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Research paper

Homeostatic effects of exercise and sleep on metabolic processes in mice with an overexpressed skeletal muscle clock

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ABSTRACT

Brain and muscle-ARNT-like factor (Bmal1/BMAL1) is an essential transcriptional/translational factor of circadian clocks. Loss of function of Bmal1/BMAL1 is highly disruptive to physiological and behavioral processes. In light of these previous findings, we examined if transgenic overexpression of Bmal1/BMAL1 in skeletal muscle could alter metabolic processes. First, we characterized *in vivo* and *ex vivo* metabolic phenotypes of muscle overexpressed mice (male and female) compared to wild-type littermates (WT). Second, we examined *in vivo* and *ex vivo* metabolic processes in the presence of positive and negative homeostatic challenges: high-intensity treadmill running (positive) and acute sleep deprivation (negative). *In vivo* measures of metabolic processes included body composition, respiratory exchange ratio (RER; VCO_2/VO_2), energy expenditure, total activity counts, and food intake collected from small animal indirect calorimetry. *Ex vivo* measure of insulin sensitivity in skeletal muscle was determined from radioassays. RER was lower for muscle overexpressed females compared to female WTs. There were no genotype-dependent differences in metabolic phenotypes for males. With homeostatic challenges, muscle overexpressed mice had lower energy expenditure after high-intensity treadmill running. Acute sleep deprivation reduced insulin sensitivity in skeletal muscle in overexpressed male mice, but not male WTs. The present study contributes to a body of evidence showing pleiotropic, non-circadian, and homeostatic effects of altered Bmal1/BMAL1 expression on metabolic processes, demonstrating a critical need to further investigate the broad and complex actions of Bmal1/BMAL1 on physiology and behavior.
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1. Introduction

Brain and muscle-ARNT-like factor (Bmal1/BMAL1) is most commonly known for its function as a positive transcriptional-translational factor of the molecular clock in the brain and periphery [1]. Its expression is essential for maintaining daily rhythms of physiology and rest/activity. The necessity of Bmal1/BMAL1 for maintaining circadian timekeeping has been identified in studies with whole-body and tissue-specific knockout (KO) mice [2–4]. Studies with whole-body and tissue-specific knockout (KO) mice have also demonstrated the essentialness of Bmal1/BMAL1 for maintaining metabolic homeostasis; compromised mitochondrial respiration [5] and sensitivity to glucose and insulin [6–9] in skeletal muscle, pancreas, and liver have been reported.

The present study is unique in that we used a transgenic overexpressed model instead of whole-body or tissue-specific knockout (KO) mice. This allowed us to study daily metabolic processes in the absence of compromised circadian timekeeping. We selected an overexpression model of Bmal1/BMAL1 specific to the skeletal muscle for several important reasons. First, this transgenic mouse line is rhythmic [3]. Second, transgenic overexpression of other gene products driven by the *Acta1* promoter has yielded several advantageous metabolic and behavioral phenotypes as reported previously in *Biochimie* [10]. Thus, we examined *in vivo* and *ex vivo* metabolic phenotypes of muscle overexpressed Bmal1/BMAL1

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(male and female) in comparison to wild-type littermates (WT).

We also aimed to determine if any changes in daily metabolic processes were amplified by positive and negative homeostatic challenges of high-intensity treadmill running (positive) and acute sleep deprivation (negative). It is well documented in rodents and humans that metabolic and endocrine function are altered in opposing directions by high-intensity exercise [11,12] and restricted sleep [13–15]. It is also known that sleep deprivation alters DNA-binding of BMAL1 [16]. Given previous findings, we hypothesize that overexpression of Bmal1/BMAL1 would be associated with changes in daily metabolic processes *in vivo* and *ex vivo*. We also hypothesize that strain-dependent differences in metabolic processes would be amplified in the presence of both positive and negative homeostatic challenges.

2. Material and methods

2.1. Transgenic mice

Adult (10–12 weeks) mice were used in all experiments. Animals were housed in a temperature-controlled vivarium (23 °C) under an entrained 12 h-12 h light-dark cycle. Food and water were provided *ad libitum*. In order to generate mice with overexpression of *Bmal1* specific to skeletal muscle, mice with a human alpha-skeletal actin gene promoter (*Acta1*) tagged to a hemagglutinin complex were bred on a CD1 background and back-crossed to C57BL/6J mice (Jackson Laboratories; Bar Harbor, ME). This line (*Acta:Bmal1*) has been used by McDearmon et al. 2006 [3] to examine the differential effects of *Bmal1* expression in brain versus skeletal muscle on circadian and homoeostatic sleep processes.

2.2. Energy expenditure and body composition

24-hr energy expenditure (EE) was measured using a 4-chamber Oxymax FAST system (Columbus Instruments, Columbus, OH) at thermoneutral housing conditions (25.9° C). Body composition was measured using an EchoMRI within 1 week of measurement of gas exchange, and mice were acclimated to the calorimetry room and chamber for at least 2 days prior to calorimetry. Mice underwent measurement for 24-hr EE as previously described in Zhang et al. 2012 [17]. Briefly, mice were enclosed in the chamber with food and water, and fresh air was provided at 3.9–4.0 should be 0.39–0.40 LPM. Chamber air was sampled at 0.3 LPM, with 120-sec reference and cage settle times, and 30-sec reference and sample measurement times, with reference air sampled every 30 min. Data from the first ≥1 of measurement were not analyzed to allow for acclimation and air equalization.

2.3. VO_{2max}

VO_{2max} measurement was also performed as previously described in Zhang et al. 2012 [17]. Briefly, mice were placed in the treadmill, which was then sealed, with fresh air provided at 0.75 LPM, and air was sampled every 10 s at 0.3 LPM. After 20 min, the treadmill was started and VO_{2max} was assessed using the following protocol: 10 m/min at 0° incline for 5 min and at 15° for 5 min; at 25° for 2 min each at 10 m/min, 15 m/min, 17 m/min. 19 m/min, 21 m/min, 23 m/min, 25 m/min, 27 m/min, and 29 m/min, or until exhaustion.

2.4. Food intake

Food intake was measured and averaged over 4 days. Mice were acclimated to wire cage bottoms for 2 days prior to being provided with pre-weighed food between 0830 and 1000. Intact and scattered food was measured, along with body weight, at the same time of day.

2.5. Insulin sensitivity

Mice were undisturbed or had been sleep-deprived in their home cages for 6 h at the time of skeletal muscle extraction (midday). For acute sleep deprivation, mice were subjected to non-stressful gentle handling described previously in Longordo et al. 2011 [18]. A separate group of *Acta:Bmal1* mice were fasted during the 6 h of sleep deprivation. Left and right soleus muscles were incubated in Krebs-Ringer bicarbonate buffer (in mM: 117 NaCl, 4.7 KCl, 24.6 $NaHCO_3$, 1.2 KH_2PO_4, 1.2 $CaCl_2$, and 2.5 $MgSO_4$) gassed with 95% O_2–5% CO_2. The first incubation was in the presence of 2 mM pyruvate for 30 min at 37 °C. Following wash, the solei were incubated with Krebs-Ringer bicarbonate buffer containing 1 mM 2-deoxy-d-[1,2–^{3}H]glucose (1.5 mCi/ml) and 7 mM d-[^{14}C] mannitol (0.45 mCi/ml). One soleus was stimulated with insulin for 10 min. The other soleus was unstimulated. Dosing for sub-optimal insulin-stimulated glucose uptake in soleus muscle was determined from the development of a dose-response curve with male WT littermates of *Acta:Bmal1* mice. Insulin doses ranged from 0.25, 0.50, 1.00, 1.50, and 2.50 μunits/μl (n = 3/concentration). The sub-optimal dose for experimentation was found to be 0.66 μunits/μl (Fig. 1). After insulin stimulation, the muscles were digested with 250 μl of 1 N NaOH at 80 °C for 10 min, and were then neutralized with 250 μl of 1 N HCl. 350 μl of scintillation liquid was added for dual-label radioactivity counting of 1 mM 2-deoxy-d-[1,2–^{3}H] glucose (1.5 mCi/ml) and 7 mM d-[^{14}C]mannitol (0.45 mCi/ml).

3. Results

3.1. Metabolic phenotyping in Bmal1/BMAL1 muscle overexpressed mice reveal sex-specific differences

In these experiments, we aimed to determine the influence of Bmal1 overexpression (in skeletal muscle; *Acta:Bmal1*) on metabolic processes *in vivo*. To examine this, we measured VO_2, respiratory exchange ratio (RER, VCO_2/VO_2), energy expenditure, total

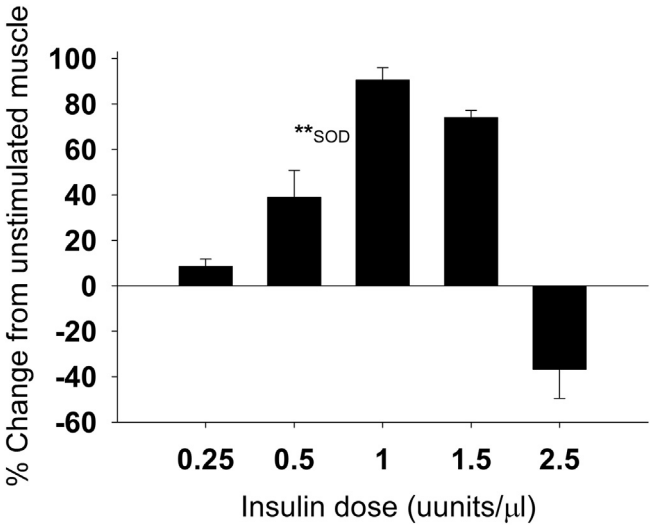

Fig. 1. Dose response curve of insulin-stimulated glucose uptake in soleus muscle. Soleus muscle extracted at midday was stimulated with insulin and subjected to radio-assay analyses with 2-deoxy-d-[1,2–^{3}H]glucose (1.5 mCi/ml) and 7 mM d-[^{14}C]mannitol. Means ± SEM % change in the extent of glucose uptake (nmol/ml) in stimulated versus unstimulated muscle extracted from the same mouse. *SOD, sub-optimal dose.