Central and peripheral contribution to heat stress intolerance in wild type and malignant hyperthermia susceptible mice

Ryan Gluck, Monica Aleman, Shane Antrobus, Gennady Cherednichenko, Wayne Wei Feng, Chelsea Crowe, Isaac Pessah

Department of Molecular Biosciences (Gluck, Antrobus, Cherednichenko, Feng, Pessah) and Department of Medicine and Epidemiology, The William R. Pritchard Veterinary Medical Teaching Hospital (Aleman, Crowe), School of Veterinary Medicine, University of California, Davis, CA

Malignant Hyperthermia (MH)
- Rare, heritable disease
- Inducible pharmacogenetic clinical syndrome affecting skeletal muscle metabolism
- Clinical signs associated with hypermetabolic state:
  - Muscle rigidity
  - Elevated core body temperature
  - Tachypnea
  - Tachycardia
  - Respiratory and metabolic acidosis

Key role in triggering muscle contractions via RYR1-mediated calcium release

RYR1 mutations are prevalent in patients with MH

Inducible pharmacogenetic clinical syndrome affecting skeletal muscle metabolism
- Rare, heritable
- Presents as MH in the setting of general anesthesia

Aim 1: Determine differences in central and peripheral responses to moderate heat stress (38°C) intolerance in wild type (WT) and malignant hyperthermia susceptible (HET) mice by continuous measurements of core body temperature, heart and respiratory rates and cortical EEG total power.

Aim 2: Determine the extent to which dietary caffeine influences heat stress intolerance using the same measurements in Aim 1.

Hypotheses
- HET mice will be more sensitive to heat stress intolerance than WT mice
- Males will be more sensitive than females
- Dietary caffeine will attenuate responses to heat stress

Approach & Methods
- Knock-in mouse model with RYR1 mutation, conferring genetic intolerance to stress-triggered MH
- Heterozygous MH susceptible mouse line “R163C-RYR1” (HET)
- 8 treatment groups of 6 mice/group
- 3 categorical variables: HET/WT & Male/Female & Caffeine/Sucrose
- Example group: HET Male Caffeine (p<0.05)
- Mice delivered to lact, allowed to habituate for 60 min
- Placed in restraint & instrumented with rectal thermometer probe
- Rest for 5 min (baseline)
- Enter thermochamber set at 38 °C [100.4°F]
- Recordings continue until adverse outcome or 60 min, whichever first
- Direct cardiac puncture for blood collection
- Dissection of skeletal muscle and brain tissues for histology

Electrophysiological Study – Measurements
- Anesthetic induction in gas chamber (2% halothane)
- Transfer to nosecone and temperature-controlled plate for instrument implementation
- Electrographic electrode placement [8] → See figure 1
  - Electroencephalogram (EEG), Electrocardiogram (ECC), Electromyogram (EMG)
- Rectal thermometer probe
- Habitate in restraint & recover from light anesthesia (baseline) → See figure 2
- Incubator temperature settings [35, 37, & 35 °C] - 3 cohorts of 6 mice/jetting
- Powered off to prevent electrical interference with electrodes

Electrographic electrode placement:
- Transfer to nosecone
- Rectal thermometer probe
- Electrodes placed

Background

Specific Aims & Approach

Results

Figure 1: Initial (T1) & Final (Tf) Temperature – HET vs. WT (aggregate data)

Figure 2: Continuous Core Body Temperature – HET (black; n=1) vs. WT (red; n=1)

Figure 3: Kaplan-Meier Survival Curve – HET vs. WT

Figure 4: Kaplan-Meier Survival Curve – Sucrose vs. Caffeine Drinking Water Cohorts

Figure 5: Kaplan-Meier Survival Curve – HET Male vs. HET Female

Figure 6: Kaplan-Meier Survival Curve – Caffeine vs. Sucrose Drinking Water Cohorts

Figure 7: Kaplan-Meier Survival Curve – Male vs. Female HET mice

Figure 8: Kaplan-Meier Survival Curve – Caffeine vs. Sucrose Drinking Water Cohorts

Figure 9: Kaplan-Meier Survival Curve – Male vs. Female HET mice

Figure 10: Kaplan-Meier Survival Curve – Caffeine vs. Sucrose Drinking Water Cohorts

Figure 11: Kaplan-Meier Survival Curve – Male vs. Female HET mice

Figure 12: Kaplan-Meier Survival Curve – Caffeine vs. Sucrose Drinking Water Cohorts

Figure 13: Kaplan-Meier Survival Curve – Male vs. Female HET mice

Figure 14: Kaplan-Meier Survival Curve – Caffeine vs. Sucrose Drinking Water Cohorts

Figure 15: Kaplan-Meier Survival Curve – Male vs. Female HET mice

Figure 16: Kaplan-Meier Survival Curve – Caffeine vs. Sucrose Drinking Water Cohorts

Figure 17: Kaplan-Meier Survival Curve – Male vs. Female HET mice

Figure 18: Kaplan-Meier Survival Curve – Caffeine vs. Sucrose Drinking Water Cohorts

Figure 19: Kaplan-Meier Survival Curve – Male vs. Female HET mice

Figure 20: Kaplan-Meier Survival Curve – Caffeine vs. Sucrose Drinking Water Cohorts

Figure 21: Kaplan-Meier Survival Curve – Male vs. Female HET mice

Figure 22: Kaplan-Meier Survival Curve – Caffeine vs. Sucrose Drinking Water Cohorts

Figure 23: Kaplan-Meier Survival Curve – Male vs. Female HET mice

Figure 24: Kaplan-Meier Survival Curve – Caffeine vs. Sucrose Drinking Water Cohorts

Figure 25: Kaplan-Meier Survival Curve – Male vs. Female HET mice

Figure 26: Kaplan-Meier Survival Curve – Caffeine vs. Sucrose Drinking Water Cohorts

Figure 27: Kaplan-Meier Survival Curve – Male vs. Female HET mice

Figure 28: Kaplan-Meier Survival Curve – Caffeine vs. Sucrose Drinking Water Cohorts

Figure 29: Kaplan-Meier Survival Curve – Male vs. Female HET mice

Figure 30: Kaplan-Meier Survival Curve – Caffeine vs. Sucrose Drinking Water Cohorts

Figure 31: Kaplan-Meier Survival Curve – Male vs. Female HET mice

Figure 32: Kaplan-Meier Survival Curve – Caffeine vs. Sucrose Drinking Water Cohorts

Figure 33: Kaplan-Meier Survival Curve – Male vs. Female HET mice

Figure 34: Kaplan-Meier Survival Curve – Caffeine vs. Sucrose Drinking Water Cohorts

Figure 35: Kaplan-Meier Survival Curve – Male vs. Female HET mice

Figure 36: Kaplan-Meier Survival Curve – Caffeine vs. Sucrose Drinking Water Cohorts

Figure 37: Kaplan-Meier Survival Curve – Male vs. Female HET mice

Figure 38: Kaplan-Meier Survival Curve – Caffeine vs. Sucrose Drinking Water Cohorts

Figure 39: Kaplan-Meier Survival Curve – Male vs. Female HET mice

Figure 40: Kaplan-Meier Survival Curve – Caffeine vs. Sucrose Drinking Water Cohorts

Figure 41: Kaplan-Meier Survival Curve – Male vs. Female HET mice

Significance
- MH susceptible mice (HET) are exquisitely sensitive to heat stress
- Dietary caffeine does not appear to influence heat stress intolerance
- Individuals with RYR1 mutations likely represent an underappreciated population with a genetic predisposition to heat stress intolerance
- Further investigation into the dysregulation of thermoregulation is extremely relevant to climate change

Acknowledgements
- Dr. Isaac Pessah
- Dr. Monica Aleman
- Shane Antrobus
- Gennady Cherednichenko
- Members of the Pessah Lab
- University of California, Davis (School of Veterinary Medicine)
- National Institutes of Health (NIH) T35 Training Grant T35GM065486-22
- National Science Foundation (NSF)
- Financial support was provided by the Students Training in Advanced Research (STAR) Program

Ongoing Analysis

Blood – Clinical Chemistry
- Electrolytes
- Metabolic analytes
- Creative kinase
- Stress response biomarkers (e.g. cortisol, heat shock proteins, DHEA)

Electrophysiological Data – central contribution
- Electroencephalograph (EEG)
- Total power
- Time to isoelectric EEG
- Respiratory rate
- Electrocardiogram (ECC)
- Heart rate
- Electromyogram (EMG)

Brain & Muscle – Histology
- Hematoxylin and Eosin (H&E), Gomori’s modified trichrome, Succinic Dehydrogenase (SDH), Periodic acid Schiff (PAS), Esterase, and Alcianblue (10, 4, 4, 6, 3, 4)

Image: Preview - Channels: 1 x = 960 (2.49 mm) y = 720 (1.86 mm) = 13 (0 )

Figure 1: Electrode Placement

Figure 2: Anesthesia Setup/Mice Recovery

Image: Preview - Channels: 1 x = 960 (2.49 mm) y = 720 (1.86 mm) = 1 (0 )

Image: Preview - Channels: 1 x = 960 (2.49 mm) y = 720 (1.86 mm) = 16 (0 )

Image: Preview - Channels: 1 x = 960 (2.49 mm) y = 720 (1.86 mm) = 10 (0 )

Image: Preview - Channels: 1 x = 960 (2.49 mm) y = 720 (1.86 mm) = 1 (0 )