Chronic Diabetes Reprograms Carbohydrate Metabolism in the Heart and Kidney: A Hyperpolarised 13C Magnetic Resonance Spectroscopy Study

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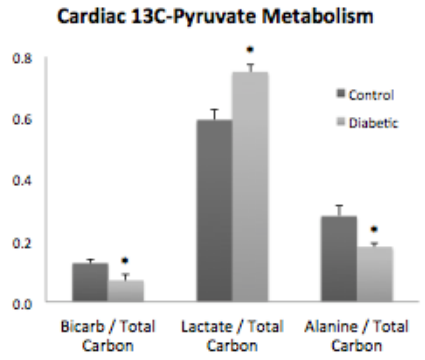
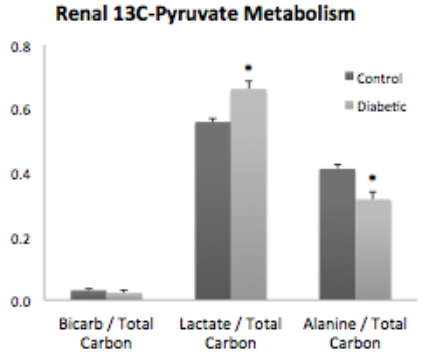
Target Audience: Clinicians and basic scientists with an interest in diabetes, heart failure, and metabolic imaging/spectroscopy.

Purpose: Diabetic cardiomyopathy is defined as the alteration of cardiac structure and function induced independently by diabetes mellitus in the absence of ischemic heart disease, hypertension, or other cardiac pathologies (1). Furthermore, diabetes is a common comorbidity in patients presenting with heart failure with preserved ejection fraction (HFpEF), and is known to be associated with the progressive left ventricular (LV) remodelling and dysfunction characteristically observed in HFpEF. Currently, HFpEF is observed in 50% of all patients with heart failure, and incidence is expected to rise (relative to heart failure with reduced ejection fraction, HFrEF) without improvements in prognosis because of a lack of specific HFpEF therapy (2). The purpose of this study was to clarify the cardiac phenotype that develops in chronically diabetic rats, with a focus on the well-characterised Goto-Kakizaki (GK) rat model. GK rats, generated via selective breeding, demonstrate reduced β-cell mass and mild hyperglycaemia after weaning (3). We tested the hypothesis that chronic diabetes would lead to GK rats displaying clinical signs and symptoms of HFpEF. Furthermore, by following hyperpolarised [1-^{13}C]pyruvate metabolism in the rat heart and kidneys simultaneously using magnetic resonance spectroscopy (MRS), we aimed to define how chronic diabetes / HFpEF reprogrammed whole-body carbohydrate utilisation.

Methods: Forty week old male Wistar and age, sex matched GK rats (*n=9* in each group) were studied. Animals underwent echocardiography at 8 weeks of age, and subsequently every 4 weeks. At 20 and 40 weeks of age, all animals also underwent metabolic caging and glycated hemoglobin (HbA1c) assessment for determination of diabetes status. MRS study of the heart and kidneys following intravenous hyperpolarised [1-^{13}C]pyruvate infusion (described below) was performed in one cohort of the animal pairs at the age of 40 weeks (*n=4)*. The second cohort of paired animals (*n=5*) underwent invasive cardiac catheterization for pressure-volume (PV) loop analysis. Cardiac and renal tissue was collected at 40 weeks from all animals, for histopathological and molecular analysis.
In vivo *MRS experiments:* Spectroscopy experiments with hyperpolarized [1-^{13}C]pyruvate (4) were performed in Wistar and matched GK rats using a 3 T MR750 scanner (GE Healthcare) and a micro-strip dual-tuned 1H-13C volume coil (8 cm ID). Sagittal gradient echo ^{1}H imaging was performed in each study for localization. Time-resolved ^{13}C MR spectroscopic data were acquired from rat hearts and kidneys at alternating time points, using an interleaved pulse-acquire pulse sequence (1.2 cm axial slice through alternately the heart or the kidneys, 20° nominal tip angle, TR□=□1 s, 5000 Hz / 2048 pts. readout, 128 spectra acquired). Data acquisition was started at the beginning of the □10 s [1-^{13}C]pyruvate infusion. All spectroscopic data were processed using the SAGETM software package (GE Healthcare). A 5 Hz Gaussian apodization was applied to the time domain data before Fourier transformation. Phasing and baseline correction were performed on the data in the spectral domain. Metabolite production was calculated using summed peak integrals of the metabolites from the first 100 spectra of each dataset, and the total carbon metabolism from each condition/organ was calculated as the sum of measured lactate, bicarbonate, and alanine production. Statistical significance was considered for P<0.05.

Results: GK rats developed diabetes by 20 weeks of age, as evidenced by significantly elevated HbA1c. At 40 weeks, GK rats had increased urinary protein excretion, LV hypertrophy, and pulmonary congestion compared with Wistar rats. PV loop assessment demonstrated impaired diastolic function but preserved systolic function. Cardiac and renal histological analysis demonstrated cardiomyocyte and glomerular hypertrophy, along with interstitial fibrosis and glomerulosclerosis.
Following infusion of hyperpolarized [1-^{13}C]pyruvate in 40 week old rats, MR spectra acquired from the cardiac slice demonstrated a 26% increase in the proportion of [1-^{13}C]pyruvate converted to [1-^{13}C]lactate (represented by the lactate/total carbon ratio). The shift towards cardiac lactate production occurred at the expense of ^{13}C-bicarbonate production (decreased by 45%) and alanine production (decreased by 36%). Hyperpolarised [1-^{13}C]pyruvate MRS acquired from the kidneys demonstrated that there was also a renal shift towards lactate production, which increased by 19%, and away from alanine production, which was reduced by 23%.





Discussion: The GK rat is a powerful model of non-obese human diabetes because of marked similarities in metabolic and inflammatory features, as well as β-cell and kidney dysfunction. Here, we show that the cardiac phenotype of the 40 week old GK rat mimics the human phenotype of heart failure, specifically HFpEF. As is the case in HFpEF patients, the GK rats had preserved systolic function (measured by echocardiography and PV loop) but showed clinical signs and symptoms of heart failure, including diastolic dysfunction, LV hypertrophy, fibrosis, pulmonary congestion, and co-morbidities including nephropathy and the metabolic risk factors inherent to diabetes.
Hyperpolarised ^{13}C MRS, applied to the heart and kidneys, revealed systemic reprogramming of carbohydrate metabolism in GK rats. In both organs, [1-^{13}C]pyruvate was channelled towards utilisation by lactate dehydrogenase (LDH) to form lactate, and shifted away from decarboxylation by pyruvate dehydrogenase (PDH) and amino acid production via alanine aminotransferase (AAT). This metabolic pattern suggests two things: firstly, that increased LDH activity and the Cori cycle may be facilitating a maladaptive increase in hepatic gluconeogenesis, and secondly, that inflammation (5) may be a culprit in the observed cardiac and renal dysfunction. Future investigations of gluconeogenesis and inflammation in the GK rat model, and whether these metabolic shifts are also features of HFpEF, may identify new targets for therapy for patients with diabetes and/or HFpEF.

Conclusions: Chronic diabetes in the GK rat is sufficient to cause heart failure, and offers a promising new disease model for heart failure with preserved ejection fraction (HFpEF). Hyperpolarised ^{13}C MRS has revealed a shift towards lactate production in the heart and kidneys of GK rats with failing hearts, which may indicate increased whole-body gluconeogenesis and cardiac and renal inflammation. Further study of the GK rat as a model of HFpEF will be important in developing the first treatments for HFpEF; this work suggests that targeting diabetic symptoms, including maladaptive metabolic reprogramming, may be an effective therapeutic strategy.

References:

1. Boudina and Abel. Circulation, 2007.
2. Zile *et al.* Circulation, 20011.
3. Akash *et al.*. Curr Diabetes Rev, 2013.
4. Chen *et al,*. MRM 2014.
5. MacKenzie *et al*, Radiology, 2011.

Acknowledgements - This study was supported by the Wellcome Trust, the Canadian Institute of Health Research, the Heart and Stroke Foundation of Canada, and GE Healthcare.