Identifying Relevant Biomarkers With Metabolomics-based Studies

Metabolomics examines the metabolites in the body on a global scale. With applications in both research and the clinic for monitoring health and disease, metabolomics offers a powerful approach for discovering and analyzing biomarkers in biological systems. In addition, the development of new analytical methods and assays using metabolomics for monitoring biomarkers help to improve existing protocols.

Metabolomics measures a set of metabolites that represent the phenotype of a biological system. The identification of discriminating metabolites between healthy and diseased systems could lead to greater understanding of disease pathology and ultimately direct research efforts in the clinic[1]. These metabolites could be used as biological markers of disease and to predict relevant clinical outcomes. Biomarker discovery is a key application of metabolomics.

Comprehensive metabolomics-based studies have been facilitated by the development of high-resolution nuclear magnetic resonance spectroscopy (NMR), mass spectrometry (MS), ultraperformance liquid chromatography (UPLC), and more sophisticated bioinformatics and analytical techniques. DHMRI offers the latest instrumentation, techniques, and software, which, combined with our expertise and experience, provides our clients and research partners the opportunity to perform comprehensive studies without the need for in-house instrumentation.

Here, we describe a study conducted by the DHMRI team and scientists at Appalachian State University, North Carolina Research Campus (ASU-NCRC). The study utilized metabolomics to investigate and identify biomarkers involved in oxidative stress and inflammation.

Discovering a biomarker: a case study

Introduction

Elevated oxidative stress and inflammatory status are correlated with increased risk for human disease. F2-isoprostanes are considered the "gold standard" measure of oxidative stress and inflammatory status[2] but, as F2-isoprostanes are present in low concentration in human plasma, they are very difficult to measure. A project by DHMRI and Prof. David C. Nieman's research group from the ASU-NCRC Human Performance Lab aimed to utilize a metabolomics study to develop an assay to quantify an alternate biomarker for oxidative stress. The researchers hoped that this new assay would serve as a more cost-effective, sensitive approach to identify elevated inflammatory status *in vivo*.

Methods and results

A clinical exercise study was conducted at the ASU-NCRC Human Performance Lab. Ten male and ten female study participants ran on a treadmill at ~70% VO_{2max} for 1.5 hours followed by 30 minutes of downhill running at ~10% VO_{2max}. Four blood samples were taken from each participant: pre-run, immediately post-run, and at one hour and 24 hours post-run.

Samples were then analyzed by DHMRI utilizing quantitative LC-MS/MS targeting 9+13 HODE, F2-isoprostanes, and other exercise-related markers of interest, including six cytokines, C-reactive Protein (CRP), creatine kinase (CK), and myoglobin (MYO). Full details of collection and analysis procedures were reported previously[3].

The data revealed that an intensive 1.5-hour run followed by 30 minutes of downhill running induced a 3.1-fold increase in plasma 9+13 HODE, with levels still elevated above pre-run levels one day later (41%) (Figure 1).

Data from the assays indicated that both 9+13 HODE and F2-isoprostanes followed the same trends in plasma concentration at the four time points (Figure 2). There is an immediate increase in plasma concentration of both 9+13 HODE and F2-isoprostanes post-run that diminishes by the final time point.

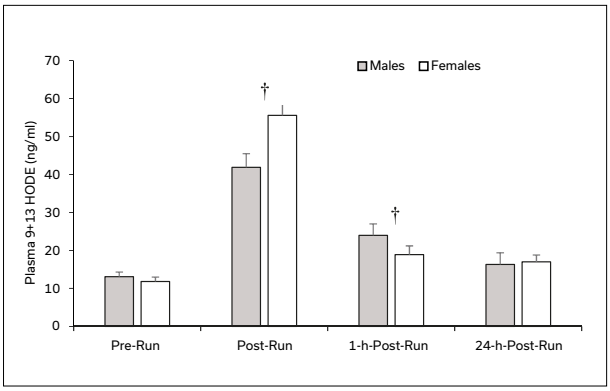

FIGURE 1

Change in plasma 9- and 13-hydroxy-octa-decadienoic acid (9+13-HODE) across four time points for male (N=10) and female (N=10) runners: pre-run, immediately post-2h run, and 1-h and 24-h run.

Discussion

While clinical studies typically target F2-isoprostanes as accurate biomarkers of oxidative stress, their low levels in human plasma make them difficult to measure. The clinical metabolomics study described herein identified two oxidized derivatives of linoleic acid, 9- and 13-hydroxy-octadecadienoic acids (9+13 HODE), as potential key biomarkers for oxidative stress and inflammation[4]. Data showed that 9+13 HODE increased 3- to 5-fold after prolonged and intense exercise. Importantly, post-exercise 9+13 HODE correlated significantly with the "gold standard" measure of oxidative stress, F2-isoprostanes[2].

Conclusions

This work demonstrated that changes in plasma 9+13 HODE over the four time points correlated with that seen for the "gold-standard" measure of F2-isoprostanes.

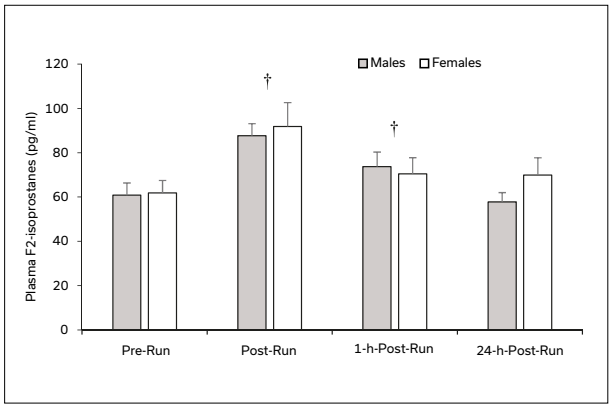

FIGURE 2

Change in plasma F2-isoprostanes across four time points for male (N=10) and female (N=10) runners: pre-run, immediatley post-2-h run, and 1-h and 24-h post-run.

In addition, the researchers found the extraction procedure for plasma 9+13 HODE (LC-MS/MS analysis) to be much easier than F2-isoprostanes extraction for GC-MS analysis.

The researchers had, therefore, identified an appropriate and more easily measured substitute biomarker for oxidative stress and inflammation. As a result, future exercise-related studies should consider measuring for oxidative stress with 9+13 HODE from plasma. Indeed, researchers at ASU-NCRC Human Performance Lab continue to work with DHMRI in many future studies using 9+13 HODE and other lipid mediators

"The DHMRI team successfully developed the 9+13 HODE assay for us. Under a service agreement, the team went on to assay 9+13 HODE and F2-isoprostanes on 200 plasma samples from an exercise study conducted at the ASU Human Performance Lab, resulting in some unique scientific discoveries.

We will continue to work with the DHMRI team in future studies using 9+13 HODE. This experience represents the type of research support that all of us at the North Carolina Research Campus are seeking."

David C Nieman, DrPH, FACSM, Director of the ASU-NCRC Human Performance Lab

Metabolomics at DHMRI

DHMRI offers a highly experienced Metabolomics Team, uniquely equipped to support customers' targeted metabolomic needs. Ready-to-run human and plant panels are available, including the one discussed here for oxidative stress using HODE. Rapid custom analytical assay and/or panel development services are also available. The team combines multiple mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy platforms, including an ultra-high field 950 MHz instrument, with a wealth of experience and expertise in metabolomic analysis and assay development.

As demonstrated in this case study, DHMRI can support study design, sample extraction through to analysis, data visualization, and interpretation - whatever your application. Our combination of instrumentation and experience makes us the ideal partner for your metabolomics research.

To find out how we can support your drug discovery program, contact us now or visit our website.

References

1. Zhang, Aihua, et al. "Metabolomics for biomarker discovery: moving to the clinic." BioMed Research International 2015 (2015).
2. Nieman, David C., et al. "Metabolomics approach to assessing plasma 13-and 9-hydroxy-octadecadienoic acid and linoleic acid metabolite responses to 75-km cycling." American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 307.1 (2014): R68-R74.
3. Nieman, David C., et al. "9- and 13-Hydroxy-octadecadienoic acids (9+13 HODE) are inversely related to granulocyte colony stimulating factor and IL-6 in runners after 2 h running." Brain, Behavior, and Immunity 56 (2016): 246-252.
4. Nieman, David C., et al. "Serum metabolic signatures induced by a three-day intensified exercise period persist after 14 h of recovery in runners. Journal of Proteome Research 12(10) (2013): 4577-4584.