Characterization of microRNAs in the heart

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INTRODUCTION.
MicroRNAs are endogenous, non-coding RNA species that regulate gene expression through inhibition of mRNA translation. Recent studies have shown that miRNAs are important for cardiac hypertrophy1 and heart failure2. However, to date, the regulation and roles of miRNAs in the diabetic human heart are still unknown.

METHODS AND RESULTS
To investigate the role(s) of microRNAs in the insulin resistant heart, we collected cardiac MRI and positron emission tomography guided left ventricular biopsies from patients with normal ventricular function with or without type II diabetes (T2DM) (n= 6 and n=7 respectively); and patients with heart failure (HF, n=6), who are insulin resistant at the myocardial level. Small RNA species were extracted (mirVana miRNA Isolation kit, Ambion). Using a TaqMan based real-time PCR method (Applied Biosystems) we determined the quantitative expression levels of 155 mature miRNAs in the bioptic samples. We identified 14 differentially regulated miRNA species (fold change > ± 2 fold, nominal P value < 0.05) between patients with T2DM as compared to the control group.

We then performed qRT-PCR for the set of differentially expressed miRNAs in the diabetic heart using samples of the HF patients. We found that a single microRNA, miRNA-223, was up-regulated in both diabetes and heart failure (fold change ≥ 2 for both, Figure 1). To determine the function of miR-223, we used adenovirus-mediated
overexpression of miR-223 in primary cultures of neonatal rat ventricular cardiac myocytes (NRVMs), and measured the uptake of tritium-labelled 2-deoxyglucose. After 48 hours of overexpression, there was a ~25% increase in glucose uptake in cells overexpressed with miR-223 (Figure 2) as compared to those expressing a control virus. Immunoblotting of samples revealed upregulation of glucose transporter 4 (GLUT4, the major insulin sensitive GLUT in the heart) by miR-223 (Figure 3).

**DISCUSSION**

Collectively, these data describe the expression of microRNAs in the diabetic human heart and show that miR-223 overexpression increases GLUT4 protein expression and glucose uptake. The direct target(s) of miRNA-223 remain to be identified, but it is likely to regulate GLUT4 through post-translational processing as GLUT4 mRNA levels are unaffected by miR-223. Our study provides new insights into the biology of the diabetic human heart and may have implications for the treatment of myocardial insulin resistance.

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**REFERENCES**