Received: 26 February 2020 | Accepted: 20 April 2020

DOI: 10.1096/fj.202000403

RESEARCH ARTICLE

THE FASEB JOURNAL

Sex-specific alterations in whole body energetics and voluntary activity in heterozygous R163C malignant hyperthermia-susceptible mice

Jennifer M. Rutkowsky | Trina A. Knotts | Paul D. Allen | Isaac N. Pessah | Jon J. Ramsey

Department of Molecular Biosciences, School of Veterinary Medicine, University of California, Davis, CA, USA

Correspondence
Jon J. Ramsey, Department of Molecular Biosciences, School of Veterinary Medicine, University of California, Davis, 1089 Vet Med Drive, Davis, CA 95616 USA.
Email: jjramsey@ucdavis.edu

Present address
Paul D. Allen, Leeds Institute of Biomedical and Clinical Sciences, University of Leeds, Leeds, UK

Funding information
HHS | National Institutes of Health (NIH), Grant/Award Number: PO1AR052354; UC Davis Mouse Metabolic Phenotyping Center, Grant/Award Number: U2CDK092993

Abstract

Malignant hyperthermia (MH) is characterized by induction of skeletal muscle hyperthermia in response to a dysregulated increase in myoplasmic calcium. Although altered energetics play a central role in MH, MH-susceptible humans and mouse models are often described as having no phenotype until exposure to a triggering agent. The purpose of this study was to determine the influence of the R163C ryanodine receptor 1 mutation, a common MH mutation in humans, on energy expenditure, and voluntary wheel running in mice. Energy expenditure was measured by indirect respiration calorimetry in wild-type (WT) and heterozygous R163C (HET) mice over a range of ambient temperatures. Energy expenditure adjusted for body weight or lean mass was increased ($P < .05$) in male, but not female, HET mice housed at 22°C or when housed at 28°C with a running wheel. In female mice, voluntary wheel running was decreased ($P < .05$) in the HET vs WT animals when analyzed across ambient temperatures. The thermoneutral zone was also widened in both male and female HET mice. The results of the study show that the R163C mutations alters energetics even at temperatures that do not typically induce MH.

KEYWORDS

body composition, energy expendtiure, metabolic rate, physical activity, thermoneutral zone

1 | INTRODUCTION

Malignant hyperthermia (MH) is a life-threatening disorder of skeletal muscle homeostasis that was first described in a family that experienced 10 deaths attributable to anesthesia.[1] A fulminant MH syndrome is characterized by an uncontrolled increase in myoplasmic free calcium (Ca^{2+}) that leads to increased metabolic rate and subsequently results in hyperthermia and muscle rigidity.[2-4] This energetic crisis can be triggered by volatile anesthetics, depolarizing muscle relaxants (eg, succinylcholine), intense exercise, and exposure to elevated environmental temperatures.[5]

Abbreviations: DEXA, dual energy X-ray absorptiometry; ER, endoplasmic reticulum; HET, heterozygous R163C ryanodine receptor 1 mutation; LCT, lower critical temperature; MH, malignant hyperthermia; RER, respiratory exchange ratio; RYR1, ryanodine receptor 1; SERCA, sarcoplasmic reticulum calcium ATPase; SR, sarcoplasmic reticulum; TNZ, thermoneutral zone; UCT, upper critical temperature; WT, wild-type.

Jennifer M. Rutkowsky and Trina A. Knotts contributed equally to the work.

The prevalence in the human population of known MH-causative mutations is 1:2750,[6] with variants in the ryanodine receptor 1 gene (*RYR1*) being responsible for the majority of MH cases.[7] Polymorphism and expressed variants within the *RYR1* gene have been estimated to occur in greater than 50% of people from MH-susceptible families, although it remains unclear how many of these confer pathogenic risks.[6,8,9]

RYR1 protein is expressed in skeletal muscle where it plays a central role in the release of Ca^{2+} from the sarcoplasmic reticulum (SR) as part of excitation-contraction coupling.[2,10] Ca^{2+} cycling is typically estimated to be responsible for ~5% of whole body resting energy expenditure,[11] and it follows that pathogenic mutations in RYR1 could stimulate resting Ca^{2+} cycling and whole animal energy expenditure. However, MH-susceptible people and mouse models are often described as showing no phenotype until exposed to a MH trigger. This is unexpected since SR Ca^{2+} leak has been reported with RYR1 mutations in both MH-susceptible people[12] and mouse models of MH,[13,14] implying that basal Ca^{2+} cycling should be affected by expression of these mutations. Skeletal muscle mitochondrial defects have also been reported in mice with RYR1 mutations[15,16] and MH-susceptible people,[17] and it would be expected that these defects could lead to alterations in whole animal energy metabolism. Little is known, however, about energy expenditure in the absence of MH-triggering agents in either MH-susceptible mice or humans. A study in humans reported that resting energy expenditure was only increased in MH-susceptible individuals during insulin stimulation.[18] However, the temperature at which these measurements were taken was not specified and the study included primarily female volunteers, limiting the ability of the investigators to determine if energy expenditure was influenced by sex. In mice, a significant increase in oxygen consumption adjusted for body weight (BW) was found in RYR1 Y522S mutants measured at 32°C,[15] but it is not known if this difference would persist at lower temperatures. Thus, only limited information is currently available about the impact of MH-susceptible mutations on whole animal energetics under conditions that typically do not induce a fulminant MH syndrome.

The purpose of this study was to determine the influence of the MH R163C RYR1, one of the more common mutations identified in humans, on energy expenditure and physical activity in male and female mice. Energy expenditure was measured in mice housed at their standard vivarium temperature (22°C) and energy expenditure was measured in animals with running wheel access at standard housing temperature and near the murine thermoneutral zone (TNZ). In addition, voluntary wheel running was measured at these temperatures. Energy expenditure was also measured in mice exposed to environmental temperatures ranging from 12 to 36°C to assess response to cold exposure and determine if the TNZ is altered in these animals.

2 | MATERIALS AND METHODS

2.1 | Animals

All wild-type (WT) and heterozygous R163C (HET) mice were obtained from a breeding colony maintained at the University of California, Davis. The HET mice were generated with a knock in vector containing a mutation of the arginine at codon 163 to cysteine (R163C) as previously described.[14] The mice were backcrossed onto C57BL/6J mice to full congenic status. Mouse genotypes were verified through PCR screening. PCR primer sequences were as follows: RyR1-WT (forward), GAG AGA AGG TTC GAG TTG GGG AT; RyR1-WT (reverse), ACT CAC CAG GTA TCG CTC AG; RyR1-R163C (forward), GAG AGA AGG TTT GCG TTG GAG AC; RyR1-R163C (reverse), ACT CAC CAG GTA TCG CTC AG.

During the studies, the mice were housed in a vivarium maintained at 20-24°C and 30%-40% relative humidity with a 12-hour light/12-hour dark cycle. Separate cohorts of mice were used for the three calorimetry experiments: (a) baseline calorimetry (male HET or WT, n = 8; female HET or WT, n = 14), (b) temperature challenge calorimetry (male HET or WT, n = 7; female HET or WT, n = 9), and (c) calorimetry with voluntary running (male HET or WT, n = 7; female HET or WT, n = 8). Separate cohorts of mice were also used for the voluntary wheel running studies in a home-cage environment (male HET or WT, n = 8; female HET or WT, n = 5). The mice in each cohort were randomly selected from more than one litter. The mice were 200-270 days of age at the start of the study, and the age spread for mice in each study cohort were within 30 days of age. All mice were provided with continuous access to water and Teklad 2918 diet (Envigo, Madison, WI), with the exception that mice used for the temperature challenge studies were food deprived for approximately 8 hours during the light cycle on the day of the calorimetry measurements. All mouse experiments were completed under protocols approved by the UC Davis Institutional Animal Care and Use Committee and were in accordance with the NIH guidelines for the Care and Use of Laboratory Animals.

2.2 | Baseline indirect respiration calorimetry

Oxygen consumption and carbon dioxide production were measured using an indirect respiration calorimeter (CLAMS, Columbus Instruments, Columbus, OH). Energy expenditure (kcal) was calculated from O_2 consumption and CO_2 production using the CLAMS Oxymax software. Respiratory exchange ratio (RER) was calculated as VCO_2/VO_2. Calorimetry chamber-mounted sensors were used to measure physical activity (infrared beam breaks in the *x* and *z*-planes) and food intake.

The calorimetry system was housed in a room maintained on a 12-hour light/dark cycle at 20-24°C. The mice were placed in acclimation chambers (calorimeter chambers not connected to the system) and housed in the calorimeter room for 24 hours. The mice were then transferred to calorimetry chambers inside a 22°C incubator and allowed to acclimate for 24 hours prior to the start of the calorimetry measurements. Calorimetry measurements were completed over a 48-hour period. Room air was drawn through the system at a rate of 500 mL/min. The oxygen and carbon dioxide analyzers were calibrated daily. A calibration gas (0.50% CO_2, 20.50% O_2, and balance nitrogen) (Airgas, Sacramento, CA) and dry room air were used to calibrate the analyzers. At the beginning and end of the experiment, the performance of the entire calorimetry system was validated by bleeding 20.00% CO_2 (balance nitrogen) standard (Airgas, Sacramento, CA) through each chamber at a regulated rate using an OxyVal gas infusion system (Columbus Instruments, Columbus, OH) and measuring recovery of CO_2 and dilution of O_2 in the exhaust flow from the chambers.

2.3 | Body composition

Following completion of the calorimetry measurements, the mice were immediately removed from the calorimeter and euthanized by CO_2 inhalation. Body composition (lean mass [LM], fat mass, and bone mineral content) was then measured by dual energy X-ray absorptiometry (DEXA) using a Lunar PIXImus II Densitometer (GE Medical Systems, Chalfont St. Giles, UK).

2.4 | Temperature challenge indirect respiration calorimetry and body temperature measurements

DST nano-T temperature recorders (Star-Oddi, Gardabaer, Iceland) were implanted intraperitoneally under anesthesia (ketamine 50-100 mg/kg IP, midazolam 4-6 mg/kg IP, and butorphanol 4-6 mg/kg IP) with flumazenil (0.5 mg/kg SC) for reversal of sedation immediately after surgery. For analgesics, animals received meloxicam (2-10 mg/kg, SC) preoperative and buprenorphine (0.05-0.1 mg/kg, SC) postoperative, if needed. Mice were moved to the calorimetry room at least 10 days after surgery.

The mice were acclimated to the calorimetry room for 24 hours while housed in acclimation chambers. The next morning food was removed from the cage prior to study to ensure that calorimetry readings were not influenced by activity related to eating. Mice in acclimation chambers were then transferred to calorimetry chambers within a temperature-controlled cabinet set at a 12°C. Actual cage temperature was measured using temperature sensors (DS1922L-F5#, iButtonLink, LLC) attached to the inside of each metabolic cage lid. Energy expenditure was evaluated by indirect respiration calorimetry in the CLAMS unit at 12°C for 60 minutes, at 18, 24, 28, and 30°C for 45 minutes each, and at 34 and 36°C for 30 minutes each. A single mouse was studied each day and gas analyzer readings were recorded every 5 seconds. Previous tests measuring temperature within the calorimeter chambers was used to select the chamber location for the studies that best matched the target temperatures. The body temperature recorders were programmed to record every 5 minutes from the 12°C until the beginning of the 30°C calorimetry measurements and for every minute for the remainder of the calorimetry measurements (30°C through 36°C). The lower critical temperature (LCT) of the TNZ was calculated using a segmental linear model of energy expenditure vs calorimeter chamber temperature with the second segment's slope = 0. Although the upper critical temperature (UCT) of the TNZ is frequently defined as the temperature where energy expenditure increases,[19] we did not consistently observe increases in energy expenditure even at temperatures where body temperature was increased. Therefore, we followed the approach suggested by Abreu-Vieira et al[20] and used an increase in body temperature to identify UCT. UCT was calculated using a segmental linear model of core body temperature vs calorimetry chamber temperature with the slope 1 = 0. Only body temperature data collected at calorimetry chamber temperatures above 28°C were used for the calculations to avoid fluctuations in body temperature that were more common at colder temperatures.

2.5 | Indirect respiration calorimetry with voluntary running

The mice were housed in acclimation chambers for 24 hours within the calorimeter room. The mice were then placed in calorimeter chambers equipped with running wheels and housed in a 22°C incubator for 24 hours. Energy expenditure and off wheel movement (x and z-plane) were measured in the CLAMS indirect respiration calorimetry system at our standard vivarium temperature of 22°C. Data were collected over 5 light/5 dark cycles (120 hours). The mice were then returned to their standard cages for 5 days. After this time period, the mice were again acclimated to the calorimeter room for 24 hours at 22°C and calorimetry chambers with running wheels for an additional 24 hours with temperature maintained at 28°C, a temperature near the TNZ. Energy expenditure and off wheel movement (x and z-plane) were then measured over 5 light/5 dark cycles at 28°C. Immediately following completion of indirect respiration calorimetry at 28°C, the mice were removed from the calorimetry chambers and body composition was measured using DEXA as described for the baseline calorimetry measurements. Wheel running was not recorded during these experiments due to

intermittent failure of the sensors on many of the wheels that prevented accurate determination of time on the wheels and distance run. However, all mice were detected running during periods when the sensors were functioning.

2.6 | Voluntary wheel running

The mice were singly housed in standard shoebox cages within an incubator set at 22°C (our standard vivarium temperature), 26°C (male mice only), or 28°C for 24 hours prior to attaching running wheels. Voluntary running was measured for 7 days at 15 seconds intervals. A 1 week "break" where mice were housed without running wheel, was scheduled between running assessments at each temperature. Running activity was measured as time and distance run during light and dark cycles. Number of exercise bouts (period of exercise ≥1 minute) and time and distance run during exercise bouts were recorded. Measurements were completed at 22°C followed by 28°C in females, and sequentially from 22°C to 26°C to 28°C in males. All males voluntarily ran on the wheels while a few female mice (equal numbers in each genotype) did not run and were excluded from analysis.

2.7 | Statistics

All statistical calculations were performed using GraphPad Prism 7 software (GraphPad Software Inc, La Jolla, CA) or using Rapid publication-ready MS-Word tables for two-way ANOVA.[21] Data are presented as means ± SE. Student *t* tests were used to test for differences between groups, and for the voluntary wheel running study a mixed effects model with temperature as a repeated measure was used to test for group differences. Analysis of covariance (ANCOVA) was used to adjust energy expenditure by BW or LM. Values were considered statistically significant when $P \leq .05$.

3 | RESULTS

3.1 | BW does not differ between HET and WT mice, although small decreases in fat mass are observed in the HET mice under some conditions

To determine body composition in mice housed at our standard vivarium temperature, measures of BW and composition were completed in male and female mice maintained at 22°C (Table 1). For females, there were no significant differences ($P > .05$) between the HET and WT animals in BW, LM, fat mass, bone mineral content, or percent body fat. In males, there was a decrease ($P < .05$) in fat mass and percent body fat in the HET compared to WT mice but the magnitude of this change was not sufficient to produce a difference in BW between the genotypes. The male HET and WT mice also did not differ ($P > .05$) in LM and bone mineral content.

To determine whether acute increases in physical activity influenced body composition, BW and composition measurements were completed in a separate cohort of male and female mice following continuous access to a running wheel for 1 week at 28°C (Table 1). There were no significant differences between the male HET and WT mice in BW, LM, fat mass, bone mineral content, or percent body fat. The female HET mice showed a trend ($P < .10$) toward a decrease in fat mass and percent body fat compared to WT animals. However, BW, LM, and bone mineral content did not differ ($P > .10$) between the female HET and WT animals. The average age of the female HET mice was 8 days older than the WT group ($P < .05$).

3.2 | Voluntary wheel running is decreased in female HET compared to WT mice

Voluntary wheel running was measured in male HET and WT mice sequentially housed at 22, 26, and 28°C with continuous access to running wheels (Table 2). There was no interaction between genotype and ambient temperature in voluntary wheel running for the male mice. When analyzed across genotypes, ambient temperature had a significant impact ($P < .05$) on number of exercise bouts (one or more minutes of continuous exercise), time spent running, and distance run with both genotypes showing the highest activity values at the intermediate temperature (26°C). However, when analyzed across temperatures, there was no significant difference between genotypes in time running, time in exercise bouts, distance run, number of exercise bouts, and distance run in exercise bouts.

Wheel running was also measured in female mice sequentially housed at 22 and 28°C with access to running wheels (Table 2). Similar to the males, there was no interaction between genotype and ambient temperature in voluntary wheel running for the female animals. However, in contrast to males, the ambient temperatures used in this study had no significant impact on wheel running when analyzed across genotypes. This lack of response to ambient temperature may reflect the fact that measurements were only completed at two temperatures in the females and the temperature that showed the greatest activity values in the males (26°C) was not included in the female study. When analyzed across temperatures, the HET females showed a decrease ($P < .05$) in time running, distance run, time in exercise bouts, and distance run in exercise bouts when compared to the WT mice.

TABLE 1 Effect of the RyR1-R163C mutation on body composition in male and female mice with or without access to a running wheel

	22°C[a]		Running wheel[b]		*P* value	
Variable	**WT**	**HET**	**WT**	**HET**	**Baseline**	**Running**
Males	*n = 8*	*n = 8*	*n = 7*	*n = 7*		
BMD (g/cm^2)	0.0498 ± 0.0007	0.051 ± 0.0018	0.0511 ± 0.0004	0.0517 ± 0.0007	0.506	0.464
BMC (g)	0.452 ± 0.016	0.445 ± 0.018	0.501 ± 0.011	0.524 ± 0.007	0.783	0.105
Bone area (cm^2)	9.07 ± 0.23	8.76 ± 0.36	9.8 ± 0.16	10.1 ± 0.08	0.488	0.099
Fat tissue (%)	21.7 ± 1.7	16.3 ± 1.5	22.2 ± 2.1	21.5 ± 1.3	0.031	0.787
Body weight (g)	32.6 ± 1.4	30.7 ± 1.5	35.1 ± 1.5	34.1 ± 1.0	0.336	0.589
Fat mass (g)	6.8 ± 0.80	4.61 ± 0.57	7.27 ± 1.02	6.89 ± 0.58	0.042	0.750
Lean mass (g)	23.8 ± 0.7	23 ± 0.6	24.5 ± 0.8	24.9 ± 0.5	0.406	0.705
Age (weeks)	29.8 ± 0.7	29.6 ± 0.7	34 ± 0.9	34 ± 0.5	0.855	0.951
Subject length (cm)	9.53 ± 0.08	9.32 ± 0.10	8.95 ± 0.08	9.05 ± 0.09	0.127	0.442
Females	*n = 10*	*n = 10*	*n = 8*	*n = 8*		
BMD (g/cm^2)	0.0686 ± 0.0126	0.076 ± 0.0141	0.0504 ± 0.0007	0.0497 ± 0.0005	0.699	0.425
BMC (g)	0.607 ± 0.122	0.67 ± 0.128	0.47 ± 0.010	0.469 ± 0.013	0.726	0.945
Bone area (cm^2)	8.67 ± 0.17	8.78 ± 0.25	9.33 ± 0.10	9.43 ± 0.18	0.733	0.634
fat tissue (%)	21.5 ± 1.4	20.2 ± 1.8	20.4 ± 1.1	17.4 ± 1.1	0.559	0.077
Body weight (g)	28 ± 1.1	28.3 ± 1.1	26.3 ± 0.8	25 ± 0.5	0.856	0.168
Fat mass (g)	5.76 ± 0.56	5.48 ± 0.68	4.87 ± 0.40	3.93 ± 0.33	0.751	0.093
Lean mass (g)	20.5 ± 0.6	20.9 ± 0.4	18.8 ± 0.4	18.5 ± 0.4	0.532	0.620
Age (weeks)	38.4 ± 1	38.1 ± 1	32.3 ± 1	33.4 ± 0	0.890	0.046
Subject length (cm)	9.25 ± 0.09	8.92 ± 0.17	9.15 ± 0.08	8.88 ± 0.12	0.102	0.079

Note: Body composition of wild type (WT) or RyR1-R163C heterozygous (HET) mice was assessed at baseline or after voluntary wheel running (running wheel). Values are mean ± SE. ($P \leq .05$ is in red, $0.1 > P > .05$ is shown in blue).

Abbreviations: BMC, bone mineral content; BMD, bone mineral density.

[a]The 22°C group includes the same mice used for the indirect calorimetry measurements at 22°C without access to running wheels.

[b]The running wheel group includes the same mice used for the indirect calorimetry measurements with access to a running wheel at 22°C (1 wk) and 28°C (1 wk), with no access to running wheels for a week between the two temperatures. The body composition measurements were performed immediately after completion of the indirect calorimetry measurements at 28°C.

3.3 | Male, but not female, HET mice show increases in energy expenditure adjusted for LM or BW when compared to WT animals

Energy expenditure was measured in male and female mice using indirect respiration calorimetry (Table 3). The mice were housed in calorimetry chambers at 22°C to determine energy expenditure at our typical vivarium temperature. A separate cohort of mice was also housed in calorimetry chambers with running wheels at 22 and 28°C to determine the impact of voluntary physical activity on energy expenditure at a typical vivarium temperature and a temperature near the murine TNZ (Table 4). Energy intake (kcal/24 hours) in the calorimetry chambers did not differ between HET and WT mice (Tables S1–S4). In males, energy expenditure (kcal/24 hours) did not differ between HET and WT mice housed at 22°C without running wheels. However, energy expenditure adjusted for LM or BW using ANCOVA (Figure 1) was increased ($P < .05$) in the HET vs WT animals. In the male mice provided with access to running wheels, energy expenditure (kcal/24 hours) did not differ between genotypes at 22°C but was increased ($P < .05$) in HET compared to WT mice at 28°C (Table 4). A similar pattern also occurred with energy expenditure adjusted for LM or BW (Figure 2) where no differences were observed between genotypes with running wheels at 22°C while energy expenditure was increased ($P < .05$) in the HET vs WT mice at 28°C.

In females, energy expenditure with or without (kcal/24 hours) adjustment for BW or LM (Figure 1 and Table 3) did not differ between HET and WT animals. Total energy expenditure (kcal/24 hours) or energy expenditure adjusted for LM or BW (Figure 2 and Table 4) also did not differ between genotypes in female mice housed with running wheels at either 22 or 28°C.

RER was measured in the mice to determine if genotype influenced substrate oxidation (Tables S1–S4). RER values were slightly higher ($P < .05$) in the male HET vs WT animals at 22°C, with or without running wheels, while RER

TABLE 2 Effect of the RyR1-R163C mutation on voluntary wheel running in male and female mice at three environmental temperatures

	22°C		26°C		28°C		*P* value		
Variable	**WT**	**HET**	**WT**	**HET**	**WT**	**HET**	**Genotype**	**Temp**	***G* × *T***
Males	*n* = 8	*n* = 8	*n* = 8	*n* = 8	*n* = 8	*n* = 6			
Total time (hr)	27.7 ± 2.8	25.5 ± 2.5	33.2 ± 2.8	27.1 ± 2.2	24.2 ± 3.3	17.8 ± 2.4	0.2102	<0.0001	0.3432
Total distance (km)	20.9 ± 2.7	19.2 ± 2.5	35.2 ± 4.4	27.2 ± 3.0	23.5 ± 5.0	15.7 ± 2.6	0.1795	<0.0001	0.2706
Time in bouts (hr)	27 ± 2.9	24.3 ± 2.7	32.3 ± 2.8	26.1 ± 2.2	23 ± 3.3	16.6 ± 2.3	0.1924	<0.0001	0.4108
Distance in bouts (km)	20.6 ± 2.8	18.9 ± 2.5	35 ± 4.4	26.8 ± 3.0	23.2 ± 5.0	15.4 ± 2.6	0.2558	<0.0001	0.2152
Bouts (#)	458 ± 32	458 ± 23	505 ± 30	441 ± 30	386 ± 32	311 ± 42	0.2692	<0.0001	0.2667
Females	*n* = 5	*n* = 5			*n* = 5	*n* = 5			
Total time (hr)	46.9 ± 1.4	34.5 ± 3.5	–	–	44.2 ± 3.8	34.2 ± 2.7	0.0135	0.5384	0.614
Total distance (km)	47.7 ± 4.5	30.2 ± 4.0	–	–	51.3 ± 6.5	33.7 ± 3.5	0.0123	0.3871	0.9928
Time in bouts (hr)	45.5 ± 1.5	33 ± 3.7	–	–	42.9 ± 3.8	32.5 ± 2.6	0.0134	0.526	0.63818
Distance in bouts (km)	47.3 ± 4.6	29.9 ± 4.0	–	–	51 ± 6.5	33.4 ± 3.4	0.0129	0.3823	0.9777
Bouts (#)	524 ± 19	438 ± 27	–	–	473 ± 27	457 ± 37	0.6361	0.0622	0.1747

Note: Running activity of wild type (WT) or RyR1-R163C hemizygote (HET) mice was assessed for 6 d at 22, 26, or 28°C. Values are mean ± SE.
G × *T* = Genotype × Temperature interaction effect. ($P \leq .05$ is in red.)

values did not differ between genotypes at 28°C with running wheels (Tables S1 and S3). In contrast to the males, there were no differences in RER between HET and WT female mice regardless of housing temperature or presence of running wheels (Tables S2 and S4).

In addition to measuring energy expenditure, the calorimetry system was also equipped with motion detectors that allowed measurement of horizontal (*x*-plane) and vertical (*z* plane) movement. We observed that voluntary wheel running was decreased in HET compared to WT female mice (Table 2), and we determined if off wheel movement within the calorimetry chambers was also influenced by genotype (Table 4). When housed at 22°C without access to a running wheel, there was no difference in *x* or *z*-plane movement between HET and WT for both male and female mice (Table 3). To determine if wheel running had an impact on off wheel activity, movement within the chamber was also measured in the groups of mice provided with continuous access to running wheels. The male HET mice showed decreased ($P < .05$) total *x*-plane and *x* ambulatory (consecutive *x*-plane beam breaks) movement off the running wheel at 28°C and a trend ($P < .10$) toward decreased *x*-plane movement at 22°C when compared to the WT mice (Table 4). These changes in activity were all driven by decreases in dark cycle activity (Table S3). In contrast to the males, the females showed no differences between genotypes in movement when off the running wheel with the HET mice only showing a trend ($P = .079$) toward increased total *x*-plane movement at 22°C (Table 4). There were no differences in average 24-hour *z*-plane movement between genotypes for either males or females. Overall, the results of these studies indicate that voluntary movement is not altered in male and female HET mice housed without access to a running wheel. However, when given access to a running wheel movement off the wheel is decreased in the HET male, but not female, mice when compared to the WT animals.

3.4 | The TNZ is expanded in HET mice with a decrease in LCT and, in males, an increase in UCT

The impact of the RyR1 R163C mutation on changes in energy expenditure in response to alterations in ambient temperature were investigated in mice housed in calorimeter chambers while ambient temperature was increased in a stepwise manner from 12 to 36°C (Figure 3). The LCT of the TNZ was determined using a segmental linear model of energy expenditure vs cage temperature to identify the temperature at the breakpoint where the slope becomes zero. The HET males showed a trend toward a 1.3°C decrease ($P = .056$) and the HET females had an approximately 1.7°C decrease ($P < .05$) in LCT compared to the WT mice (Table 5). The UCT of the TNZ was determined using a segmental linear model of body temperature vs cage temperature to identify the cage temperature at the breakpoint where slope begins to increase. There was no difference in UCT between genotypes in the female mice, whereas the male HET mice showed a 1.6°C increase ($P < .05$) in UCT compared to WT animals (Table 5, Figure S1). The shifts in LCT and/or UCT in the HET mice resulted in these animals having a broader TNZ than the WT mice.

The slopes of energy expenditure vs cage temperature were also determined above and below the TNZ to examine the influence of the RyR1 R163C mutation on energetic response to heat and cold stress. As expected, slope was

TABLE 3 Effect of the RyR1-R163C mutation on average 24-hour energy expenditure (kcal) and physical activity in male and female mice housed at 22°C without access to running wheels

Variable	WT	HET	*P* value
Males	*n = 8*	*n = 8*	
Energy expenditure (kcal/24 h)	12.4 ± 0.39	13.1 ± 0.36	.200
Avg 24 h XTOT (#beam breaks/24 h)	36 200 ± 4070	32 400 ± 3420	.487
Avg 24 h XAMB (#beam breaks/24 h)	14 700 ± 2420	12 000 ± 1730	.378
Avg 24 h ZTOT (#beam breaks/24 h)	4550 ± 920	5570 ± 1120	.490
Females	*n = 14*	*n = 14*	
Energy expenditure (kcal/24 h)	12.6 ± 0.40	12.9 ± 0.35	.596
Avg 24 h XTOT (#beam breaks/24 h)	42 000 ± 2100	44 600 ± 3550	.525
Avg 24 h XAMB (#beam breaks/24 h)	16 900 ± 983	17 300 ± 1930	.844
Avg 24 h ZTOT (#beam breaks/24 h)	5680 ± 465	5610 ± 934	.945

Note: Energy expenditure and voluntary motion were measured in wild type (WT) and RyR1-R163C hemizygote (HET) mice housed in indirect respiration calorimeter chambers. Values are mean ± SE.

Abbreviations: XAMB, number of consecutive *x*-axis IR beam breaks; XTOT, number of *x*-axis infrared (IR) beam breaks; ZTOT = number of *z*-axis infrared (IR) beam breaks.

increased ($P < .01$) in the HET mice above UCT, consistent with the increased risk of hyperthermia in these animals with heat exposure. However, there was no difference in slope below LCT in the HET vs WT mice, suggesting that there is no overt impairment in the ability of the HET mice to increase energy expenditure in response to cold stress.

4 | DISCUSSION

Maintenance of a Ca^{2+} gradient between the SR lumen and cytoplasm in skeletal muscle is achieved through the coordinated function of the sarcoplasmic reticulum calcium ATPase (SERCA) and ryanodine receptor Ca^{2+} channel (RYR1). Inherent changes in the efficiency of calcium cycling across the SR due to RYR1-mediated Ca^{2+} leak is likely to elicit global influences on muscle cell Ca^{2+} dynamics across the myoplasm and other organelles, in particular mitochondria, and contribute to myopathic changes.[4,22] Skeletal muscle expressing RYR1 mutations that confer MH susceptibility have also been shown to have chronically elevated cytoplasmic Ca^{2+} at rest, increased RYR1-mediated Ca^{2+} leak, depleted SR stores, and abnormal plasmalemmal Ca^{2+} fluxes.[23] Accordingly, long-term changes in global Ca^{2+} dynamics and compensatory mechanisms that limit muscle damage are likely to impose a demand on skeletal muscle and whole animal energy expenditure. MH caused by mutations in the RYR1[5,24] demonstrates the severe energetic consequences that can occur with defects in this system for maintaining cellular Ca^{2+} gradients. However, humans and mice with mutations in the RyR1 are often described as having no overt phenotypic expression without exposure to anesthesia or heat stress. The purpose of the present study was to determine if energy expenditure and body composition are altered in mice expressing the human MH RYR1 R163C mutation without an anesthesia trigger. The impact on BW and composition of MH mutations has primarily been studied in pigs where the mutation generated interest because it was linked with increased LM.[25,26] However, there have also been reports that body fat content is lower in MH-susceptible pigs,[27,28] and it has been shown that the RYR affects lipid storage in fat cells of *Drosophila* by modulating ER Ca^{2+} levels.[29] It remains to be determined if a similar mechanism for modulating fat storage is operational in mammalian tissue. In our study, there were no differences in BW and LM between the HET and WT mice but there was a significant difference or trend toward a decrease in fat mass in some cohorts of the HET mice. These results are consistent with the idea that the R163C mutation may be associated with a slight decrease in body fat. However, the magnitude of these changes are small and the influence of RYR1 mutations on fat deposits is not sufficient to produce a consistent, overt body composition phenotype.

Physical activity can be a major contributor to daily energy expenditure, and exercise intolerance in MH-susceptible people[30] could have a substantial impact on daily physical activity. To determine if the RYR1 R163C mutation alters voluntary activity, HET and WT mice were given access to running wheels. Female HET mice spent less time running and ran shorter distances than WT animals, while no differences in voluntary wheel running was observed between genotypes for males. A previous study reported decreased voluntary wheel running in mice with the MH RYR1 Y522S mutation,[31] but these results were for a mixed cohort of male and female mice and did not provide information about sex differences. Our study indicates that decreased wheel running in the HET mice is limited to females, and this may reflect the fact that the WT female mice run substantially more than WT male animals. This high wheel running activity in the females may help expose deficits in voluntary activity. The

TABLE 4 Effect of the RyR1-R163C mutation and voluntary wheel running on average 24-hour energy expenditure and physical activity in male and female mice

	22°C + RW		28°C + RW		*P* value	
Variable	**WT**	**HET**	**WT**	**HET**	**22°C + RW**	**28°C + RW**
Males	*n* = 7	*n* = 7	*n* = 7	*n* = 7		
Energy expenditure (kcal/24 h)	12 ± 0.31	12.2 ± 0.30	8.35 ± 0.23	9.02 ± 0.17	0.632	0.035
Avg 24 h XTOT (#beam breaks/24 h)	34 000 ± 4520	22 300 ± 4050	32 700 ± 2200	25 200 ± 1240	0.077	0.012
Avg 24 h XAMB (#beam breaks/24 h)	16 900 ± 2960	8860 ± 2290	16 500 ± 1550	11 700 ± 800	0.053	0.018
Avg 24 h ZTOT (#beam breaks/24 h)	19 600 ± 5100	9250 ± 4470	16 600 ± 2890	8960 ± 521	0.286	0.134
Females	*n* = 8	*n* = 8	*n* = 8	*n* = 8		
Energy expenditure (kcal/24 h)	11.1 ± 0.47	11.4 ± 0.25	8.18 ± 0.37	8.57 ± 0.32	0.534	0.436
Avg 24 h XTOT (#beam breaks/24 h)	18 700 ± 5980	36 500 ± 7270	32 100 ± 6310	38 500 ± 5120	0.079	0.441
Avg 24 h XAMB (#beam breaks/24 h)	7050 ± 3000	14 100 ± 3130	14 600 ± 3080	17 600 ± 2560	0.125	0.464
Avg 24 h ZTOT (#beam breaks/24 h)	7370 ± 4280	13 300 ± 3500	17 000 ± 3710	13 600 ± 2150	0.299	0.445

Note: Energy expenditure and voluntary motion were measured in wild type (WT) or RyR1-R163C hemizygote (HET) mice housed in indirect respiration calorimeter chambers equipped with running wheels (RW).Values are mean ± SE. ($P \leq .05$ is in red, $0.1 > P > .05$ is shown in blue).

Abbreviations: XAMB, number of consecutive *x*-axis IR beam breaks; XTOT, number of *x*-axis infrared (IR) beam breaks; ZTOT = number of *z*-axis infrared (IR) beam breaks.

results of our study indicate that low intensity activity, such as normal movement about the cage, is not altered in HET and WT mice with the exception that male HET mice show decreased off wheel movement when housed with a running wheel. Thus, exercise affects both male and female HET mice with the females showing less voluntary running and the males exhibiting less postexercise activity. Mitochondrial defects have been reported in skeletal muscle from both the RYR1 R163C[16] and Y522S[15] mutations and it is possible that these impairments may limit activity that requires a high rate of oxidative metabolism. Rhabdomyolysis has been reported in MH-susceptible people following exercise,[32] and muscle injury may provide an explanation for decreased off wheel movement in the male HET mice. Access to running wheels was sufficient to trigger an MH episode and death in two of the male mice at 28°C, and heat production with exercise may cause discomfort or induce heat-dissipating activities, such as licking, that limit running or postexercise activity. It has been reported that treadmill running in RYR1 Y522S mice at 34°C induces a lethal crisis, suggesting that exercise induces mechanisms similar to those triggered by heat stress or halogenated anesthetics in susceptible individuals.[3] The results of our study are consistent with the idea that RYR1 mutations can influence exercise-related activity and trigger MH episodes with voluntary activity at temperatures as low as 28°C.

Calcium cycling is a significant contributor to resting skeletal muscle energy expenditure,[4,11,33] and it follows that mutations resulting in disruption of the balance between SR Ca^{2+} sequestration and SR Ca^{2+} efflux via leak and depletion of sequestered Ca^{2+} in the SR could have a noticeable impact on energy expenditure. This is especially important since elevated Ca^{2+} leak is predicted to influence plasmalemmal Ca^{2+} both at rest and during EC coupling.[34] In support of this idea, the results of our study showed that energy expenditure adjusted for LM or BW was significantly increased in HET male mice at 22°C without running wheels and 28°C with running wheel access. Oxygen consumption adjusted for BW has been shown to be increased at 37°C in mice with the RYR1 Y522S mutation.[13] Similarly, an increase in BW-adjusted oxygen consumption was reported in Y522S mutant vs WD mice at 32°C.[15] The results of our study are consistent with the idea that LM and BW-adjusted energy expenditure is increased in MH RYR1 mutant male animals even at temperatures below or near the TNZ (22 and 28°C). However, the magnitude of the increase in energy expenditure in the HET vs WD mice is not large and significant differences between genotypes disappeared with running wheel access at 22°C. This reflects that the RYR1 mutation is likely altering calcium cycling that contributes to resting muscle energy expenditure and resting energy expenditure is a relatively small contributor to total

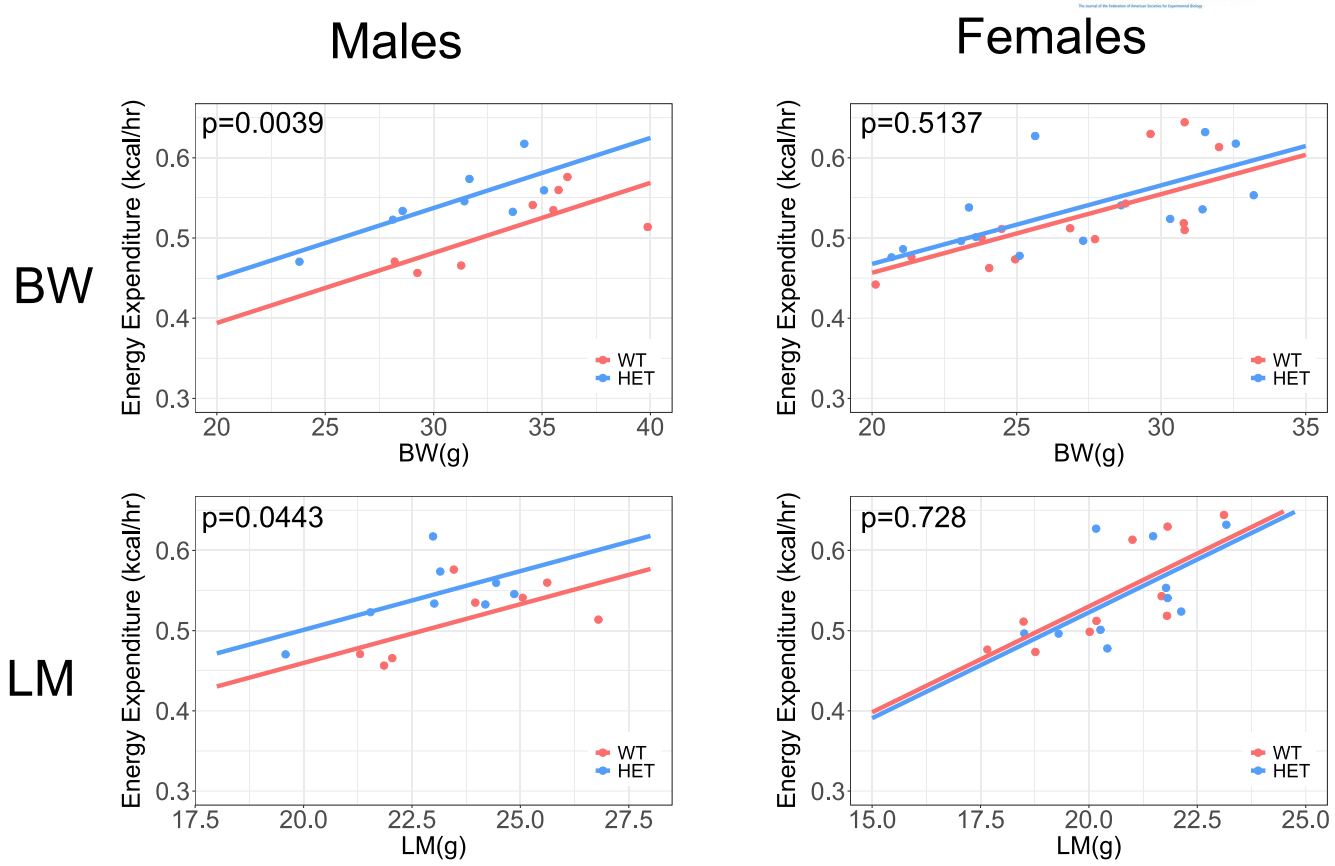

FIGURE 1 Energy expenditure adjusted for lean mass (LM) or body weight (BW) using of covariance (ANCOVA). Energy expenditure in wild-type (WT) and RyR1-R163C heterozygote (HET) male and female mice at 22°C without access to running wheels

energy expenditure (approximately 30%) at typical vivarium temperatures, such as 22°C.[20] Increased physical activity with running wheel access could, therefore, be sufficient to obscure differences in energy expenditure between genotypes when exercise is promoted. If the RYR1 mutation is influencing resting energy expenditure, energy expenditure differences would become more pronounced at temperatures near thermoneutral where resting energy expenditure becomes the major contributor to total energy expenditure. Our data is consistent with this idea since significant increases in energy expenditure, even without adjustment for LM or BW, were apparent in the HET vs WD male mice at 28°C.

The present study also showed that energy expenditure changes induced by the RYR1 mutation are sex dependent. Increases in energy expenditure only occurred in the male HET mice, and no differences in energy expenditure were observed between genotypes in females. In humans, it has been reported that resting energy expenditure is the same between MH-susceptible and MH-negative individuals.[18] However, this study used primarily female volunteers. If the energy expenditure results in the HET mice translate to humans, it is possible that differences in energy expenditure between MH-susceptible and negative people would be missed without including sufficient numbers of male subjects. There is evidence that prevalence or susceptibility to MH is increased in male humans[35,36] and mice.[37] Furthermore, it has been reported that odds of developing MH are greater in males than females even with similar exposures to anesthetics.[7] Our study also shows that energy expenditure, at temperatures that do not trigger MH, is influenced by sex with differences between HET and WT mice restricted to male animals. These results raise the possibility that MH RYR1 mutations have a sex-dependent impact on energy expenditure that may contribute to the increased susceptibility to MH in males. The mechanisms leading to sex difference in physiological measures in individuals with MH RYR1 mutations is not known, and it remains to be determined if allele-specific differences in RYR1 mRNA expression[38] or other potential mechanisms are influenced by sex.

Mitochondrial dysfunction resulting in decreases in mitochondrial respiration has been reported in both MH-susceptible humans[17] and RYR1 R163C mutant mice.[16] Alterations in morphology resulting in swollen and misshapen mitochondria also occur in MH RYR1 mutant mice.[15] If mitochondrial abnormalities are common and consistent components of MH RYR1 mutations, the results of our study would indicate that these defects are not sufficient to alter whole animal energy expenditure. This may reflect the fact that skeletal muscle is responsible for less than a third of resting energy expenditure,[39] and RYR1 mutation-induced

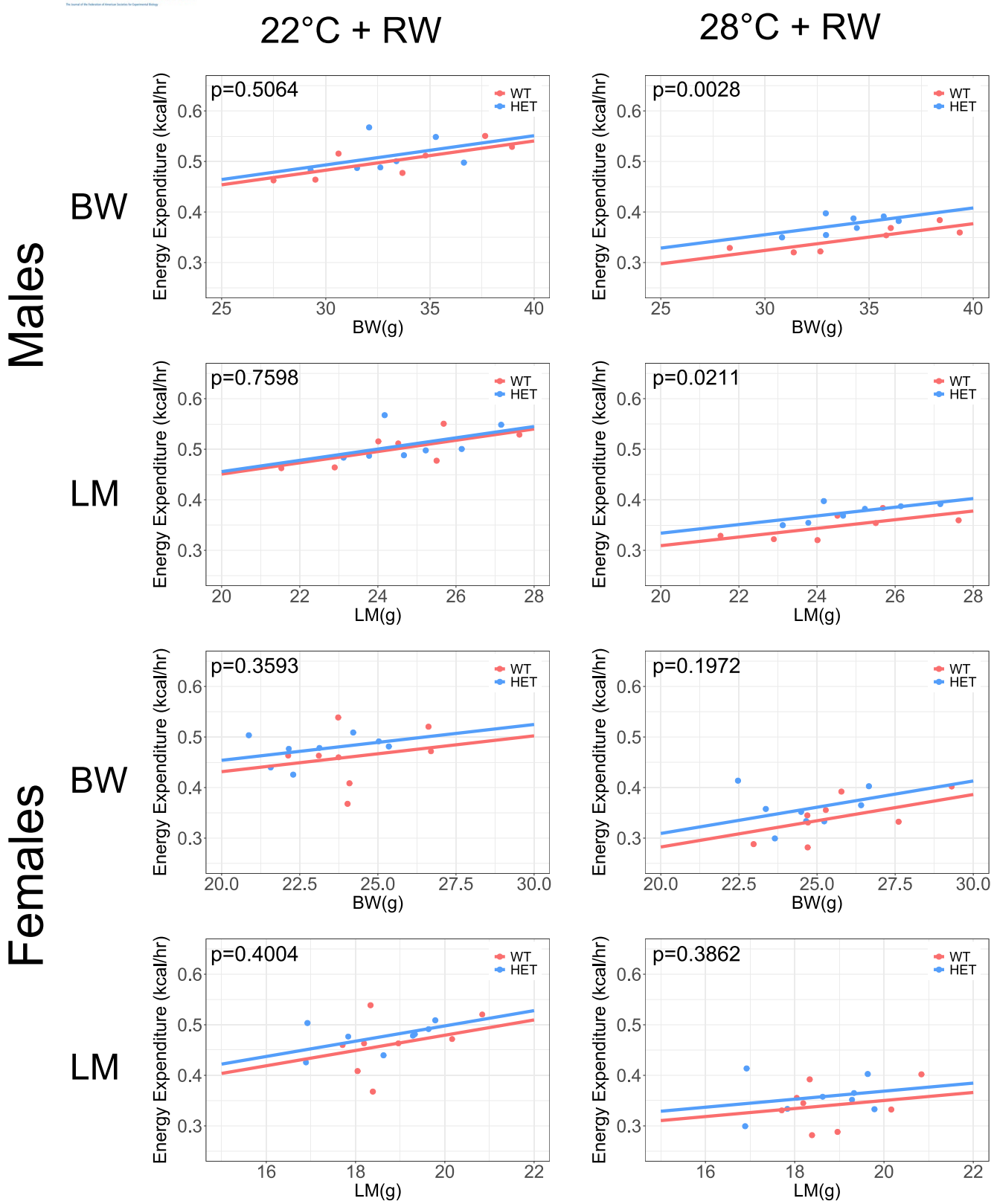

FIGURE 2 Energy expenditure adjusted for lean mass (LM) or body weight (BW) using of covariance (ANCOVA). Energy expenditure in wild-type (WT) or RyR1-R163C heterozygote (HET) male and female mice housed at 22°C or 28°C with running wheels (+ RW)

defects in muscle mitochondrial function may not be of sufficient magnitude to alter energy expenditure at the level of the whole animal. It is possible that mitochondrial dysfunction with RYR1 mutations will limit muscle capacity for oxidative metabolism and this may only impact whole animal energy expenditure during intense exercise. Nonetheless, the results

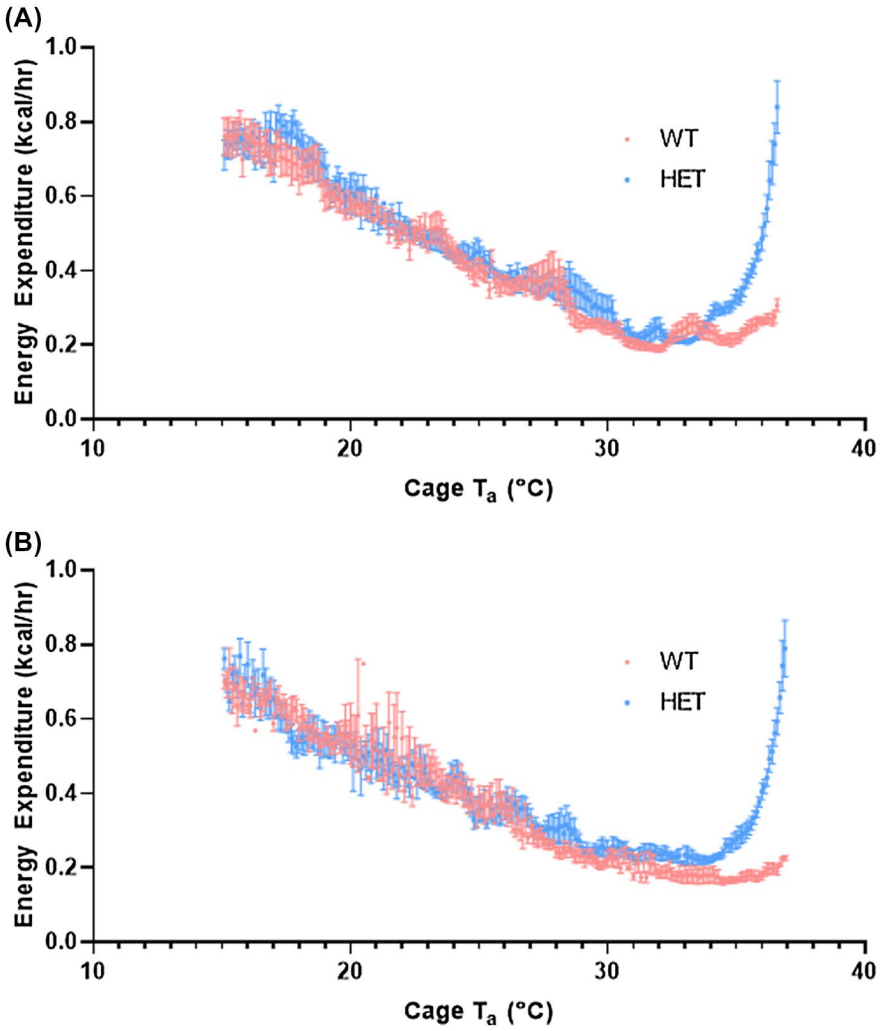

FIGURE 3 Effect of ambient temperature (T_a) on energy expenditure in male (A) and female (B) wild-type (WT) or RyR1-R163C heterozygote (HET) mice. Values are mean ± SE

TABLE 5 Thermal neutral zone and energetic response to cold or heat stress in male and female RyR1-R163C heterozygous (HET) and wild type (WT) mice

Variable	WT	HET	*P* value
Males	*n* = 7	*n* = 7	
LCT	30.6 ± 0.2	29.3 ± 0.6	.056
Slope < LCT	−0.0345 ± 0.0019	−0.038 ± 0.0032	.363
UCT	31.69 ± 0.32	33.31 ± 0.23[a]	.003
Slope > UCT	0.5457 ± 0.0184	1.2427 ± 0.0613	>.001
Females	*n* = 9	*n* = 9	
LCT	30.73 ± 0.5	29.07 ± 0.53	.036
Slope < LCT	−0.0303 ± 0.0024	−0.0296 ± 0.0023	.836
UCT	32.09 ± 0.36[b]	32.16 ± 0.5	.904
Slope > UCT	0.55 ± 0.0487	0.8446 ± 0.0796	.009

Note: Values are mean ± SE. ($P \leq .05$ is in red, $0.1 > P > .05$ is shown in blue).
Abbreviations: LCT, lower critical temperature; UCT, upper critical temperature.

[a]Six HET mice were used to calculate UCT.

[b]Eight WT mice were used to calculate UCT.

of the present study indicate that metabolic alterations in the HET mice do not decrease whole animal energy expenditure, even when the mice are housed with running wheels.

Previous studies in mouse models of MH have measured oxygen consumption in animals at warm environmental temperatures (>32°C),[13,15,40] and there is a lack of information about energetic response in RYR1 mutant mice exposed to a range of temperatures. Thus, we measured energy expenditure in HET mice at temperatures ranging from 12 to 36°C and constructed Scholander plots to determine the effect of ambient temperature on energy expenditure and identify the TNZ.[41] The LCT of the TNZ for all the groups of animals were consistent with values previously reported for laboratory mice,[42,43] however, the HET females had a significant decrease and the HET males a strong trend ($P = .056$) toward a decrease in LCT vs WT animals. The HET males, but not females, also showed a significant increase in the UCT of the TNZ. Overall, these changes resulted in a wider TNZ in the HET mice. The width of the TNZ is dependent on basal energy expenditure and ability to conserve or dissipate heat. An increase in

basal energy expenditure alone could theoretically induce a shift in the TNZ to the left, but this would not be consistent with a widening of the TNZ and we observed no increases in energy expenditure in the female HET mice that would suggest this mechanism is viable. Changes in the LCT and UCT in the HET mice may instead reflect alterations in the activation of mechanisms to conserve or transfer heat, such as changes in posture or vascular tone. While research has primarily focused on RYR1 function in skeletal muscle, RYR1 is also expressed in several brain regions[44] and it has been reported to play a role in voltage-induced Ca^{2+} release in hypothalamic neurons.[45] This raises the possibility that RYR1 mutations could influence regulation of body heat through mechanisms in tissues other than skeletal muscle.

The energy expenditure measurements below TNZ also provided information about acute response to cold in the HET mice. The RYR1 mutation did not alter body insulation since the slope of energy expenditure vs ambient temperature below the TNZ, a measure of insulation,[42] did not differ between genotypes. The HET and WD mice both increased energy expenditure by more than three-fold in response to cold exposure when compared to TNZ. The acute cold-induced increase in energy expenditure in mice previously housed at ~22°C is due to a combination of shivering (skeletal muscle) and non-shivering (brown adipose) thermogenesis.[42] The similar energy expenditure increase observed in HET and WD mice suggests that the RYR1 mutation does not impair these tissue thermogenic responses to acute cold exposure.

A challenge with our TNZ studies was determining the UCT. The UCT is often defined as the ambient temperature where heat stress induces an increase in energy expenditure.[19] However, similar to reports in other strains of mice,[20] we did not observe an increase in energy expenditure prior to an increase in core body temperature. Thus, we used the point where body temperature increases to define UCT. This approach likely provides a slight overestimation of UCT since active evaporative water loss, another common indicator of UCT,[19] would almost certainly have begun by this point. The UCT values in our study, however, show that the TNZ in mice is narrow (only ~1°C in WT animals). This is important to consider for studies designed to occur at TNZ since room temperature gradients could easily move some animals into heat or cold stress.

In conclusion, the RYR1 mutation significantly altered energetics in the HET mice. Energy expenditure, adjusted for either LM or BW, showed an increase in HET male mice but not female animals. However, female HET mice had decreased voluntary wheel running. The RYR1 mutation also induced changes in thermic response to ambient temperature and widened the TNZ. The results of the study show that the RYR1 R163C mutation alters whole animal energetics even at temperatures that do not typically induce MH. Alterations in energetics in individuals harboring RYR1 mutations likely impact physiological function in ways beyond increasing susceptibility to MH.

ACKNOWLEDGEMENTS

This work was supported by a National Institutes of Health grant PO1AR052354 (to PDA and INP) and the UC Davis Mouse Metabolic Phenotyping Center (U2CDK092993). We would also like to thank Todd Tolentino and Leslie Stewart for their technical expertise.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

J.M. Rutkowsky, T.A. Knotts, and J.J. Ramsey designed the research. J.M. Rutkowsky, P.D. Allen, I.N. Pessah, and J.J. Ramsey wrote the paper. J.M. Rutkowsky and T.A. Knotts performed the research. J.M. Rutkowsky, T.A. Knotts, and J.J. Ramsey analyzed the data. P.D. Allen and I.N. Pessah provided the mice.

REFERENCES

1. Denborough MA, Forster JF, Lovell RR, Maplestone PA, Villiers JD. Anaesthetic deaths in a family. *Br J Anaesth*. 1962;34:395-396.
2. Lanner JT, Georgiou DK, Joshi AD, Hamilton SL. Ryanodine receptors: structure, expression, molecular details, and function in calcium release. *Cold Spring Harb Perspect Biol*. 2010;2:a003996.
3. Michelucci A, Paolini C, Boncompagni S, Canato M, Reggiani C, Protasi F. Strenuous exercise triggers a life-threatening response in mice susceptible to malignant hyperthermia. *FASEB J*. 2017;31:3649-3662.
4. Periasamy M, Herrera JL, Reis FCG. Skeletal muscle thermogenesis and its role in whole body energy metabolism. *Diabetes Metab J*. 2017;41:327-336.
5. Canato M, Capitanio P, Reggiani C, Cancellara L. The disorders of the calcium release unit of skeletal muscles: what have we learned from mouse models? *J Muscle Res Cell Motil*. 2015;36:61-69.
6. Riazi S, Kraeva N, Hopkins PM. Malignant hyperthermia in the post-genomics era: new perspectives on an old concept. *Anesthesiology*. 2018;128:168-180.
7. Ibarra Moreno CA, Hu S, Kraeva N, et al. An assessment of penetrance and clinical expression of malignant hyperthermia in individuals carrying diagnostic ryanodine receptor 1 gene mutations. *Anesthesiology*. 2019;131(5):983-991.
8. Merritt A, Booms P, Shaw MA, et al. Assessing the pathogenicity of RYR1 variants in malignant hyperthermia. *Br J Anaesth*. 2017;118:533-543.
9. Miller DM, Daly C, Aboelsaod EM, et al. Genetic epidemiology of malignant hyperthermia in the UK. *Br J Anaesth*. 2018;121:944-952.
10. Takeshima H, Iino M, Takekura H, et al. Excitation-contraction uncoupling and muscular degeneration in mice lacking functional skeletal muscle ryanodine-receptor gene. *Nature*. 1994;369:556-559.
11. Rolfe DF, Brown GC. Cellular energy utilization and molecular origin of standard metabolic rate in mammals. *Physiol Rev*. 1997;77:731-758.
12. Cully TR, Choi RH, Bjorksten AR, Stephenson DG, Murphy RM, Launikonis BS. Junctional membrane Ca(2+) dynamics in human muscle fibers are altered by malignant hyperthermia causative RyR mutation. *Proc Natl Acad Sci U S A*. 2018;115:8215-8220.

13. Lanner JT, Georgiou DK, Dagnino-Acosta A, et al. AICAR prevents heat-induced sudden death in RyR1 mutant mice independent of AMPK activation. *Nat Med*. 2012;18:244-251.
14. Yang T, Riehl J, Esteve E, et al. Pharmacologic and functional characterization of malignant hyperthermia in the R163C RyR1 knock-in mouse. *Anesthesiology*. 2006;105:1164-1175.
15. Durham WJ, Aracena-Parks P, Long C, et al. RyR1 S-nitrosylation underlies environmental heat stroke and sudden death in Y522S RyR1 knockin mice. *Cell*. 2008;133:53-65.
16. Giulivi C, Ross-Inta C, Omanska-Klusek A, et al. Basal bioenergetic abnormalities in skeletal muscle from ryanodine receptor malignant hyperthermia-susceptible R163C knock-in mice. *J Biol Chem*. 2011;286:99-113.
17. Chang L, Daly C, Miller DM, et al. Permeabilised skeletal muscle reveals mitochondrial deficiency in malignant hyperthermia-susceptible individuals. *Br J Anaesth*. 2019;122:613-621.
18. Freymond D, Deriaz O, Frascarolo P, Reiz S, Jequier E, Urwyler A. In vivo whole-body resting energy expenditure and insulin action in human malignant hyperthermia. *Anesthesiology*. 2000;93:39-47.
19. Gordon CJ. Thermal physiology of laboratory mice: defining thermoneutrality. *J Therm Biol*. 2012;37:654-685.
20. Abreu-Vieira G, Xiao C, Gavrilova O, Reitman ML. Integration of body temperature into the analysis of energy expenditure in the mouse. *Mol Metab*. 2015;4:461-470.
21. Assaad HI, Hou Y, Zhou L, Carroll RJ, Wu G. Rapid publication-ready MS-Word tables for two-way ANOVA. *SpringerPlus*. 2015;4:33.
22. Allard B. From excitation to intracellular Ca(2+) movements in skeletal muscle: basic aspects and related clinical disorders. *Neuromuscul Disord*. 2018;28:394-401.
23. Reddish FN, Miller CL, Gorkhali R, Yang JJ. Calcium dynamics mediated by the endoplasmic/sarcoplasmic reticulum and related diseases. *Int J Mol Sci*. 2017;18.
24. Kushnir A, Betzenhauser MJ, Marks AR. Ryanodine receptor studies using genetically engineered mice. *FEBS Lett*. 2010;584:1956-1965.
25. Aalhus JL, Jones SDM, Robertson WM, Tong AKW, Sather AP. Growth-characteristics and carcass composition of pigs with known genotypes for stress susceptibility over a weight range of 70-kg to 120-kg. *Anim Prod*. 1991;52:347-353.
26. Leach LM, Ellis M, Sutton DS, McKeith FK, Wilson ER. The growth performance, carcass characteristics, and meat quality of halothane carrier and negative pigs. *J Anim Sci*. 1996;74:934-943.
27. Hartmann S, Otten W, Kratzmair M, Seewald MJ, Iaizzo PA, Eichinger HM. Influences of breed, sex, and susceptibility to malignant hyperthermia on lipid composition of skeletal muscle and adipose tissue in swine. *Am J Vet Res*. 1997;58:738-743.
28. Lucke JN, Hall GM, Lister D. Anaesthesia of pigs sensitive to malignant hyperthermia. *Vet Rec*. 1977;100:45-48.
29. Bi J, Wang W, Liu Z, et al. Seipin promotes adipose tissue fat storage through the ER Ca(2)(+)-ATPase SERCA. *Cell Metab*. 2014;19:861-871.
30. Thompson SJ, Riazi S, Kraeva N, et al. Skeletal muscle metabolic dysfunction in patients with malignant hyperthermia susceptibility. *Anesth Analg*. 2017;125:434-441.
31. Rouviere C, Corona BT, Ingalls CP. Oxidative capacity and fatigability in run-trained malignant hyperthermia-susceptible mice. *Muscle Nerve*. 2012;45:586-596.
32. Dlamini N, Voermans NC, Lillis S, et al. Mutations in RYR1 are a common cause of exertional myalgia and rhabdomyolysis. *Neuromuscul Disord*. 2013;23:540-548.
33. Smith IC, Bombardier E, Vigna C, Tupling AR. ATP consumption by sarcoplasmic reticulum Ca(2)(+) pumps accounts for 40–50% of resting metabolic rate in mouse fast and slow twitch skeletal muscle. *PLoS ONE*. 2013;8:e68924.
34. Rios E. The cell boundary theorem: a simple law of the control of cytosolic calcium concentration. *J Physiol Sci*. 2010;60:81-84.
35. Britt BA, Kalow W. Malignant hyperthermia: a statistical review. *Can Anaesth Soc J*. 1970;17:293-315.
36. Islander G, Rydenfelt K, Ranklev E, Bodelsson M. Male preponderance of patients testing positive for malignant hyperthermia susceptibility. *Acta Anaesthesiol Scand*. 2007;51:614-620.
37. Yuen B, Boncompagni S, Feng W, et al. Mice expressing T4826I-RYR1 are viable but exhibit sex- and genotype-dependent susceptibility to malignant hyperthermia and muscle damage. *FASEB J*. 2012;26:1311-1322.
38. Grievink H, Stowell KM. Allele-specific differences in ryanodine receptor 1 mRNA expression levels may contribute to phenotypic variability in malignant hyperthermia. *Orphanet J Rare Dis*. 2010;5:10.
39. Ramsey JJ, Harper ME, Weindruch R. Restriction of energy intake, energy expenditure, and aging. *Free Radic Biol Med*. 2000;29:946-968.
40. Knoblauch M, Dagnino-Acosta A, Hamilton SL. Mice with RyR1 mutation (Y524S) undergo hypermetabolic response to simvastatin. *Skelet Muscle*. 2013;3:22.
41. Scholander PF, Hock R, Walters V, Johnson F, Irving L. Heat regulation in some arctic and tropical mammals and birds. *Biol Bull*. 1950;99:237-258.
42. Cannon B, Nedergaard J. Nonshivering thermogenesis and its adequate measurement in metabolic studies. *J Exp Biol*. 2011;214:242-253.
43. Speakman JR, Keijer J. Not so hot: optimal housing temperatures for mice to mimic thermoneutral environment of humans. *Mol Metab*. 2013;2:5-9.
44. Giannini G, Conti A, Mammarella S, Scrobogna M, Sorrentino V. The ryanodine receptor/calcium channel genes are widely and differentially expressed in murine brain and peripheral tissues. *J Cell Biol*. 1995;128:893-904.
45. De Crescenzo V, Fogarty KE, Lefkowitz JJ, et al. Type 1 ryanodine receptor knock-in mutation causing central core disease of skeletal muscle also displays a neuronal phenotype. *Proc Natl Acad Sci U S A*. 2012;109:610-615.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the Supporting Information section.

How to cite this article: Rutkowsky JM, Knotts TA, Allen PD, Pessah IN, Ramsey JJ. Sex-specific alterations in whole body energetics and voluntary activity in heterozygous R163C malignant hyperthermia-susceptible mice. *The FASEB Journal*. 2020;34:8721–8733. https://doi.org/10.1096/fj.202000403