Articles in PresS. Am J Physiol Endocrinol Metab (January 7, 2014). doi:10.1152/ajpendo.00555.2013

Leanness and Heightened Non-Resting Energy Expenditure: Role of Skeletal Muscle Activity Thermogenesis

Authors:

Chaitanya K.Gavini[1], Sromona Mukherjee[2], Charu Shukla[2], Steven L. Britton[3], Lauren G. Koch[3], Haifei Shi[4], Colleen M. Novak[1, 2]

[1] School of Biomedical Sciences, Kent State University, Kent, Ohio, USA.

[2] Department of Biological Sciences, Kent State University, Kent, Ohio, USA.

[3] Department of Anesthesiology, University of Michigan, Ann Arbor, Michigan, USA.

[4] Physiology and Neuroscience, Department of Biology, Miami University, Ohio, USA.

Running Head: **Skeletal Muscle and NEAT**

Corresponding author:

Correspondence should be addressed to Chaitanya K. Gavini, School of Biomedical Sciences, Kent State University, PO Box 5190, A328/A330 Cunningham Hall, Kent, OH 44242, USA.

Tel: +1 330 672 2304; fax: +1 330 670 3713; email: cgavini@kent.edu

ABSTRACT

A high-calorie diet accompanied by low levels of physical activity (PA) accounts for the widespread prevalence of obesity today. Yet, some people remain lean even in this obesogenic environment. Here, we investigate the cause for this exception. A key trait that predicts high PA in both humans and laboratory rodents is intrinsic aerobic capacity. Rats artificially selected as high capacity runners (HCR) are lean and consistently more physically active than their low-capacity runner (LCR) counterparts; this applies to both males and females. Here, we demonstrate that HCR show heightened total energy expenditure (TEE) and hypothesize that this is due to higher non-resting energy expenditure (NREE; includes activity EE). After matching for body weight and lean mass, female HCR consistently had heightened non-resting EE, but not resting EE, compared to female LCR. Because of the dominant role of skeletal muscle in non-resting EE, we examined muscle energy use. We found that lean female HCR had higher muscle heat dissipation during activity, explaining their low economy of activity and high activity EE. This may be due to the amplified skeletal muscle expression levels of proteins involved in EE, and reduced expression levels of proteins involved in energy conservation, in HCR relative to LCR. This is also associated with an increased sympathetic drive to skeletal muscle in HCR compared to LCR. We find little support for the hypothesis that resting metabolic rate is correlated with maximal aerobic capacity if body size and composition are fully considered; rather, the critical factor appears to be activity thermogenesis.

Keywords: Non-exercise activity thermogenesis (NEAT), high and low-capacity runners, energy expenditure, obesity

INTRODUCTION

Obesity is increasing worldwide and underlies innumerable associated health problems that lead to decreased quality of life and increased mortality [3, 17, 19, 24, 27, 77]. While the contribution of diet to the obesogenic environment is unquestioned [70], a sedentary lifestyle also has deleterious effects on body weight (BW) and health [8, 14, 33]. An individual's level of daily physical activity (PA) is a biologically regulated, heritable trait that tends to be associated with leanness [4, 46, 47]. We have found that a key feature predicting high PA is intrinsic aerobic capacity; this holds true for both humans and laboratory rodents [36, 37, 39, 58, 64, 96]. Rats that have been selectively bred for high aerobic endurance treadmill running capacity (high-capacity runners; HCR) are consistently more physically active than their low-endurance counterparts selectively bred as low-capacity runners (LCR). Aerobic capacity is a strong predictor of early morbidity and mortality in both men and women [39, 58]; in fact, after age, it is the strongest predictor of longevity in men [58]. This provides support for the theory that greater capacity for energy transfer at all levels of biological organization underlies reduced risk for complex disease and diminished longevity (aerobic hypothesis). Consistent with this, HCR have a low risk of obesity and associated metabolic disorders, as well as enhanced longevity [34, 94]; even though they were not selected for leanness or obesity *per se*, HCR and LCR reliably display lean and obese phenotypes, and this is likely to stem from bioenergetic mechanisms underlying complex disease [35]. We exploit these traits of a lean, high-endurance phenotype to uncover the mechanisms through which a significant fraction of human population (roughly one-third [18]) avoids becoming obese even in the present day obesogenic environment.

The preponderance of evidence points to an individual's "spontaneous" daily activity as an important predictor of obesity resistance [29, 47, 48, 68]. Energy expenditure (EE) associated with both voluntary exercise and non-exercise activity thermogenesis (NEAT) is a vital contributor to both weight maintenance and weight loss [48]. NEAT accounts for the bulk of activity-associated EE in most individuals [46], and the ability to increase NEAT in the face of additional caloric intake has been shown to be the key trait distinguishing people who resist gaining fat [48]. A person's NEAT is determined by their amount of PA, the load they carry, and the fuel cost of that activity. Even though NEAT plays an important role in overall EE, little is known about the mechanisms that might explain the variance in NEAT among individuals. Human NEAT is equivalent to all activity thermogenesis in animals in the absence of exercise [62].

LCR are known to have elevated adiposity and low PA, even in the absence of dietary challenge, whereas HCR resist becoming obese [63, 64]. Here, we tested the hypothesis that heightened PA and, therefore, NEAT meaningfully augment total energy expenditure (TEE). The dominant contributors to TEE are body size and composition; differences in body sizes between lean and obese phenotypes have hampered accurate comparisons of EE and how this relates to obesity propensity [9, 91]. In order to determine the role of NEAT in this lean, high-capacity phenotype and rule out the confounding effect of BW and composition [49], we measured TEE in male rats as well as a population of female rats of the lean, high-capacity (i.e., HCR) and obesity-prone, low-capacity (i.e., LCR) phenotypes that were equivalent in BW or lean mass. Next, using statistical multi-linear modeling, we determined which component(s) of TEE – resting or non-resting EE – differed between lean vs. obesity-prone rats. Heightened NEAT implicates altered

skeletal muscle energy use. Therefore, we tested the hypothesis that calories are dissipated during PA as heat energy, and investigated several potential molecular mechanisms that could account for this additional EE. Lastly, we explored the possibility that differences in activity thermogenesis were accompanied by altered sympathetic nervous system (SNS) outflow to skeletal muscle.

MATERIALS AND METHODS

Adult female HCR/LCR rats (selection generation 25, N=13/group; 10-12 months of age and overlapping in body weights) and adult males from generation 27 (N=16), were obtained from the University of Michigan. Each rat was housed individually on a 12:12 light:dark cycle with lights on at 0700 EST. Rats received rodent chow (5P00 MRH 3000, T.R. Last Co. Inc) and water *ad libitum.* All studies were conducted according to the rules and approval of the Kent State University IACUC.

We verified that the weight-matched females selected for this study were generally representative of the phenotypes of generation 25 female rats by comparing results of the aerobic-capacity treadmill phenotyping analysis conducted for each rat at 11 weeks of age, including body weight and best time, distance, and speed, and work performed. T-tests were used to compare the females studied here relative to all females of generation 25.

Body composition

We measured body composition using EchoMRI-700 (Echo Medical Systems, Houston, TX) to determine the fat and lean mass (in grams) of each rat the day before test. This did not interfere with temperature transponder function (see transponder implantation below).

Measurement of total energy expenditure (TEE) and its components

After body composition determination, adult female HCR and LCR rats were measured for TEE and 24-hr PA using small animal indirect calorimetry (4-chamber Oxymax FAST system, Columbus Instruments, Columbus, OH). Though there are sex and estrous-cycle-related differences in metabolism, the phenotypic differences in activity we documented are robust and seen regardless of sex [63] and of any variance related to the estrous cycle, which has minimal effects on either resting or non-resting EE [21]. Rats were acclimated to the calorimetry chamber (7.5 in x 12 in x 9 in) and room for 24-48 hrs prior to testing; this is sufficient to avoid novelty-induced increases in PA (data not shown). On the day of calorimetry, rats were weighed and placed in the chamber with food and water; the chamber was then sealed. The calorimeter was calibrated using primary gas standards. Air was pumped into the chamber at 1.9-3.1 lpm, depending on the weight of the rat, and chamber air was sampled at 0.4 lpm. Measurement of gas exchange took place every 30 sec throughout the 24-hr period except for a 3.5-min room-air reference and settle period after each 60-sample interval. PA data were collected using infrared beam-break counts, collected every 10 sec uninterrupted throughout the 24-hr period, in the X and Z axes; the first hour of data was not included in the analysis. Data collected from 1200 (EST) on day 1 through 1200 on day 2 were analyzed. EE data (VO2, VCO2, RER, kcal/hr) were averaged, and PA data were expressed as mean beam breaks per minute. BW and food intake

(FI) data indicate that rats were in homeostatic energy balance during EE measurements. For male rats, FI was similar during the EE measurement (20.58±0.64; HCR, 21.41±0.77; LCR, 19.19±1.02) compared to normal daily FI (20.53±0.69; unpaired, 2-tailed *t*-test *p*-value=0.96; HCR, 20.86±0.66; LCR, 19.98±1.55), and BW did not change significantly compared to home-cage days (unpaired, 2-tailed *t*-test *p*-value=0.972). For females, average BW was slightly lower after calorimetry; this is not surprising given that their initial BW is measured at the peak of the daily rhythm (early light phase), and the second measurement is taken at the nadir (late light phase). Change in BW did not differ between groups (HCR, -1.84±1.13g; LCR, -1.94±0.094g; *t*-test *p*-value = 0.95). Female FI was normal for their size and sex, and HCR ate significantly more than did LCR for the duration of calorimetry (HCR, 19.93±1.24g; LCR, 15.60±0.86g; *t*-test *p*-value = 0.01).

Next, we separated the non-resting EE and resting EE components of TEE data described above using Columbus Instruments software (CLAX). Using the data set described above, resting EE was defined as the lowest level of EE, excluding the lowest 5 episodes (i.e., short sequences of consecutive data points) to prevent potential bias due to variation in gas exchange values that can occur during the switch from sample to reference air measurement. We did not use a consistent time period to define resting EE as others have done [21, 52, 53] because the high-resolution activity (every 10 sec) and EE (every 30 sec) recordings revealed that rats were rarely inactive for 30 consecutive minutes or more, and that these periods did not reliably fall at the same time of day among rats. Moreover, these sedentary periods were often marked by heightened residual EE from recent PA. This same method was used to define resting EE during the treadmill test (see below). Because each rat had two separate resting EE values calculated within a short period

of time (one for 24-hr EE, one before treadmill walking), we compared the two values for each rat to validate our method. The two resting EE measurements were very similar: resting EE from the 24-hr calorimetry for HCR was 1.23±0.04 kcal/hr, and resting EE from the 2-hr measurement was 1.29±0.04 kcal/hr; for LCR, the values were 1.19±0.05 kcal/hr and 1.18±0.06 kcal/hr, respectively. Non-resting EE is defined as (TEE) – (resting EE).

As previously reported [63], resting and physical activity EE were directly assessed by measuring gas exchange once every 10 sec during a treadmill activity test. At least one day after a 15-min treadmill acclimation period, rats were placed in the treadmill and allowed to acclimate without food for 2 hrs. Given the time of day, two hours without food is likely to be sufficient to avoid the thermic effect of food (TEF) from what little the rats may have eaten prior to this time during the light phase of the cycle [28]; TEF could not be quantified during the 24-hr EE measurement, however. Over the course of this 2-hr period, rats showed a predictable pattern of behavior: PA and EE rise markedly then fall gradually, reaching a steady state. After the 2-hr resting period, the treadmill was started at 7 m/min for 30 min, during which time steady-state activity EE data were collected. Rats that did not show adequate resting or activity (walking backward on the treadmill, sitting on the treadmill or shocker) were measured at a later date.

All EE data from both males and females were analyzed using *t*-tests, ANOVA, and analysis of covariance (ANCOVA), male and females were not directly compared to each other, however. Because our previous studies suggested the hypothesis that EE was elevated in HCR, we compared resting and non-resting EE between weight-matched or lean-mass-matched HCR and LCR using a 1-tailed paired *t*-test. An unpaired 1-tailed *t*-test was used to compare treadmill gas

exchange values between groups based on our previous results. Lastly, for both male and female rats, TEE, resting EE, and non-resting EE were analyzed using analysis of covariance (ANCOVA) with either body mass or lean mass as the covariate; interaction terms were removed from the analysis if not significant. While lean mass is regarded as the best covariate for analyzing resting EE or total EE [91], activity EE is better evaluated using body weight [76]. Thus resting and non-resting EE were analyzed using each lean mass and body weight as covariates in separate analyses to allow full comparison.

Statistical Modeling

EE data were subjected to modeling using multiple linear regression to determine what parameters/factor(s) best predicted TEE, resting EE, and non-resting EE in HCR compared to LCR. For each of the six combinations of phenotype (HCR, LCR) and EE (TEE, resting EE, non-resting EE), we included BW, fat mass, lean mass, and activity level (horizontal activity counts) as predictors in the initial analysis. To more effectively narrow the relevant predictors of EE, and to avoid over-fitting of the data as well as overlap in predictors (i.e., fat and lean mass as components of BW), the data were then re-analyzed after removing any non-significant factors from the analysis. Analysis was done using MS Excel 2010 (www.microsoft.com).

Transponder Implantation

Adult female HCR/LCR (n=7/group, a subset of actual group mentioned above) rats were anesthetized using isoflurane. A short incision was made on both hind legs. Sterile temperature transponders IPTT-300 (Bio Medic Data Systems, Inc.) were implanted adjacent to the gastrocnemius (gastroc) muscle group of both hind limbs to measure the heat generated by

skeletal muscle during activity. Care was taken to place the transponders so as not to disrupt locomotor function. Rats were allowed to recover for a week before the graded treadmill test was performed. Implant placement was examined during tissue harvest and data from inaccurately placed transponders were omitted from final analyses.

Graded treadmill test

To determine skeletal muscle heat dissipation during controlled PA, we measured the temperatures of the gastroc muscle group using a graded treadmill exercise test. The rats were acclimated to the treadmill for 10 minutes in the days prior to the test as well as immediately before the test. Gastroc temperatures in each leg were recorded at baseline and at set intervals during a 5-level graded treadmill test. Starting at 7 m/min, 0° incline, temperature was measured at 2, 5, and 10 minutes, 15 minutes (9 m/min at 0° incline), 20 minutes (9 m/min, 10° incline), 25 minutes (11 m/min, 10° incline), and 30 minutes (11 m/min, 20° incline). The test was stopped at end of 35 minutes; however some LCR were not able to complete all 5 levels of activity.

Muscle gene expression

Skeletal muscle (gastroc and quadriceps (quad)) was collected from adult female HCR/LCR rats (from a separate group, generation 25, N = 8 or 7/group) after euthanasia by rapid decapitation without anesthetic agents after several weeks without exercise. Muscle samples were homogenized and total mRNA was extracted using Ambion ribopure kit following manufacturer's instructions. The purity of mRNA was measured using NANODROP (ND-1000) (Nanodrop technologies) and A260/280 ratio to be ranging from 1.8 – 2.1.This mRNA was used to prepare cDNA using an Applied Biosystems kit and thermal cycling at 25°C for 10 minutes,

48°C for 30 minutes, 95° C for 5 minutes and holding at 4°C. The cDNA was used for quantifying the expression of uncoupling proteins 2 and 3 (UCP2, UCP3), ATP-dependent potassium channel (K^+_{ATP} subunits Kir6.1, Kir6.2), Mediator of RNA polymerase II transcription subunit 1 (MED1), and glyceraldehyde 3-phosphate dehydrogenase (GAPDH, used as a control). The relative expression was calculated using comparative Ct method (ΔCt). Data are expressed as a percent expression using the HCR as the reference value (defined as 100%), and groups were compared using a 2-tialed t-test.

Western blotting

UCP2, UCP3, and the K^+_{ATP} subunits Kir 6.1 and Kir 6.2 (predominantly found in skeletal muscle) and MED1 were examined in skeletal muscle (gastroc and quad) from adult female HCR/LCR rats (same group of generation 25 rats used for muscle gene expression, N = 5 or 4/group), using actin as a loading control for protein expression. Skeletal muscle samples were homogenized with ice-cold RIPA buffer (Thermo Scientific) containing a protease inhibitor cocktail (Roche Diagnostics). The supernatant from the homogenization and subsequent centrifugation was used for the analysis. Equal quantities of supernatant and sample buffer (150mM tris-HCl pH 6.8, Trizma-base for pH, 6% SDS, 30% glycerol, 0.03% pyronin-Y, DTT) were mixed and tubes were heated at 90°C for 3 minutes. Samples containing equal quantity of protein were loaded on to a gradient gel (4-15%) (Bio Rad) and electrophoresed using SDS running buffer (0.384M glycine, 0.05M Trizma bas, 0.1% SDS) at constant voltage (150V) for 30 minutes. The gel was blotted on to a PVDF membrane using semi-wet blotting apparatus and Otter et al. transfer buffer (49.6mM Trizma base, 384mM glycine, 17.5% methanol, 0.01% SDS) at constant current (400mAmp). The blot was incubated overnight in a blocking solution of 5%

milk (Blotto) in 1xPBST (Phosphate buffered saline; 84mM sodium hydrogen phosphate, 16mM sodium dihydrogen phosphate, 100mM sodium chloride, tween20), then rinsed using 1xPBST. Primary antibodies for UCP2, UCP3, Actin, MED1 (Abcam Inc. ab67241, ab3477, ab1801, ab60950 respectively), and for Kir 6.1, Kir 6.2 (Santa Cruz Biotechnology, Inc. sc-11224 and sc-11230 respectively) were diluted in blocking solution at ratio of 1:1000 (Kir 6.2, Kir 6.1, and MED1 at 1:500) and incubated with the blot overnight. Secondary antibodies were diluted in blocking solution in the ratio 1:5000 and incubated for 1 hour at room temperature. After washing, the blots were developed using a chemiluminiscence detector using an Amersham kit (GE Healthcare, UK). The expression levels relative to actin were plotted as a percent of the reference value (with HCR as 100%), and groups were compared using a 2-tialed t-test.

Norepinephrine turnover (NETO)

Adult male HCR/LCR rats (generation 30, N= 13 HCR, 12 LCR) were used to assess sympathetic drive to skeletal muscle (including quad, lateral and medial gastroc, and soleus) and brown adipose tissue (BAT). They were individually housed on a 12:12 light: dark cycle with *ad libitum* access to standard rodent chow and water and acclimated to daily handling for a week. Level of sympathetic drive to peripheral tissues was determined by norepinephrine (NE) turnover (NETO) method using α-methyl-*p*-tyrosine (aMPT) [78]. aMPT is a competitive inhibitor of tyrosine hydroxylase, the rate limiting enzyme in catecholamines biosynthesis. After aMPT administration, the endogenous tissue levels of NE decline at a rate proportional to the initial NE concentrations. The rats were divided into 2 sub-groups per group (aMPT, control). On the day of study, rats receiving aMPT were injected with aMPT (125 mg aMPT/Kg of BW, 25 mg/ml) and with a booster dose at same concentration 2 hours later. All rats were euthanized by rapid

decapitation between 1200 and 1500, 4 hours after first aMPT injection. Tissues were rapidly dissected and snap-frozen in liquid nitrogen.

Briefly, tissue was thawed and homogenized in a solution containing dihydroxybenzylamine (DHBA, internal standard) in 0.2M perchloric acid (PCA) with 1 mg/ml ascorbic acid (AA). Following centrifugation for 15 min at 7,500 *g* at 4°C, catecholamines were extracted from the homogenate with alumina and were eluted into the PCA/AA. The catecholamines were assayed using an HPLC system with electrochemical detection (Coulochem III), MDTM mobile phase and reverse phase MD 150x3.2 column. NETO in quad, lateral and medial gastroc, BAT and soleus were calculated using the following formula [78]:

$k = (\lg[NE]0 - \lg[NE]4)/(0.434 \times 4)$

$K = k[NE]0$

k is the constant rate of NE efflux (also known as fractional turnover rate),

[NE]0 is the initial NE concentration or from 0-hr group (control),

[NE]4 is the final NE concentration or from 4-hr group (aMPT), and

K = NETO.

Difference in NETO of HCR and LCR tissues were compared using 1-tailed, unpaired t-test for each tissue.

RESULTS

Rats of the lean, high-activity phenotype have higher TEE and non-resting EE, but not consistently high resting EE.

Results of t-tests supported the assertion that the weight-matched female HCR and LCR measured in this study were both representative of the larger populations (all other female HCR and LCR from generation 25). Compared to other generation 25 female HCR, the female HCR in this study weighed significantly more (+9.6 g; $p=0.03$), but showed equivalent performance on all aerobic capacity variables (best time, distance, and speed; $p>0.30$ for all), with the exception of work (Joules), which was greater in the weight-matched HCR ($p<0.05$), a consequence of their larger BW. The LCR in this study weighed significantly less than the other female LCR of generation 25 (-13.7 g; $p<0.01$), but there were no other differences between this group and other female LCR in generation 25 in work, best time, distance, or speed during the treadmill test.

As shown in Figure 1, female HCR have significantly higher TEE when rats were matched for either BW (Figure 1A) or for lean mass (Figure 1B), or when analyzed as a group (Figure 1C). To parse the components of TEE (i.e., resting EE + non-resting EE), we first validated our method to estimate resting EE from the 24-hr calorimetry dataset using the 2 hr treadmill test described above [63]. The two methods of calculating resting EE did not significantly differ (t = -0.64; p = 0.53), supporting the validity and accuracy of our resting EE calculation from TEE data. After matching for BW, total energy cost of activity (treadmill walking) was higher in HCR (3.09±0.10 kcal/hr) compared to the LCR (2.88±0.08 kcal/hr).

Resting EE over the 24-hr test did not differ between female HCR and LCR, neither as a group (without matching; Figure 1C) or when matched for lean mass (Figure 1B). Non-resting EE was significantly higher in female HCR in every analysis performed—after matching for either lean mass or BW, or without matching (Figure 1). The heightened non-resting EE in the lean, high-

capacity phenotype (HCR) was not secondary to a higher workload as fat mass and/or total mass were higher in the LCR in each analysis. PA, both horizontal and ambulatory activity, was also higher in female HCR than LCR in every analysis performed (Figure 1). ANCOVA analyses of EE data from female HCR/LCR revealed that BW or lean mass were the dominant determinants of resting EE, as resting EE was consistently higher in rats with greater BW or lean mass (Figure 2). Lastly, non-resting EE increased with higher BW or lean mass, and HCR consistently had higher non-resting EE compared to LCR, even after BW or composition were considered using ANCOVA (Figure 2).

Table 2 contains the results of the multiple linear regression analyses of TEE and its components in female HCR and LCR. In general, lean mass was the dominant predictor of all EE variables; lean mass tended to be a better predictor in LCR than in HCR, however. PA also significantly contributed to predicting TEE and non-resting EE in both lines of rats. Overall, there was more unaccounted-for variance in TEE and resting EE in HCR compared the LCR.

As illustrated in Figure 2, ANCOVA analysis of EE data from male HCR/LCR revealed that, as in females, both BW and lean mass strongly affected TEE, resting EE, and non-resting EE. As with the females, male HCR showed greater TEE relative to LCR with either BW or lean mass as the covariate. Non-resting EE was also higher in male HCR compared to male LCR with either BW or lean mass as the covariate. Unlike in females, however, the male HCR also showed a slight but significant elevation in resting EE compared to LCR.

High activity energy expenditure is accompanied by skeletal muscle energy dissipation as heat.

Our analysis of EE clearly implicated non-resting EE (composed primarily of activity EE), more than resting EE, as the predominant contributor to the heightened TEE seen in the lean phenotype. This, along with previous data showing decreased economy of activity in HCR [63], strongly implicated differential skeletal muscle energy use as an underlying cause of differences in non-resting EE and, therefore, TEE. The question remained as to how the HCR dispose of these additional calories. Here, we tested the hypothesis that HCR ultimately expend calories through heat dissipation in skeletal muscle. This hypothesis was tested by measuring gastroc temperature during a graded exercise treadmill test equalizing workload between rats (matched by body weight). HCR showed significantly higher gastroc temperatures, and their maximal rise in temperature was significantly higher in HCR compared to LCR (Figure 3), demonstrating that HCR have heightened skeletal muscle heat dissipation compared to LCR.

Lean, high-activity rats have higher expression of mRNA and proteins involved in energy expenditure, and lower expression of energy conserving processes in both quadriceps and gastrocnemius.

To determine the source of the calorie use and heat dissipation in HCR skeletal muscle, we examined mRNA and protein expression of molecular endpoints known to alter energy use in quad and gastroc of HCR and LCR; for energy conservation, MED1 [11] and K^+_{ATP} (subunits Kir6.1, Kir6.2) [2, 61]; for energy expenditure, we examined UCP2 and UCP3 [72]. The mRNA content of UCP2 and UCP3 were found to be higher in HCR compared to LCR; that of potassium channel subunits Kir6.1 and 6.2, as well as MED1, were higher in LCR compared to

HCR (Figure 4). Protein expression levels of proteins involved in energy expenditure (UCP2, 3) were found to be higher in HCR compared to LCR in both quad and gastroc. Protein expression levels of MED1 were higher in LCR compared to HCR in both quad and gastroc (Figure 4). No differences were found in protein levels of K^+_{ATP} subunits Kir6.1 and 6.2 in the quad.

Lean rats have elevated SNS drive to skeletal muscle.

As illustrated in Figure 5, compared to LCR, HCR had higher NETO in several skeletal muscle groups, including quad (5C), medial (5A) and lateral gastroc (5B), and soleus (5D) indicating higher sympathetic drive to skeletal muscle, potentially modulating their economy of activity. Higher NETO was also found in interscapular BAT in HCR (104.08± 9.8) compared to LCR (29.17± 2.35; one-tailed, unpaired *t*-test *p*-value<0.0001).

DISCUSSION

It is known that lean people and lean animals have higher daily PA levels [29, 47, 64, 65] and that this is a biologically regulated trait [4, 46]. It was not known, however, whether or not this physical activity energy expenditure, or NEAT, meaningfully contributes to TEE. Here, we use a rat model of a lean phenotype to demonstrate that lean rats show heightened TEE, and that this is primarily due to elevated non-resting EE. Taken together with previous studies [63, 64], we have shown that the amplified NEAT characteristic of this lean phenotype is predominantly due to increased daily activity levels combined with increased fuel cost of that activity. The increased calorie use may be secondary to heightened function of muscle UCPs, combined with decreased function of K^+_{ATP} and MED1, potentially driven by sympathetic outflow to skeletal muscle.

390 Comparing EE in animals where the phenotypes differ in BW or composition has been an area of
391 disagreement, causing some confusion due to overcorrection for BW or misinterpretation of data
392 [9, 66, 91]. One strategy to compare EE between lean vs. obesity-prone groups is to obtain
393 individuals of each phenotype that overlap in BW or lean mass. Here, we used this strategy to
394 compare TEE and its components in HCR and LCR. This necessitated using female rats, as the
395 male HCR and LCR have minimal overlap in weight between groups; the weight-matched
396 females accurately represented their phenotype except for BW. Relative to female LCR, female
397 rats of the lean, high-capacity phenotype (HCR) did not show significantly elevated resting EE.
398 Instead, the lean, high-capacity rats consistently displayed significantly heightened non-resting
399 EE (Figure 2). This persisted when the groups were matched for BW or lean mass, or without
400 matching (Figure 1, Table 1). This was consistent with ANCOVA analyses, which demonstrated
401 that non-resting EE was consistently higher in female HCR, even after BW and lean mass were
402 considered as covariates, without any detectable difference in resting EE (Figure 2).
403
404 Though we are not able to compare weight-matched male HCR and LCR, the results of
405 ANCOVA analysis from male rat EE data were similar to females. BW and lean mass are the
406 strongest determinants of TEE and each of its components (resting and non-resting EE), and
407 male HCR had higher non-resting EE than LCR even after lean mass and BW were considered in
408 the analyses. Unlike results from female rats, however, male HCR had slightly but significantly
409 elevated resting EE compared to male LCR even after lean mass was factored into the analysis.
410 Overall, this gives little support for the hypothesis that there is a meaningful deficiency in resting
411 metabolic rate in the obese. Rather, our results support the assertion that non-resting EE,
412 including physical activity EE, is consistently elevated in the lean phenotype associated with

high aerobic capacity, regardless of sex, even after individual differences in body mass and composition are taken into account. This is in line with elevated activity EE observed in other models of leanness [42].

Though ANCOVA can be used to account for group differences in BW or lean mass, [91] even this method is not universally accepted [55, 97]. We used regression analyses to model the contribution of body mass and its components, as well as PA, to TEE as well as to resting and non-resting EE in female HCR and LCR. As shown in Table 2, the predictive factors more effectively modeled EE in LCR compared to HCR; models including lean mass and PA best predicted TEE in both HCR and LCR, though lean mass appeared to be more important in LCR, and activity in HCR. Lean mass was the single best predictor of resting EE in both HCR and LCR. PA level was a more important predictive factor for non-resting EE. In LCR, the ability of PA to predict non-resting EE was partially dependent on lean mass, which appeared to be the critical factor determining non-resting EE in LCR. This was not the case for HCR, where PA alone significantly predicted non-resting EE, and this was augmented by considering workload (i.e., BW). This suggests the possibility of an additional factor that contributes to non-resting EE in HCR that was not accounted for in our analyses, a factor which alters the efficiency or economy of locomotion [63], making activity EE less predictable in HCR than in LCR. It is conceivable that economy of activity is differentially modulated in HCR than in LCR, potentially by sympathetic outflow, resulting in greater variance in activity EE in HCR. The ability to use PA levels to predict non-resting EE was likely to be hampered by the uniquely low variance in daily activity when considering only HCR or only LCR [63, 64]. Differences in thermic effect of food may also contribute to inter-individual variance in EE.

Several investigations have addressed the extent to which EE, particularly basal metabolic rate (BMR) or related measures of resting or sleeping metabolic rates, differs among individuals, and how this contributes to the tendency to become obese [30, 68]. Answering this question requires accurate measurement of BMR as well as precise consideration of the well-known major determinants of BMR, namely body mass (primarily lean mass) and age [22, 23, 53, 67, 87]. There is no consensus regarding exactly how much BMR varies in the population after accounting for these factors, but most studies estimate lean mass alone accounts for 72% or more of inter-individual variance in BMR; fat mass and age also independently predict BMR [16]. Therefore, it is not a surprise that studies meticulously measuring BMR and body composition find little unaccounted-for variance in BMR that could explain obesity propensity [30, 68]. Our statistical modeling of EE reaffirmed the dominance of lean mass as determinants of TEE and resting EE (Table 2); obesity-resistant rats had minor (males) or undetectable (females) differences in resting EE (Figures 1-2). Non-resting EE, on the other hand, is consistently elevated in lean rats. These analyses also supported the importance of PA level as a determinant of TEE and non-resting EE, particularly non-resting EE in HCR. As we have established previously, in both males and females, HCR are consistently more active than LCR [63, 64]. We now show that this difference persists even when BW and composition are nearly identical (in female rats; Figure 1), indicating that lower daily PA levels in LCR are not due to the any difficulty in locomotion incurred by greater body mass, but are inherent to the obesity-prone, low aerobic capacity phenotype. This is true of both ambulatory and stereotypical PA (Table 1). This reflects what many studies have established regarding leanness in humans, namely the link

458 between high aerobic capacity, high PA and NEAT, and favorable metabolic health [15, 32, 47,
459 48, 63, 64, 82].
460
461 NEAT (i.e., activity EE) is the component of TEE that consistency differs between lean and
462 obesity-prone individuals, and this is elevated in obesity-resistant rats through higher levels of
463 PA combined with decreased fuel economy of activity [46, 63] (Figure 1; Table 1). Skeletal
464 muscle is the major contributor to activity EE, and also significantly impacts resting EE [98].
465 Therefore, the logical next step was to investigate cellular and molecular mechanisms that
466 differentially regulate NEAT in skeletal muscle of these phenotypes. Specifically, what is the
467 fate of the "extra" calories being burned during NEAT in HCR [63]? In an attempt to answer this
468 question, we hypothesized that excess calories used during the less-economical NEAT in the
469 lean, high-capacity phenotype are being dissipated as heat, analogous to the fuel inefficiency that
470 occurs during BAT thermogenesis [43, 54, 71]. Hind-limb gastroc muscle temperature was
471 significantly higher in HCR than LCR during physical activity of equivalent workload (treadmill
472 speed and incline, similar BW (Figure 3)). This supports the hypothesis that the "wasted"
473 calories utilized for non-resting EE are being used by muscle, specifically for NEAT, and at least
474 some of the energy is being dissipated as heat. This is consistent with the work of others
475 demonstrating relatively inefficient coupling in HCR mitochondria specifically in HCR skeletal
476 muscle [44, 45, 60, 84, 92]. This is also consistent with reports that, relative to LCR, HCR have
477 enhanced muscle glucose uptake, glucose and lipid oxidation, and higher muscle glycogen [69].
478 Obesity-related differences in walking or running economy in humans is a matter of some
479 contention. Studies in both athletes and non-athletes, however, support the idea that those with

480 higher VO_{2max} (i.e., aerobic capacity) have lower economy of activity [50, 75]. This relative
481 inefficiency may be a trade-off for enhanced aerobic capacity.
482
483 We predicted that the lean vs. obesity-prone phenotypes would exhibit differential expression of
484 molecular mediators of energy consumption in myocytes. We examined K^+_{ATP} channels, which
485 are important in determining the metabolic state of the cell by maintaining potential gradient for
486 ATP synthesis [2, 61]. Given that mice deficient in of K^+_{ATP} channels are lean and have lower
487 fuel economy of activity [2], similar to HCR, we predicted that lean, high-NEAT HCR would
488 show low expression of K^+_{ATP} channels in skeletal muscle compared to obesity-prone LCR.
489 Consistent with the hypothesis, Kir6.2, the predominant subtype of the K^+_{ATP} channel in muscle
490 [1, 12, 61, 79], showed dampened levels of expression in gastroc of HCR (Figure 4). In addition,
491 a component of mediator cofactor complex, MED1 [38, 95], also showed lower levels of
492 expression in the muscle of HCR than LCR, particularly in gastroc (Figure 4). This component
493 of mediator cofactor complex is associated with several nuclear receptors involved in the
494 transcription of genes involved in fatty acid oxidation [38, 95], and is hypothesized to mediate
495 myocyte fuel conservation [11]. Low levels of both MED1 and K^+_{ATP} channels are consistent
496 with the compromised economy of activity and increased heat generation of HCR muscle, likely
497 through altered control of fatty acid oxidation [2]; assessments of channel function are needed to
498 directly test this hypothesis, however.
499
500 Similar to BAT, skeletal myocytes express uncoupling proteins (UCP2 and 3). UCPs play an
501 important role in uncoupling ATP generation and in proton leak across mitochondrial
502 membranes, opposing the function of K^+_{ATP} channels in the cell. Here, we find that lean rats have

503 consistently heightened expression of both UCP2 and UCP3 in skeletal muscle (both quad and
504 gastroc) compared to the obesity-prone LCR (Figure 4), consistent with what has been reported
505 by others in male rats [44, 45, 84]. There is no consensus on the role of UCPs, especially UCP 3,
506 in skeletal muscle, though they are hypothesized to facilitate fatty acid translocation or
507 mitigation of reactive oxygen species [7, 13, 25, 26, 59, 74]. It is unknown whether the
508 uncoupling affects efficiency or thermogenesis. Altogether, the data support a theoretical model
509 in which the myocytes of the lean phenotype have increased use of metabolic fuels, particularly
510 fatty acids, through heightened UCP function along with reduced ability to conserve fuel through
511 MED1 and K^{+}_{ATP} channels.
512
513 Our findings using a contrasting genetic model system support a role for differential activity-
514 related skeletal muscle thermogenesis in maintaining leanness, and identify potential molecular
515 mechanisms underlying this; the data also identify a potential source of these differences. It is
516 possible that the central nervous system modulates muscle fuel efficiency through the SNS in a
517 fashion analogous to what has been documented in other systems such as BAT [10]. As
518 illustrated in Figure 5, compared to LCR, HCR were found to have significantly higher skeletal
519 muscle NETO, an indicator of SNS drive [5]. It is possible that muscle fuel uptake and utilization
520 is modulated through the SNS, controlled by central nervous system effectors [73, 80], possibly
521 including brain orexigenic and anorexigenic peptides that are known to act in the paraventricular
522 nucleus to affect muscle uncoupling protein levels [40, 41, 93]. Brain melanocortins have been
523 found to impact muscle lipid mobilization and glucose uptake [73, 89, 90] and the lean and
524 obesity-prone rats show differences in central melanocortins [81], a system recognized to
525 modulate SNS drive [31, 83]. Taken together with previous evidence, these data are consistent

with a model in which brain systems (e.g., melanocortins) modulate SNS outflow and myocyte beta adrenoreceptors to increase myocyte glucose and fatty acid uptake and utilization, amplifying energy expenditure of activity (i.e., NEAT) [56, 80, 86, 88, 89]. Similar to skeletal muscle, we also found that NETO was higher in brown adipose tissue of HCR compared to LCR. Effects of BAT thermogenesis on EE would not be expected to preferentially affect non-resting EE, however, as was found in our female HCR.

The data described here support a theoretical model where the modulation of metabolic fuel use of skeletal myocytes, potentially through enhanced SNS drive, results in increased or decreased fuel efficiency during locomotion. This predominantly impacts non-resting EE, specifically NEAT, rather than resting or basal metabolism, particularly in female rats (Figure 3). This has implications for how we consider metabolism when attempting to prevent or treat obesity. Targeting of pathways maximizing skeletal muscle energy use during physical activity may take advantage of already-existing mechanisms which are endogenously employed to a greater extent in naturally lean people.

PERSPECTIVES

Apart from the implications regarding obesity and human health, these data can also be viewed form an evolutionary perspective. The lean, high-NEAT rats were originally derived through artificial selection for intrinsic aerobic capacity [36]. This selective breeding was based on the hypothesis that differences in aerobic capacity underlie the root causes of complex disease [57]. Our results not only support the link between oxygen use and disease (specifically obesity and metabolic disease), but also speak to the hypothesis that endothermy evolved through selection

549 for maximal aerobic capacity, and that resting or basal metabolism is necessarily elevated in
550 association with aerobic capacity [6, 85]. Our data do not support the supposition that maximal
551 and resting metabolic rates are inexorably linked, aside from the fact that both resting and
552 maximal metabolic rates increase along with body size and lean mass. The HCR were selected
553 specifically for high maximal aerobic capacity and LCR for low capacity [36], yet HCR and
554 LCR have nearly identical resting metabolic rates once differences in lean mass were effectively
555 factored out using lean-mass-matched female rats (Figure 2). Maximal aerobic capacity and
556 resting metabolism must be at least somewhat independently modulated [20]. Lastly, it is
557 conceivable that the original selection for maximal aerobic capacity was associated with
558 heightened resting metabolism, but the two aspects of metabolism have since been dissociated.
559

560 **ACKNOWLEDGEMENTS**

561 We acknowledge Dr. Werner Geldenhuys for assistance with the statistical modeling and
562 associated text, and Antonio A Nunez for a critical reading of the manuscript. We also
563 acknowledge the expert care of the rat colony provided by Molly Kalahar and Lori Gilligan.
564 Contact L.G.K. (lgkoch@med.umich.edu) or S.L.B. (brittons@umich.edu) for information on
565 the LCR and HCR rats: these rat models are maintained as an international collaborative resource
566 at the University of Michigan, Ann Arbor.
567

568 **GRANTS**

569 NIH R01NS055859, NIH R15DK097644, and AHA GIA 410805 to CMN, and AHA
570 11PRE7320029 to CS. The LCR-HCR rat model system was funded by the National Center for
571 Research Resources grant R24 RR017718 and is currently supported by the Office of Research

572 Infrastructure Programs/OD grant ROD012098A (to LGK. and SLB) from the NIH. SLB was
573 also supported by NIH RO1 DK077200.
574

575 **DISCLOSURES**

576 None.
577

578 **FIGURE LEGENDS**

579 **Figure 1:** High capacity runners (HCR) have heightened total energy expenditure (TEE) and
580 non-resting energy expenditure (NREE), but not resting energy expenditure (REE), when
581 matched for (A) body weight (BW), (B) lean mass (LM), and/or (C) by group. Daily physical
582 activity was higher in HCR when matched for BW, LM, or by group. HCR and LCR show
583 significant differences in fat mass (FM) but not in LM or BW when analyzed by group (C) or
584 when weight matched (A).*$p<0.05$ (HCR≠LCR). All data are means ± SEM. (N = 8/group for
585 BW and lean mass matched, N=13/group for TEE by group)
586

587 **Figure 2:** In all rats, non-resting energy expenditure (NREE) increased along with body weight
588 (A) and lean mass (B); NREE was significantly higher in the lean phenotype (high-capacity
589 runners, HCR) compared to low-capacity runners (LCR), even after lean mass and body weight
590 were factored out. Resting EE (REE) also increased with body weight (C) and lean mass (D), but
591 did not significantly differ between female HCR and LCR. (N = 13/group) *$p<0.05$, HCR>LCR.
592

593 **Figure 3: High-capacity runners (HCR) have heightened muscle heat dissipation during**
594 **physical activity.** (A) Mean gastrocnemius (gastroc) temperature during a graded treadmill
595 exercise test. HCR show higher gastroc temperatures throughout the test period compared to

low-capacity runners (LCR) with exception of resting. (B) Maximal rise in gastroc temperature was significantly higher in HCR compared to LCR during the graded treadmill test. *p<0.05 (HCR>LCR). All data are means ± SEM. (N=7/group)

Figure 4: Compared to low-capacity runners (LCR), high-capacity runners (HCR) have lower expression of molecular mediators of energy conservaiton in skeletal muscle [quadriceps (quad) and gastrocnemius (gastroc)], including (A, F) Mediator of RNA polymerase II transcription subunit 1(MED1), and subunits of the ATP-gated K^+ channels (K^+_{ATP}) Kir6.1 (B) and Kir6.2 (C); (G, H) Kir6.1 and Kir6.2 protein levels were also higher in gastroc, but not quad. (D, E) Uncoupling protein 2 and 3 (UCP2, UCP3) mRNA expression and (I, J) protein levels were significantly higher in skeletal muscle of HCR compared to LCR. *p<0.05 (HCR>LCR for UCP2, UCP3; and HCR<LCR for Kir6.1, Kir6.2, and MED1). All data are means ± SEM. (N=7-8 for mRNA expression, N= 4-5 for protein expression)

Figure 5: Compared to low-capacity runners (LCR), high-capacity runners (HCR) have elevated sympathetic drive indicated by higher norepinephrine turnover (NETO) in skeletal muscle groups: (A) medial gastrocnemius, (B) lateral gastrocnemius (C) quadriceps, and (D) soleus. *p<0.05 (HCR>LCR). All data are means ± SEM. (N= 7 HCR, 6 LCR)

Table 1: Physical activity and energy expenditure in rats of the lean (HCR) and obesity-prone (LCR) phenotype.

HCR, High capacity runners; LCR, Low capacity runners. *p<0.05 (HCR≠LCR).

Table 2: Results of statistical modeling using multiple linear regression analysis to determine which factors (body mass, lean mass, fat mass, physical activity) best predicted energy expenditure; each component was modeled separately (total energy expenditure, TEE; resting energy expenditure REE; non-resting energy expenditure, NREE) was modeled separately for each the lean (high-capacity runners, HCR) and obesity-prone (low-capacity runners, LCR) phenotypes.

REFERENCES

1. **Aguilar-Bryan L., Bryan J.** Molecular biology of adenosine triphosphate-sensitive potassium channels. Endocr. Rev. (1999) 20, 101–135
2. **Alekseev AE, Reyes S, Yamada S, Hodgson-Zingman DM, Sattiraju S, Zhu Z, Sierra A, Gerbin M, Coetzee WA, Goldhamer DJ, Terzic A, Zingman LV.** Sarcolemmal ATP-sensitive K^+ channels control energy expenditure determining body weight. Cell Metabolism. 2010; 11(1):58–69.
3. **Allison D.B., Fontaine K.R., Manson J.E., Stevens J and Van Itallie T.B.** Annual deaths attributable to obesity in the United States, JAMA, 282 (1999) 1530-8.
4. **Annemiek MCP Joosen, Marij Gielen, Robert Vlietinck, and Klaas R Westerterp.** Genetic analysis of physical activity in twins, Am J Clin Nutr 2005; 82:1253–9.
5. **Bartness TJ, Vaughan CH, Song CK.** Sympathetic and sensory innervation of brown adipose tissue. Int J Obes (Lond) 2010; 34(Suppl 1):S36–S42.
6. **Bennet AF, Ruben JA.** Endothermy and activity in vertebrates. Science (1979) 206: 649–654. doi: 10.1126/science.493968.

7. **Bezaire, V., E.L. Seifert, and M.E. Harper.** Uncoupling protein-3: clues in an ongoing mitochondrial mystery. FASEB J, 2007. **21**(2): p. 312-24.
8. **Brown D.W., Brown D.R., Heath G.W., Balluz L, Giles W.H., Ford E.S and Mokdad A.H.** Associations between physical activity dose and health-related quality of life, Med Sci Sports Exerc, 36 (2004) 890-6.
9. **Butler A.A., Kozak L.P.** A recurring problem with the analysis of energy expenditure in genetic models expressing lean and obese phenotypes. Diabetes. 2010; 59:323–329. doi: 10.2337/db09-1471.
10. **Cannon, Barbara, and Jan Nedergaard.** Brown Adipose Tissue: Function and Physiological Significance. Physiol Rev 84: 277–359, 2004; 10.1152/ physrev. 00015.2003.
11. **Chen W, Zhang X, Birsoy K, Roeder RG.** A muscle-specific knockout implicates nuclear receptor coactivator MED1 in the regulation of glucose and energy metabolism. Proceedings of the National Academy of Sciences of the United States of America. 2010; 107:10196–10201.
12. **Coetzee WA, Amarillo Y, Chiu J, Chow A, Lau D, McCormack T, Moreno H, Nadal MS, Ozaita A, Pountney D, Saganich M, Vega-Saenz de Miera E, Rudy B.** Molecular diversity of K^+ channels. Ann NY Acad Sci (1999) 868:233–285.
13. **Costford SR, Seifert EL, Bézaire V, F Gerrits M, Bevilacqua L, Gowing A, Harper ME.** The energetic implications of uncoupling protein-3 in skeletal muscle. Appl Physiol Nutr Metab, 2007. **32**(5): p. 884-94.

14. **Danaei G, Ding EL, Mozaffarian D, Taylor B, Rehm J, Murray CJ, Ezzati M.** The preventable causes of death in the United States: comparative risk assessment of dietary, lifestyle, and metabolic risk factors. PLoS Med, 2009. 6(4): p. e1000058.
15. **Ekelund U, Brage S, Franks PW, Hennings S, Emms S, Wareham NJ.** Physical activity energy expenditure predicts progression toward the metabolic syndrome independently of aerobic fitness in middle-aged healthy caucasians: the medical research council ely study. Diabetes Care. 2005; 28(5):1195–1200.
16. **Ferraro R, Ravussin E.** Fat mass in predicting resting metabolic rate. Am J Clin Nutr 1992; 56: 460(letter).
17. **Flegal K.M, Carroll M.D, Ogden C.L and Johnson C.L.** Prevalence and trends in obesity among US adults, 1999-2000, JAMA, 288 (2002) 1723-7.
18. **Flegal KM, Carroll MD, Kit BK, Ogden CL.** Prevalence of obesity and trends in the distribution of body mass index among US adults, 1999-2010. JAMA, 2012. 307(5): p. 491-7.
19. **Fontaine K.R, Redden D.T, Wang C, Westfall A.O and Allison D.B.** Years of life lost due to obesity, JAMA, 289 (2003) 187-93.
20. **Gębczyński, A.K. & Konarzewski, M.** Metabolic correlates of selection on aerobic capacity in laboratory mice: a test of the model for the evolution of endothermy. Journal of Experimental Biology (2009), 212, 2872–2878.
21. **Giles ED, Jackman MR, Johnson GC, Schedin PJ, Houser JL, MacLean PS.** Effect of the estrous cycle and surgical ovariectomy on energy balance, fuel utilization, and physical activity in lean and obese female rats. Am J Physiol Regul Integr Comp Physiol, 2010. 299(6): p. R1634-42.

685 22. **Goldberg, A. P., Dengel, D. R. and Hagberg, J. M.** Exercise physiology and aging. In
686 Handbook of the Biology of Aging, fourth edition (E. L. Schneider and J. W. Rowe), pp.
687 331-354. San Diego: Academic Press, 1996.
688 23. **Gonzalez, E., Messi, M. L. and Delbono, O.** The specific force of single intact extensor
689 digitorum longus and soleus mouse muscle fibers declines with aging. J. Membr. Biol.
690 (2000) 178, 175-183.
691 24. **Haffner. S and Taegtmeyer. H.** Epidemic obesity and the metabolic syndrome,
692 Circulation, 108 (2003) 1541-5.
693 25. **Harper ME, Dent RM, Bezaire V, Antoniou A, Gauthier A, Monemdjou S,**
694 **McPherson R.** UCP3 and its putative function: consistencies and controversies. Biochem
695 Soc Trans, 2001. **29**(Pt 6): p. 768-73.
696 26. **Harper, M.E., K. Green, and M.D. Brand.** The efficiency of cellular energy
697 transduction and its implications for obesity. Annu Rev Nutr, 2008. **28**: p. 13-33.
698 27. **Heo M, Allison D.B, Faith M.S, Zhu S and Fontaine K.R.** Obesity and quality of life:
699 mediating effects of pain and comorbidities, Obes Res, 11 (2003) 209-16.
700 28. **Iossa, S.; Lionetti, L.; Mollica, M. P.; Barletta, A.; Liverini, G.,** Thermic effect of
701 food in hypothyroid rats. *J Endocrinol* 1996**,** *148* (1), 167-74.
702 29. **Johannsen DL, Welk GJ, Sharp RL, Flakoll PJ.** Differences in daily energy
703 expenditure in lean and obese women: the role of posture allocation. Obesity (Silver
704 Spring) (2008) 16: 34–39.
705 30. **Jonathan Webber.** Energy balance in obesity, Proceedings of the Nutrition Society
706 (2003), 62, 539–543

31. **Kathleen G. Mountjoy.** Distribution and Function of Melanocortin Receptors within the Brain. In: Melanocortins: Multiple Actions and Therapeutic Potential. pp 29-48, 2010. DOI 10.1007/978-1-4419-6354-3_3

32. **Katzmarzyk, P. T.** Physical activity, sedentary behavior, and health: Paradigm paralysis or paradigm shift? Diabetes, (2010). 59, 2717-2725.

33. **Klein. S.** The national obesity crisis: a call for action, Gastroenterology, 126 (2004) 6.

34. **Koch L.G, Kemi O.J, Qi N, Leng S.X, Bijma P, Gilligan L.J, Wilkinson J.E, Wisloff H, Hoydal M.A, Rolim N, Abadir P.M, Van Grevenhof I, Smith G.L, Burant C.F, Ellingsen O, Britton S.L, Wisloff U.** Intrinsic Aerobic Capacity Sets a Divide for Aging and Longevity**.** Circulation Research, 2011; DOI: 10.1161/ CIRCRESAHA.111. 253807.

35. **Koch LG, Britton SL, Wisloff U.** A rat model system to study complex disease risks, fitness, aging, and longevity. Trends Cardiovasc Med. 2012;22:29–34.

36. **Koch, Lauren Gerard, and Steven L. Britton.** Artificial selection for intrinsic aerobic endurance running capacity in rats. Physiol Genomics 5: 45–52, 2001.

37. **Kodama S, Saito K, Tanaka S, Maki M, Yachi Y, Asumi M, Sugawara A, Totsuka K, Shimano H, Ohashi Y, Yamada N, Sone H.** Cardiorespiratory fitness as a quantitative predictor of all-cause mortality and cardiovascular events in healthy men and women: a meta-analysis. JAMA, 2009. 301(19): p. 2024-35.

38. **Kodera Y, Takeyama K, Murayama A, Suzawa M, Masuhiro Y, Kato S.** Ligand type-specific interactions of peroxisome proliferator-activated receptor γ with transcriptional coactivators. J Biol Chem 275: 33201– 33204, 2000.

39. **Kokkinos P, Myers J, Kokkinos JP, Pittaras A, Narayan P, Manolis A, Karasik P, Greenberg M, Papademetriou V, Singh S.** Exercise capacity and mortality in black and white men. Circulation 117: 614–622, 2008.

40. **Kotz CM, Wang C, Levine AS, Billington CJ.** Urocortin in the hypothalamic PVN increases leptin and affects uncoupling proteins-1 and -3 in rats. American Journal of Physiology—Regulatory Integrative and Comparative Physiology. 2002;282(2):R546–R551.

41. **Kotz CM, Wang CF, Briggs JE, Levine AS, Billington CJ.** Effect of NPY in the hypothalamic paraventricular nucleus on uncoupling proteins 1, 2, and 3 in the rat. Am J Physiol Regul Integr Comp Physiol. 2000;278:R494–R498.

42. **Kotz,C.M., Teske,J.A., and Billington,C. J.** (2008). Neuroregulation of nonexercise activity thermogenesis and obesity resistance. Am. J. Physiol. Regul. Integr. Comp. Physiol. 294, R699–R710.

43. **Leslie P. Kozak.** Brown Fat and the Myth of Diet-Induced Thermogenesis Cell Metabolism 11, April 7, 2010 DOI 10.1016/j.cmet.2010.03.009

44. **Lessard SJ, Rivas DA, Chen ZP, van Denderen BJ, Watt MJ, Koch LG, Britton SL, Kemp BE, Hawley JA.** Impaired skeletal muscle beta-adrenergic activation and lipolysis are associated with whole-body insulin resistance in rats bred for low intrinsic exercise capacity. Endocrinology, 2009. **150**(11): p. 4883-91.

45. **Lessard SJ, Rivas DA, Stephenson EJ, Yaspelkis BB 3rd, Koch LG, Britton SL, Hawley JA**. Exercise training reverses impaired skeletal muscle metabolism induced by artificial selection for low aerobic capacity. Am J Physiol Regul Integr Comp Physiol, 2011. **300**(1): p. R175-82.

46. **Levine J.A and Kotz C.M.** NEAT – non-exercise activity thermogenesis – egocentric & geocentric environmental factors vs. biological regulation, Acta Physiol Scand 2005, 184, 309–318

47. **Levine JA, Lanningham-Foster LM, McCrady SK, Krizan AC, Olson LR, Kane PH, Jensen MD, Clark MM.** Interindividual variation in posture allocation: possible role in human obesity. Science (2005) 307: 584–586.

48. **Levine, J.A., Eberhardt, N.L. & Jensen, M.D.** Role of nonexercise activity thermogenesis in resistance to fat gain in humans. Science (1999b) 283, 212–214.

49. **Levine, James A.** Nonexercise activity thermogenesis (NEAT): environment and biology. Am J Physiol Endocrinol Metab 286: E675–E685, 2004; 10.1152/ ajpendo.00562.2003.

50. **Lucía A, Hoyos J, Pérez M, Santalla A, Chicharro JL.** Inverse relationship between VO2max and economy/efficiency in world-class cyclists. Med Sci Sports Exerc, 2002. **34**(12): p. 2079-84.

51. **MacLean PS, Higgins JA, Johnson GC, Fleming-Elder BK, Donahoo WT, Melanson EL, Hill JO.** Enhanced metabolic efficiency contributes to weight regain after weight loss in obesity-prone rats. Am J Physiol Regul Integr Comp Physiol, 2004. **287**(6): p. R1306-15.

52. **MacLean PS, Higgins JA, Johnson GC, Fleming-Elder BK, Peters JC, Hill JO.** Metabolic adjustments with the development, treatment, and recurrence of obesity in obesity-prone rats. Am J Physiol Regul Integr Comp Physiol, 2004. **287**(2): p. R288-97.

53. **Mark A. Chappell, Enrico L. Rezende and Kimberly A. Hammond.** Age and aerobic performance in deer mice (2003) The Journal of Experimental Biology 206, 1221-1231

54. **Michael J Stock, Nancy J Rothwell.** Role of brown adipose tissue thermogenesis in overfeeding: a review Journal of the Royal Society of Medicine Volume 76 January 1983.

55. **Miller, G.A. and J.P. Chapman.** Misunderstanding analysis of covariance. J Abnorm Psychol, 2001. **110**(1): p. 40-8.

56. **Miyaki T, Fujikawa T, Kitaoka R, Hirano N, Matsumura S, Fushiki T *&* Inoue K** *(*2011*).* Noradrenergic projections to the ventromedial hypothalamus regulate fat metabolism during endurance exercise. Neuroscience 190*,* 239–250.

57. **Molly S. Bray.** Genomics, genes, and environmental interaction: the role of exercise. J. Appl. Physiol. 88: 788–792, 2000.

58. **Myers J, Prakash M, Froelicher V, Do D, Partington S, Atwood JE**. Exercise capacity and mortality among men referred for exercise testing. N Engl J Med (2002) 346:793–801

59. **Nabben, M. and J. Hoeks.** Mitochondrial uncoupling protein 3 and its role in cardiac- and skeletal muscle metabolism. Physiol Behav, 2008. **94**(2): p. 259-69.

60. **Naples SP, Borengasser SJ, Rector RS, Uptergrove GM, Morris EM, Mikus CR, Koch LG, Britton SL, Ibdah JA, Thyfault JP**. Skeletal muscle mitochondrial and metabolic responses to a high-fat diet in female rats bred for high and low aerobic capacity. Appl Physiol Nutr Metab 35: 151–162, 2010.

61. **Nichols, C.G.** KATP channels as molecular sensors of cellular metabolism. Nature (2006). 440, 470–476

62. **Novak CM, Burghardt PR, Levine JA**: The use of a running wheel to measure activity in rodents: relationship to energy balance, general activity, and reward. Neurosci Biobehav Rev 2012, 36:1001–1014.

63. **Novak CM, Escande C, Burghardt PR, Zhang M, Barbosa MT, Chini EN, Britton SL, Koch LG, Akil H, Levine JA.** Spontaneous activity, economy of activity, and resistance to diet-induced obesity in rats bred for high intrinsic aerobic capacity, Horm. Behav. (2010), doi:10.1016/j.yhbeh.2010.03.013

64. **Novak CM, Escande C, Gerber SM, Chini EN, Zhang M, Britton SL, Koch LG, and Levine JA.** Endurance capacity, not body size, determines physical activity levels: role of skeletal muscle PEPCK. PLoS ONE 4: e5869, 2009.

65. **Novak, C.M. and J.A. Levine.** Central neural and endocrine mechanisms of non-exercise activity thermogenesis and their potential impact on obesity. J Neuroendocrinol, 2007. **19**(12): p. 923-40.

66. **Packard, G.C. and T.J. Boardman.** The misuse of ratios, indices, and percentages in ecophysiological research. Physiol Zool, 1988. **61**(1): p. 1-9.

67. **Piers, Leonard S., Mario J. Soares, Leanne M. McCormack, and Kerin O'Dea.** Is there evidence for an age related reduction in metabolic rate? J. Appl. Physiol. 85(6):2196–2204, 1998.

68. **Ravussin E, Lillioja S, Knowler WC, Christin L, Freymond D, Abbott WGH, Boyce V, Howard BV & Bogardus C.** Reduced rate of energy expenditure as a risk factor for bodyweight gain. New England Journal of Medicine (1988) 318, 467–472.

69. **Rivas DA, Lessard SJ, Saito M, Friedhuber AM, Koch LG, Britton SL, Yaspelkis BB 3rd, Hawley JA.** (2011) Low intrinsic running capacity is associated with reduced

skeletal muscle substrate oxidation and lower mitochondrial content in white skeletal muscle. American Journal of Physiology. Regulatory, Integrative and Comparative Physiology 300: R835–43.

70. **Roberts SB, McCrory MA, Saltzman E.** The influence of dietary composition on energy intake and body weight. J Am Coll Nutr 2002; 21: 140S–145S.

71. **Rothwell, N.J., and Stock, M.J.** A role for brown adipose tissue in diet-induced thermogenesis, Nature (1979) 281, 31–35.

72. **Rousset S, Alves-Guerra MC, Mozo J, Miroux B, Cassard-Doulcier AM, Bouillaud F, Ricquier D.** The biology of mitochondrial uncoupling proteins. Diabetes 53: S130–S135, 2004.

73. **Ruben Nogueiras, Petra Wiedmer, Diego Perez-Tilve, Christelle Veyrat-Durebex, Julia M. Keogh, Gregory M. Sutton, Paul T. Pfluger, Tamara R. Castaneda, Susanne Neschen, Susanna M. Hofmann, Philip N. Howles, Donald A. Morgan, Stephen C. Benoit, Ildiko Szanto, Brigitte Schrott, Annette Schürmann, Hans-Georg Joost, Craig Hammond, David Y. Hui, Stephen C. Woods, Kamal Rahmouni, Andrew A. Butler, I. Sadaf Farooqi, Stephen O'Rahilly, Françoise Rohner-Jeanrenaud, Matthias H. Tschöp.** The central melanocortin system directly controls peripheral lipid metabolism J Clin Invest. 2007; 117(11):3475–3488 doi:10.1172/JCI31743

74. **Samec, S., J. Seydoux, and A.G. Dulloo.** Interorgan signaling between adipose tissue metabolism and skeletal muscle uncoupling protein homologs: is there a role for circulating free fatty acids? Diabetes, 1998. **47**(11): p. 1693-8.

75. **Sawyer BJ, Blessinger JR, Irving BA, Weltman A, Patrie JT, Gaesser GA.** Walking and running economy: inverse association with peak oxygen uptake. Med Sci Sports Exerc, 2010. **42**(11): p. 2122-7.

76. **Schoeller, D. A.; Jefford, G.,** Determinants of the energy costs of light activities: inferences for interpreting doubly labeled water data. *Int J Obes Relat Metab Disord* 2002, *26* (1), 97-101.

77. **Sheehan M.T and Jensen M.D.** Metabolic complications of obesity. Pathophysiologic considerations, Med Clin North Am, 84 (2000) 363-85, vi.

78. **Shi H, Bowers RR, Bartness TJ.** Norepinephrine turnover in brown and white adipose tissue after partial lipectomy. Physiol Behav. 2004;81:535–42. doi: 10.1016/j.physbeh.2004.02.023.

79. **Shieh, C.C., Coghlan, M., Sullivan, J.P. and Gopalakrishnan, M.** Potassium channels: molecular defects, diseases, and therapeutic opportunities. Pharmacol Rev (2000) 52: 557–594.

80. **Shiuchi T, Haque MS, Okamoto S, Inoue T, Kageyama H, Lee S, Toda C, Suzuki A, Bachman ES, Kim YB, Sakurai T, Yanagisawa M, Shioda S, Imoto K, Minokoshi Y.** Hypothalamic orexin stimulates feeding-associated glucose utilization in skeletal muscle via sympathetic nervous system. Cell Metab, 2009. **10**(6): p. 466-80.

81. **Shukla C, Britton SL, Koch LG and Novak CM.** Region-specific differences in brain melanocortin receptors in rats of the lean phenotype. Neuroreport. 2012; 23: 596-600. (PMID: 22643233)

82. **Simmons RK, Griffin SJ, Steele R, Wareham NJ, Ekelund U. the ProActive Research Team.** Increasing overall physical activity and aerobic fitness is associated

with improvements in metabolic risk: cohort analysis of the ProActive trial. Diabetologia. 2008; 51:787–794. doi: 10.1007/s00125-008-0949-4.

83. **Sohn, Jong-Woo; Harris, Louise E.; Berglund, Eric D.; Liu, Tiemin; Vong, Linh; Lowell, Bradford B.; Balthasar, Nina; Williams, Kevin W.; Elmquist, Joel K.** Melanocortin 4 Receptors Reciprocally Regulate Sympathetic and Parasympathetic Preganglionic Neurons. Cell vol. 152 issue 3 January 31, 2013. p. 612-619

84. **Stephenson EJ, Stepto NK, Koch LG, Britton SL, Hawley JA.** Divergent skeletal muscle respiratory capacities in rats artificially selected for high and low running ability: a role for Nor1? J Appl Physiol, 2012. **113**(9): p. 1403-12.

85. **Swanson DL, Thomas NE, Liknes ET, Cooper SJ.** Intraspecific Correlations of Basal and Maximal Metabolic Rates in Birds and the Aerobic Capacity Model for the Evolution of Endothermy. PLoS ONE (2012) 7(3): e34271. doi:10.1371/journal.pone.0034271.

86. **Tanaka T, Masuzaki H, Yasue S, Ebihara K, Shiuchi T, Ishii T, et al.** Central melanocortin signaling restores skeletal muscle AMP-activated protein kinase phosphorylation in mice fed a high-fat diet. Cell Metab. 2007;5:395–402.

87. **Thompson, L. V. and Brown, M.** Age-related changes in contractile properties of single skeletal fibers from the soleus muscle. J. Appl. Physiol. (1999) 86, 881-886.

88. **Toda C, Shiuchi T, Kageyama H, Okamoto S, Coutinho EA, Sato T, Okamatsu-Ogura Y, Yokota S, Takagi K, Tang L, Saito K, Shioda S, Minokoshi Y.** Extracellular signal-regulated kinase in the ventromedial hypothalamus mediates leptin-induced glucose uptake in red-type skeletal muscle. Diabetes. 2013 Jul; 62(7):2295-307. doi: 10.2337/db12-1629.

89. **Toda C, Shiuchi T, Lee S, Yamato-Esaki M, Fujino Y, Suzuki A, Okamoto S, Minokoshi Y**. Distinct effects of leptin and a melanocortin receptor agonist injected into medial hypothalamic nuclei on glucose uptake in peripheral tissues. Diabetes. 2009; 58(12):2757–2765.

90. **Tomohiro Tanaka Hiroaki Masuzaki Shintaro Yasue, Ken Ebihara , Tetsuya Shiuchi, Takako Ishii, Naoki Arai, Masakazu Hirata, Hiroshi Yamamoto, Tatsuya Hayashi, Kiminori Hosoda, Yasuhiko Minokoshi, Kazuwa Nakao.** Central Melanocortin Signaling Restores Skeletal Muscle AMP-Activated Protein Kinase Phosphorylation in Mice Fed a High-Fat Diet (2007) Cell Metabolism, 5 (5), pp. 395-402. doi: 10.1016/ j.cmet. 2007. 04.004

91. **Tschöp MH, Speakman JR, Arch JR, Auwerx J, Brüning JC, Chan L, Eckel RH, Farese RV Jr, Galgani JE, Hambly C, Herman MA, Horvath TL, Kahn BB, Kozma SC, Maratos-Flier E, Müller TD, Münzberg H, Pfluger PT, Plum L, Reitman ML, Rahmouni K, Shulman GI, Thomas G, Kahn CR, Ravussin E.** A guide to analysis of mouse energy metabolism. Nat. Methods. 2012; 9:57–63.

92. **Tweedie C, Romestaing C, Burelle Y, Safdar A, Tarnopolsky MA, Seadon S, Britton SL, Koch LG, Hepple RT.** Lower oxidative DNA damage despite greater ROS production in muscles from rats selectively bred for high running capacity. Am J Physiol Regul Integr Comp Physiol, 2011. **300**(3): p. R544-53.

93. **Wang C., Billington C. J., Levine A. S., Kotz C. M.** (2000). Effect of CART in the hypothalamic paraventricular nucleus on feeding and uncoupling protein gene expression. Neuroreport 11 3251–3255.

94. **Wisløff U, Najjar SM, Ellingsen O, Haram PM, Swoap S, Al-Share Q, Fernström M, Rezaei K, Lee SJ, Koch LG, Britton SL.** Cardiovascular risk factors emerge after artificial selection for low aerobic capacity. Science, 2005. 307(5708): p. 418-20.

95. **Yuan CX, Ito M, Fondell JD, Fu ZY, Roeder RG.** The TRAP220 component of a thyroid hormone receptor- associated protein (TRAP) coactivator complex interacts directly with nuclear receptors in a ligand-dependent fashion. Proc Natl Acad Sci U S A (1998) 95: 7939–7944.

96. **Zhan, Wen-Zhi, John G. Swallow, Theodore Garland, Jr., David N. Proctor, Patrick A. Carter, and Gary C. Sieck.** Effects of genetic selection and voluntary activity on the medial gastrocnemius muscle in house mice. J. Appl. Physiol. 87(6): 2326–2333.

97. **Zinbarg RE, Suzuki S, Uliaszek AA, Lewis AR.** Biased parameter estimates and inflated Type I error rates in analysis of covariance (and analysis of partial variance) arising from unreliability: alternatives and remedial strategies. J Abnorm Psychol, 2010. 119(2): p. 307-19.

98. **Zurlo F, Larson K, Bogardus C, Ravussin E**. Skeletal muscle metabolism is a major determinant of resting energy expenditure. J Clin Invest 1990; 86:1423– 1427.

Figure 1

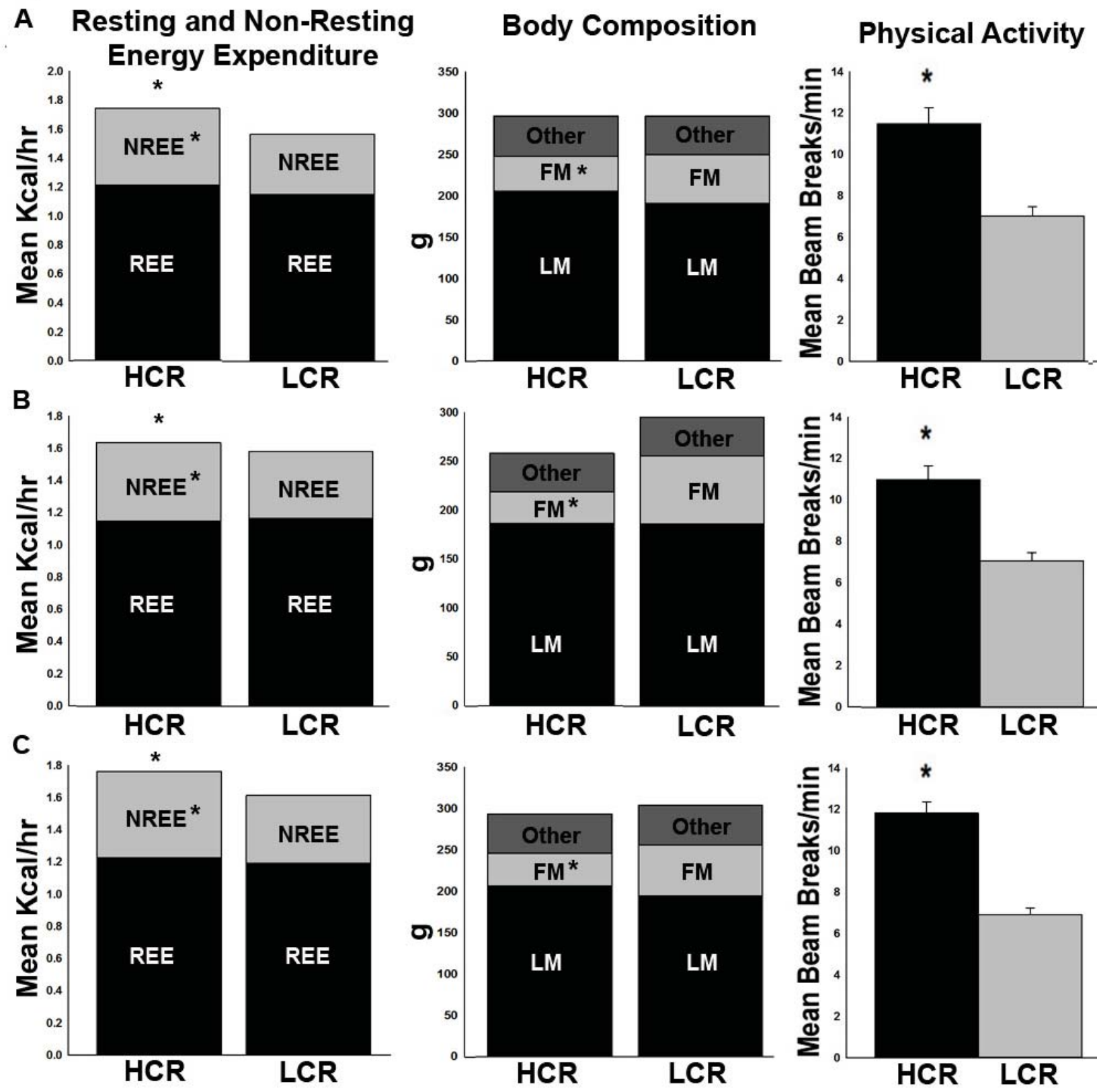

A
Resting and Non-Resting Energy Expenditure
Body Composition
Physical Activity
Mean Kcal/hr
NREE *
NREE
REE
REE
HCR
LCR
g
Other
FM *
FM
LM
LM
Mean Beam Breaks/min
B
C

Figure 2

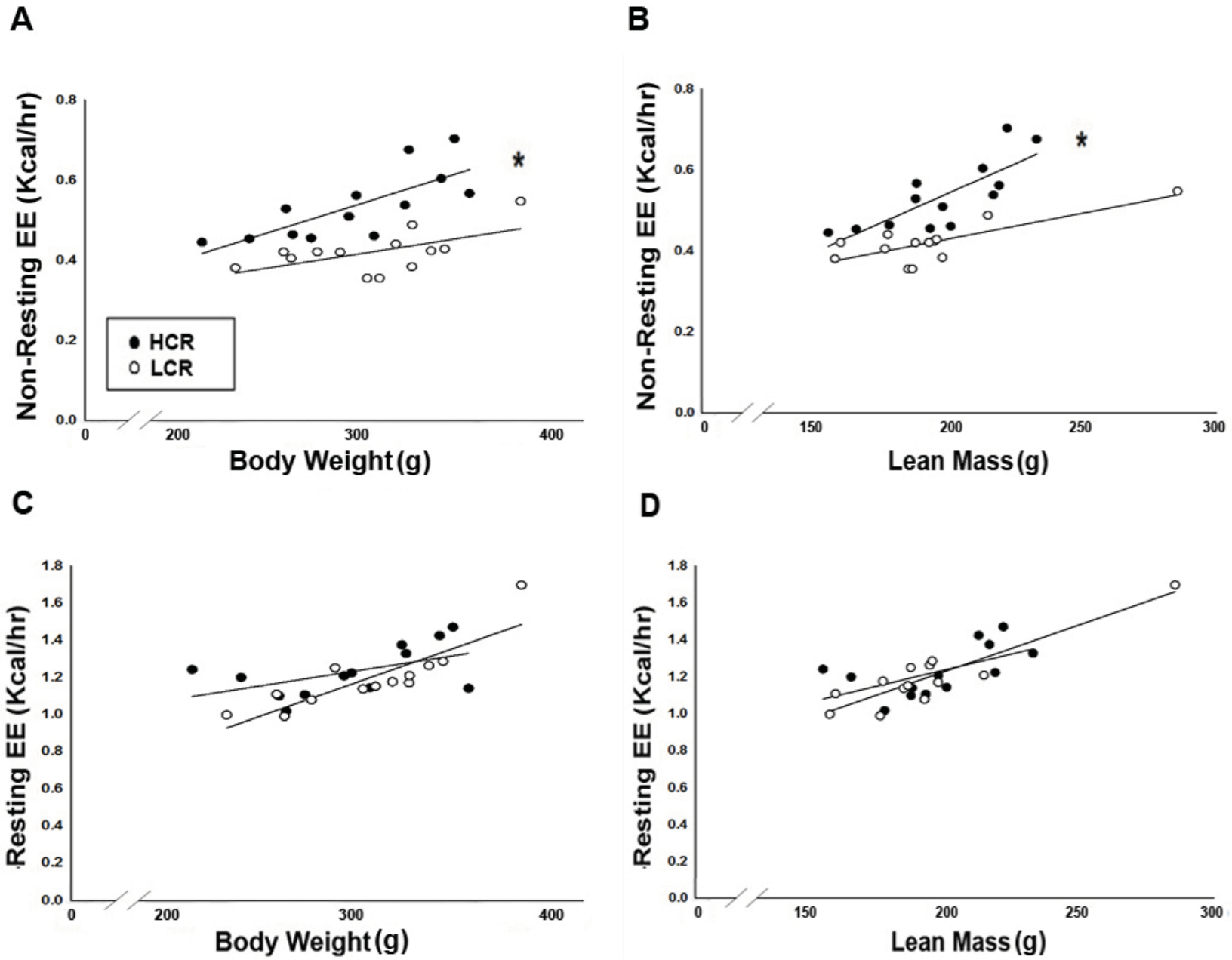
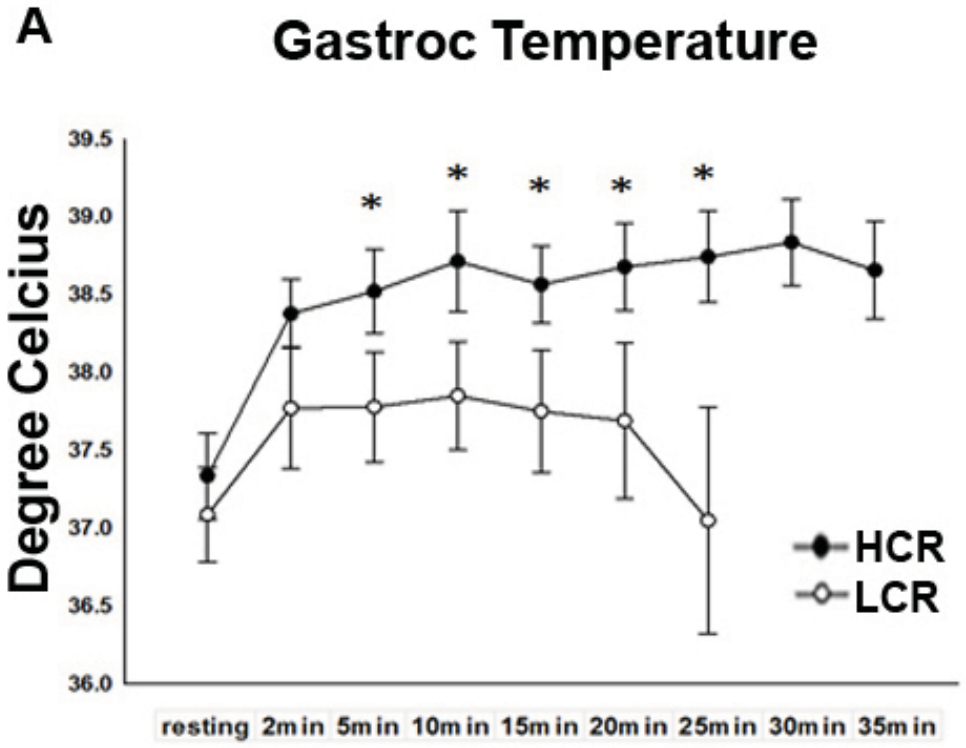

A
B
C
D
Non-Resting EE (Kcal/hr)
Resting EE (Kcal/hr)
Body Weight (g)
Lean Mass (g)
HCR
LCR
*
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
0
150
200
250
300
400

Figure 3


A
Gastroc Temperature
Degree Celcius
39.5
39.0
38.5
38.0
37.5
37.0
36.5
36.0
*
*
*
*
*
HCR
LCR
resting
2min
5min
10min
15min
20min
25min
30min
35min

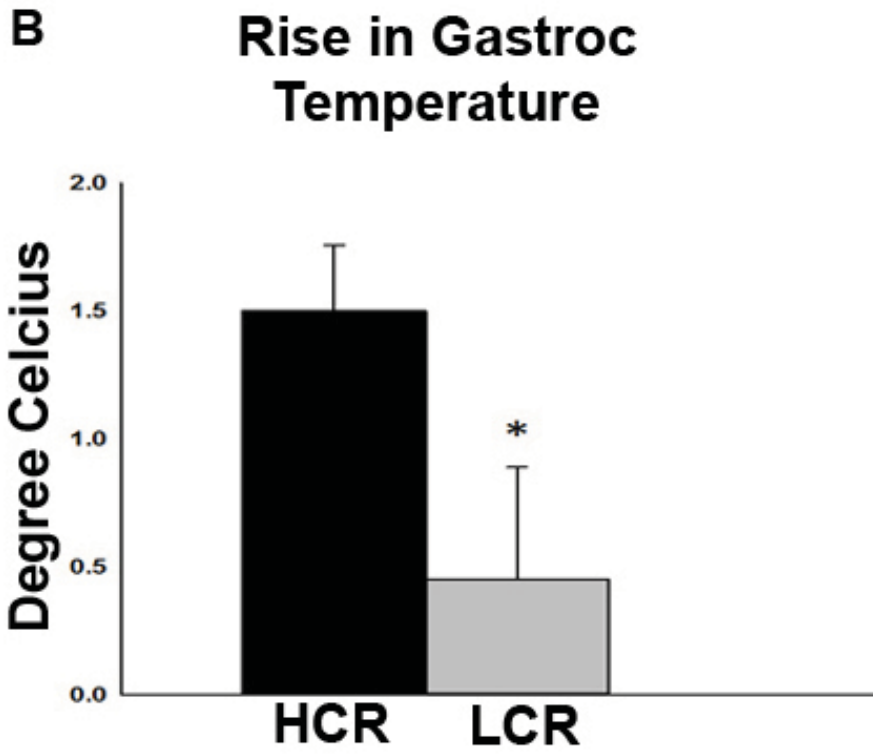

B
Rise in Gastroc Temperature
Degree Celcius
2.0
1.5
1.0
0.5
0.0
*
HCR
LCR

Figure 4

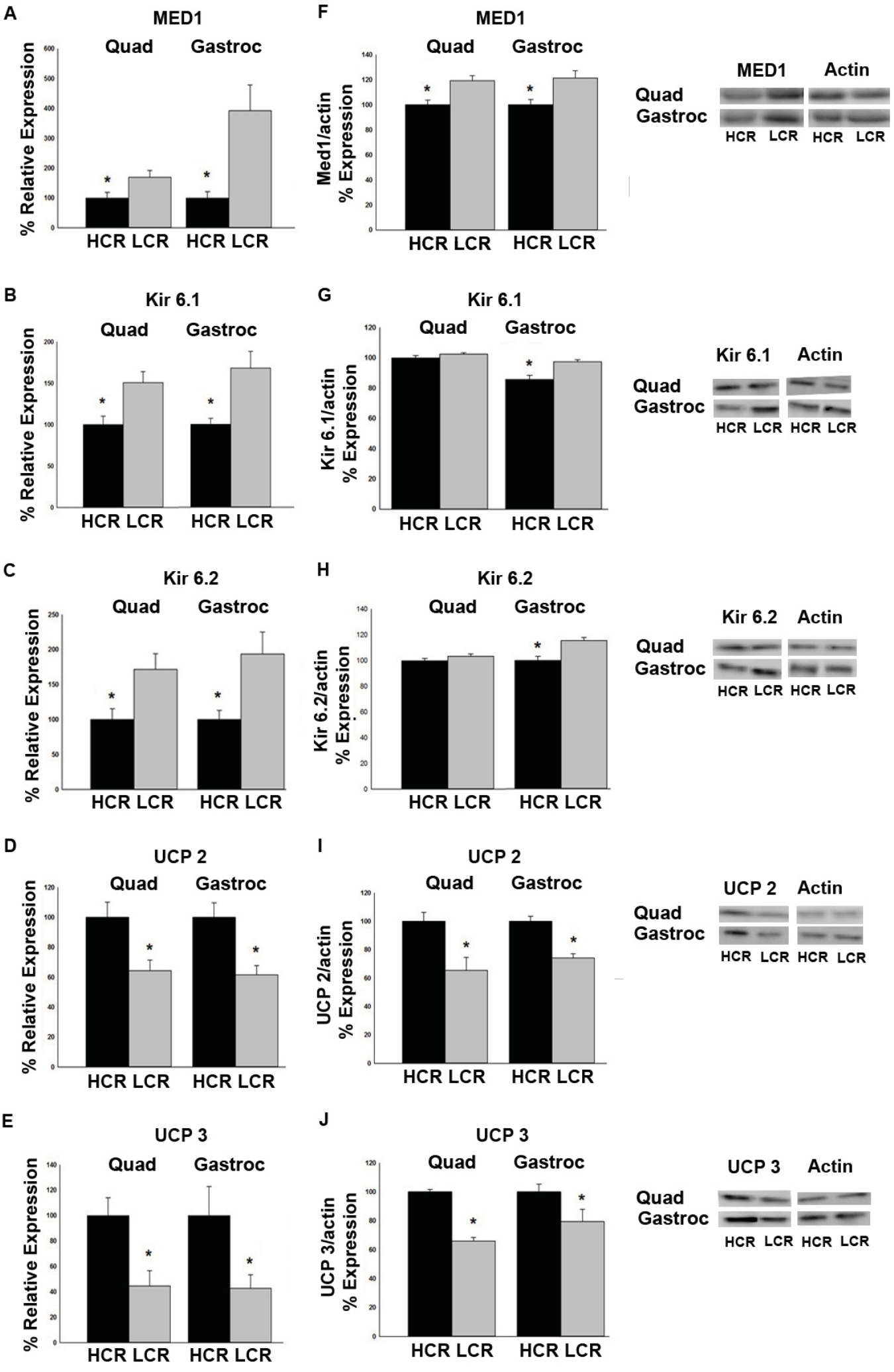

A
MED1
Quad
Gastroc
% Relative Expression
HCR LCR HCR LCR
F
MED1
Quad
Gastroc
Med1/actin
% Expression
HCR LCR HCR LCR
MED1
Actin
Quad
Gastroc
HCR LCR HCR LCR
B
Kir 6.1
Quad
Gastroc
% Relative Expression
HCR LCR HCR LCR
G
Kir 6.1
Quad
Gastroc
Kir 6.1/actin
% Expression
HCR LCR HCR LCR
Kir 6.1
Actin
Quad
Gastroc
HCR LCR HCR LCR
C
Kir 6.2
Quad
Gastroc
% Relative Expression
HCR LCR HCR LCR
H
Kir 6.2
Quad
Gastroc
Kir 6.2/actin
% Expression
HCR LCR HCR LCR
Kir 6.2
Actin
Quad
Gastroc
HCR LCR HCR LCR
D
UCP 2
Quad
Gastroc
% Relative Expression
HCR LCR HCR LCR
I
UCP 2
Quad
Gastroc
UCP 2/actin
% Expression
HCR LCR HCR LCR
UCP 2
Actin
Quad
Gastroc
HCR LCR HCR LCR
E
UCP 3
Quad
Gastroc
% Relative Expression
HCR LCR HCR LCR
J
UCP 3
Quad
Gastroc
UCP 3/actin
% Expression
HCR LCR HCR LCR
UCP 3
Actin
Quad
Gastroc
HCR LCR HCR LCR

Figure 5

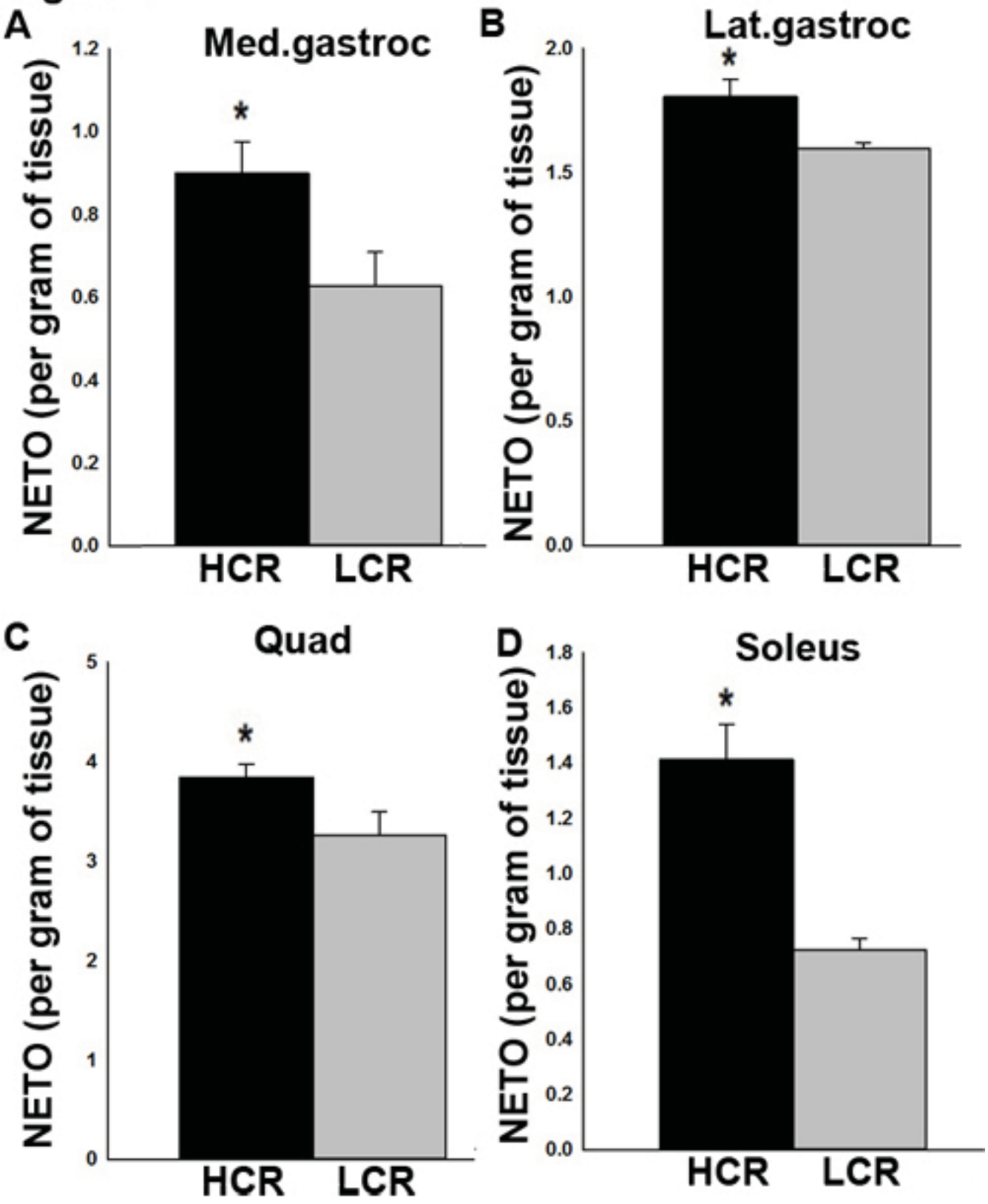

A
Med.gastroc
NETO (per gram of tissue)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
HCR
LCR
B
Lat.gastroc
NETO (per gram of tissue)
2.0
1.5
1.0
0.5
0.0
*
HCR
LCR
C
Quad
NETO (per gram of tissue)
5
4
3
2
1
0
*
HCR
LCR
D
Soleus
NETO (per gram of tissue)
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
HCR
LCR

Table 1: Physical activity and energy expenditure in rats of the lean (HCR) and obesity-prone (LCR) phenotype.

		All rats					Matched by body weight						Matched by lean mass				
		Body weight (g)	Lean mass (g)	Fat Mass (g)	Total counts	Ambul-atory counts	Body weight (g)	Lean mass (g)	Fat mass (g)	Total counts	Ambul-atory counts	Stereot-ypical counts	Body weight (g)	Lean mass (g)	Fat mass (g)	Total counts	Ambul-atory counts
female	HCR	293.4 ± 12.8	206.27 ± 7.92	*39.39 ± 4.29	*11.82 ± 0.55	*4.93 ± 0.35	295.99 ± 11.5	*204.96 ± 6.91	*41.85 ± 5.58	*11.49 ± 0.75	*4.86 ± 0.5	*1.88 ± 0.01	*264.74 ± 11.44	188.95 ± 6.3	*32.77 ± 3.97	*10.99 ± 0.69	*4.44 ± 0.42
	LCR	303.3 ± 12.0	194.26 ± 8.32	61.32± 6.16	6.92 ± 0.31	2.7 ± 0.23	296.06 ± 11.9	190.33 ± 5.73	58.66 ± 6.7	7.04 ± 0.43	2.9 ± 0.34	0.77 ± 0.14	307.89 ± 12.13	189.12 ± 6.11	70.2 ± 7.74	7.09 ± 0.38	2.94 ± 0.33
male	HCR	*414.7 ± 12.2	*292.6 ± 9.2	*59.2 ± 6.1	*6.4 ± 0.17	*2.11 ± 0.09	–	–	–	–	–	–	–	–	–	–	–
	LCR	610.5 ± 42.6	339.5 ± 20.3	193.4 ± 19.4	4.7 ± 0.28	1.53 ± 0.09	–	–	–	–	–	–	–	–	–	–	–

HCR, High capacity runners; LCR, Low capacity runners. *$p<0.05$, HCR≠LCR

Table 2: Results of statistical modeling using multiple linear regression analysis to determine which factors (body mass, lean mass, fat mass, physical activity) best predicted energy expenditure; each component was modeled separately (total daily energy expenditure, TDEE; resting energy expenditure REE; non-resting energy expenditure, NREE) was modeled separately for each the lean (high-capacity runners, HCR) and obesity-prone (low-capacity runners, LCR) phenotypes.

		LCR			**HCR**		
		Predictive factors	r^2	Significance of model or factor (*p*)	Best predictor	r^2	Significance of model or factor (*p*)
TDEE	Best predictive model	Lean mass + physical activity	0.878	<0.001	Lean mass + physical activity	0.637	0.006
	Within model	Lean mass		<0.001	Lean mass		0.093
		Physical activity		0.099	Physical activity		0.091
REE	Best predictive model	All factors	0.880	<0.001	Lean mass	0.347	0.034
	Best single predictor	Lean mass	0.819	<0.001			
NREE	Best predictive model	Lean mass + physical activity	0.791	<0.001	Body weight + physical activity	0.782	<0.001
	Within model	Lean mass		<0.001	Body weight		0.010
		Physical activity		0.012	Physical activity		0.011