Day to Day and Leg to Leg Variation in Gene Expression

Zohal Alizai, Robert Shute, Dustin Slivka
University of Nebraska at Omaha, School of Health and Kinesiology, Omaha, NE

Exercise Physiology Lab

ABSTRACT

BACKGROUND: Skeletal muscle biopsies are extensively used in research to determine the effects of exercise. Usually, sequential biopsies are taken before and after exercise in order to determine the influence of exercise on gene expression. Often it is not clear if the results are due to exercise or as a result of damage cause by the biopsy to the muscle. Previous studies have proven that multiple biopsies in the same leg cause stress to the muscle and such stress leads to inflammation and other response pathways that alter the gene expression. **PURPOSE**: The purpose of this study is to determine the day to day variance in skeletal muscle gene expression in the same leg and between legs to establish an experimental design that eliminates artifacts and reduces the number of biopsies needed for a study. **METHODS**: Eight participants had a muscle biopsy taken from the vastus lateralis muscle on three separate occasions approximately 1 week apart after repeating the same diet and exercise for 24 hours prior. Legs were randomized and altered on subsequent weeks. Genes related to mitochondrial development and stable reference were measured using real-time PCR. The first biopsy was defined as stable reference control condition and gene expression was normalized using $2^{-\Delta\Delta CT}$ and $2^{-\Delta CT}$ method. **Results:** No difference was found in gene expression from day to day variation or between legs for ERRa ($p = 0.85$), GABPA ($P = 0.85$), MEF2a ($P = 0.86$), NRF1 ($P = 0.97$), PPARG ($P = 0.38$), PGC1a ($p = 0.88$), SIRT1 ($p = 0.299$), TFAM ($p = 0.99$), and VEGF ($p = 0.61$). For FNDC5 there was a difference between legs, trial 1 compared to trial 2 ($p = 0.04$), but trial 2 compared to trial 3 did not show a difference ($p = 0.906$) and not within the same leg trial 1 to trial 3 ($p = 0.946$). The reference genes B2M ($p = 0.005$) and CYC ($p = 0.002$) were different between biopsies; GAPDH and RPS18 were not different between biopsies ($p > 0.05$). **Conclusion:** While many genes are statistically not different between legs and between days, researchers need to determine the amount of error that is acceptable in each study design.

PURPOSE

The purpose of this study is to determine the day to day variance in skeletal muscle gene expression in the same leg and between legs to establish an experimental design that eliminates artifacts and reduces the number of biopsies needed for a study.

METHODS

Eight healthy college age volunteers had a muscle biopsy taken from the *vastus lateralis* on three separate occasions separated by 7 days. Before the biopsies were taken, all the subjects were instructed to not exercise and keep the same diet one day prior to the biopsy. Two biopsies were taken from one leg and a third biopsy was taken from the other. This will allow comparisons between and within legs on different days. A total of 24 muscle biopsies were analyzed to test the expression of genes using real time PCR. Genes related to mitochondrial development were ERRa, GABPA , MEF2a, NRF1, PPARG, PGC1a, SIRT1, TFAM, VEGF, and FNDC5 and stable reference genes B2M, CYC, GAPDH and RPS18. Gene expression was expressed using the $2^{-\Delta\Delta CT}$ and $2^{-\Delta CT}$ method. The stable reference control condition for $2^{-\Delta\Delta CT}$ was defined as the first biopsy. A one-way repeated measures analysis of variance (ANOVA) was used to determine statistical significance. All data is presented as mean ± standard error.

RESULTS

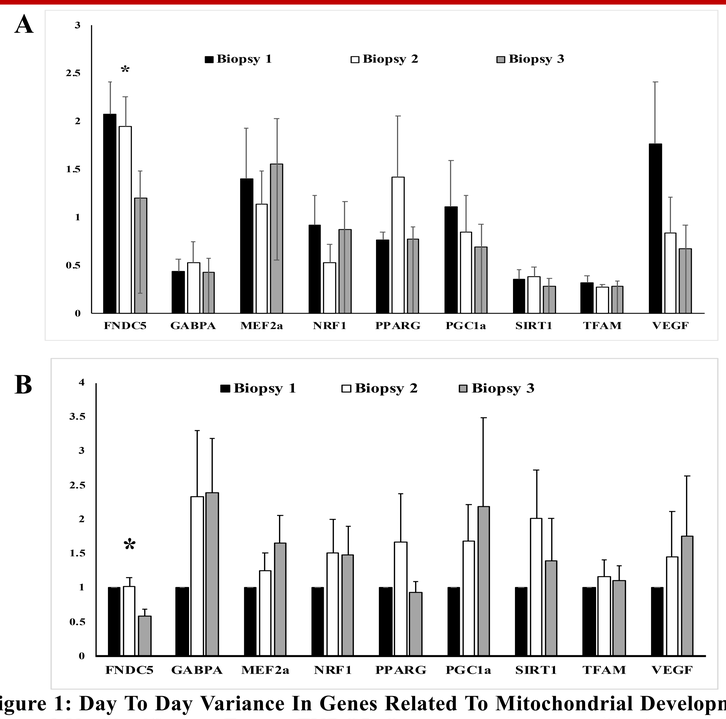

Figure 1: Day To Day Variance In Genes Related To Mitochondrial Development Showed No Significance Except FNDC5. Gene expression was utilized using the $2^{-\Delta CT}$ method for Figure 1A and $2^{-\Delta\Delta CT}$ for Figure 1B. The stable reference control condition was defined as the first biopsy. No difference was found in gene expression from day to day variation or between legs for ERRa, GABPA, MEF2a, NRF1, PPARG, PGC1a, SIRT1, TFAM, and VEGF ($p > 0.05$). FNDC5 was different between legs (biopsy 1 compared to biopsy 2; $p=0.04$), but biopsy 3 was similar to biopsies 1 and 2 ($p > 0.05$). * $p < 0.05$ from biopsy 1. Values are mean ± SE in relative units.

RESULTS

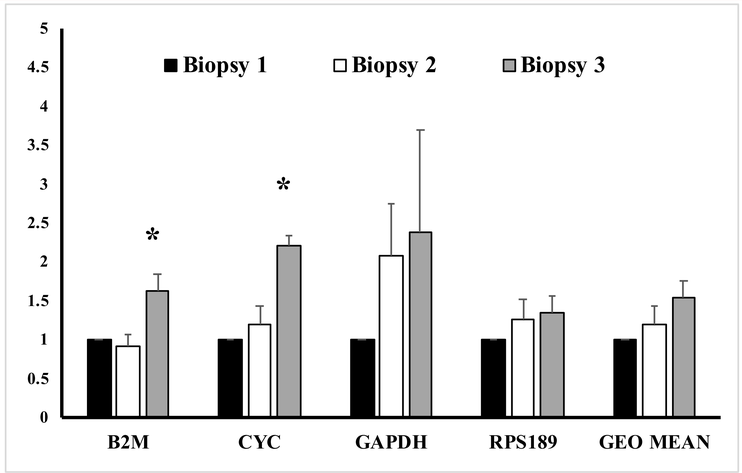

Figure 2: Day To Day Variance In Stable Reference Gene Expression Between Legs and Within Legs were Significant For B2M and CYC. Gene expression was utilized using the $2^{-\Delta\Delta CT}$ method. The stable reference control condition was defined as the first biopsy. Biopsy 3 had higher expression than biopsies 1 and 2 for B2M and CYC ($p < 0.05$). GAPDH and RPS18 were not different between biopsies ($p > 0.05$). *$p < 0.05$ from biopsies 1 and 2. Values are mean ± SE in relative units.

CONCLUSION AND FUTURE DIRECTION

- Genes related to mitochondrial biogenesis are not statistically different between legs or between days except for FNDC5 which was different between biopsy 1 and 2 but not different than biopsy 3.
- Two of the four stable reference genes were stable between biopsies and two were higher on biopsy 3 than the other biopsies. However, by using the geometric mean of all four reference genes these differences disappear.
- While many genes are statistically not different between legs and between days, researchers need to determine the amount of error that is acceptable in each study design.
- 12 more subjects are currently being analyzed to expand the sample size.
- These data will aid researchers utilizing the muscle biopsy technique in the best study design in terms of the appropriate timing of the biopsy in relation to subsequent biopsies.

FUNDING

This work was supported by the National Institute for General Medical Science (NIGMS P20GM103427), Nebraska IDeA Networks of Biomedical Research Excellence (INBRE)