Influence of Metformin and Insulin on Myocardial Substrate Selection by 13C MRS

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PURPOSE: Diabetes mellitus is a major cause of cardiovascular morbidity and mortality. Treatment of Type 2 diabetes may involve dietary modifications, oral agents and insulin. Insulin sensitizing agents, such as the biguanide metformin, appear to improve outcomes in diabetic patients with coronary artery disease and heart failure [1,2]. Metformin may also improve cardiac morbidity in patients without diabetes mellitus, a finding supported by experimental models of myocardial infarction or ischemia [3,4]. These effects appear to be unrelated to improved glycemic control and seem to involve alterations in myocardial metabolism. The effects of metformin on myocardial substrate selection under these conditions have not previously been evaluated but may explain improved cardiovascular outcomes in these patients. We hypothesize that metformin treatment influences myocardial substrate selection by reducing fatty acid oxidation and that these alterations in myocardial metabolism may explain improved outcomes in this patient population. We evaluated this hypothesis in a small animal isolated heart perfusion model.

METHODS: Groups of male Sprague-Dawley rat hearts (n=4 per group) were equilibrated with a Langendorff apparatus by perfusion with Krebs-Heinseleit buffer containing physiologic concentrations of unlabeled fatty acids (.35mM), acetoacetate (.17mM), lactate (1.2mM), pyruvate (.12mM) and glucose (5.5mM) and either no additives (Control), 500mM metformin (Metformin), 10 units/L insulin (Insulin), or both insulin and metformin (Metformin + Insulin). After stabilization, hearts were perfused for an additional 30 minutes with buffer containing the same additives and Carbon-13 (^{13}C) labelled substrates (U-^{13}C fatty acids, 1,3-^{13}C acetoacetate, 3-^{13}C lactate, 3-^{13}C pyruvate and unlabeled glucose) to enable determination of relative substrate oxidation. Additional experiments were performed with U-^{13}C labelled glucose and unlabeled fatty acids to allow for accurate quantification of glucose and endogenous substrate oxidation.

Developed pressure, heart rate, coronary flow, inflow and outflow pO_2 were measured. MVO_2 was calculated from coronary flow and pO_2 data. After the final 30 minutes of perfusion, hearts were removed and freeze clamped in liquid nitrogen. Cardiac tissue was extracted with perchloric acid and lyophilized. Extracts were reconstituted in D_2O and high-resolution proton decoupled ^{13}C NMR spectra were obtained on a 14.1T Varian Inova spectrometer. Fractional substrate oxidation was determined by glutamate isotopomer analysis under non-steady-state conditions [5]. Groups were compared by one-way analysis of variance. Differences between groups, when present, were determined by Tukey's test for multiple comparisons.

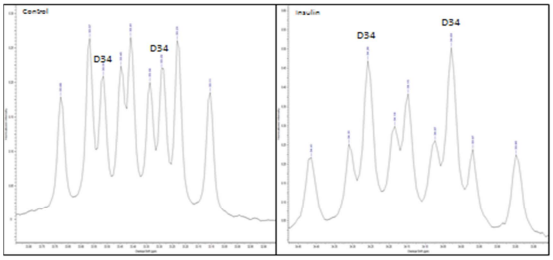
RESULTS: No major functional differences were noted among groups. Fractional substrate utilization is illustrated in **Table 1**. Fatty acid oxidation appeared reduced in the Metformin+Insulin group but this difference did not reach statistical significance. Fatty acid oxidation was otherwise similar in all other groups.

Table 1 – Fractional Substrate Contribution to Oxidative Metabolism

Group	Ketones	Lactate/Pyruvate	Fatty Acids	Glucose	Endogenous Substrates
Control	0.26±0.07	0.14±0.07	0.44±0.06	0.004±0.004	0.16±0.05
Metformin	0.25±0.02	0.13±0.03	0.43±0.05	0.03±0.04	0.16±0.02
Insulin	0.01±0.01*	0.42±0.06*	0.44±0.03	Not detected	0.13±0.02
Metformin+ Insulin	0.31±0.01	0.17±0.002	0.37±0.01	Not detected	0.15±0.03

***-p<.05 vs all other groups**

Figure 1 – Sample Glutamate C4 Multiplets



Note differences in C4 D34 doublets in insulin treated animals compared to control animals, indicative of increased lactate/pyruvate oxidation

Glucose oxidation was minimal, independent of the presence of metformin, insulin or both in the perfusate. The contribution of the unlabeled fraction to oxidative metabolism was almost completely due to oxidation of endogenous substrates. The addition of insulin completely eliminated myocardial ketone oxidation and resulted in a corresponding increase in the fractional utilization of lactate/pyruvate. See **Figure 1** for sample spectra. Adding metformin to insulin completely reversed this effect.

DISCUSSION: Consistent with prior reports, fatty acids are the preferred myocardial substrate and the fractional utilization of fatty acids appears similar when insulin, metformin, or both are added to the perfusate. The presence of insulin alone alters myocardial substrate selection resulting in elimination of ketone oxidation and a corresponding increase in lactate/pyruvate oxidation by the heart. The addition of metformin to insulin containing buffer yields an oxidation profile similar to control hearts.

CONCLUSION: Metformin alone does not appear to affect myocardial substrate preferences at the concentrations and conditions evaluated in this study. It, however, appears to reverse the inhibition of ketone oxidation noted in insulin treated hearts by an unknown mechanism. Further studies investigating this effect and its clinical impact on cardiovasular outcomes are warranted.

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