AICAR prevents fat gain following the cessation of voluntary physical activity

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Running Title: AICAR prevents fat gain accompanying inactivity

Key words: wheel lock, Cyclin A1, AICAR, physical inactivity, fat growth

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NEW FINDINGS

What is the central question of this study?  
We investigated whether AICAR could prevent acute increases in body fat and changes in omental and subcutaneous adipose tissue following the sudden transition from physical activity to physical inactivity.

What is the main finding and its importance?  
AICAR prevented fat gains following the transition from physical activity to inactivity to levels comparable to rats that remained physical activity. AICAR and continuous physical activity produced depot-specific changes in cyclin A1 mRNA and protein that associated with
the prevention of fat gain. These findings suggest targeting AMPK signaling could oppose rapid adipose mass growth.

ABSTRACT

The transition from physical activity to inactivity is associated with drastic increases in ‘catch-up’ fat that in turn foster the development of many obesity-associated maladies. We tested if 5-Aminoimidazole-4-carboxamide ribonucleotide (AICAR) treatment would prevent gains in body fat following the sudden transition from a physically active state to an inactive state by locking a voluntary running wheel. Male, Wistar rats were either sedentary (SED), or given wheel access for 4 weeks, at which time rats with wheels continued running (RUN), either had their wheel locked (WL), or had WL with daily AICAR injection (WL+AICAR) for 1 week. RUN and WL+AICAR prevented gains in body fat compared to SED and WL (p < 0.001). Cyclin A1 mRNA, a marker of cell proliferation, was decreased in omental (OMAT), but not subcutaneous (SAT) adipose tissue, in RUN and WL+AICAR compared to SED and WL (p < 0.05). Both cyclin A1 mRNA and protein positively associated with gains in fat mass (p < 0.05). Cyclin A1 mRNA in OMAT, but not SAT, negatively correlated with p-AMPK levels (p < 0.05). Differences in fat gain and OMAT mRNA and protein levels were independent of changes in food intake and in differences in select hypothalamic mRNAs. These findings suggest that AICAR treatment prevents acute gains in adipose tissue following physical inactivity to levels of rats that continuously run, and that together continuous physical activity and AICAR could, at least initially under these conditions, exert similar inhibitory effects on adipogenesis in a depot-specific manner.

Abbreviations: Arc, arcuate nucleus of the hypothalamus; AICAR, 5-Aminoimidazole-4-carboxamide ribonucleotide; AMPK, AMP-activated protein kinase; OMAT, omental adipose
INTRODUCTION

Childhood physical inactivity is a major environmental factor driving increases in childhood obesity, which is associated with future adverse health outcomes such as type 2 diabetes (T2D) and cardiovascular disease (CVD) (Manco & Dallapiccola, 2012). While greater than 70% and 95% of girls and boys 6 to 8 years old, respectively, met recommended physical activity guidelines, less than 15% and 43% of girls and boys 12 to 14 years old, respectively, met the same requirements (Nader et al., 2008; Dumith et al., 2011). Similarly, the prevalence of obesity increased from ~12% in children 2-5 years of age to ~18% among adolescents 12-19 years of age (Ogden et al., 2012). Together, these trends highlight a growing public health crisis in youth, as physical inactivity and obesity are associated with increased risk of premature mortality, numerous chronic diseases, and diminished quality of life (Booth et al., 2012).

Adipose tissue is highly plastic and responds rapidly to periods of nutritional overabundance (Rutkowski et al., 2009). Expansion of adipose tissue, especially with visceral depots, by adipocyte hypertrophy promotes a pro-inflammatory phenotype that is simultaneous to the development of metabolic dysfunction and T2D and CVD risk (Antos & Potter, 2007; Rutkowski et al., 2009). Importantly, energy depletion early in life may influence fat growth later in life. Dulloo (Dulloo, 2008) reports that in most cases, large decreases in body weight followed by weight recovery during infancy or childhood are accompanied by increases in body fat, specifically visceral fat, at a disproportionately faster rate than growth of lean tissue.
Since its development by Rhodes et al. (Rhodes et al., 2003), the rodent wheel lock (WL) model has been an invaluable tool to study how the transition from physical activity to physical inactivity effects different organ systems. In juvenile rats, our lab has shown drastic increases in visceral adiposity in rats following 1 week of WL, as compared to both rats with continued free wheel-access and rats never exposed to running wheels, highlighting the influences of ending reduced caloric expenditure on adipose tissue expansion and metabolism in maturing rats (Kump & Booth, 2005a; Company et al., 2013; Ruegsegger et al., 2015).

One enzyme central to changes in energy state is AMP-activated protein kinase (AMPK). In addition to being a ‘master regulator’ of metabolism (Hardie, 2007), AMPK inhibits early clonal expansion or pre-adipocytes, a process essential for adipogenesis, and prevents the expression of late adipogenic markers and enzymes essential for lipid synthesis and storage in mature adipocytes (Habinowski & Witters, 2001; Zhou et al., 2009). Pharmacological activation of AMPK with 5-aminoimidazole-4-carboxamide-1-beta-D-ribofuranoside (AICAR) reduces fat mass in rodents (Winder et al., 2000; Narkar et al., 2008), and AMPK activation during exercise decreases triglyceride synthesis in white adipose tissue (Park et al., 2002; Kump & Booth, 2005b). However, the effect of AICAR on mediating the detrimental effect of physical inactivity on adipose tissue growth and function is less well studied.

Therefore, the aim of this study to determine whether AICAR could prevent increases in body fat following 7 days of WL in juvenile rats, as previously observed in our model (Company et al., 2013; Ruegsegger et al., 2015), to levels comparable to rats with continuous wheel access. Additionally, we assessed mRNA expression in omental (OMAT) and subcutaneous adipose tissue (SAT) associated with hypoxia (Vegf, Hif1α), cell proliferation and differentiation (Cdk2, Cyclin A1, Cyclin D1), and metabolism (Adiponectin, Leptin), as well as energy homeostasis-regulating mRNAs in the hypothalamus (Pomc, Cart, Agrp, Npy,

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We hypothesized increases in fat growth accompanying WL, as compared to continuously running rats, would be attenuated by AICAR and that this attenuation would associate with reductions in adipogenic mRNA expression in OMAT and SAT and reduced orexigenic mRNA expression in the hypothalamus.

METHODS

Experimental Animals and Design

All animal protocols were approved by the Institutional Animal Care and Use Committee at the University of Missouri and complied with the National Institutes of Health’s Guide for the Care and Use of Laboratory Animals. The investigators understand the ethical principles under which Experimental Physiology operates and the work presented complies with their animal ethics checklist. Male Wistar rats (n= 29) (Charles River, Raleigh, NC) were maintained in a 12:12-h light/dark cycle at 21-22°C, and food (Formulab Diet 5008, Purina) and water were provided ad libitum. At 28 days of age rats (n = 21) were introduced to voluntary running wheels (circumference: 1.062 m), with an additional group pair-housed without assess to voluntary running wheels (SED, n = 8). Running distance was recorded using Sigma Sport BC 800 bicycle computers (Cherry Creek Cyclery, Foster Falls, VA). At 49 d, rats with voluntary running wheels either maintained access to unlocked running wheels (RUN, n = 6) for the next 7 days, or had a stainless steel rod was placed through the running wheel, thus prohibiting the running wheel from turning during the same time period. The wheel-lock model in our lab has been described previously (Kump & Booth, 2005a). Following wheel-lock, separate cohorts of rats received either vehicle (sterile 0.9% saline) (WL, n = 7), or AICAR (500mg/kg) (WL+AICAR, n = 8), via subcutaneous injection once daily for 7 days at 0700 h. Additionally, SED and RUN rats also received subcutaneous sterile 0.9% saline injection during the same timeframe. All rats were sacrificed by CO₂.

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asphyxiation at 1200-1400 h, approximately 5-7 h after the final AICAR or vehicle injections, when 56-days-old. Body weight and food intake were recorded weekly. Body composition was assessed at the time of WL and on the day of sacrifice.

Body composition assessment and tissue collection

Body composition was assessed twice using a dual-energy X-ray absorptiometry (DXA) machine calibrated for rats (QDR 4500A; Hologic, Bedford, MA). Seven days before sacrifice, rats were anesthetized with isoflurane before the DXA scan. All scans were repeated on the day of sacrifice were performed immediately after CO₂ asphyxiation, which was immediately followed by tissue removal. During sacrifices, gastrocnemius, soleus, triceps, and OMAT were removed and weighed. SAT from the dorsolumbar region was also removed but not weighed. Tissue from the arcuate nucleus of the hypothalamus (Arc) was extracted using a 2 mm-thick punch tool and brain sectioning apparatus (Braintree Scientific). Tissue plugs from 2 mm-thick coronal brain slices were identified per a rat brain atlas (Paxinos & Watson, 1998). We chose to analyze transcripts from the Arc due to their important roles in regulating appetite and energy balance (Morton & Schwartz, 2001; Williams & Schwartz, 2005). Tissues were stored at −80°C until processing.

RNA isolation, cDNA synthesis, and qRT-PCR

OMAT, SAT, and Arc RNA isolation, cDNA synthesis, and qRT-PCR were performed as previously described (Ruegsegger et al., 2015). Gene-specific primers were constructed using PrimerExpress3.0 software (Applied Biosystems) (Table 1). mRNA expression values were quantified using the 2^ΔΔCt method, whereby ΔCT = 18S Ct – target gene Ct. One SAT mRNA sample was removed due to poor RNA quality.
Western Blotting

Western blotting was performed on OMAT and SAT samples as previously described (Ruegsegger et al., 2015). Equal loading was verified by Ponceau S (Sigma). Primary antibody for phospho-AMPKα1/2 (Thr 172) (1:500; Santa Cruz Biotechnology), AMPKα1/2 (1:500; Santa Cruz Biotechnology), phospho-ACC (Ser 79) (1:500; Santa Cruz Biotechnology), phospho-HSL (Ser 565) (1:1000; Cell Signaling), and cyclin A1 (1:500; Signalway Antibody) were diluted in tris-buffered saline with Tween 20 (TBST) with 5% bovine serum albumin and applied to membranes overnight at 4°C. Next, HRP-conjugated secondary antibody (1:1,000; Cell Signaling) was applied to membranes for 1 h at room temperature in TBST with 5% non-fat milk. Prior to exposure, ECL substrate (Pierce Biotechnology) was applied for 5 minutes. Band densitometry was obtained using a Kodak 4000R Imager and Molecular Imagery Software (Kodak Molecular Imaging Systems).

Statistical Analysis

Data were analyzed used SigmaPlot 12.0 (Systat Software) with an alpha-value of 0.05. Values are reported as mean ± SD. Outcome measures for between-group comparison were assessed with one-way analysis of variance (ANOVA). Changes in body weight, food intake, and running distance over the duration of the study were assessed by two-way [Age x Treatment] ANOVA. Holm-Sidak post hoc analysis were performed when appropriate. Pearson correlations were used to assess relationships between OMAT and SAT mRNA expression, changes in fat mass and body fat percentage, and OMAT and SAT p-AMPK levels. No significant correlations were present when data were analyzed by individual groups. Therefore, all Pearson correlations were calculated encompassing all experimental animals.
RESULTS

**Animal characteristics**

Body weight was increased in SED rats compared to the other experimental groups in the final 3 weeks of the study ($F_{3,150} = 14.46, p < 0.001$) (Fig. 1A). No group differences were observed in food intake for the study duration ($F_{3,106} = 1.81, p = 0.15$) (Fig. 1B), or running distance up to the point of wheel lock ($F_{3,72} = 1.24, p = 0.30$) (Fig. 1C). Animal characteristics at the time of sacrifice are presented in Table 2. Notably, both RUN and WL+AICAR treatments resulted in decreases in OMAT mass ($F_{3,25} = 8.66, p < 0.001$) and OMAT mass normalized to body weight ($F_{3,25} = 9.30, p < 0.001$) compared to both SED and WL. From the time of WL until study termination, increases in body fat percentage ($F_{3,25} = 7.07, p < 0.001$) (Fig. 2A) and fat mass ($F_{3,25} = 7.65, p < 0.001$) (Fig. 2B) were attenuated in both RUN and WL+AICAR compared to both SED and WL. However, their differences in fat percentage and mass were independent of their differences lean mass ($F_{3,25} = 0.53, p = 0.66$) or food intake ($F_{3,25} = 0.96, p = 0.43$) (data not shown).

**OMAT and SAT mRNA and protein expression**

OMAT and SAT mRNA expression data are shown in Figure 3. Surprisingly, no differences in SAT mRNA expression were observed (Fig. 3C). In OMAT, Hif1α mRNA expression was decreased in WL ($F_{3,25} = 5.04, p = 0.007$), while adiponectin mRNA expression was decreased in WL+AICAR ($F_{3,25} = 8.03, p < 0.001$) (Fig. 3A). Additionally in OMAT, leptin mRNA expression was decreased in WL and RUN compared to SED, and further decreased in WL+AICAR ($F_{3,25} = 8.02, p < 0.001$). Intriguingly, cyclin A1 mRNA expression in OMAT was decreased in RUN and WL+AICAR compared to SED and WL ($F_{3,25} = 3.87, p = 0.021$), and was positively correlated with changes in fat mass from the time of WL to the end of the study for all groups ($r = 0.37, p = 0.03$) (Fig. 3B).
observations concerning cyclin A1 mRNA expression in SAT were not observed (Fig. 3D).

Also in OMAT, Hif1α mRNA expression was negatively correlated with changes in fat mass (\(r = -0.39, p = 0.04\)) and cyclin A1 mRNA expression (\(r = -0.58, p = 0.001\)) (data not shown).

Similar to its mRNA expression, cyclin A1 protein in OMAT was increased in WL (\(F_{3,25} = 8.004, p < 0.001\)) (Fig. 3E) while no differences in cyclin A1 protein were detected in SAT (\(F_{3,25} = 0.23, p = 0.87\)) (Fig. 3G). Additionally, cyclin A1 protein in OMAT was positively correlated with increases in fat mass from the time of WL until study termination (\(r = 0.62, p < 0.001\)) (Fig. 3F). Similar observations were not observed in SAT (\(r = 0.18, p = 0.33\)) (Fig. 3H).

**Downstream markers of AMPK activity and correlations with mRNA expression**

Surprisingly, one-way ANOVA did not detect between-group differences in the p-AMPK/AMPK ratio (\(F_{3,25} = 1.87, p = 0.16\)), p-HSL (\(F_{3,25} = 0.18, p = 0.91\)), or p-ACC (\(F_{3,25} = 0.37, p = 0.77\)) protein levels in OMAT (Fig. 4A). Similarly, no differences in proteins levels in SAT were observed for the p-AMPK/AMPK ratio (\(F_{3,25} = 1.72, p = 0.18\)), p-HSL (\(F_{3,25} = 0.41, p = 0.74\)), or p-ACC (\(F_{3,25} = 1.06, p = 0.38\)) (Fig. 4B). We speculate these negative findings could be attributed to the 5-7 h delay between the final period of voluntary wheel running or AICAR injection and tissue collection. It was shown that p-AMPK Thr\(^{172}\) concentration significantly peaked at 30 min post-exercise in the vastus lateralis muscle of 23-yr-old males after they performed 3 sets of 8 repetitions of maximal eccentric knee contractions, but p-AMPK Thr\(^{172}\) was not significantly increased at 15 or 60 min post-exercise in these subjects (Gehlert et al., 2012). Despite no group differences in the above measures of AMPK activity, we next assessed relationships between p-AMPK, p-HSL, and p-ACC protein with mRNA expression in OMAT or SAT given findings that AMPK is an upstream regulator of cell cycle-related (Zhuang & Miskimins, 2008), and Hif1α (Lee et al.,...
2003), gene expression. In OMAT, p-AMPK protein was negatively correlated with OMAT cyclin A1 mRNA expression \((r = -0.42, p = 0.02)\) (Fig. 4C), positively correlated with OMAT Hif1α mRNA expression \((r = 0.49, p < 0.01)\) (Fig. 4D), and trended to negatively correlate with body fat percentage at the time of sacrifice \((r = -0.34, p = 0.07)\) (Fig. 4E). In contrast to OMAT, p-AMPK levels in SAT were not correlated with SAT cyclin A1 mRNA expression \((r = -0.03, p = 0.86)\) (Fig. 4F), SAT Hif1α mRNA expression \((r = -0.14, p = 0.47)\) (Fig. 4G), or body fat percentage at the time of sacrifice \((r = 0.13, p = 0.52)\) (Fig. 4H). No significant relationships were observed between p-HSL or p-ACC protein and mRNA expression in OMAT or SAT.

Hypothalamic mRNA expression

One-way ANOVA found no differences in Arc, Pomc, Cart, Agrp, Npy, or Lepr mRNA expression among the four groups, as shown in Figure 5.

DISCUSSION

Here, we report novel findings that AICAR treatment prevents gains in fat mass accompanying the 1-week transition from physical activity to physical inactivity. Additionally, the effect of AICAR on fat gain following WL was similar to the effect of continuous running on fat gain during the same period. The prevention of fat gain in WL+AICAR and RUN groups, compared to WL condition, was associated with reductions in OMAT, but not SAT, cyclin A1 mRNA expression and protein content, permitting the hypothesis for reductions in cell division and proliferation in OMAT, but not SAT. Further, p-AMPK levels in OMAT negatively correlated with OMAT cyclin A1 mRNA expression and trended to correlate with decreased body fat percentage at the time of sacrifice, while similar responses were not observed in SAT. Additionally, the prevention of fat gain in RUN
and WL+AICAR occurred independent of both changes in food intake during the final week of the study and expressions of 5 Arc mRNAs, suggesting changes in energy intake were not the primary determinant of changes in body fat in this experiment. While associative, these findings collectively suggest a hypothesis that cyclin A1 mRNA and protein expression could partially mediate the separate effects of AICAR supplementation and continuous physical activity on the continued hindrance of OMAT mass expansion.

Our observation that AICAR treatment in conjunction with WL prevented gains in omental, and total body, fat masses is similar to previous studies demonstrating the AICAR reduced intra-abdominal fat mass (Winder et al., 2000; Buhl et al., 2002; Narkar et al., 2008). Furthermore, the short duration of our study (1 week) compared to previous reports (4-8 weeks) suggests even short treatments of AICAR may produce drastic changes in adipose tissue phenotype in physically active rats undergoing detraining. Gaidhu et al. (Gaidhu et al., 2011) reported that AICAR-treated rats that never had running, or any type of added physical activity, during their experiment had increased dark-cycle energy expenditure by 8 and 16% after 4 and 8 weeks, respectively. However, prevention of fat increase with AICAR treatment differed as Gaidhu et al. (Gaidhu et al., 2011) examined fat gain in growing male rats, whereas we tested fat regain. In the final week of our current study, fat masses remained similar in the rat group that voluntarily ran when compared to the group receiving AICAR treatment undergoing that detraining week after weeks of running. A limitation of the current study was the use of group housing in SED rats and single housing in the other three groups, which could have influenced changes in fat gain, as previously data demonstrate less variability in fat gain in single vs. group-housed mice (Nagy et al., 2002). However, given the considerable difference in fat gain between the WL and WL+AICAR groups, which were both single housed, we speculate AICAR was the predominant effector of the inhibiting fat
growth, rather than the single vs. group housing effect, because the WL only group tended to gain a greater percentage of body fat than the SED group.

To further explore potential differences in adipose tissue phenotype, we analyzed selective mRNA expression in OMAT and SAT. Intriguingly, our mRNA findings hint that RUN and AICAR treatment might reduce cell proliferation of OMAT compared to WL, as detailed next. Cyclin A1 protein functions to promote cell proliferation and survival by controlling critical points of the cell cycle, and its mRNA is indicative of cell proliferation (Girard et al., 1991; Pagano et al., 1992). Here, cyclin A1 mRNA and protein in OMAT were decreased in RUN and WL+AICAR compared to WL and negatively correlated with decreases in fat mass from the time of WL, suggesting continuous exercise and AICAR may similarly inhibit physical inactivity-induced proliferation and/or differentiation of preadipocytes. Both AICAR (Habinowski & Witters, 2001; Dagon et al., 2006; Lee et al., 2011) and exercise training (Sakurai et al., 2010) have been shown to inhibit preadipocytes differentiation and adipogenesis. However, the results of the current study are limited in that we did not assess adipocyte hypertrophy or hyperplasia. Given previous observations following WL in 56 d-old rats (Company et al., 2013), we speculate that RUN and WL+AICAR could have prevented fat growth compared to WL via the prevention of adipocyte hyperplasia. Surprisingly, no differences in cyclin D1 or Cdk2 mRNA, two additional mRNAs involved in cell cycle regulation and cell proliferation, were observed between groups in OMAT. Another supportive observation is that the down-regulation of Hif1α mRNA by WL in OMAT in our study is consistent with previous reports demonstrating that knockdown of Hif1α protein enhances adipogenesis (Wagegg et al., 2012).

AMPK is hypothesized to be a physiological regulator of adipogenesis (Dagon et al., 2006; Daval et al., 2006). We observed a negative correlation between p-AMPK levels and

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activity or pharmacological treatment) may initially have greater effects on OMAT rather than on SAT, at least with concern for the mRNAs presented herein. This notion is in line with several reports demonstrating that exercise training (Despres et al., 1991; Mourier et al., 1997; Ross et al., 2004; Chaston & Dixon, 2008) and the anti-obesity drugs orlistat and sibutramine (Kamel et al., 2000; Kelley et al., 2004) preferentially reduce visceral adipose tissue compared to SAT. Future research is needed to clarify depot-specific effects of physical inactivity or activity and AICAR treatment on adipose tissue growth and function.

In the current study, differences in adipose tissue gain between WL, RUN, and WL + AICAR occurred independent of changes in food intake. Rapid increases in body fat, despite decreases in food intake to control levels, in WL have been previously observed (Company et al., 2013; Ruegsegger et al., 2015), highlighting the importance of energy expenditure on adipocyte expansion during endurance types of exercise. Consistent with lack of change in food intake, we did not find differences appetite control mRNAs in the Arc. These findings are in contrast to previous findings demonstrating that chronic AICAR treatment (Gaidhu et al., 2011) and voluntary physical activity (Krawczewski Carhuatanta et al., 2011) enhance leptin signaling in the Arc. We speculate that the levels of voluntary running and time course of AICAR injection performed in the current study may have been below a threshold needed to stimulate adaptations in Arc mRNA expression. Additionally, because cage activity was not assessed in the current study, the possible influence changes in cage-activity following AICAR treatment on fat gain cannot be ignored, as chronic AICAR treatment has been shown to increase ambulatory activity (Gaidhu et al., 2011).

In conclusion, our data suggests that 1-week of WL produces rapid increases in fat gain that are prevented by AICAR supplementation. Moreover, the inhibition of body fat increase accompanying AICAR mirrored attenuations in body fat accompanying continuous voluntary wheel running, and was associated with the down-regulation of cyclin A1 mRNA.
and protein in OMAT in both RUN and WL+AICAR. While catch-up enlargement of adipose tissue upon the cessation of physical activity may be a natural survival adaptation to accrue adipose tissue in times of ‘feast and famine’ (Neel, 1962), this adaptation is mal-adaptive in modern society where food intake is often ad libitum and potentially leads to rapid increases in adipose tissue-initiated low-grade inflammation (Ruegsegger et al., 2015). AICAR also has an AMPK-independent inhibition of inflammation (Jhun et al., 2004; Kuo et al., 2008; Boss et al., 2016), which could also abate gains in inflammatory adipose tissue when long periods of inactivity occur after physical activity. While this study underscores the importance of continuous physical activity levels to sustain a healthy adipose tissue phenotype, these data also suggest that AICAR supplementation may prevent gains in adipose tissue during periods of physical inactivity. As the majority of weight loss is regained in obesity (Anderson et al., 2001; Weiss et al., 2007), MacLean et al. (Maclean et al., 2011) have concluded that strategies to counter weight regain will need to be as comprehensive as the “biological adaptations they are attempting to counter.” Future studies could pursue the current studies intriguing evidence that targeting AMPK signaling could have beneficial effects in opposing rapid adipose mass growth in the face of forced reductions in physical activity during medically-induced periods of physical inactivity.

AUTHOR CONTRIBUTIONS
GNR and FWB designed the study. GNR, JAS, KBG, TEC, and FWB performed the experiments, analyzed the data, and performed statistical analyses. GNR and FWB wrote the manuscript. All authors read and approved the final manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify are listed.
CONFLICT OF INTEREST
The authors disclose no conflicts of interest.

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### Table 1: qRT-PCR primers.

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<th>Gene</th>
<th>Forward (5'-3')</th>
<th>Reverse (5'-3')</th>
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<td>18S</td>
<td>GCCGCTAGAGGTTGAATTTCTTG</td>
<td>CATTCCTGGCAAATGCTTTCG</td>
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<td>Adiponectin</td>
<td>TCCCTCCACCAAGGAAACT</td>
<td>GGCACGCTGCAGCAAGGT</td>
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<td>Agrp</td>
<td>TGACATCATAACCCGAAACA</td>
<td>AATTTCACATGCTCCATTTG</td>
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<tr>
<td>Ce2k</td>
<td>ACTAAACCAGTGCCCCCCACCTT</td>
<td>ACCACAGGTGAAAGGCTT</td>
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<td>Cyclin A1</td>
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<td>Cyclin D1</td>
<td>AAGTGTGACCCGGACTGC</td>
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<td>Vegf</td>
<td>GGAGGATGTCCTCCTTCCTGGA</td>
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### Table 2: Animal Characteristics

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<th>RUN</th>
<th>WL+AICAR</th>
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<tr>
<td>Body weight (g)</td>
<td>357.0 ± 23.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>322.7 ± 23.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>323.3 ± 22.2&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>318.9 ± 17.2&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>Body fat (%)</td>
<td>12.1 ± 2.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.6 ± 1.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.3 ± 1.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.9 ± 1.2&lt;sup&gt;b,c&lt;/sup&gt;</td>
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<td>Fat mass (g)</td>
<td>43.0 ± 7.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31.3 ± 6.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>20.2 ± 4.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>25.3 ± 4.8&lt;sup&gt;b,c&lt;/sup&gt;</td>
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<td>Lean mass (g)</td>
<td>314.0 ± 26.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>291.4 ± 18.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>303.1 ± 25.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>293.6 ± 14.7&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>OMT (g)</td>
<td>0.51 ± 0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.46 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.29 ± 0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.30 ± 0.10&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Gastrocnemius (g)</td>
<td>1.84 ± 0.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.73 ± 0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.79 ± 0.12&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.74 ± 0.10&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Soleus (g)</td>
<td>0.20 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.19 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.22 ± 0.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.19 ± 0.02&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Triceps (g)</td>
<td>1.42 ± 0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.34 ± 0.13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.44 ± 0.14&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.39 ± 0.09&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>OMT/body weight (g/kg)</td>
<td>1.43 ± 0.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.42 ± 0.25&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.89 ± 0.18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.95 ± 0.28&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Gastrocnemius/body weight (g/kg)</td>
<td>5.15 ± 0.19</td>
<td>5.36 ± 0.35</td>
<td>5.54 ± 0.28</td>
<td>5.46 ± 0.29</td>
</tr>
<tr>
<td>Soleus/body weight (g/kg)</td>
<td>0.55 ± 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.58 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.68 ± 0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.60 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Triceps/body weight (g/kg)</td>
<td>3.98 ± 0.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.14 ± 0.35&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>4.47 ± 0.28&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.34 ± 0.29&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Absolute food intake (kcal/day)</td>
<td>122.2 ± 3.5</td>
<td>123.5 ± 10.4</td>
<td>125.3 ± 6.1</td>
<td>115.3 ± 8.3</td>
</tr>
<tr>
<td>Relative food intake (g/day/BW) (kcal/kg/day)</td>
<td>349.2 ± 13.7</td>
<td>382.6 ± 27.6</td>
<td>390.1 ± 43.4</td>
<td>361.1 ± 11.0</td>
</tr>
</tbody>
</table>

Values are mean ± SD (n = 6-8 per group). Data are analyzed with one-way ANOVA. Different letters designate statistically significant differences.
FIGURE LEGENDS

Figure 1. Changes in body weight (A), food intake (B), and voluntary wheel running distance (C) for SED (black), WL (gray), RUN (red), and WL + AICAR (blue). Voluntary running wheels were locked at the end of wk 4. Values are mean ± SD (n = 6-8 per group). Symbols: * SED vs. WL, # SED vs. WL+AICAR, & SED vs. RUN (p < 0.05). Note: data includes one rat that ran an exceptionally high distance in the RUN group, contributing to large SD.
Figure 2. Changes in body fat percentage (A) and fat mass (B) during the 7-day period from the time of wheel lock until the termination of the experiment. Values are mean ± SD (n = 6-8 per group). Different letters signify statistically significant differences (p < 0.05).
Figure 3. mRNA expression in omental adipose tissue (OMAT) (A) and subcutaneous adipose tissue (SAT) (C). Cyclin A1 mRNA expression in OMAT positively correlated significantly with changes in fat mass during the 7 day period from the time of wheel lock until the termination of the experiment (B). In SAT, cyclin A1 mRNA expression was not correlated with changes in fat mass (D). Similarly, cyclin A1 protein was increased in WL in OMAT (E) and positively correlated significantly with changes in fat mass from the time of wheel lock until study termination (F). No differences in cyclin A1 protein were detected in SAT (G) and cyclin A1 protein in SAT did not correlate with changes in fat mass from the time of wheel lock until study termination (H). (I) Representative cyclin A1 Western blot bands for OMAT and SAT. Values are mean ± SD (n = 6-8 per group). Different letters designate statistically significant differences (p < 0.05).
Figure 4. No differences in the p-AMPK/AMPK ratio, p-HSL, or p-ACC protein expression were detected in omental adipose tissue (OMAT) (A) or subcutaneous adipose tissue (SAT) (B). However, p-AMPK protein expression in OMAT was negatively correlated significantly with cyclin A1 (C) and positively correlated significantly with Hif1α (D) mRNA expression in OMAT, and tended to negatively associate with body fat percentage at sacrifice (E). p-AMPK protein expression in SAT did not correlate with cyclin A1 (F) or Hif1α (G) mRNA expression in SAT, or with body fat percentage at sacrifice (H). (I) Representative p-AMPK, AMPK, p-HSL, and p-ACC Western blot bands for OMAT and SAT. Values are mean ± SD (n = 6-8 per group). Different letters designate statistically significant differences (p < 0.05). Note that in panels C-H, outlier data points are denoted with an asterisk (*). Pearson correlations were performed with and without (asterisk by r-value) these data points.
Figure 5. mRNA expression in the arcuate nucleus of the hypothalamus (Arc) did not differ between groups. Values are mean ± SD (n = 6-8 per group).