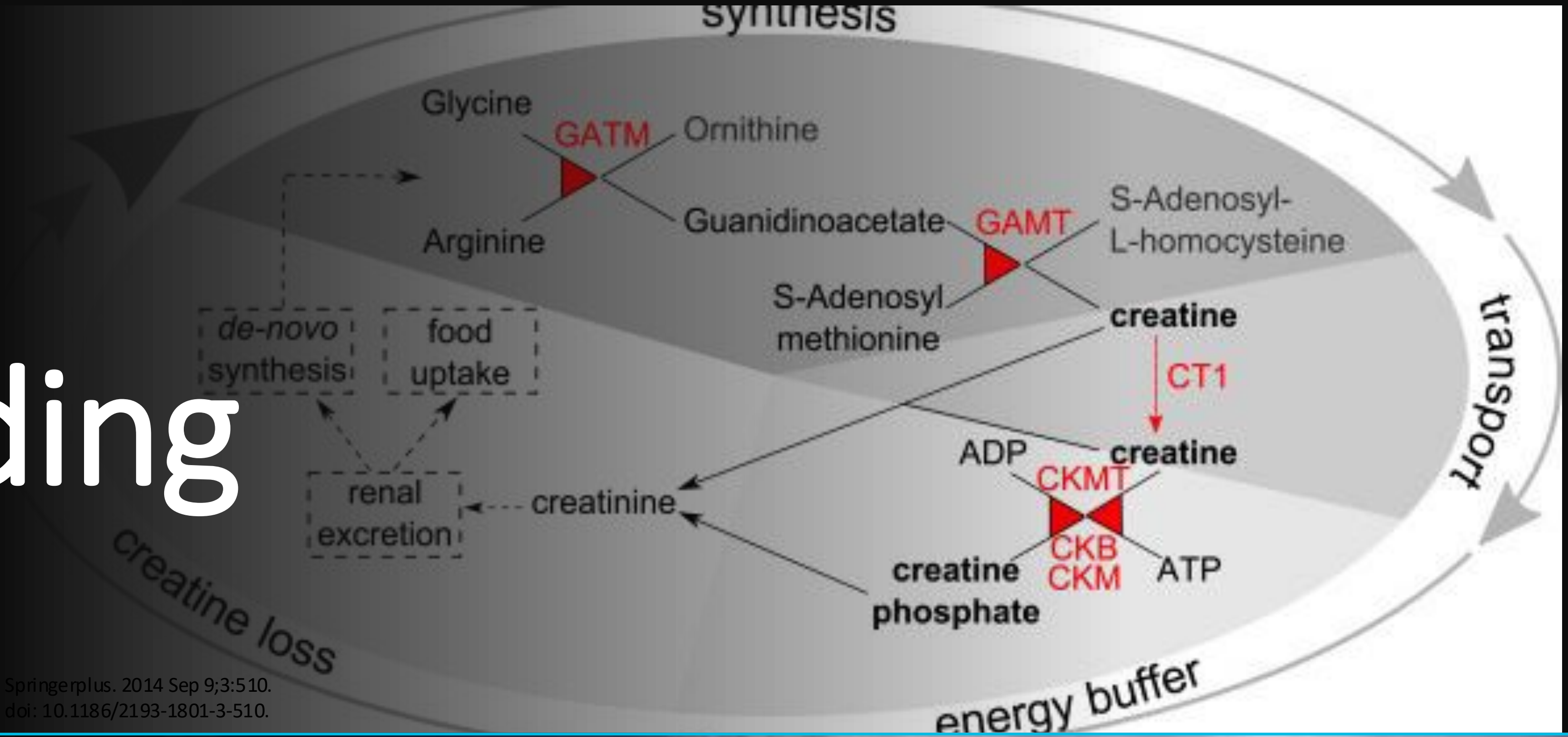
  Subjective data indicate that it may be temporal – even Sherpas can get AMS!

  <u>High level of fitness does not prevent it</u>.

  Can it be both?

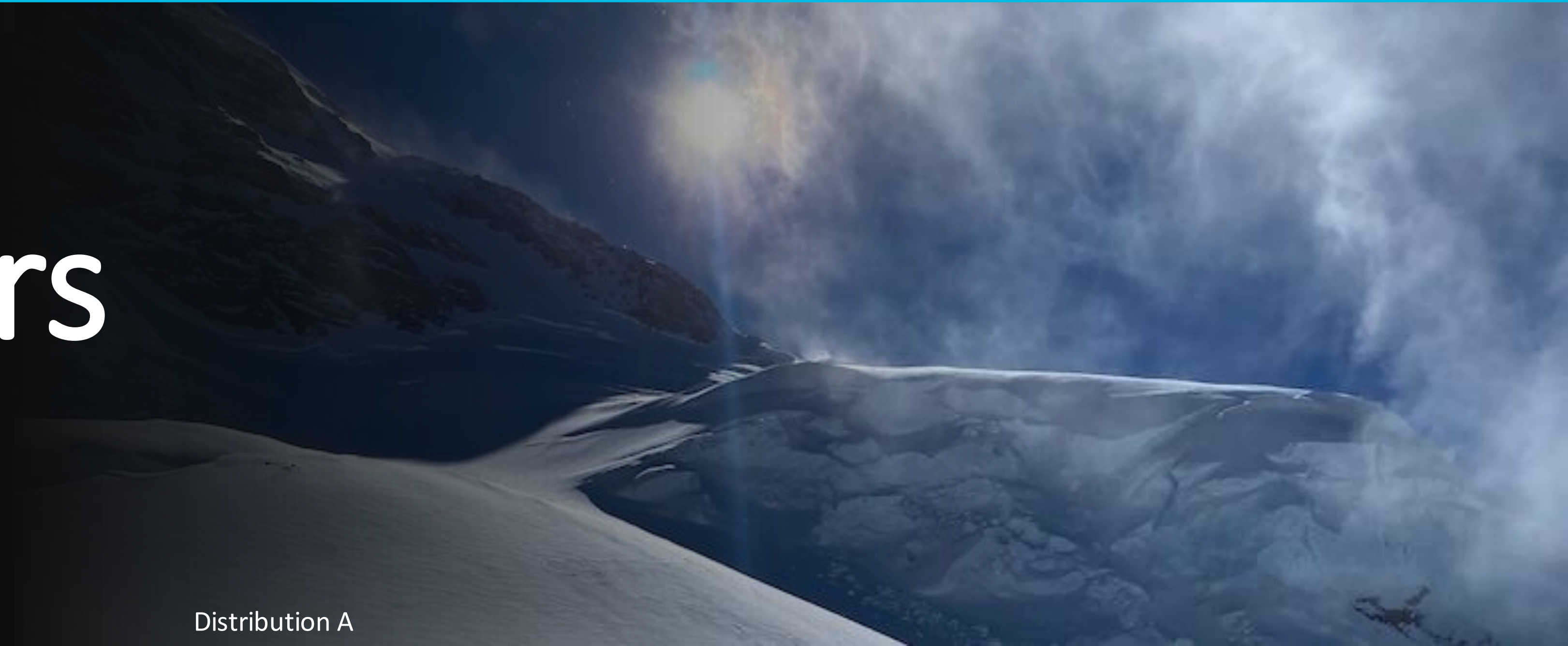


Understanding Outliers and Confounders



Springerplus. 2014 Sep 9;3:510.

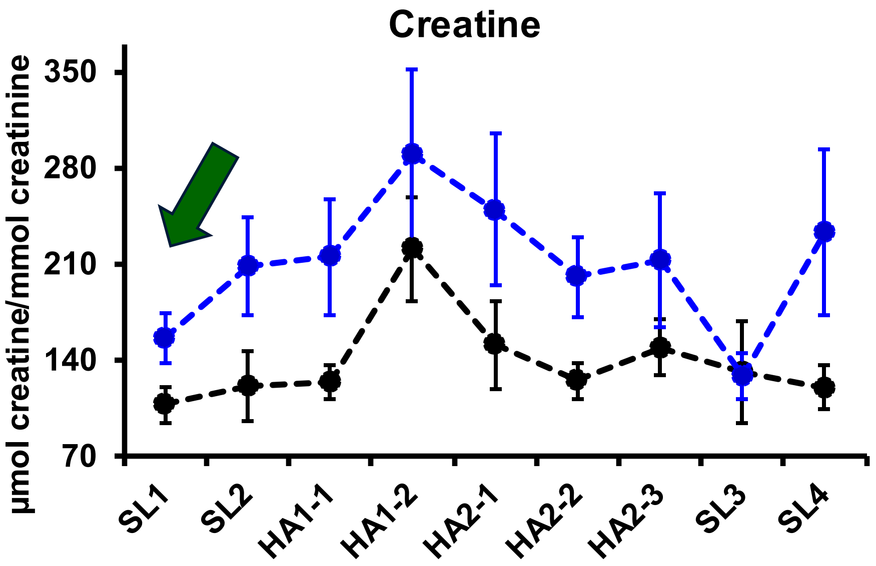
doi: 10.1186/2193-1801-3-510.

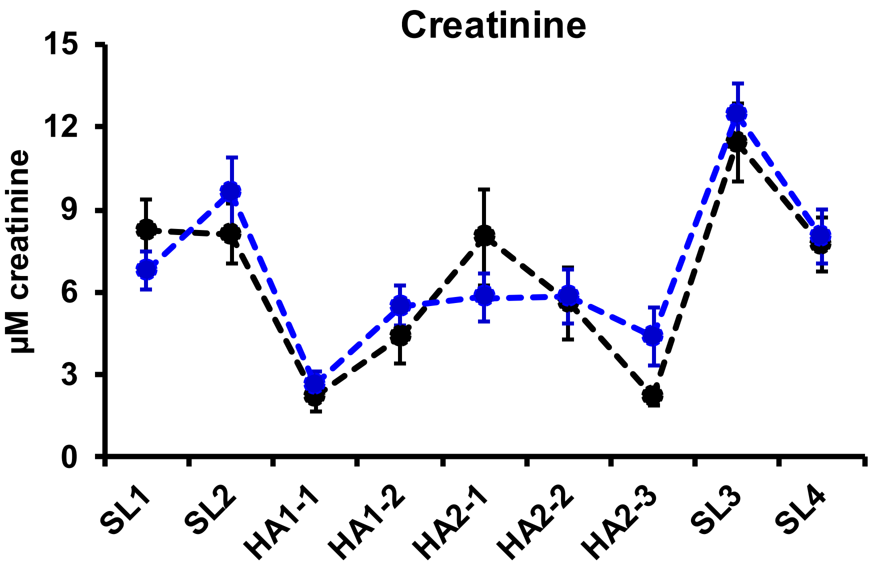


Examining AMSPA Biomarker Response in Females

Characterizing Possible Confounders:

Contraception use and Urinary Clearance Levels of Creatine and Creatinine



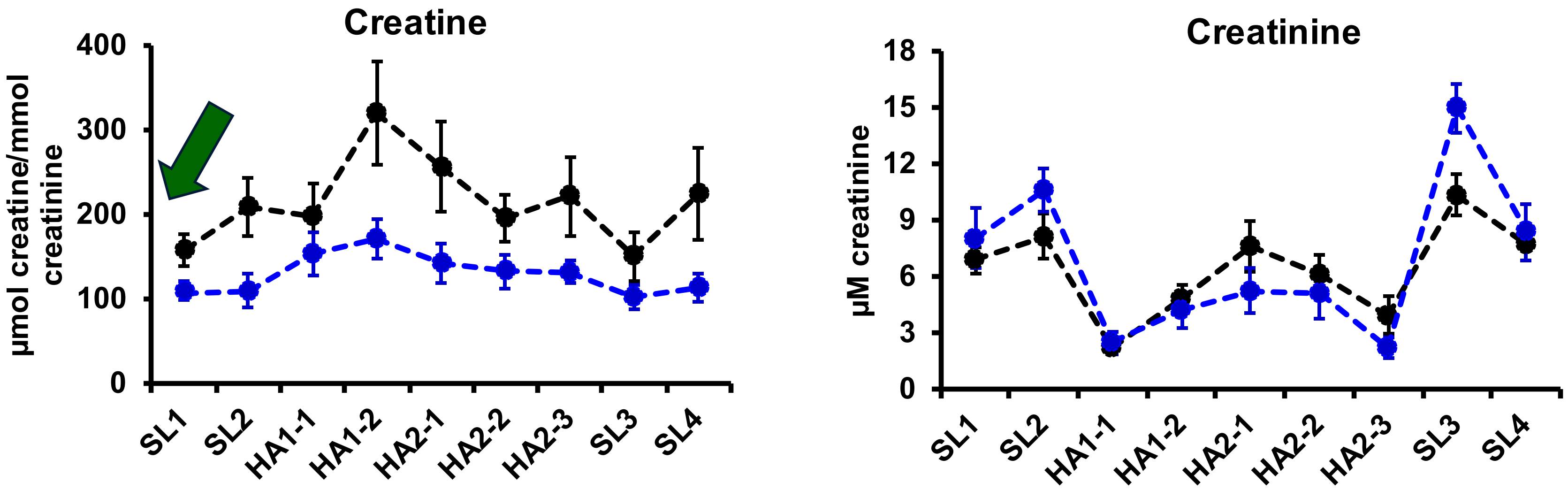


Legend

- **No contraceptive use** — N = 13
- **Use of contraceptive** — N = 17

Examining AMSPA Biomarker Response in Females

Characterizing Possible Confounders:
Females with Regular vs. Irregular Menstrual Cycles



Legend
- **Regular menstrual cycle** N = 18
- **Irregular menstrual cycle** N = 9

Understanding Outliers – AMS Symptoms and Classifiers

Cerebral AMS (AMS-C)

- Feel sick
  Headache
- Hungover
  Dizzy
- Vision dim
  Feel faint
- Feel weak
- Coordination
  off
- Sick to stomach
- Lightheaded
- Lost appetite

Respiratory AMS (AMS-R)

- Hard to breathe
- Short of breath
- Stomachache
- Sick to stomach
- Backache
- Nosebleed
- Nose stuffed
- Stomach craps
- Depressed
- Poor sleep
- Headache

The Environmental Symptoms Questionnaire (ESQ) Development and Application

Sampson JB, Kobrick JL, and Johnson RF.
USARIEM Technical report 93-10529
March 1993

- Self Reporting Symptoms
- Symptom Clustering
- Weighted
- Doesn't always align with Lake Louise Scores

But There Are Other ESQ Item Weights and Factor Divisors:

Cold Stress (AMS-COLD)

Fatigue (AMS-F)

Exertion (AMS-E)

Distress (AMS-D)

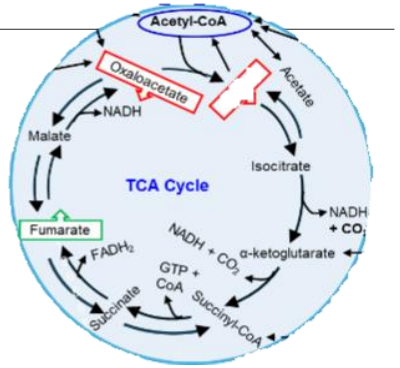
Alertness (AMS-A)

Muscle Discomfort (AMS-M)

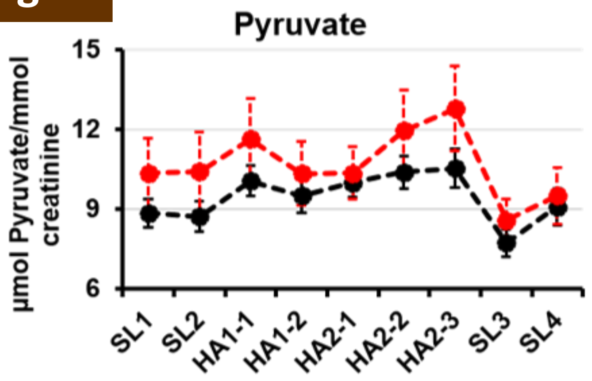
Ear-Nose-Throat (AMS-ENT)

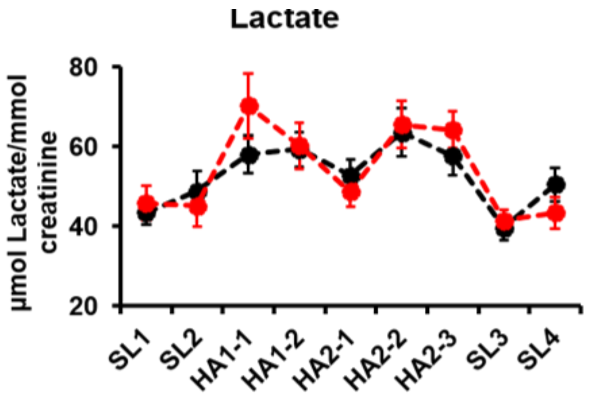
Changes in Metabolites Associated with Energy Production

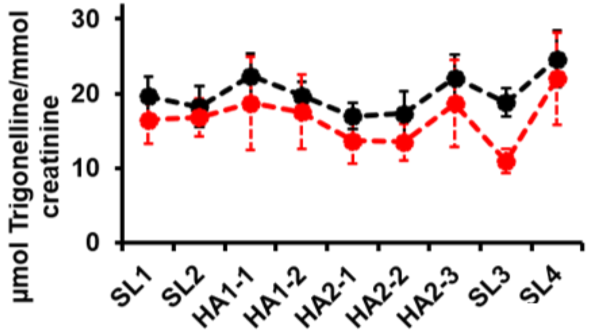
- Examining Metabolite Alterations based on AMS-F and AMS-COLD scores
- Comparing No AMS vs Severe AMS
- Male + Female Data Combined



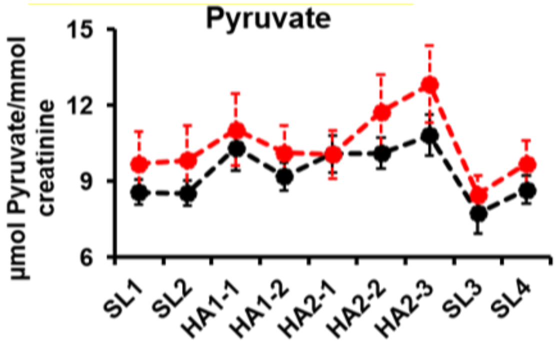
ESQ AMS-F Fatigue

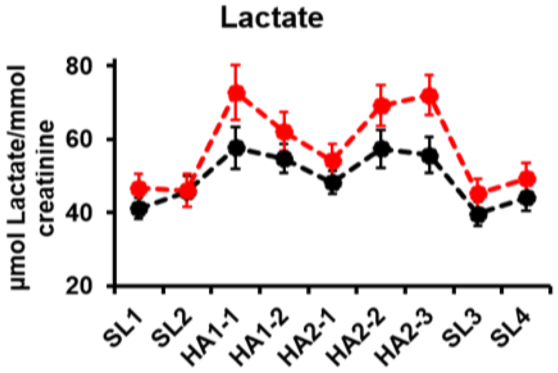


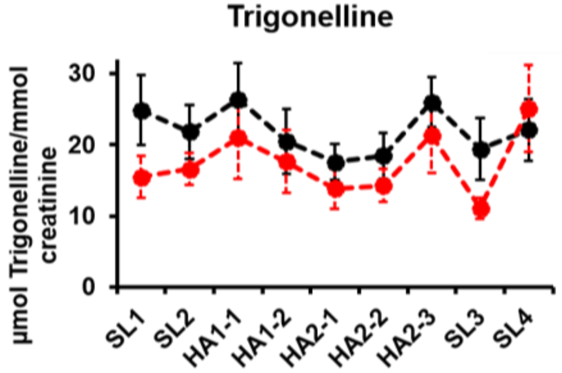




ESQ AMS-COLD
Cold Stress



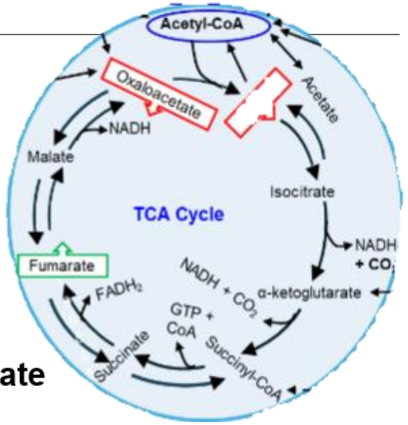




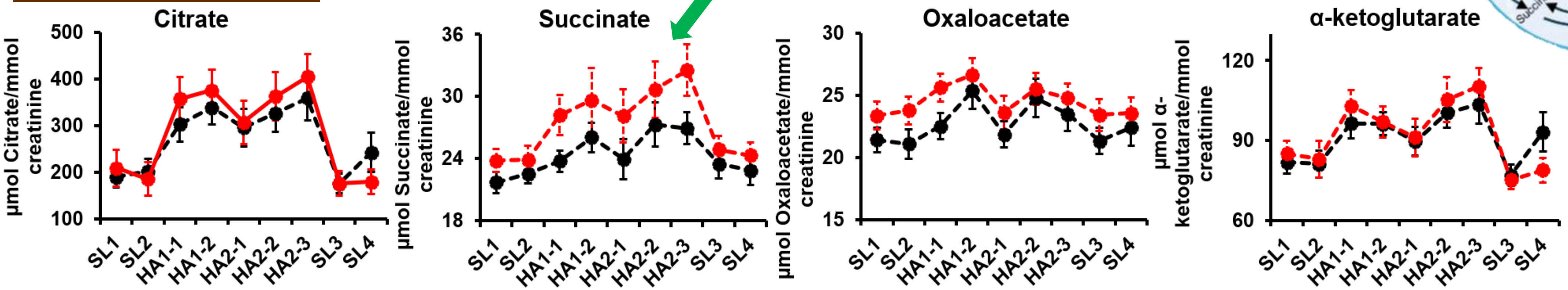
Legend
- ● NoAMS
- ● sAMS

TCA Cycle Intermediate Metabolite Changes

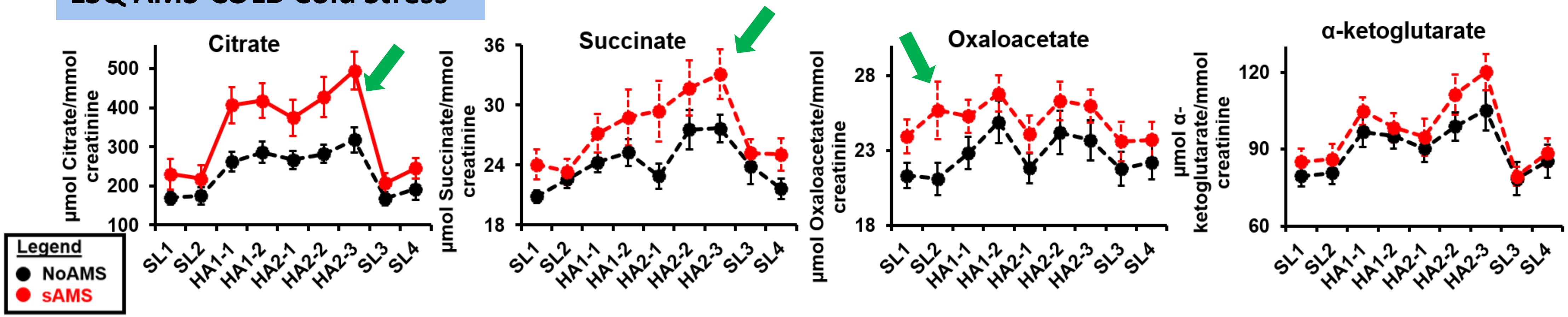
- Examining Metabolite Alterations based on AMS-F and AMS-COLD scores
- Comparing No AMS vs Severe AMS
- Male + Female Data Combined



ESQ AMS-F Fatigue

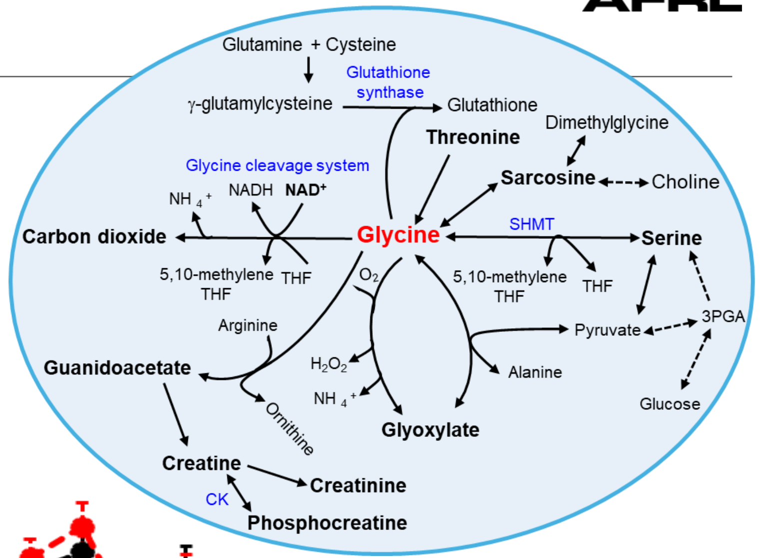


ESQ AMS-COLD Cold Stress



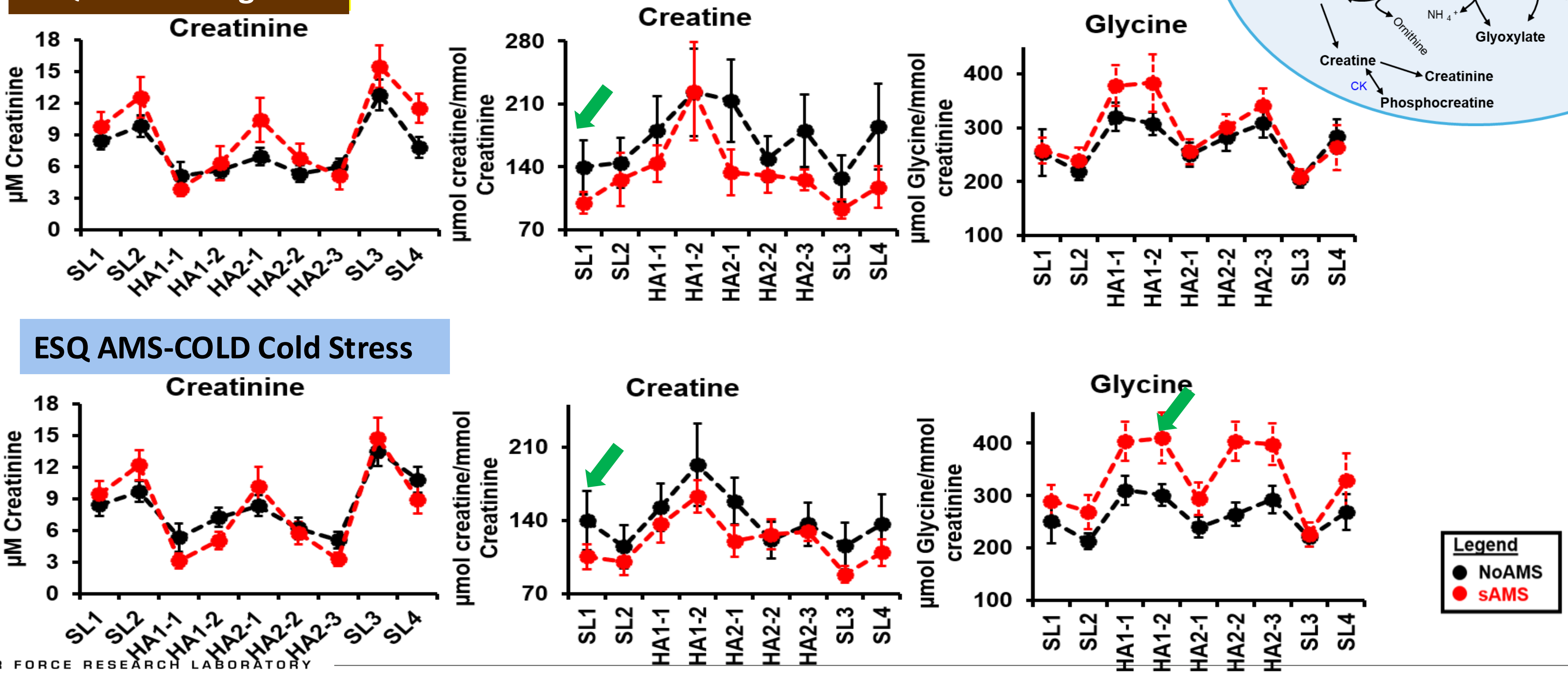
Creatine Metabolism Changes

- Examining Metabolite Alterations based on AMS-F and AMS-COLD scores
- Comparing No AMS vs Severe AMS
- Male + Female Data Combined



ESQ AMS-F Fatigue

ESQ AMS-COLD Cold Stress



Predictive Value of AMSPA With AMS Risk

How Predictive Is AMSPA with Regards to sAMS Risk?

Sensitivity refers to a biomarker's ability to correctly identify individuals with a disease or condition (true positives).

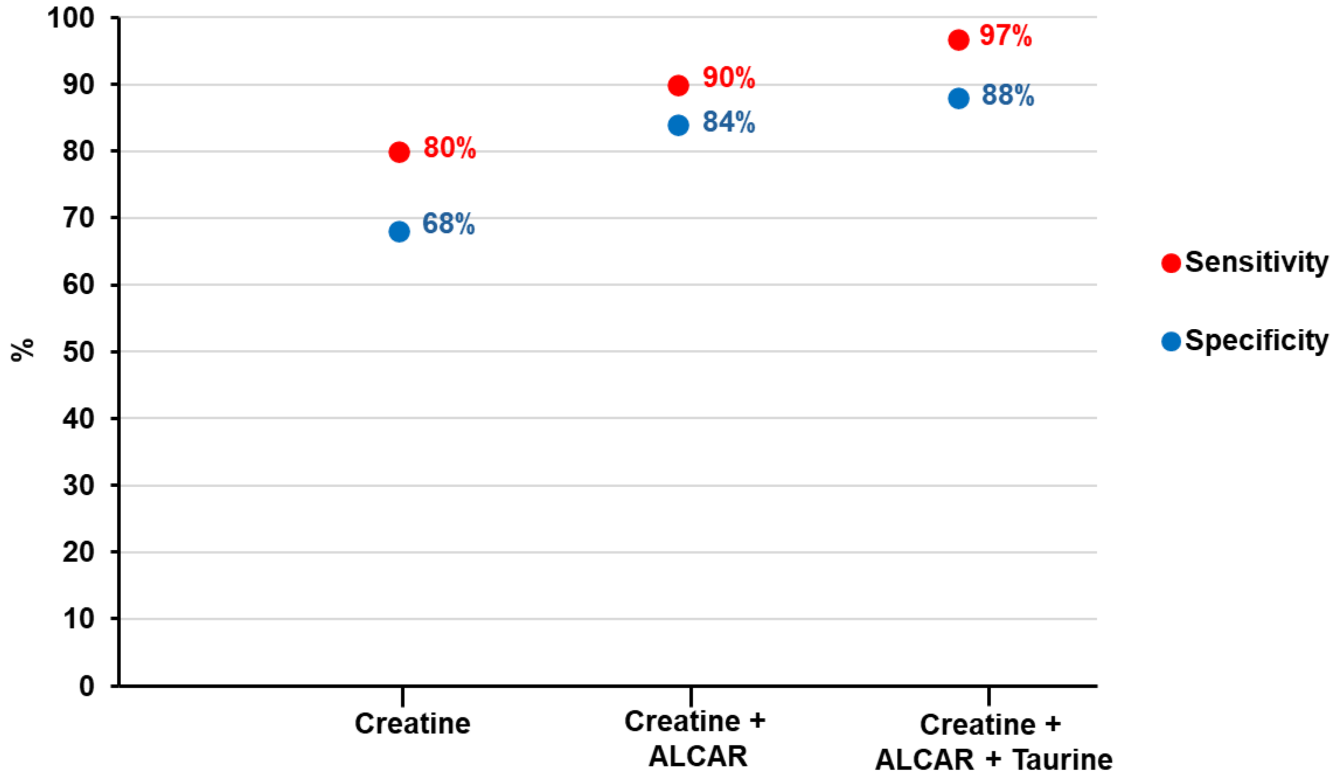
Specificity measures how well it excludes the presence of a disease, i.e. how well it will identify a large portion of individuals without the disease.

Urinary

- **Creatine,**
- **Creatine + Acetylcarnitine (ALCAR)**
- **Creatine+ ALCAR + Taurine**

Sensitivity and Specificity for sAMS Incidence Prediction at Sea Level prior to High Altitude Exposure.

Sensitivity and specificity values were calculated using data from 3 human altitude studies.



High sensitivity is important for detecting sAMS **risk.**

High specificity is important for avoiding false positives, thus unnecessary treatment or interventions individuals who are not at risk of sAMS.

Questions?

AFRL

NEW!

Susceptibility to mild and severe acute mountain sickness is associated with distinct urine metabolite profiles.

Sibomana I, Beidleman BA, Karl JP, Figueiredo PS, Landspurg SD, Staab JE and Mauzy CA
Front. Physiol. 2026 17:1704360.

Urinary Metabolites as Predictors of Acute Mountain Sickness Severity.

Sibomana I, Foose DP, Raymer ML, Reo NV, Karl JP, Berryman CE, Young AJ, Pasiakos SM and Mauzy CA
Front Physiol. 2021 12:709804

Acute Mountain Sickness Predictive Assay (AMSPA)

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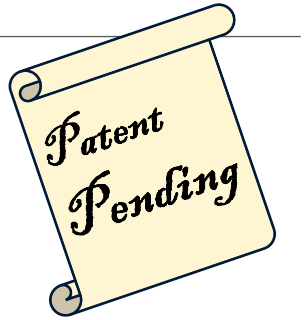

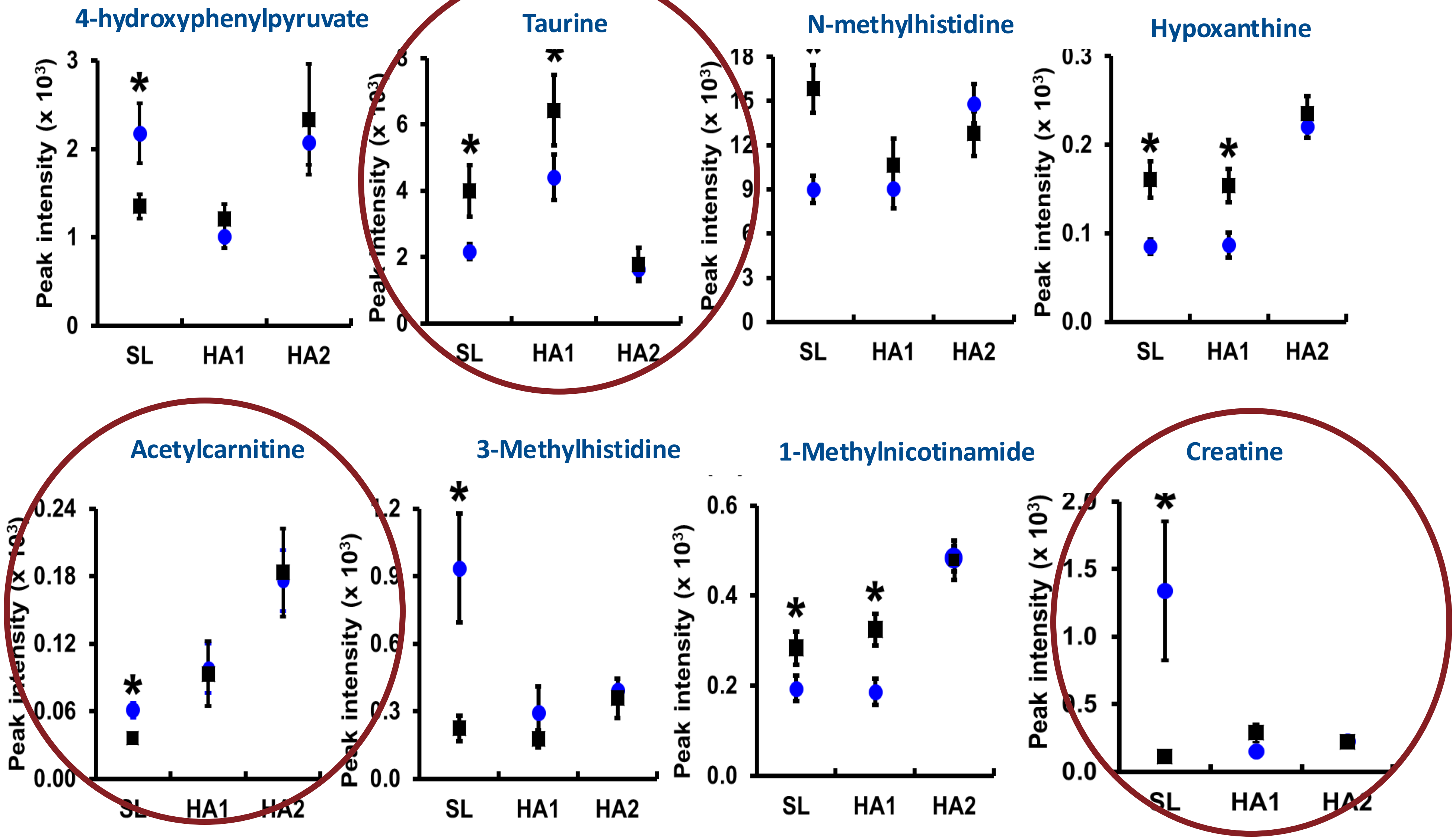

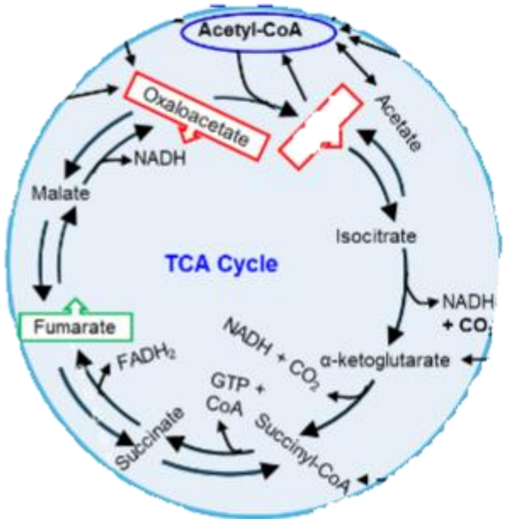
Acetyl-CoA
Oxaloacetate
Acetate
NADH
Malate
Isocitrate
TCA Cycle
NADH
+ CO2
Fumarate
NADH + CO2
α-ketoglutarate
FADH2
GTP +
CoA
Succinate
Succinyl-CoA