Investigating the Effects of Hyperthyroidism on Cardiac Metabolism Using Hyperpolarized Magnetic Resonance

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Introduction

Hyperthyroidism, caused by an increase in the thyroid hormone (T3), results in cardiac hypertrophy with a concomitant increase in heart rate, myocardial contractility and cardiac output. Basal metabolic rate is elevated and cardiac fatty acid catabolism is increased. Thyroid hormone is a transcription factor which regulates aspects of growth, development and energy metabolism in multiple organs, including the heart. Studies using both freshly prepared cardiac myocytes and isolated mitochondrial preparations have reported an increase in pyruvate dehydrogenase kinase (PDK) activity in response to elevated T3 concentration [1,2]. However, to date, it has not been possible to elucidate how the increase in PDK activity affects pyruvate dehydrogenase (PDH) flux *in vivo*, nor whether PDH inhibition contributes to the pathology of the hyperthyroid heart. The development of hyperpolarization enhanced magnetic resonance spectroscopy (MRS) has enabled the visualisation of real time metabolite flux through specific enzymes *in vivo* [3]. Using [1-^{13}C]pyruvate it is possible to rapidly and non-invasively obtain a measure of PDH flux by quantifying the [1-^{13}C]bicarbonate signal. In this study a combined *in vivo* hyperpolarized ^{13}C MRS and *ex vivo* ^{1}H-nuclear magnetic resonance (NMR) spectroscopy approach has been used to investigate the effect of hyperthyroidism on cardiac metabolism.

Methods

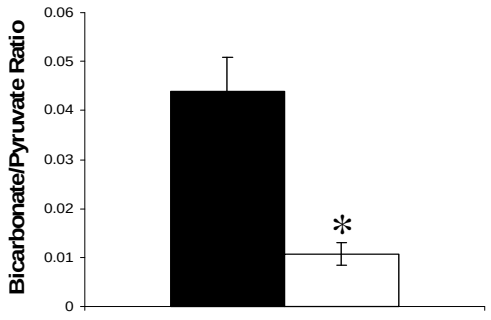
Twelve male Wistar rats were examined at baseline using hyperpolarized ^{13}C MRS. Rats were then injected (i.p) with either saline (0.9%) or T3 (0.2 mg/kg body weight/day) for 7 days after which time rats were examined again by the hyperpolarized MR protocol. Rats were sacrificed after 24 hrs and hearts removed for metabolite profiling. Saphenous blood was sampled at days 0 and 7 for biochemical analysis.

MRS: [1-^{13}C]pyruvate was hyperpolarized and dissolved as previously described [4]. One millilitre of 80 mM hyperpolarized [1-^{13}C]pyruvate solution was injected over 10 s via a tail vein catheter into an anaesthetised rat positioned in a 7 T MR scanner. Spectra were acquired for 1 min following injection with 1 s temporal resolution, and signal was localised to the heart via the use of a surface coil. Spectra were quantified and maximum [1-^{13}C]bicarbonate/[1-^{13}C]pyruvate was used as a direct measure of PDH flux.

Metabolite profiling: Metabolites were extracted using a methanol: chloroform: water protocol as previously described [5]. Metabolites were reconstituted in 600 µl D_2O and analysed using a 500 MHz NMR spectrometer (ns = 128, T = 37 °C). Spectra were integrated into 0.04 ppm 'buckets' and normalised to total integral area. Each integral region was independently analysed using a Student's t-test.

Results

Using hyperpolarized ^{13}C MRS, 7 days of T3 administration was observed to cause a 76 % decrease in PDH flux *in vivo* (Fig. 1). This was accompanied by a 4-fold increase in circulating non-esterified fatty acids, as well as a 40 % increase in β-hydroxybutyrate (BHB), and a 25 % decrease in glucose concentrations ($p < 0.05$). *Ex vivo* ^{1}H-NMR spectroscopy of cardiac tissue extracts identified a number of additional significant metabolic changes consistent with perturbations in glycolysis, Krebs cycle metabolism and energy homeostasis (Table 1).



Metabolite	Representative integral region	Percentage metabolite concentration relative to control values (%)	P-value
Taurine	δ 3.26-3.30	118 ± 9	0.02
Lactate	δ 1.30-1.34	140 ± 10	0.04
Alanine	δ 1.46-1.50	160 ± 20	< 0.01
Glutamate	δ 2.38-2.42	130 ± 20	0.03
Malate	δ 2.66-2.70	130 ± 20	0.06
BHB	δ 1.18-1.22	140 ± 20	0.02
Creatine	δ 3.02-3.06	64 ± 3	< 0.01
Glutamine	δ 2.46-2.50	75 ± 5	< 0.01
Glucose	δ 5.22-5.26	73 ± 7	< 0.01
Glycogen	δ 5.38-5.42	20 ± 10	< 0.01

Fig.1: [1-^{13}C]bicarbonate/[1-^{13}C]pyruvate, used as a measure of PDH flux, at baseline (black) and after 7 days of T3 administration (white). ($p = 0.03$).
Table 1: Summary of significant metabolite differences in cardiac tissue extracts analysed using ^{1}H-NMR spectroscopy.

Discussion

Using hyperpolarized ^{13}C MRS it is now possible to visualise real time metabolism *in vivo*. In this study, 7 days administration of T3 was seen to reduce cardiac PDH flux by 76 %. Thus, hyperpolarized [1-^{13}C]pyruvate has suggested that PDH inhibition may contribute to the pathology of the hyperthyroid heart. Using *ex vivo* methods this decrease in PDH flux was seen to be associated with an increase in glutamate and malate indicating increased Krebs cycle flux. This is likely to be caused by an increase in acetyl CoA concentration secondary to enhanced fatty acid oxidation, a well documented effect of elevated T3 levels. Increased acetyl CoA would also account for the observed decrease in PDH flux as it can both allosterically inhibit PDH and activate PDK. Other alterations in the hyperthyroid heart include an increase in glycolysis, evidenced by increased alanine and lactate, as well as a decrease in total creatine and an increase in BHB which provide evidence of perturbed energy homeostasis. Taken together these findings suggest that the hyperthyroid heart can be characterised by an increase in fatty acid catabolism and glycolysis, as well as a marked decrease in PDH flux.

References

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