EXERCISE AND SPORT SCIENCES REVIEWS

AMERICAN COLLEGE of SPORTS MEDICINE www.acsm.org

ESSR Journal Club

Covered Article: "Identifying Novel Signaling Pathways: An Exercise Scientists Guide to Phosphoproteomics" by Gary M. Wilson, Rocky Blanco, Joshua J. Coon, and Troy A. Hornberger. *Exercise and Sport Sciences Reviews*. 46(2), April 2018.

1) You have designed an experiment that will include the phosphoproteomic analysis of human muscle tissues obtained both before and after a bout of high-intensity exercise. You have chosen to include a baseline sample along with samples from four time points collected after the onset of exercise. In total, you will collect 20 samples from four subjects. Discuss the different ways in which you could generate quantitative data along with the specific techniques that could be used to maximize coverage both within and between individual samples.

2) Your find that ~600 of the nearly 10,000 identified phosphopeptides were significantly altered as determined by a two-tailed student's t-test. What are the consequences of thinking that all of these phosphorylation sites are biologically relevant and what further tests you could perform to validate the phosphoproteomic data?

3) You want to annotate your data to identify the cellular processes that are associated with the significantly altered phosphoproteins. What resources are available to perform this type of analysis? What background list should your regulated processes be scored against?

4) Sequence analysis has revealed that the motif xxx(S/T)Pxxx is overrepresented within the group of phosphorylation sites that were altered by exercise. What conclusions can be made from this finding? What resources are available to further explore and visualize these data?

5) We have provided a supplementary activity with the article that takes you through data processing with the Perseus software suite and PHOTON. The following questions will help to gauge your comprehension of the procedures and plots that were generated.
    a. Most of the significantly altered phosphopeptides are located on the right of the parabolic lines in the volcano plot. What does this mean about the relative abundance of these phosphopeptides between the two samples conditions? What do the parabolic lines represent?
    b. When visualizing the volcano plots, you can scroll through the list of categorical KEGG pathways and identified members will appear in red. Which KEGG pathways, other than MAPK signaling, appear to be enriched with significantly altered phosphopeptides? What types of experiments could you perform to further validate these findings?
    c. The data were normalized to 'Z-scores' before performing hierarchical clustering. What does this type of normalization accomplish and why does it make sense to do this before hierarchical clustering?
    d. What information does the PHOTON network analysis provide? How does PHOTON differ from other programs that generate network diagrams?

