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

UC DAVIS VETERINARY MEDICINE
Students Training in Advanced Research

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## Background

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
- *Triggered by*: volatile anesthetics, depolarizing muscle relaxants, exertional & environmental stress
- *Evaluation of susceptibility*: in vitro contracture testing of muscle biopsies with halothane and caffeine

Ryanodine Receptor Type 1 (RYR1)
- *Function*: key role in triggering muscle contractions via mediation of calcium release
- Dozens of known RYR1 mutations are causative for MH susceptibility
  - R163C = prevalent inherited pathogenic gene variant in humans

## Specific Aims & Approach

Aim 1: Determine differences in central and peripheral responses to moderate heat stress (38ºC) 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 aggravate adverse 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 (n=6)
- Mice delivered to lab, allowed to habituate for 60 min
- Placed in restraint & instrumented with rectal thermometer probe
  - Rest for 5 min (baseline)
- Enter thermostatic chamber set at 38 ºC (100.4 ºF)
- 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

### *Electrographic Study – Modifications*
- Anesthetic induction in gas chamber (2% halothane)
- Transfer to nosecone and temperature-controlled plate for instrument implementation
- Electrographic electrode placement [8] - *See Image 1*
  - Electroencephalogram (EEG), Electrocardiogram (ECG), Electromyogram (EMG)
- Rectal thermometer probe
- Habituate in restraint & recover from light anesthesia (baseline) – *See Image 2*
- Incubator temperature settings (39, 37, & 35 ºC) - 3 cohorts of 6 mice/setting
  - Powered off to prevent electrical interference with electrodes

## Results

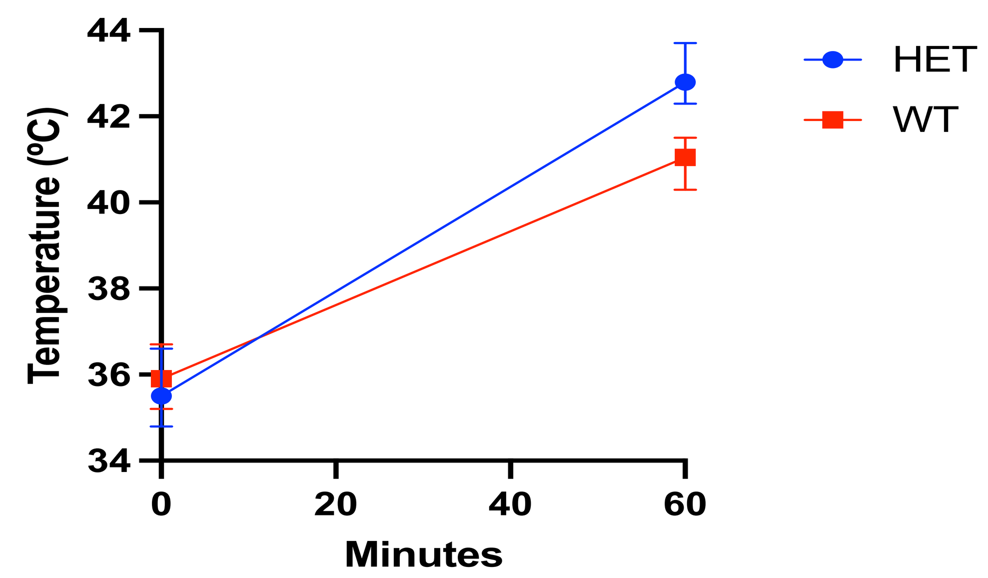


Figure 1. Initial ($T_i$) & Final ($T_f$) Temperature – *HET vs. WT (aggregate data)*

Change in Temperature ($T_f$ - $T_i$):
- HET (7.2 ºC) and WT (5.3 ºC)
  - $p < 0.001$

Temperature$_{final}$
- HET (42.7 ºC) and WT (41.0 ºC)
  - $p < 0.001$

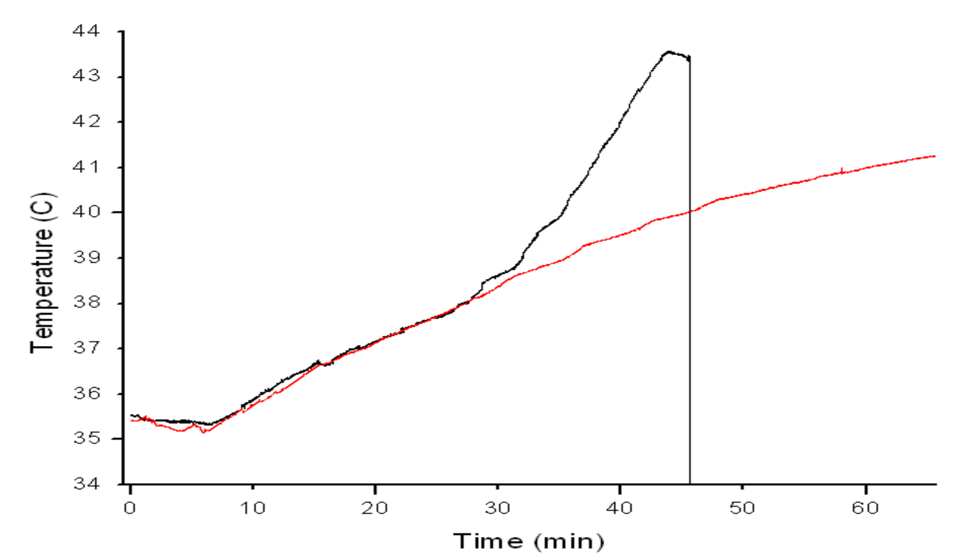


Figure 2. Continuous Core Body Temperature – HET (black; n=1) vs. WT (red; n=1)

WT mice:
- Monotonic rise in core body temperature (0.124 ºC/min)

HET mice:
- Biphasic rise in core body temperature
- Steep second phase (0.366 ºC/min) - *not* seen in WT animals

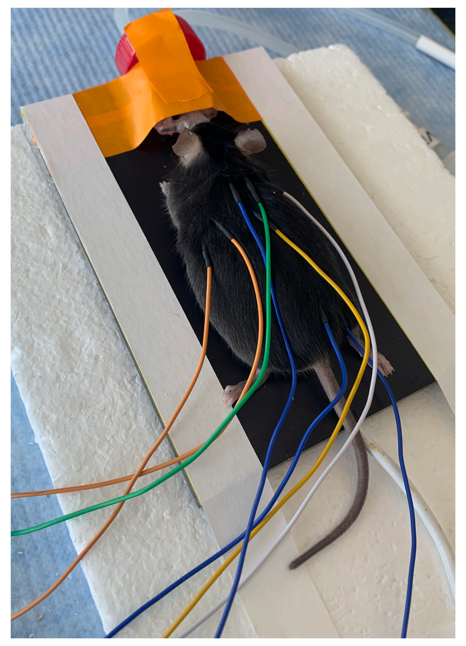

Image 1. Electrode Placement

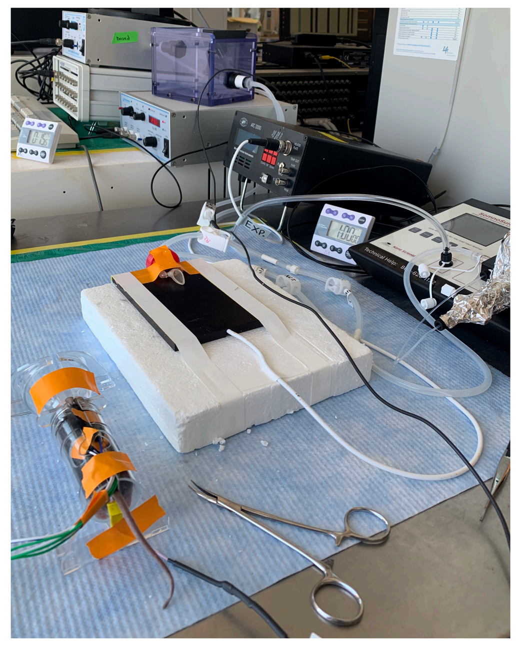

Image 2. Anesthetic Setup/Mouse Recovery

## Results

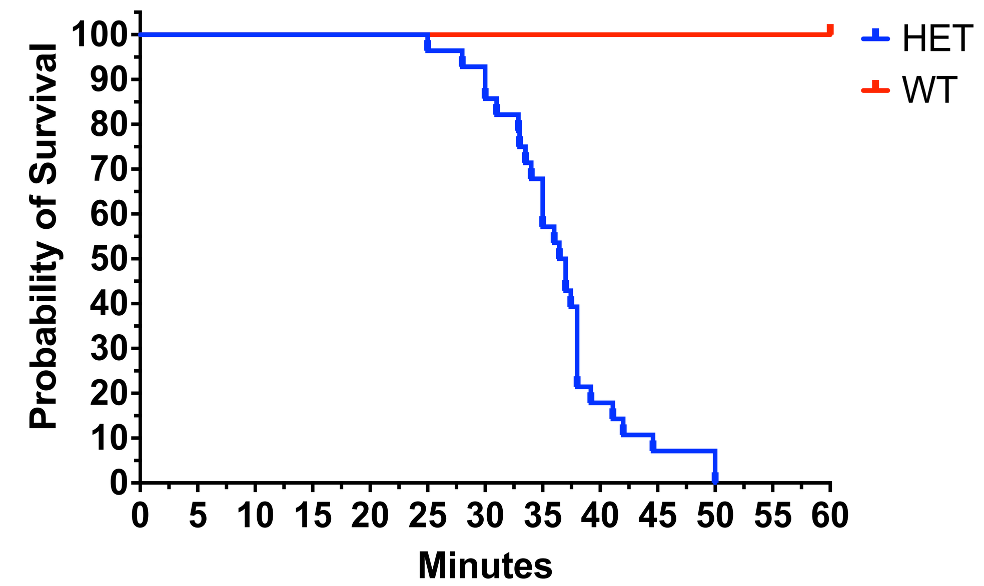


Figure 3. Kaplan-Meier Survival Curve – *HET vs. WT*

- HET mice are extremely sensitive to heat stress intolerance
  - Median survival = 36.7 min
- All WT mice survived 60 minutes of heat stress exposure - 38 ºC

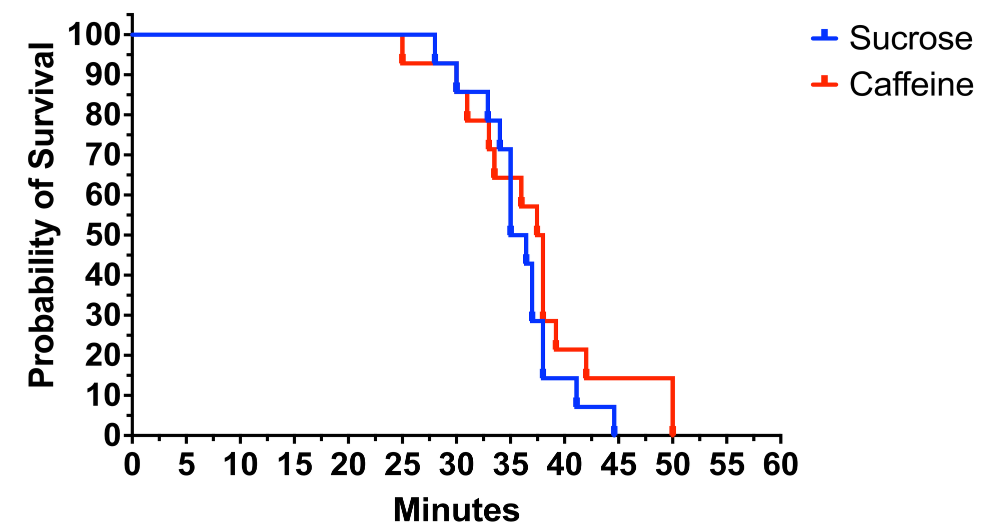


Figure 4. Kaplan-Meier Survival Curve – *Sucrose vs. Caffeine Drinking Water Cohorts*

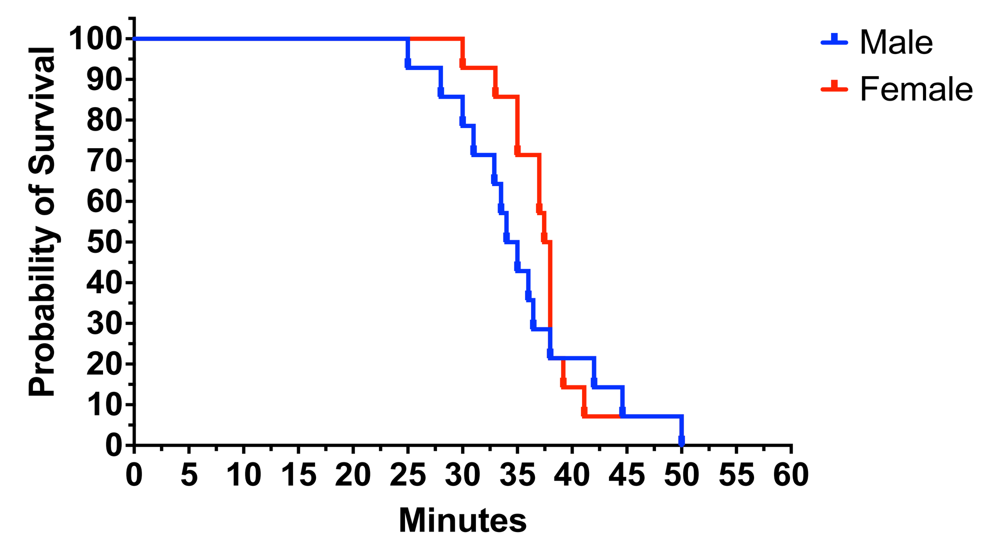


Figure 5. Kaplan-Meier Survival Curve – *HET Male vs. HET Female*

HET Median Survival:
- Caffeine (37.7 min) and Sucrose (35.7 min)
- Male (34.5 min) and Female (37.7 min)

No statistically significant difference in survival between:
- Caffeine and Sucrose HET mice ($p = 0.51$)
- Male and Female HET mice ($p = 0.14$)

## Ongoing Analysis

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

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

Brain & Muscle – Histology
- Hematoxylin and Eosin (H&E), Gomori's modified trichome, Succinic Dehydrogenase (SDH), Periodic acid Schiff (PAS), Esterase, and ATPase (10.2, 4.6, 4.3)

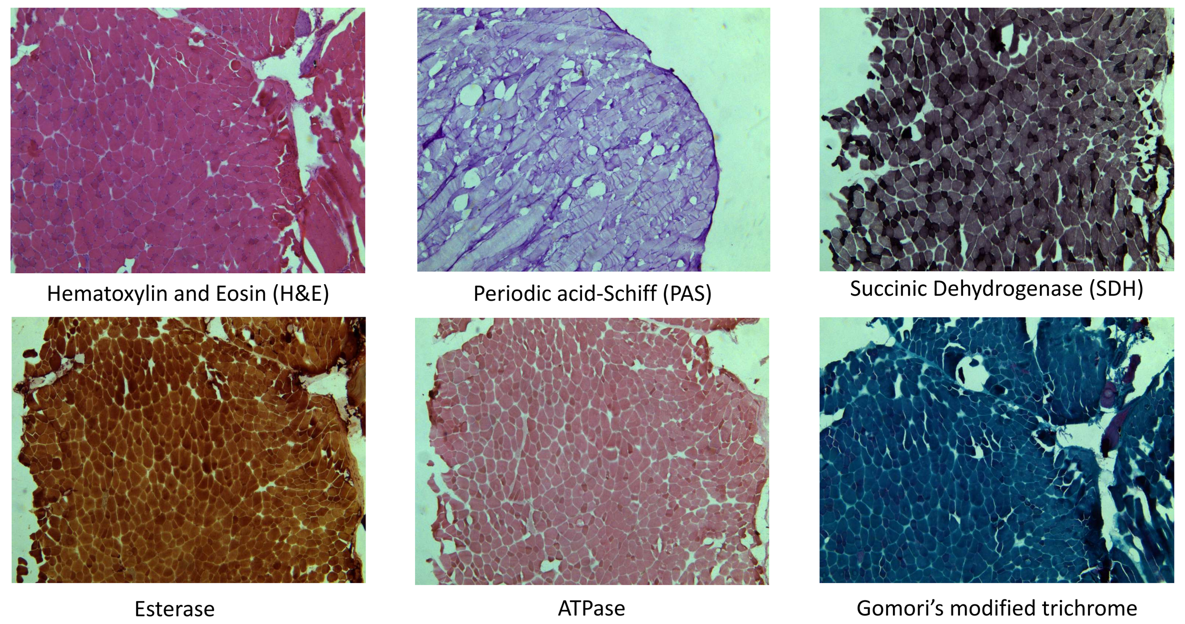


Image 3. Muscle Biopsy Stains

## 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

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