Effect of Short Term Exercise and High Fat Diet on Skeletal Muscle miR133a

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INTRODUCTION

Micro RNAs (miR) are small non-coding RNA that regulate gene expression at the post-transcriptional level. miR133a is abundant in cardiac and skeletal muscle. In skeletal muscle, miR133a is best known for its regulatory role in myogenesis and differentiation. Nie (2016) found that muscle miR133a expression increased after acute exercise and with 12w of treadmill exercise training in mice. Knockdown of miR133a in transgenic mice resulted in blunted skeletal muscle mitochondrial biogenesis and function in response to exercise training (Nie, 2016) suggesting a role for miR133a in regulating the normal skeletal muscle metabolic adaptive response to exercise. Among other miR, skeletal muscle miR133a is reported as downregulated in insulin-resistant muscle. Insulin resistance in mice fed a high-fat diet is detectable after 3 days on diet (Lee, 2011). In this study, voluntary, rather than forced, exercise was employed to test whether miR133a expression is regulated early in the adoption of increased daily physical activity.

HYPOTHESIS

The working hypothesis for this pilot study was that miR133a is upregulated early in the adoption of exercise, in the form of voluntary, daily activity on rodent cage wheels.

The secondary hypothesis was that simultaneous adoption of a high-fat diet would oppose the upregulation of miR133a expression.

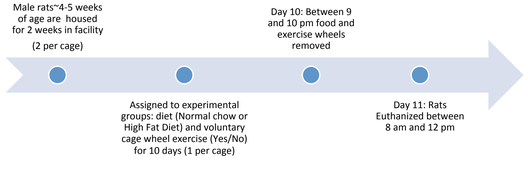
METHODS

Methods

- Four groups of 6 Harlan Sprague Dawley (Envigo) male rats were placed on diet and exercise plans for 10 days: normal chow [Control], normal chow + exercise; high-fat diet and high-fat diet + exercise. High-fat diet was TestDiet 58Y1 Blue; 60% kcal from fat. Normal chow diet was Envigo Teklad 2018; 18% kcal from fat.
- Rats were euthanized via CO_2 inhalation during the morning of Day 11, and tissues removed, snap frozen in liquid N_2 and stored at -80C.
- RNA was extracted from the right soleus of each rat according to ThermoFisher PureLink miRNA Isolation Kit (Catalog Number K157001)
- Reverse Transcription and Real Time PCR performed according to Applied Biosystems Taqman Universal Master Mix II Protocol without UNG on a StepOnePlus Real Time PCR System
- Normalization was performed using miR191 ($\Delta C_T = C_T$ miR133a-C_T miR191) and then converted to the linear form $2^{-\Delta CT}$. Values are expressed as fold-change from the average of the $2^{-\Delta CT}$ values for the Control (normal diet-no cage wheel) group.

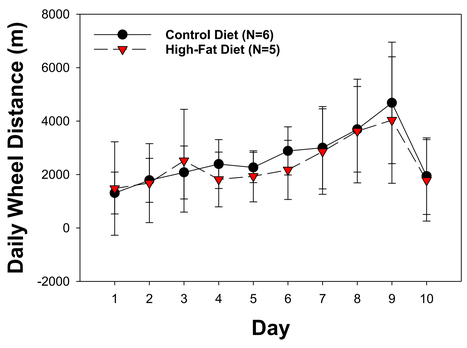
Data Analysis

- Using the EXPLORE function in SPSS 25 on the $2^{-\Delta CT}$ values, one outlier each (value > 1.5x the interquartile range) was identified in the Control and in the High-Fat Diet group. Outliers were removed prior to statistical analyses for miR133a relative expression.
- One rat originally assigned to the HFD-Exercise group had zero activity on the wheel recorded over the ten days. The data from this rat was transferred to the HFD (no exercise) group.



FIGURES

Figure 1

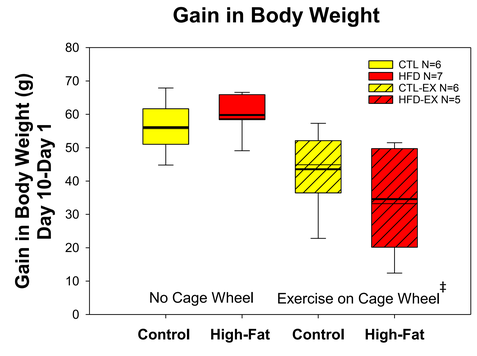


High-fat diet had no effect on daily or cumulative distance run on the cage wheels.

The decrease in distance run during day 10 is due to removal of cage wheels between 9-10 p.m.

Distance was measured with a Cateye bike computer attached to the cage wheel. Repeated measures ANOVA revealed that distance run increased over time (P=0.001) with no interaction between DIET and Distance (P=0.6). Cumulative distance over the exercise period was similar in the animals on the normal chow (26 km [95%CI: 15-37km]) and the high-fat diet (24km [95%CI: 12-36 km]). Values in the graph are mean [SD].

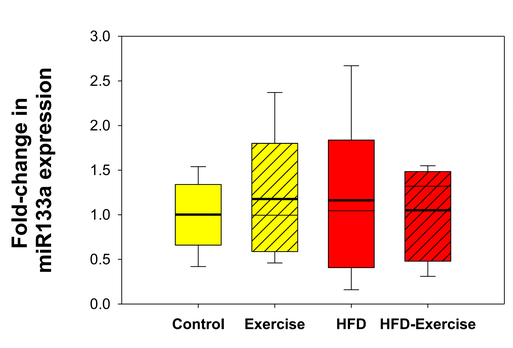
Figure 2



High-fat diet had no effect on the gain in body weight over 10 days. Exercise on the cage wheels decreased body weight gain.

Two-way ANOVA revealed no interaction between DIET and EXERCISE status (P=0.16), and that exercise‡ (P<0.005) but not diet status (P=0.5) affected body weight gain over 10 days. The thick line in the box plot represents the mean and the thin line represents the median. Whiskers are 10th and 90th percentiles.

Figure 3

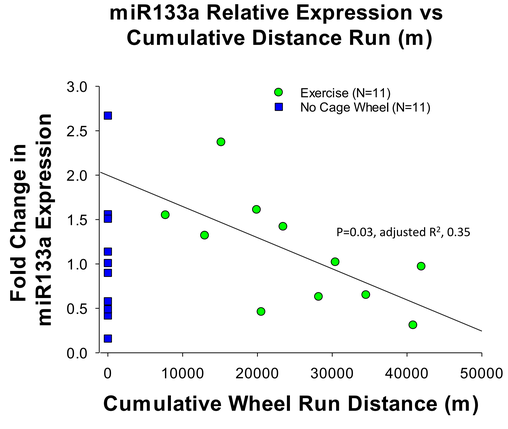


Neither a high-fat diet nor voluntary exercise on a cage wheel affected the relative expression of miR133a in rat soleus muscle.

Two-way ANOVA revealed no DIET x EXERCISE interaction (P=0.2) and no main effect of DIET (P=0.9) or EXERCISE (P=0.9) on relative expression of miR133a. Expression of the data as $2^{-\Delta Ct}$ rather than as fold-change from Control group did not affect the relationship. Mean value, thick line. Median, thin line. Whiskers represent 10th and 90th percentiles.

Controlling for cumulative distance (ANCOVA) revealed no effect of DIET on miR133a expression (P=0.5) in the rats housed with a cage wheel.

Figure 4



Increased exercise distance over the 10 day period was associated with lower levels of miR133a relative expression. However, the range of the miR133a values is clearly within the range observed for the rats that did not engage in wheel exercise.

Controlling for cumulative distance (ANCOVA) revealed no effect of DIET on miR133a relative expression (P=0.5) in the rats housed with a cage wheel. Thus, the groups were combined for the regression analysis.

Linear regression revealed a significant inverse relationship (P=0.032) with adjusted R^2 of 0.35. Expression of the data as $2^{-\Delta Ct}$ rather than as fold-change from Control group did not affect the relationship.

RESULTS

1. **High-fat diet had no effect on daily or cumulative distance run on the cage wheels.**
2. **High-fat diet had no effect on the gain in body weight over 10 days. Exercise on the cage wheels decreased body weight gain.**
3. **Neither a high-fat diet nor voluntary exercise on a cage wheel affected the expression of miR133a in rat soleus muscle.**
4. **Increased exercise distance over the 10 day period was associated with lower levels of miR133a expression, and accounted for 35% of the variability in miR133a expression in the rats allowed access to a cage wheel.**

DISCUSSION and CONCLUSION

The expression of miR133a was rather variable in the young adult rats housed without cage wheels (range in fold change: -6 to +2.7). However, a novel design element of this study is that the rats allowed access to cage wheels self-selected their exercise volume, which resulted in a 5-fold range in total distance run over the 10 d period. This allowed us to evaluate the relationship between voluntary wheel distance run and miR133a expression in the soleus muscle. Cumulative distance run accounted for 1/3 of the variability in miR133a expression in the group of rats allowed access to a cage wheel. Thus, the degree of daily activity volume and/or intensity are factors likely to affect miR133a expression in recruited skeletal muscle fibers during the adaptation phase to chronically increased levels of physical activity. Pathways that miR133a is thought to directly or indirectly regulate that are relevant to exercise adaptations include muscle regeneration (Ultimo, 2018), mitochondrial biogenesis and function (Nie, 2016 and Wüst, 2018) and insulin sensitivity (Esteves, 2017).

In conclusion, miR133a expression in oxidative (soleus) rat hindlimb muscle was unaffected by short term exposure to a high-fat diet and/or exercise. However, the amount of exercise (cumulative distance) was a factor that partially accounted for the variability in miR133a expression in exercised muscle.

References:

Esteves JV et al. J Diabetes Res. 2017 ID 7267910; Lee YS et al. Diabetes 60: 2474-2483, 2011; Nie Y et al. FASEB J 30: 3745-3758, 2016; Ultimo S et al. Oncotarget 9: 17220-17237, 2018; Wüst S et al. Cell Metabol. 27: 1-14, 2018

ACKNOWLEDGEMENTS

Funding for the study was provided by the Office of Research and Sponsored Programs at Midwestern University (MWU). Sowmya Anjur was supported by MWU Dept of Physiology and the APS 2018 Frontiers in Physiology Research Community Leaders Professional Development Fellowship. Adam Van Handel was supported by the Federal Work-Study Program.