MESSAGE FROM THE GRAYSON-JOCKEY CLUB RESEARCH FOUNDATION

MicroRNAs as Novel Biomarkers or Insulin Resistance

Grayson-Jockey Club Research Foundation

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ONE SENTENCE SUMMARY: In an effort to improve the diagnosis, management, and treatment of the many horses and ponies who suffer from metabolic disorders related to insulin dysregulation, we will investigate the ability of novel, small, gene-regulating molecules called microRNAs to be used as convenient and accurate biomarkers for equine insulin resistance.

Insulin resistance occurs when circulating concentrations of the hormone insulin fail to exert their normal biologic response, setting the stage for serious metabolic complications, acute or chronic illness, and even death. Normally, in response to a meal or feeding challenge, the combined effect of increased blood glucose concentrations and action from hormones will stimulate insulin secretion from the pancreas. In a healthy horse this insulin enters the bloodstream and initiates a cascade of events at the cellular level to shuttle circulating glucose into tissues; primarily in skeletal muscle and the liver, where it is stored as glycogen, and in adipose tissue where it is stored as fat. As a result, circulating levels of glucose are kept within a narrow window compatible with normal metabolic functioning.

However, in some animals, the system breaks down, causing IR. Exact mechanisms for how, why, and when IR occurs remain unknown. A multitude of factors might play a part. Disturbances in insulin effectiveness might affect systems that regulate immunology, circulation, reproduction, growth, and more. For this reason, IR in horses is associated with a number of debilitating disorders, including equine metabolic syndrome, pituitary pars, intermedia dysfunction, and laminitis.

Our current diagnosis of IR is done most accurately by performing tests such as the euglycemic hyperinsulinemic clamp or the frequently sampled intra-venous glucose tolerance test with the minimal model analysis. However, these tests are expensive and labor intensive, requiring large numbers of samples, significant time commitment, and complicated analytical modeling.

Since their discovery in 1993, microRNAs (miRNAs) have emerged as exciting key regulators of biological processes and promising biomarkers for disease. These small (~22 nucleotides in length) RNA molecules bind to specific mRNA targets and either promote their degradation or stop their ability to code for proteins. In most species there are few miRNAs relative to the number of mRNAs they might regulate. For example, in the horse there might be more than 25,000 genes and associated mRNAs, but the number of miRNAs is predicted to be ~1000. This is because one miRNA might regulate hundreds of mRNAs, and thus single miRNAs might have a substantial influence. For this reason, differential miRNA expression patterns between healthy and diseased animals are especially rich in information.

MiRNA profiling has become a powerful tool as miRNAs have been shown to be incredibly robust in circulation and are measurable to a much higher sensitivity than cell-free circulating proteins. For this reason, miRNA profiling is of great interest to scientists wishing to develop biomarkers for diverse biomedical applications, including cancer, autoimmune, cardiovascular, and metabolic diseases. In human metabolic research, for example, miRNAs have already been identified that can separate groups of patients into clinically-relevant classes, such as pre-diabetic or type 1 diabetic cohorts, and a large suite of miRNAs has been implicated in aberrant beta-cell development and functioning.

In this study we apply this novel technology to insulin resistance, a complex and causative factor in the development of several debilitating medical conditions with large economic impact to the horse industry. Identification of miRNAs responsible for "fine tuning" expression of vast numbers of target genes is a cost-effective method that will not only provide new and useful information on pathogenesis of equine disease but also give insight into biomarkers associated with IR processes and progression.

We accomplished this by investigating the circulating miRNA profile of insulin resistant horses. We hypothesized that insulin resistant horses would have a different circulating miRNA profile than those that were healthy. Twelve Thoroughbred/Thoroughbred-cross and 12 Welsh/Dartmoor non-pregnant mares were evaluated for insulin sensitivity using frequent sampling intravenous glucose tolerance tests. Serum samples were collected for miRNA profiling. Mares were divided into healthy or IR groups and their miRNA profiles compared. MiRNA profiles of horses and ponies were also compared. Results demonstrated that 13 miRNAs were differently expressed between IR and IS horses, 15 between IR and IS ponies, 17 between horses and ponies, and 10 between IR and IS animals (horses and ponies combined) ($p<0.05$). Finally, 8 miRNAs were proposed as potential regulators of equine insulin resistance. In addition to our preliminary investigation, this study suggests potential miRNA profiling is a potential new tool to be used to further understand the mechanisms involved in equine insulin resistance and associated conditions. BH

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