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Reduced contextually induced muscle thermogenesis in rats with calorie restriction and lower aerobic fitness but not monogenic obesity

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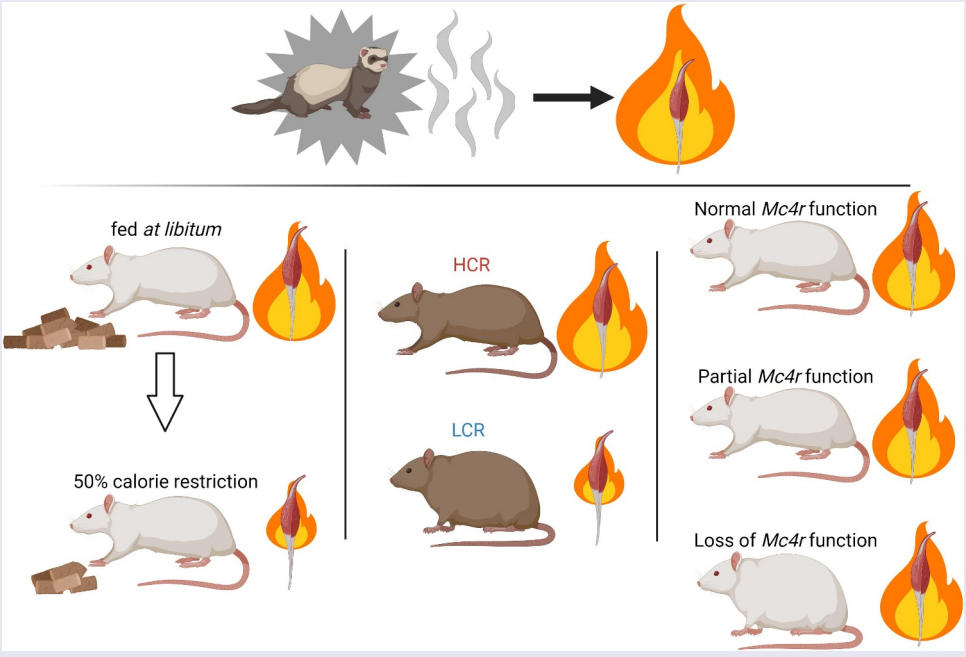
ABSTRACT

We have previously identified predator odor as a potent stimulus activating thermogenesis in skeletal muscle in rats. As this may prove relevant for energy balance and weight loss, the current study investigated whether skeletal muscle thermogenesis was altered with negative energy balance, obesity propensity seen in association with low intrinsic aerobic fitness, and monogenic obesity. First, weight loss subsequent to 3 wk of 50% calorie restriction suppressed the muscle thermogenic response to predator odor. Next, we compared rats bred based on artificial selection for intrinsic aerobic fitness – high- and low-capacity runners (HCR, LCR) – that display robust leanness and obesity propensity, respectively. Aerobically fit HCR showed enhanced predator odor-induced muscle thermogenesis relative to the less-fit LCR. This contrasted with the profound monogenic obesity displayed by rats homozygous for a loss of function mutation in *Melanocortin 4 receptor* (*Mc4r*$^{K3a,4X/K314X}$ rats), which showed no discernable deficit in thermogenesis. Taken together, these data imply that body size or obesity *per se* are not associated with deficient muscle thermogenesis. Rather, the physiological phenotype associated with polygenic obesity propensity may encompass pleiotropic mechanisms in the thermogenic pathway. Adaptive thermogenesis associated with weight loss also likely alters muscle thermogenic mechanisms.



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Introduction

With the progression of the obesity epidemic [1], increasing interest has focused on methods that can decrease caloric intake as well as amplify caloric expenditure. Potential strategies being considered have widened beyond those involving direct manipulation of food intake and increasing physical activity, however, to include activating thermogenesis (reviewed by [2,3]). Strategies to combat obesity by engaging thermogenic mechanisms have focused on brown adipose tissue thermogenesis and "browning" of white adipose to induce or amplify caloric expenditure (both reviewed by [4]); less consideration is commonly given to the potential thermogenic role of skeletal muscle. Indeed, muscle comprises a large proportion of body mass in both men and women [5] and contributes 40% [6] to resting energy expenditure in humans. Evidence supports a sizable role of skeletal muscle glucose uptake in homeostatic acute cold-induced thermogenesis [7] as well as in thermogenic hypermetabolism to maintain temperature homeostasis in an aquatic mammal [8,9].

Recently, we reported that skeletal muscle exhibits a strong thermogenic response in rats and mice exposed to predator threat [10]. This predator odor-induced muscle thermogenesis cannot be accounted for by elevated physical activity alone, and amplifies thermogenesis during physical activity [10]. Caloric expenditure is elevated by 40% or more in association with predator threat in rats [10]. Like cold-induced thermogenesis in brown fat and white adipose browning, the muscle thermogenic response is similarly reliant on sympathetic neural activation and beta-adrenergic receptor activation [10]. While this may provide a promising additional avenue to amplify energy expenditure, it is important to discern if the muscle thermogenic response interacts with energetic challenge. That is, does obesity or the weight loss associated with negative energy balance diminish the ability of predator threat to induce muscle thermogenesis?

Predation risk is known to interact with energy balance on several levels. The presence of predators alters the energy balance of prey species. For example, voles lose weight when forced to share an ecological niche with the odor of their natural predators, weasels [11,12]. Predator threat changes multiple aspects of behavior and physiology that impact energy balance, including caloric intake [11–13], physical activity, and energy expenditure [12], and as described above, energy expenditure stemming from muscle thermogenesis [10]. All of this interacts with ecological and other factors including sex, season, and trade-offs with missed opportunities that affect risk and survival [12,14,15]. Altogether, animals can lessen their risk for predation by shedding grams of body weight, prioritizing mitigating predation risk over starvation risk [13]. Less is known, however, regarding how energetic challenge might alter the response to predator threat, including the muscle thermogenic response. Revealing these mechanisms may allow them to be exploited for therapeutic purposes. Though predation is not a threat to modern humans, the innate responsiveness to predator threat is retained in humans and is able to strengthen fear memory [16]. In humans and non-human primates, predator threat may be detected through olfactory along with other sensory modalities, and the neural substrates mediating behavioral responses to these threat stimuli are highly conserved [16,17]. This is an important consideration in the context of potential therapeutic application given how human obesity is associated with relatively low brown adipose tissue thermogenesis [18,19], and the difficulty in harnessing brown fat thermogenesis to promote weight loss [3,20]. Conversely, negative energy balance induces adaptations that lower energy expenditure (reviewed in [21]). This adaptive thermogenic response with reduced weight includes diminished caloric expenditure during physical activity [22,23]. Lower core body temperature with food restriction, associated with lower heat production [24,25], may also encompass an adaptive thermogenic response in muscle heat generation.

Here, we addressed this question from two angles in rats. First, we examined how negative energy balance and weight loss impacted predator odor-induced muscle thermogenesis. Next, we considered how obesity or obesity propensity might alter muscle thermogenesis using two rat

models. Predator odor-induced muscle thermogenesis was measured in a contrasting rat model of disease risk based on high and low intrinsic aerobic fitness, wherein low aerobic fitness is associated with obesity propensity and metabolic disease [26]. Next, the muscle thermogenic response was examined in a monogenic model for profound obesity using rats homozygous for a loss of function mutation (K314X) in *Melanocortin 4 receptor* (*Mc4r*$^{K314X/K314X}$; HOM) alongside rats heterozygous for the mutation (*Mc4r*$^{+/K314X}$; HET) and wild-type rats with intact MC4R function (*Mc4r*$^{+/+}$; WT) [27,28]. Muscle thermogenesis in this rat model is particularly interesting given the role of melanocortin 4 receptor (MC4R) in modulating sympathetic nervous system (SNS) outflow at multiple levels [29], and given the ability of central activation of melanocortin receptors with melanotan-II to increase muscle thermogenesis [30]. As we have demonstrated that the rat muscle thermogenic response is reliant on intact SNS nerves and beta-adrenergic receptor activation [10], we predicted that loss of MC4R function could have a substantial effect on the ability of predator threat to engage this sympathetically driven muscle thermogenic response.

Materials and methods

Animals, surgery, and predator odor.

These experiments examined (1) adult, male Sprague Dawley rats (N = 8) from Envigo (Indianapolis, IN), starting at 8 wk old; (2) female HCR/LCR (generation 39, N = 16; n = 8/selected line) from the University of Toledo; and (3) male and female WT, HET, and HOM littermate offspring (N = 5/genotype/sex) that resulted from mating of HET rats at Kent State University. WT, HET, and HOM animals were weaned at postnatal day 21 and group-housed (2–3 per cage); ear punches were obtained at weaning and processed using polymerase chain reaction to identify genotype [31]. The HCR and LCR were part of a larger study that utilized viral vector (AAV)-mediated expression of a designer receptor construct; the rats described here received the control construct (AAV-SF1-mCherry (AAV8)) along with i.p. injections of a sterile-saline vehicle 1 h before odor presentation. At ~3 months of age, rats underwent five treadmill-running trials to assess aerobic capacity phenotype. All rats were housed at 22–25°C and had free access to water and fed *ad libitum* unless otherwise noted and were housed individually with environmental enrichment at the time of surgical transponder placement. All rats were fed Prolab RMH 3000 diet with 26% of kcal from protein, 14% from fat, and 60% from carbohydrates. Odor exposures and treadmill assessments took place in the middle of the light phase at ambient temperatures of 22–25°C. Treadmill-walking muscle thermogenesis was assessed to ensure differences in thermogenesis did not stem from reduced muscle contractile thermogenesis. All rats were exposed to the treadmill [32] at walking speed (7 m/min) for 5 min at least 1 d prior to measurement to assure compliance with the walking protocol. Though walking compliance was encouraged using a footshock, pre-exposure to the treadmill procedure minimized footshock during assessments. All breeding and procedures were approved by the Kent State University Institutional Animal Care and Use Committee.

Rats underwent surgical implantation of sterile IPTT-300 temperature transponders (Bio Medic Data Systems, Seaford, DE) bilaterally into the gastrocnemius muscle group, as previously described [10,33]. Briefly, rats were anesthetized using inhaled isoflurane (3–5% for induction, 1–2.5% for maintenance), and a small incision was made in each hindlimb. The transponders were injected, and incisions were closed. Ketoprofen (5 mg/kg, s.c.) and/or buprenorphine (0.01 mg/kg; s.c.) were administered for analgesia. Euthanasia procedures included CO_2 asphyxiation and transcranial perfusion after terminal anesthesia.

After at least 2 wk of recovery from surgery, rats were habituated to the experimental procedure (i.e. moved to the testing room, exposed to towel stimulus with no odor, and transponder temperatures measured using the reader) with no experimental stimuli [10,34]. For measurement of the predator odor-induced thermogenic response, rats were exposed to a small fragment (~2X2 inches) of a towel that had been housed with ferrets for 2–3 wk (Marshall Bioresources, North

Rose, NY), or a "blank" odorless, control towel. Temperatures were measured before odor presentation as well as periodically through 2–6 h after odor exposure. Each rat was measured under both conditions in random order.

Predator odor and muscle thermogenesis before and after 50% calorie restriction

Predator odor-induced muscle temperatures were measured in male Sprague Dawley rats, along with muscle temperature in response to the control odor, in random order, as previously reported [10]. Briefly, rats were exposed to control odor or predator odor counterbalanced in random order, separated by 48 h; muscle temperatures were measured bilaterally at baseline before odor presentation, then again 10, 20, 30, 60, and 90 min after the control or predator odor stimulus presentation. Muscle temperatures reported are the average of the right and left gastrocnemius muscle group, except for one rat in which only the left-leg transponder was functional. Muscle temperature was also measured during graded treadmill walking following the protocol reported by Gavini et al. (2014) [33]. Briefly, muscle temperatures were measured, while rats walked for 2, 5, and 10 min at 7 m/min (0° incline), then 15 min at 9 m/min and 0° incline, and at 20 min at 9 m/min and 10° incline. Many of the rats refused to walk after 15 min, with 5 (before 50% CR) and 4 (after 50% CR) rats completing 20 min of treadmill walking.

Before the onset of food restriction, but after baseline muscle thermogenesis measures, baseline food intake was calculated. Then, rats were subjected to 50% calorie restriction (50% CR, based on 50% of baseline intake) for 3 wk; body weights were measured daily. Subsequently, rats were again assessed for their muscle thermogenic response to predator odor, control odor, and treadmill walking. EchoMRI (EchoMRI, Houston, TX) [35] measurement of fat and lean mass were conducted 10 d before the onset of CR, then again on day 19 of CR (where food was restricted starting on day 0). Muscle thermogenesis in response to predator and control odors was measured on days 20 and 22 of CR, and treadmill-walking muscle thermogenesis was measured on day 23 of CR.

Predator odor and muscle thermogenesis in aerobically fit high-capacity runners and low-fitness low-capacity runners

HCR and LCR were habituated to the context of odor presentation three times prior to exposure to predator or control odors. Female HCR and LCR were presented with control odor or predator odor in random order, and muscle temperatures were measured over 6 h after stimulus presentation.

Predator odor and muscle thermogenesis in rats with monogenic obesity resulting from loss of MC4R function

Muscle thermogenesis in association with monogenic obesity was examined using HOM and HET rats compared to WT controls. Rats underwent EchoMRI magnetic resonance spectroscopy for measurement of fat and lean mass [35]. After transponder implantation and habituation to the testing procedure, muscle thermogenesis was measured after exposure to control odor and predator odor, in random order, in 30 male and female rats (N = 5/genotype/sex). Baseline measurements were taken before odor presentation, immediately after the odor (i.e. towel) was placed in the cage at 0 min, and throughout 120 min of constant exposure to ferret and control odors. On separate days, muscle thermogenesis was also measured during treadmill walking at a constant speed of 7 meters/min for 35 min at 0° incline to promote compliance in the obese HOM rats. The rats were compliant with the treadmill-walking protocol with the exception of the HOM rats, only one of which finished the 35 min of walking; all but one rat finished at least 20 min of walking in each condition.

Statistical analyses

Data are presented as mean ± SEM, with statistical significance at $p < 0.05$. For all repeated-measures ANOVAs, the Huynh-Feldt correction was used when the assumption of sphericity was violated. To examine the effect of negative energy balance from food restriction (before vs after 50% CR) on predator odor-induced muscle thermogenesis (control vs ferret odor, baseline plus 5 timepoints),

average right and left muscle temperatures were compared using a 3-way (2X2X6) repeated-measures analysis of variance (ANOVA). Subsequent follow-up tests consisted of 2 × 6 ANOVAs comparing control vs predator odor before CR, and also after CR, as well as 2 × 6 ANOVAs examining control odor-induced thermogenesis before compared to after CR, and ferret odor-induced thermogenesis before vs after CR. For treadmill-walking thermogenesis, there was no odor presented, so treadmill-walking contractile thermogenesis was measured using a 2 × 5 ANOVA to analyze the impact of food restriction (before vs after 50% CR) on muscle temperatures before and during treadmill walking. Though the rats subjected to CR could walk on the treadmill for up to 25 min, many rats walked only 15 min; therefore, data analyses were conducted on 5 time-points (baseline, and treadmill walking for 2 min, 5 min, 10 min, and 15 min).

Muscle thermogenic response of HCR and LCR was compared using a three-way mixed ANOVA, with selected line (HCR and LCR) as the between-animal independent variable, and odor (control vs ferret odors) and time as the within-animal independent variables. Separate follow-up 2-way ANOVAs compared HCR and LCR thermogenic responses to control odor and predator odor separately. Similarly, a three-way mixed ANOVA compared the muscle thermogenic response of WT, HET, and HOM rats. For body weight and composition in the WT, HET, and HOM rats, a 3 × 2 ANOVA was used to compare body weight, fat mass, and lean mass in the male and female rats between genotypes. Follow-up tests between genotypes used the LSD test, with unpaired t-tests used for within-sex analyses (1-tailed).

Though time of day, recent activity, ambient temperature [36], habituation to the environment, and potentially genetic background can all add variance to muscle physiology and thermoregulation [10,30,34], differences in baseline muscle temperature likely stemmed from differences in transponder placement between investigators. This was mitigated by calculation of baseline-corrected temperature area under the curve (AUC), computed using the trapezoidal method correcting for baseline temperature in each rat, as described previously [10]. Muscle temperature AUCs with control and predator odors before and after 50% CR were compared using a 2 × 2 ANOVA. Temperature AUCs with control and predator odor for WT, HET, and HOM rats were compared using a 2 × 3 ANOVA. For WT, HET, and HOM rats, AUC was calculated using the second baseline measurement (i.e. muscle temperature just before exposure to the odor stimulus). Treadmill-walking temperature AUCs were compared using a two-tailed paired t-test. Temperature AUCs were compared within-rat using paired t-tests (50% CR) or repeated-measures ANOVAs (HCR/LCR and WT/HET/HOM rats), with paired (within rat) or unpaired (between group) t-tests used for follow-up comparisons.

Results

Calorie restriction-induced weight loss reduced the thermogenic response to predator odor.

As shown in Table 1, body composition changes with weight loss over 24 d of food restriction, where 50% CR induced an 18.5% weight loss (± 0.7%, range of 16.6% to 22.2%, 88 g to 112 g); initial body weights ranged from 487 g to 603 g and final body weights ranged from 391 g to 503 g. As shown in Figure 1a, predator odor significantly increased rat gastrocnemius muscle temperature (significant main effects of odor and time). While there was no main effect of 50% CR, there was a significant 3-way ANOVA. Follow-up ANOVAs revealed that predator odor significantly increased muscle temperature both before CR and after CR (Supplemental Table 1). The muscle thermogenic response to control-odor presentation did not significantly change over 50% CR, but predator odor-induced thermogenesis showed a significant interaction where the temperature after 50% CR was lower than before CR. This was mirrored by the muscle-temperature AUC (Figure 1b). At both 60 and 90 min after odor presentation, there was a significant interaction where the thermogenic response to predator odor changed over 50% CR. Although predator odor increased muscle temperature AUCs significantly more than control odor both before and after 50% CR, temperature AUCs after predator-odor presentation were

Table 1. Body weight and composition before and after 50% calorie restriction (CR).

		Before 50% CR	After 50% CR	Change
Body Weight (g)	**Day 0–24**	536 ± 14	437 ± 13	−99 ± 3
	Day −10-19	537 ± 15	455 ± 13	−83.6 g ± 3
Fat mass (g)		74.7 g ± 10	42.8 g ± 8.5	−31.9 g ± 2.5
Lean mass (g)		357.4 g ± 10.9	312.1 g ± 8.5	−45.2 g ± 4.4

Mean ± SEM; 50% CR significantly decreased body weight, fat mass, and lean mass (*p < 0.05).
Fat and lean mass measured 10 d before onset of CR and on the 19th d of CR. N = 8 adult male Sprague Dawley rats.

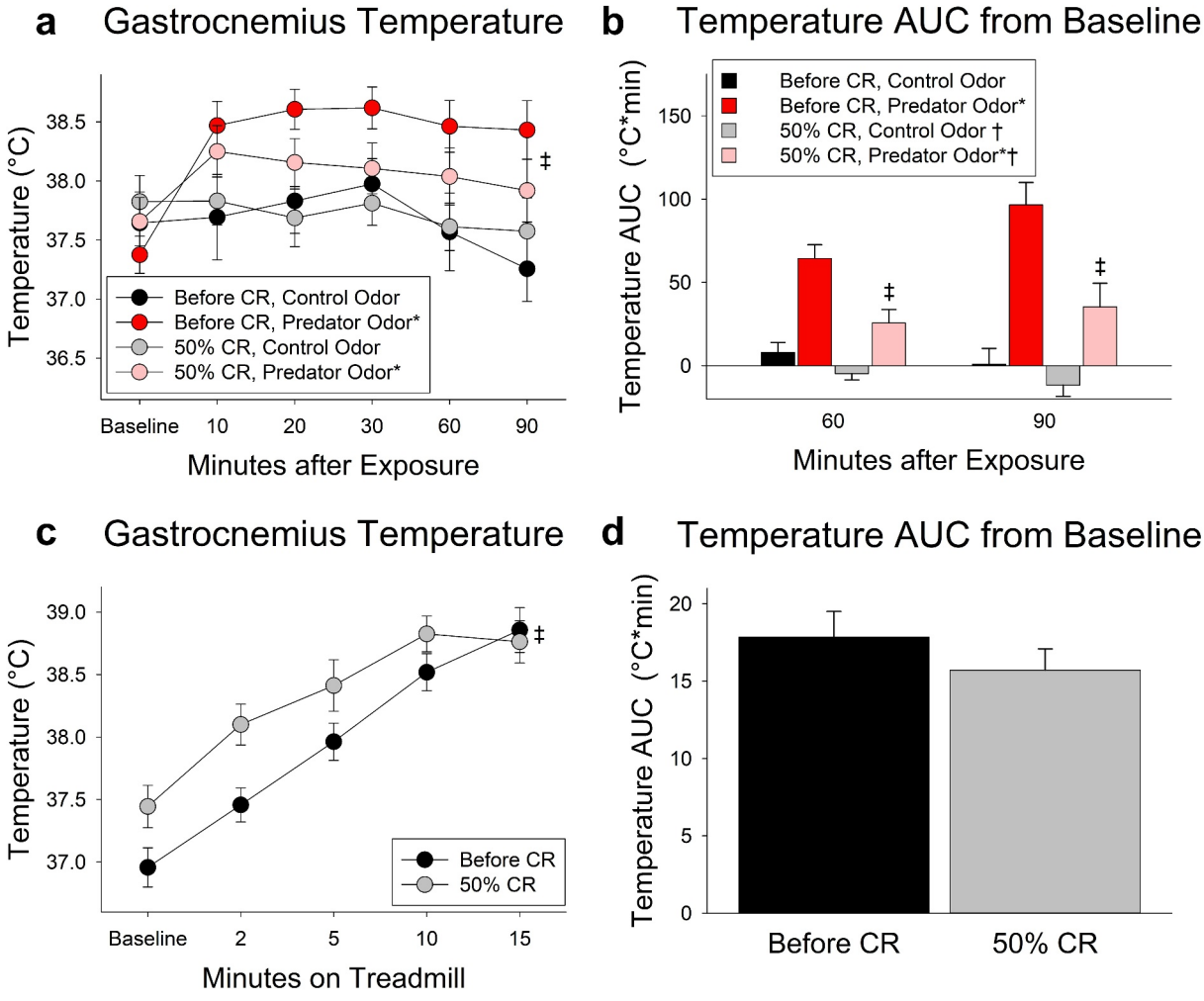

Figure 1. Three weeks of food restriction decreased the muscle thermogenic response to predator odor. (a) Gastrocnemius muscle temperature rapidly increased after exposure to predator (ferret) odor, and this significantly decreased after 21 d of 50% calorie restriction (CR). (‡significant interaction where the thermogenic response to predator, but not control, odors significantly decreased with 50% CR; *significant main effect of predator odor; $p < 0.05$) (b) Baseline-corrected temperature area under the curve (AUC) at 60 or 90 min after exposure (†significant main effect of CR; *significant main effect of predator odor; $p < 0.05$) (c) Muscle temperature increased over time as rats walked on treadmills, with no decrement after CR and no difference in the maximum temperature reached, and no difference in temperature AUC from baseline (d). (‡significant interaction between CR and time on treadmill; $p < 0.05$; N = 10).

significantly higher after 50% CR compared to before CR. In contrast, control-odor temperature AUC did not significantly change over CR.

Treadmill-walking contractile thermogenesis increased over time spent walking on the treadmill (Figure 1c). There was no significant effect of CR;

however, there was a significant interaction. Interestingly, two-tailed paired t-tests revealed significantly higher muscle temperature at 2 min of walking after CR compared to before CR, but not at any other time-point. The baseline-corrected treadmill-walking temperature AUC did not differ before compared to after 50% CR (Figure 1d).

Low-fitness rats prone to obesity showed a reduced thermogenic response to predator odor.

As shown in Table 2, LCR weighed significantly more than HCR in both control ($p = 0.023$) and predator odor exposure ($p = 0.033$; two-tailed unpaired t-test), with no difference between control and predator odor-exposed conditions in either HCR ($p = 0.551$) or LCR ($p = 0.913$; two-tailed paired t-test). This is consistent with their phenotype (high and low aerobic fitness), as was the HCRs' relatively elevated time spent running and distance run on the treadmill during phenotype assessment (Table 2).

The 3-way ANOVA revealed that HCR and LCR showed significantly different thermogenic responses to predator odor over time (Figure 2a). Since all main effects and interactions were significant, separate follow-up 2-way ANOVAs were conducted showing a significant difference between HCR and LCR muscle temperatures (i.e. main effect) and an interaction between selected line (i.e. HCR and LCR) and time (6 h after exposure) after predator-odor exposure but not after control-odor exposure (Supplemental Table 2). Both HCR and LCR showed significantly higher temperatures after predator odor than control odor (i.e. main effect), with a significant interaction between odor (i.e. predator odor vs control) and time (6 h of exposure) only in HCR. The maximal temperature elevation after ferret odor above control exposure in HCR was 1.38°C at 25 min, compared to a 0.52°C increase in LCR at 20 min.

The baseline-corrected AUC analyses for 120 min and 360 min of exposure both showed a significant elevation of muscle temperature with predator odor compared to the control odor, as well as a significant difference between HCR and LCR, and a significant interaction where HCR and LCR responded differently to predator odor and control odor (Figure 2b). Follow-up 2-tailed t-tests revealed that HCR temperature AUCs were significantly higher than LCR after predator odor but not control odor. Moreover, HCR showed a significant change in temperature AUCs when comparing control and predator odor exposures, whereas LCR did not.

Rats with loss of MC4R function showed no deficit in predator odor-induced muscle thermogenesis.

As shown in Table 3, body weight and composition all significantly differed by genotype and sex, where males had higher body weight, fat mass, and lean mass than females. For body weight and fat mass, the LSD test showed that each genotype significantly differed from every other genotype, with the HOM

Table 2. Body weight and aerobic capacity differences in high-capacity runners (HCR) and low-capacity runners (LCR).

Selected line	Exposure	Body weight (g)	Best time (min)	Best distance (m)	Best speed (m/min)	Work (J)
HCR	Control	†268 ± 7 (230–297)	†79.6 ± 2.6 (64.0–87.1)	†2355 ± 131 (1632–2724)	†49.3 ± 1.4 (42–53)	†1007.0 ± 65 (666.9–1217.0)
	PO	†270 ± 8 (226–300)				
LCR	Control	321 ± 19 (248–409)	16.6 ± 0.8 (13.4–19.6)	227 ± 14 (172–281)	17.8 ± 0.4 (16–19)	118.0 ± 8.9 (78.9–154.5)
	PO	321 ± 20 (297–405)				

Mean ± SEM (range in parentheses). There was no difference between control and predator-odor (PO) exposed rats within selected line (2-tailed paired t-test). Aerobic capacity phenotyping took place at ~3 months of age and consisted of 5 graded treadmill trials. HCR had significantly longer maximal running time (best time), maximal distance traveled (best distance), highest speed attained (best speed), and vertical work performed (work). †Significantly different than LCR ($p < 0.05$, 2-tailed unpaired t-tests) N = 8 female HCR and 8 female LCR.

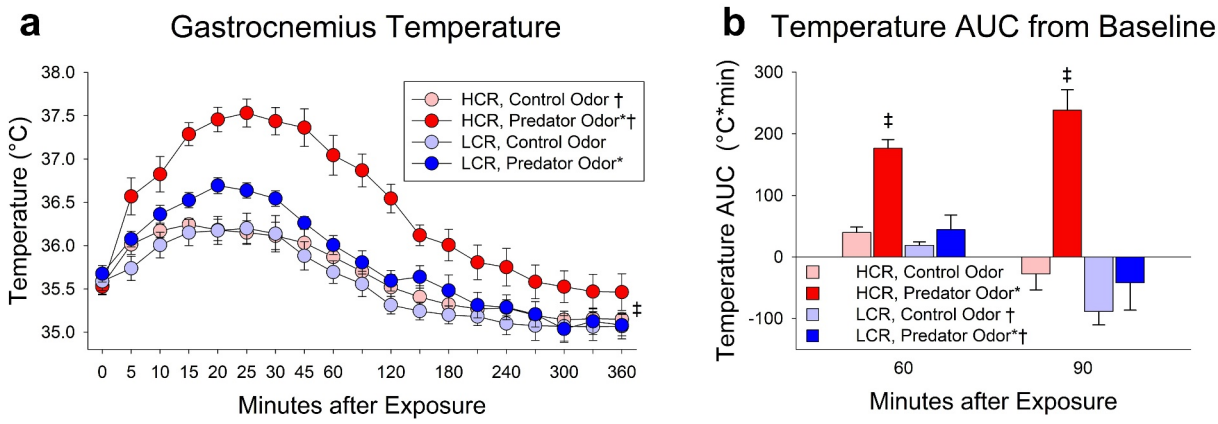

Figure 2. Predator odor induces a greater muscle thermogenic response in lean high-capacity runners (HCR) compared to obesity-prone low-capacity runners (LCR). (a) Exposure to predator (ferret) odor rapidly increased muscle temperature in HCR and LCR, with a significantly higher amplitude in HCR. (‡significant 3-way interaction between fitness phenotype, odor, and time; †significant main effect of fitness phenotype; *significant main effect of predator odor; $p < 0.05$) (b) Temperature area under the curve (AUC) from baseline was also significantly increased after 60 and 90 min of odor exposure in HCR compared to LCR. (‡significant interaction between fitness phenotype and odor; †significant main effect of fitness phenotype; *significant main effect of predator odor; $p < 0.05$; N = 7 HCR + 7 LCR).

Table 3. Body weight and composition of rats deficient in MC4R and the heterozygous and wild-type controls. Mean ± SEM.

Genotype†	Sex*	Body weight (g)	Fat mass (g)	Lean mass (g)
Mc4$^{+/+}$	**Female**	278.7 ± 5.4	45.0 ± 4.3	176.8 ± 3.4
	Male	488.0 ± 24.4	98.7 ± 14.5	304.2 ± 8.0
Mc4r$^{+/K314X}$	**Female**	323.1 ± 22.2	78.1 ± 17.2	183.5 ± 6.5
	Male	553.6 ± 25.3	143.1 ± 9.7	322.5 ± 14.8
Mc4r $^{K314X/K314X}$	**Female**	508.2 ± 16.8	235.6 ± 10.8	202.8 ± 6.9
	Male	743.0 ± 11.4	297.6 ± 8.0	349.1 ± 4.5

WT, *Mc4*$^{+/+}$; HET, *Mc4r*$^{+/K314X}$; HOM, *Mc4r*$^{K314X/K314X}$
†Significant main effects of genotype: body weight and fat mass, HOM > HET > WT; for lean mass, HOM > HET and WT.
*Significant main effect of sex: body weight, fat mass, and lean mass, male > female ($p < 0.05$).
N = 5 females and 5 males/genotype.

rats having significantly higher body weights and fat mass than HET rats, which were in turn significantly higher than WT rats; one-tailed t-tests showed that these same differences were found in both male and female rats of each genotype. Lean mass showed less divergence between genotypes, with the HOM rats having significantly more lean mass than HET rats and WT rats. T-tests of male and female rats separately revealed that both male and female HOM rats had more lean mass than WT rats, and female ($p = 0.037$), but not male ($p = 0.062$), HOM rats had more lean mass than HET rats; Neither male nor female HET rats had more lean mass than WT rats ($p > 0.100$).

Temperature data were first subjected to a 4-way ANOVA including all factors (genotype, sex, predator or control odor, and change in temperature over 120 min); significant effects were limited to time (change in temperature over 120 min) and the interaction between odor (control vs predator odor) and time; no significant main effect or interactions were found for sex, therefore, the analysis was collapsed over sex and subjected to a 3-way ANOVA to probe the factors of genotype, odor, and time (change over 120 min). Again, there was a main effect where temperature changed over 120 after odor presentation, and a significant interaction between odor and time where exposure to predator and control odors resulted in a different change in temperature over time. There was no significant main effect of genotype and no significant interaction between genotype and odor presentation nor 3-way

interaction; the interaction between temperature (change over 120 min) and genotype did not reach significance. The analyses of the baseline-control temperature AUC yielded similar results. A 2X2X3 ANOVA (odor stimulus, sex, genotype) yielded a significant main effect of odor on temperature; no main effect of sex or interactions with sex were found, so this factor was dropped from the analysis. The resulting 2 × 3 ANOVA revealed a significant main effect of odor, where predator odor presentation resulted in a significantly higher temperature AUC than control-odor presentation, but no other main effects or interactions (Supplemental Table 3); the main effect of genotype did not reach significance ($p = 0.093$).

Muscle temperatures during treadmill walking and treadmill-walking temperature AUCs were analyzed using mixed ANOVAs; no effects of sex and no sex-related interactions were found, so sex was dropped as a factor from the analyses. The analyses were complicated by the incomplete treadmill walking by all but one of the male HOM rats after both predator- and control-odor exposures (all other rats completed the full 35 min of treadmill walking). To enable inclusion of male HOM in the analysis, the full 35 min treadmill walking were supplemented by separate analyses of shorter datasets comprised 15 min (N = 4 HOM males) and 20 min (N = 3 HOM males) treadmill walking. In all analyses, temperatures increased with time spent walking on the treadmill, but no other main effects or interactions were significant. In short, walking on the treadmill increased muscle temperatures, and neither treadmill-walking contractile thermogenesis nor temperature AUCs were significantly altered by sex, genotype, or predator odor.

Discussion

Like other aspects of energy balance, muscle thermogenesis is modulated by energetic challenge, even when the thermogenesis is provoked by an allostatic stimulus such as predator threat. Negative energy balance and weight loss suppressed predator odor-induced thermogenesis; however, even after substantial weight loss, rats still showed a marked thermogenic response to predator threat (Figure 1). This implies that muscle thermogenesis could be harnessed to promote energy expenditure even in states of reduced weight or negative energy balance, albeit with some deficit. The ability of predator threat to provoke muscle thermogenesis also differed based on underlying aerobic fitness, where rats artificially selected for high aerobic capacity show an elevated thermogenic response compared to low-fitness rats (Figure 2). The suppressed thermogenic response in obesity-prone rats does not appear to stem from or be dependent on obesity itself, however, as monogenic obesity resulting from loss of MC4R function led to no detectable deficit in thermogenesis (Figure 3). Altogether, the muscle thermogenesis seen with predator threat, while impacting energy balance due to the resultant caloric expenditure [10], may serve a function outside of energy balance, for example, promoting escape from predators [10,12,13].

Three weeks of 50% CR induced a marked weight loss, which was accompanied by a dampening of the ability of predator threat to induce muscle thermogenesis (Figure 1). Contrary to our previous report [37], CR did not suppress muscle contractile thermogenesis during treadmill walking in these rats (Figure 1c). This implies that changes in load *per se* do not meaningfully contribute to muscle thermogenesis, at least within a range of ~100 g, consistent with our prior evidence [10]. Also, this implies that muscle has the capacity to generate ample heat even during CR, but that thermogenic mechanisms are less engaged by the predator-threat context during CR compared to *ad libitum* feeding. Similarly, CR may have differential impact on contractile thermogenesis compared to non-shivering thermogenesis in muscle. The rats were in negative energy balance during thermogenic assessments. Thus, the adaptive thermogenic suppression of the muscle response demonstrated here could be reliant on sustained negative energy balance [38], the weight-reduced state (e.g. even if the absence of suppressed intake relative to expenditure), or both. This suppressed muscle thermogenesis stands in contrast to the ability of calorie restriction to enhance white adipose tissue "browning" to adopt a thermogenic profile [39,40]. We did not consider the potential impact of shorter- or longer-term food restriction or weight loss nor

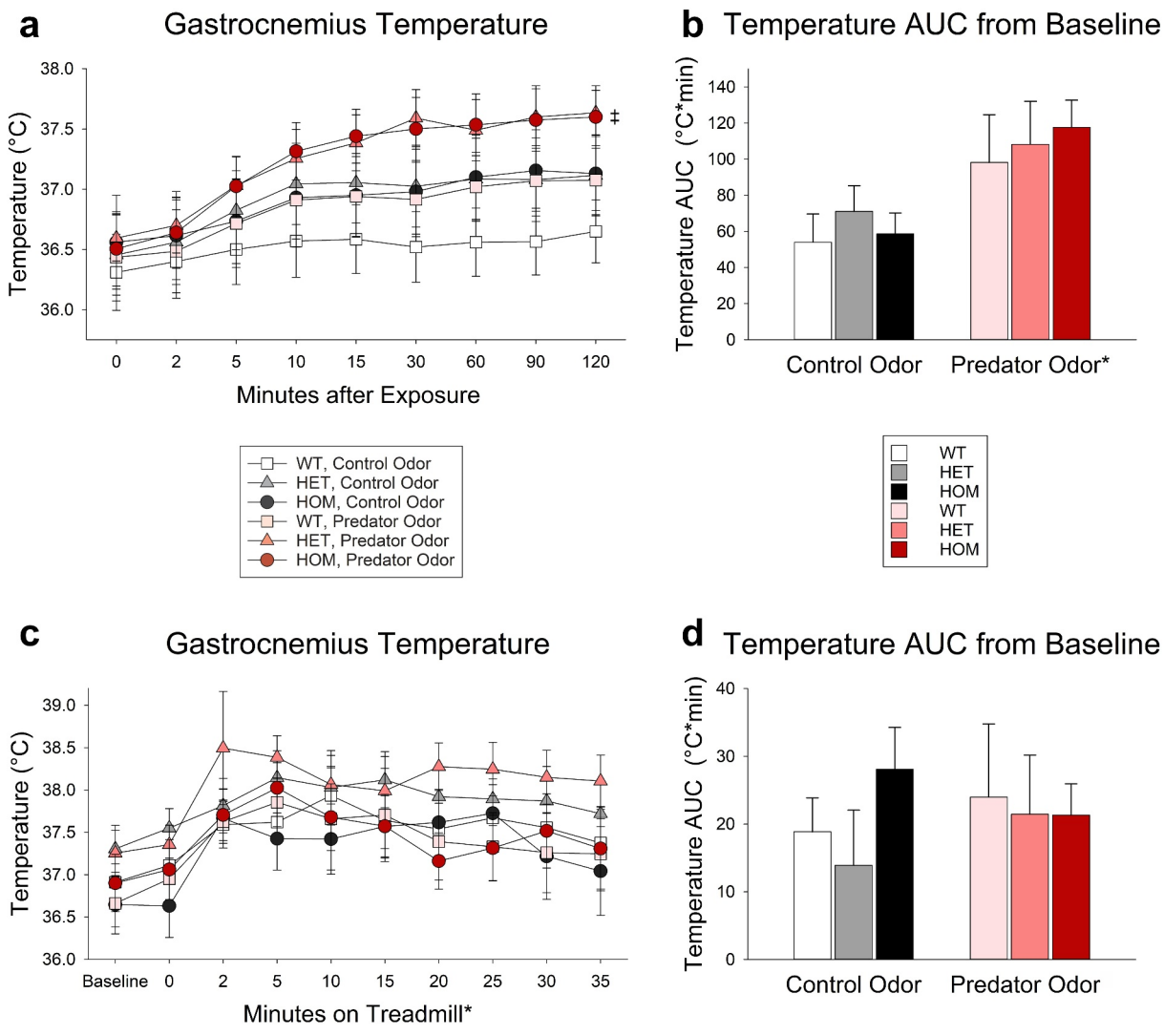

Figure 3. Obese rats lacking functional melanocortin 4 receptor (HOM, *Mc4r*$^{K314X/K314X}$) do not show deficits in predator odor-induced muscle thermogenesis relative to rats heterozygous for the mutation (HET, *Mc4r*$^{+/K314X}$) or wild-type rats (WT, *Mc4*$^{+/+}$). (a) Exposure to predator odor increased muscle (gastrocnemius) temperature relative to control odor, and there was no significant difference between HOM, HET, and WT rats, despite the profound obesity seen in HOM rats. (‡significant interaction between odor and time; $p < 0.05$) (b) Temperature area under the curve (AUC) from baseline was significantly higher with predator odor compared to control odor (*significant main effect of predator odor; $p < 0.05$), with no effect of genotype. (N = 5 females and 5 males per genotype) (c) Treadmill walking increased muscle temperature, with no significant differences between genotype and no effect of odor. (*significant main effect of time on treadmill; $p < 0.05$) (d) Treadmill-walking temperature AUC did not significantly change with exposure to predator odor or genotype. (N = 5 females and 5 males per genotype; for treadmill, N = 4 male HOM).

other dietary challenge like a high-fat diet, on muscle thermogenesis, however.

The suppressed muscle thermogenesis could stem from one or more of several potential mechanisms during food restriction. Lower leptin levels concomitant with fat loss impair the thermogenic response to cold exposure in mice [41]. Like muscle thermogenesis [10], brain control of brown adipose tissue (BAT) thermogenesis is regulated centrally and controlled by SNS outflow [42]. Human skeletal muscle shows biochemical adaptations to weight reduction, along with increased muscle work efficiency [43–45], and much of this is countered by leptin treatment [46,47]. In addition, recovery from weight loss alters iodothyronine deiodinase actions in muscle to favor lower T3 and a local hypothyroid state in muscle without changing systemic thyroid hormones [48–50]. Given the importance of thyroid hormone actions in thermogenesis and

metabolism, this may be relevant to muscle thermogenesis as well.

The metabolic challenge of obesity has the potential to alter non-shivering thermogenesis, at least in BAT. For example, in humans, BAT and cold-induced thermogenesis are lower with higher body mass index [19,51]. Febrile heat response, on the other hand, is intact in different rat models of monogenic obesity [52]. Here, we examined predator odor-induced muscle thermogenesis in rats with loss of MC4R function that shows profound obesity [27,53–55]. Surprisingly, we detected no deficit in predator odor-induced muscle thermogenesis in HOM rats (Figure 3). In fact, there was a non-significant trend toward a higher (rather than lower) thermogenic response in the obese HOM rats compared to their leaner WT counterparts. This was unexpected due to the known role of MC4R in multiple aspects of thermogenic control [56,57], including muscle thermogenesis [30]. We have previously demonstrated that activation of brain melanocortin receptors induces muscle thermogenesis [30]. Moreover, MC4R located in sympathetic preganglionic neurons is involved in the control of body weight, energy expenditure, and thermogenesis [29,58], and the full muscle thermogenic response to predator threat is reliant on intact sympathetic neuronal innervation of muscle [10]. Yet, predator odor-induced thermogenesis was evident in HOM rats compared to WT rats, suggesting that MC4R is dispensable for allostatic induction of muscle thermogenesis. There remains the possibility that melanocortin peptides could exert their actions through other receptors, including MC3R and MC5R, which are present in the brain and modulate metabolic endpoints [59–62]. Moreover, HOM rats show evidence of compensatory overexpression of hypothalamic *Mc3r* [27,55], suggesting the possibility of adaptations in the brain and subsequent behavioral and physiological responses resulting from life-long absence of MC4R signaling. Acute loss of MC4R signaling could influence the normal thermogenic response to predator threat in genetically intact animals. Altogether, despite the contribution of MC4R to the central and endocrine stress response [63] and SNS preganglionic neuron activity [29], the relevance of the brain agouti-related peptide/melanocortin system to integrating internal state with environmental cues including contextual fear [64] and the ability of melanocortin receptor agonists to promote moderate muscle thermogenesis [30,33], this receptor does not appear to be critical for the ability of predator threat to stimulate muscle thermogenesis.

Whereas monogenic obesity had no discernable impact on muscle thermogenesis, the metabolically relevant trait of aerobic capacity was a significant determinant of muscle thermogenic response to predator odor. Rats with high intrinsic aerobic fitness showed an elevated muscle thermogenic response compared to low-fitness rats (Figure 2). This is consistent with prior demonstration of elevated activity thermogenesis in high-fitness rats [30,65]. As differences in metabolic or thermogenic contributions of brain, sympathetic outflow, and muscle have all been established [33,65–67], this phenotypic difference in muscle thermogenesis could originate from any or all of these levels of the pathway that ultimately control muscle thermogenesis. Centrally, this elevated thermogenic response to predator odor may be a part of a distinct profile of response to predation risk in the high-fitness HCR, where a predator odor is a more salient conditioning stimulus [68]. HCR also show relatively elevated sympathetic outflow to muscle, as indicated by increased norepinephrine turnover [33,65]. Lastly, the high-capacity runners also show elevated muscle expression of some proteins relevant to thermogenesis [33,65], as well as lower sarco-endoplasmic reticulum Ca^{2+} ATPase (SERCA) coupling efficiency [69]. Altogether, this implies that elevated muscle thermogenic capacity may be part of the phenotype of high aerobic capacity or cardiorespiratory fitness, which is associated with lower disease risk in adults and children [70–76]. Given the importance of muscle sarco/endoplasmic reticulum Ca^{2+} ATPase uncoupling to both predator odor-induced muscle thermogenesis [69] and resistance to running fatigue [77], it is conceivable that a shared mechanism connects elevated running capacity and muscle thermogenic potential.

Exposure to predator threat alters behavior and endocrine outcomes along with thermogenesis [10,78–80]. Previous investigation considered potential confounders including general stress, aversion, and novelty, also demonstrating elevated

muscle thermogenesis in the presence of predator threat even when physical activity is held constant [10]. These considerations are relevant given the differential spontaneous physical activity and stress response of WT and HOM rats [27,55,63], HCR and LCR rats [33,68,81,82], and food deprived rats [37,83]. While exposure to predator odor makes rats more active, the relatively minor increase in activity seen does not lead to large elevations in muscle temperature in other contexts [10,30]. Therefore, increased physical activity is unlikely to be a major contributor to predator odor-induced muscle thermogenesis or the differences detected here with food deprivation or phenotype. Though restraint stress induces a relatively minor increase in muscle temperature in rats [10], it is conceivable that this could vary with sex, as sex-dependent effects of MC4R genotype have been observed related to HPA axis tone [31]. Sex and the accompanying size difference are unlikely to meaningfully affect muscle thermogenesis. Both male and female rats exhibit muscle thermogenesis with predator threat, with no detectable change over the estrous cycle [10]. Lastly, evidence to date does not suggest that size differences between sexes or genotypes, or changes in body size with food deprivation, affect muscle thermogenesis. Here, female rats are consistently smaller than males but not cooler, and the obese HOM rats do not have higher muscle temperatures than their HET or WT counterparts; lastly, consistent with previous evidence [10], rats that increase body weight over time do not see a corresponding increase in muscle temperature.

Altogether, these studies demonstrate that contextually induced muscle thermogenesis is modulated by energy availability as well as aerobic fitness and obesity propensity but is not diminished by obesity *per se*. Though weight loss suppresses predator odor-induced muscle thermogenesis, the weight-reduced rats still showed significant muscle thermogenesis, implying that this thermogenesis might be harnessed to increase caloric expenditure even in conditions of energetic adaptation. Similarly, rats with monogenic obesity stemming from loss of MC4R function showed no discernable deficit in contextually induced muscle thermogenesis. Though rats with low intrinsic aerobic fitness that were obesity prone (but not obese) showed less thermogenic capacity than their high-fitness counterparts, the muscle thermogenic response was still sizable – ~1°C – therefore potentially exploitable for weight loss. The difference in muscle thermogenesis with disparate aerobic fitness may reflect altered function of one or more mechanisms underlying muscle non-shivering thermogenesis [69]. Altogether, augmenting muscle thermogenesis may be one way to amplify energy expenditure and combat weight gain.

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Abbreviations

50% CR – 50% calorie restriction; ANOVA – analysis of variance; AUC – area under the curve; BAT – brown adipose tissue; CR – calorie restriction; HCR – high-capacity runners; HET – rats heterozygous for the mutation ($Mc4r^{+/K314X}$); HOM – rats homozygous for the mutation ($Mc4r^{K314X/K314X}$); MC4R – melanocortin 4 receptor; LCR – low-capacity runner; SERCA – sarco/endoplasmic reticulum Ca^{2+} ATP-ase; WT – wild-type rats with intact MC4R function ($Mc4r^{+/+}$).

References

[1] Hales CM, Carroll MD, Fryar CD, et al. Prevalence of obesity and severe obesity among adults: United States, 2017-2018. NCHS Data Brief. 2020;Feb(360):1–8.

[2] Li H, Wang C, Li L, et al. Skeletal muscle non-shivering thermogenesis as an attractive strategy to combat obesity. Life Sci. 2021 Mar;15(269):119024.

[3] Larson CJ. Translational pharmacology and physiology of brown adipose tissue in human disease and treatment. Handb Exp Pharmacol. 2019;251:381–424.

[4] Gaspar RC, Pauli JR, Shulman GI, et al. An update on brown adipose tissue biology: a discussion of recent findings. Am J Physiol Endocrinol Metab. 2021 Mar 1;320(3):E488–E495.

[5] Janssen I, Heymsfield SB, Wang ZM, et al. Skeletal muscle mass and distribution in 468 men and women aged 18-88 yr. J Appl Physiol. 1985 2000 Jul;89 (1):81–88.

[6] Zurlo F, Larson K, Bogardus C, et al. Skeletal muscle metabolism is a major determinant of resting energy expenditure. J Clin Invest. 1990 Nov;86(5):1423–1427.

[7] Blondin DP, Labbe SM, Phoenix S, et al. Contributions of white and brown adipose tissues and skeletal muscles to acute cold-induced metabolic responses in healthy men. J Physiol. 2015 Feb 1;593(3):701–714.

[8] Wright T, Sheffield-Moore M. Skeletal muscle plasticity and thermogenesis: insights from sea otters. Temperature. 2022;9(2):119–121. DOI:10.1080/23328940.2021.2004048

[9] Wright T, Davis RW, Pearson HC, et al. Skeletal muscle thermogenesis enables aquatic life in the smallest marine mammal. Science. 2021 Jul 9;373 (6551):223–225.

[10] Gorrell E, Shemery A, Kowalski J, et al. Skeletal muscle thermogenesis induction by exposure to predator odor. J Exp Biol. 2020 Apr 16;223(8). DOI:10.1242/jeb.218479.

[11] Liesenjohann T, Eccard JA. Foraging under uniform risk from different types of predators. BMC Ecol. 2008;8:19.

[12] Tidhar WL, Bonier F, Speakman JR. Sex- and concentration-dependent effects of predator feces on seasonal regulation of body mass in the bank vole Clethrionomys glareolus. Horm Behav. 2007 Nov;52 (4):436–444.

[13] Monarca RI, da Luz Mathias M, Wang D, et al. Predation risk modulates diet-induced obesity in male C57BL/6 mice. Obesity (Silver Spring). 2015 Oct;23 (10):2059–2065.

[14] Eccard JA, Liesenjohann T. The importance of predation risk and missed opportunity costs for context-dependent foraging patterns. PLoS One. 2014;9(5):e94107.

[15] Carlsen M, Lodal J, Leirs H, et al. The effect of predation risk on body weight in the field vole, Microtus agrestis. Oikos. 1999;87(2):277–285.

[16] Taylor JE, Lau H, Seymour B, et al. An evolutionarily threat-relevant odor strengthens human fear memory. Front Neurosci. 2020;14:255.

[17] Montardy Q, Kwan WC, Mundinano IC, et al. Mapping the neural circuitry of predator fear in the nonhuman primate. Brain Struct Funct. 2021 Jan;226 (1):195–205.

[18] van Marken Lichtenbelt W. Brown adipose tissue and the regulation of nonshivering thermogenesis. Curr Opin Clin Nutr Metab Care. 2012 Nov;15(6):547–552.

[19] van Marken Lichtenbelt WD, Vanhommerig JW, Smulders NM, et al. Cold-activated brown adipose tissue in healthy men. N Engl J Med. 2009 Apr 9;360 (15):1500–1508.

[20] Dinas PC, Nikaki A, Jamurtas AZ, et al. Association between habitual physical activity and brown adipose tissue activity in individuals undergoing PET-CT scan. Clin Endocrinol (Oxf). 2015 Jan;82(1):147–154.

[21] Doucet E, McInis K, Mahmoodianfard S. Compensation in response to energy deficits induced by exercise or diet. Obes Rev. 2018 Dec;19(Suppl 1):36–46.

[22] Doucet E, Imbeault P, St-Pierre S, et al. Greater than predicted decrease in energy expenditure during exercise after body weight loss in obese men. Clin Sci (Lond). 2003 Jul;105(1):89–95.

[23] Rosenbaum M, Leibel RL. Models of energy homeostasis in response to maintenance of reduced body weight. Obesity (Silver Spring). 2016 Aug;24 (8):1620–1629.

[24] Reinhardt M, Schlogl M, Bonfiglio S, et al. Lower core body temperature and greater body fat are components of a human thrifty phenotype. Int J Obes (Lond). 2015 Oct;40(5):754–60.

[25] Calonne J, Arsenijevic D, Scerri I, et al. Low 24-hour core body temperature as a thrifty metabolic trait driving catch-up fat during weight regain after caloric restriction. Am J Physiol Endocrinol Metab. 2019 Oct 1;317(4):E699–E709.

[26] Koch LG, Britton SL. Theoretical and biological evaluation of the link between low exercise capacity and disease risk. Cold Spring Harb Perspect Med. 2018 2;8:Jan.

[27] Mul JD, van Boxtel R, Bergen DJ, et al. Melanocortin receptor 4 deficiency affects body weight regulation, grooming behavior, and substrate preference in the rat. Obesity (Silver Spring). 2012 Mar;20(3):612–621.

[28] da Silva AA, Spradley FT, Granger JP, et al. Brain-mediated antidiabetic, anorexic, and cardiovascular actions of leptin require melanocortin-4 receptor signaling. J Neurophysiol. 2015 Apr 1;113 (7):2786–2791.

[29] Sohn JW, Harris LE, Berglund ED, et al. Melanocortin 4 receptors reciprocally regulate sympathetic and parasympathetic preganglionic neurons. Cell. 2013 Jan 31;152(3):612–619.

[30] Gavini CK, Jones WC 2nd, Novak CM. Ventromedial hypothalamic melanocortin receptor activation: regulation of activity energy expenditure and skeletal muscle thermogenesis. J Physiol. 2016 Sep 15;594(18). 5285–5301.

[31] Chaffin AT, Fang Y, Larson KR, et al. Sex-dependent effects of MC4R genotype on HPA axis tone: implications for stress-associated cardiometabolic disease. Stress. 2019 Sep;22(5):571–580.
[32] Kunstetter AC, Barbosa NHS, Moraes MM, et al. Pre-exercise exposure to the treadmill setup changes the cardiovascular and thermoregulatory responses induced by subsequent treadmill running in rats. Temperature. 2018;5(2):109–122. DOI:10.1080/23328940.2017.1388343
[33] Gavini CK, Mukherjee S, Shukla C, et al. Leanness and heightened nonresting energy expenditure: role of skeletal muscle activity thermogenesis. Am J Physiol Endocrinol Metab. 2014 Mar;306(6):E635–47.
[34] Watts CA, Haupt A, Smith J, et al. Measuring Skeletal Muscle Thermogenesis in Mice and Rats. J Vis Exp. 2022;27(185). DOI:10.3791/64264.
[35] Nixon JP, Zhang M, Wang C, et al. Evaluation of a quantitative magnetic resonance imaging system for whole body composition analysis in rodents. Obesity (Silver Spring). 2010 Aug;18(8):1652–1659.
[36] Andrade MT, Goulart KNO, Barbosa NHS, et al. Core body temperatures of rats subjected to treadmill exercise to fatigue or exhaustion: The journal Temperature toolbox. Temperature. 2023. DOI:10.1080/23328940.2022.2115274.
[37] Almundarij TI, Gavini CK, Novak CM. Suppressed sympathetic outflow to skeletal muscle, muscle thermogenesis, and activity energy expenditure with calorie restriction. Physiol Rep. 2017;5(4).
[38] Nunes CL, Casanova N, Francisco R, et al. Does adaptive thermogenesis occur after weight loss in adults? A systematic review. Br J Nutr. 2021 Mar;25:1–19.
[39] Fabbiano S, Suarez-Zamorano N, Rigo D, et al. Caloric restriction leads to browning of white adipose tissue through type 2 immune signaling. Cell Metab. 2016 Sep 13;24(3):434–446.
[40] Corrales P, Vivas Y, Izquierdo-Lahuerta A, et al. Long-term caloric restriction ameliorates deleterious effects of aging on white and brown adipose tissue plasticity. Aging Cell. 2019 Jun;18(3):e12948.
[41] Steiner AA, Romanovsky AA. Leptin: at the crossroads of energy balance and systemic inflammation. Prog Lipid Res. 2007 Mar;46(2):89–107.
[42] Contreras C, Nogueiras R, Dieguez C, et al. Traveling from the hypothalamus to the adipose tissue: the thermogenic pathway. Redox Biol. 2017 Aug;12:854–863.
[43] Vidal-Puig A, Rosenbaum M, Considine RC, et al. Effects of obesity and stable weight reduction on UCP2 and UCP3 gene expression in humans. Obes Res. 1999 Mar;7(2):133–140.
[44] Rosenbaum M, Vandenborne K, Goldsmith R, et al. Effects of experimental weight perturbation on skeletal muscle work efficiency in human subjects. Am J Physiol Regul Integr Comp Physiol. 2003 Jul;285(1):R183–92.
[45] Goldsmith R, Joanisse DR, Gallagher D, et al. Effects of experimental weight perturbation on skeletal muscle work efficiency, fuel utilization, and biochemistry in human subjects. Am J Physiol Regul Integr Comp Physiol. 2010 Jan;298(1):R79–88.
[46] Rosenbaum M, Goldsmith R, Bloomfield D, et al. Low-dose leptin reverses skeletal muscle, autonomic, and neuroendocrine adaptations to maintenance of reduced weight. J Clin Invest. 2005 Dec;115(12):3579–3586.
[47] Baldwin KM, Joanisse DR, Haddad F, et al. Effects of weight loss and leptin on skeletal muscle in human subjects. Am J Physiol Regul Integr Comp Physiol. 2011 Nov;301(5):R1259–66.
[48] De Andrade PB, Neff LA, Strosova MK, et al. Caloric restriction induces energy-sparing alterations in skeletal muscle contraction, fiber composition and local thyroid hormone metabolism that persist during catch-up fat upon refeeding. Front Physiol. 2015;6:254.
[49] Calonne J, Isacco L, Miles-Chan J, et al. Reduced skeletal muscle protein turnover and thyroid hormone metabolism in adaptive thermogenesis that facilitates body fat recovery during weight regain. Front Endocrinol (Lausanne). 2019;10:119.
[50] Di Munno C, Busiello RA, Calonne J, et al. Adaptive thermogenesis driving catch-up fat is associated with increased muscle type 3 and decreased hepatic type 1 iodothyronine deiodinase activities: a functional and proteomic study. Front Endocrinol (Lausanne). 2021;12:631176.
[51] Vijgen GH, Bouvy ND, Teule GJ, et al. Brown adipose tissue in morbidly obese subjects. PLoS One. 2011 Feb 24;6(2):e17247.
[52] Ivanov AI, Kulchitsky VA, Romanovsky AA. Does obesity affect febrile responsiveness? Int J Obes Relat Metab Disord. 2001 Apr;25(4):586–589.
[53] Mul JD, Begg DP, Alsters SI, et al. Effect of vertical sleeve gastrectomy in melanocortin receptor 4-deficient rats. Am J Physiol Endocrinol Metab. 2012 Jul 1;303(1):E103–10.
[54] Mul JD, Seeley RJ, Woods SC, et al. Angiotensin-converting enzyme inhibition reduces food intake and weight gain and improves glucose tolerance in melanocortin-4 receptor deficient female rats. Physiol Behav. 2013 Sep 10;121:43–48.
[55] Almundarij TI, Smyers ME, Spriggs A, et al. Physical activity, energy expenditure, and defense of body weight in melanocortin 4 receptor-deficient male rats. Sci Rep. 2016 Nov 25;6:37435.
[56] Song CK, Vaughan CH, Keen-Rhinehart E, et al. Melanocortin-4 receptor mRNA expressed in sympathetic outflow neurons to brown adipose tissue: neuroanatomical and functional evidence. Am J Physiol Regul Integr Comp Physiol. 2008 Aug;295(2):R417–28.
[57] Vaughan CH, Shrestha YB, Bartness TJ. Characterization of a novel melanocortin receptor-containing node in the SNS outflow circuitry to brown adipose tissue involved in thermogenesis. Brain Res. 2011 9;Sep(1411):17–27.

[58] Berglund ED, Liu T, Kong X, et al. Melanocortin 4 receptors in autonomic neurons regulate thermogenesis and glycemia. Nat Neurosci. 2014 Jul;17(7):911–913.
[59] Sutton AK, Goforth PB, Gonzalez IE, et al. Melanocortin 3 receptor-expressing neurons in the ventromedial hypothalamus promote glucose disposal. Proc Natl Acad Sci U S A. 2021;18(15).
[60] Butler AA, Girardet C, Mavrikaki M, et al. A life without hunger: the Ups (and Downs) to modulating melanocortin-3 receptor signaling. Front Neurosci. 2017;11:128.
[61] Begriche K, Girardet C, McDonald P, et al. Melanocortin-3 receptors and metabolic homeostasis. Prog Mol Biol Transl Sci. 2013;114:109–146.
[62] Xu Y, Guan X, Zhou R, et al. Melanocortin 5 receptor signaling pathway in health and disease. Cell Mol Life Sci. 2020 Oct;77(19):3831–3840.
[63] Ryan KK, Mul JD, Clemmensen C, et al. Loss of melanocortin-4 receptor function attenuates HPA responses to psychological stress. Psychoneuroendocrinology. 2014 Apr;42:98–105.
[64] Padilla SL, Qiu J, Soden ME, et al. Agouti-related peptide neural circuits mediate adaptive behaviors in the starved state. Nat Neurosci. 2016 May;19(5):734–741.
[65] Gavini CK, Britton SL, Koch LG, et al. Inherently lean rats have enhanced activity and skeletal muscle response to central melanocortin receptors. Obesity (Silver Spring). 2018 May;26(5):885–894.
[66] Shukla C, Koch LG, Britton SL, et al. Contribution of regional brain melanocortin receptor subtypes to elevated activity energy expenditure in lean, active rats. Neuroscience. 2015 Dec;03(310):252–267.
[67] Shukla C, Britton SL, Koch LG, et al. Region-specific differences in brain melanocortin receptors in rats of the lean phenotype. Neuroreport. 2012 Jul 11;23(10):596–600.
[68] Burghardt PR, Flagel SB, Burghardt KJ, et al. Risk-assessment and coping strategies segregate with divergent intrinsic aerobic capacity in rats. Neuropsychopharmacology. 2011 Jan;36(2):390–401.
[69] Heemstra LA, Koch LG, Britton SL, et al. Altered skeletal muscle sarco-endoplasmic reticulum Ca(2+)-ATPase calcium transport efficiency after a thermogenic stimulus. Am J Physiol Regul Integr Comp Physiol. 2022;323(5):R628–R637.
[70] Kodama S, Saito K, Tanaka S, et al. Cardiorespiratory fitness as a quantitative predictor of all-cause mortality and cardiovascular events in healthy men and women: a meta-analysis. JAMA. 2009 May 20;301(19):2024–2035.
[71] Goran MI. Metabolic precursors and effects of obesity in children: a decade of progress, 1990-1999. Am J Clin Nutr. 2001 Feb;73(2):158–171.
[72] Artero EG, Jackson AS, Sui X, et al. Longitudinal algorithms to estimate cardiorespiratory fitness: associations with nonfatal cardiovascular disease and disease-specific mortality. J Am Coll Cardiol. 2014 Jun 3;63(21):2289–2296.
[73] Kokkinos P, Myers J, Faselis C, et al. Exercise capacity and mortality in older men: a 20-year follow-up study. Circulation. 2010 Aug 24;122(8):790–797.
[74] Myers J, Prakash M, Froelicher V, et al. Exercise capacity and mortality among men referred for exercise testing. N Engl J Med. 2002 Mar 14;346(11):793–801.
[75] McGavock JM, Torrance BD, McGuire KA, et al. Cardiorespiratory fitness and the risk of overweight in youth: the healthy hearts longitudinal study of cardiometabolic health. Obesity (Silver Spring). 2009 Sep;17(9):1802–1807.
[76] Ladenvall P, Persson CU, Mandalenakis Z, et al. Low aerobic capacity in middle-aged men associated with increased mortality rates during 45 years of follow-up. Eur J Prev Cardiol. 2016 Sep;23(14):1557–1564.
[77] Sopariwala DH, Pant M, Shaikh SA, et al. Sarcolipin overexpression improves muscle energetics and reduces fatigue. J Appl Physiol (1985). 2015 Apr 15;118(8):1050–1058.
[78] Campeau S, Nyhuis TJ, Sasse SK, et al. Acute and chronic effects of ferret odor exposure in Sprague-Dawley rats. Neurosci Biobehav Rev. 2008 Sep;32(7):1277–1286.
[79] Masini CV, Sauer S, White J, et al. Non-associative defensive responses of rats to ferret odor [Comparative Study]. Physiol Behav. 2006 Jan 30;87(1):72–81.
[80] Masini CV, Sauer S, Campeau S. Ferret odor as a processive stress model in rats: neurochemical, behavioral, and endocrine evidence. Behav Neurosci. 2005 Feb;119(1):280–292.
[81] Novak CM, Escande C, Burghardt PR, et al. Spontaneous activity, economy of activity, and resistance to diet-induced obesity in rats bred for high intrinsic aerobic capacity. Horm Behav. 2010 Aug;58(3):355–367.
[82] Novak CM, Escande C, Gerber SM, et al. Endurance capacity, not body size, determines physical activity levels: role of skeletal muscle PEPCK. PLoS One. 2009;4(6):e5869.
[83] Smyers ME, Bachir KZ, Britton SL, et al. Physically active rats lose more weight during calorie restriction. Physiol Behav. 2015 Feb;139:303–313.