Effect of initial hepatic glycogen concentration and resulting plasma glucose concentration on the route of glycogen synthesis in vivo in Wistar rats: a 13C NMR Study

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INTRODUCTION

Several studies have been performed in which the use of a metabolic tracer provides information on the pathway of glycogen synthesis; and the effect of the physiological state on the relative contributions from the different pathways has been investigated[1-5]. The aim of this study was to use non-invasive *in vivo* ^{13}C MRS to investigate the effect of the initial hepatic glycogen concentration and the plasma glucose concentration on the relative contributions of the direct and indirect pathways to glycogen synthesis following an infusion of [1-^{13}C]glucose in Wistar rats. We used a combination of two different fasting periods to vary the basal hepatic glycogen and two glucose infusion rates to vary the glucose uptake. Characterisation of the synthetic route of glycogen deposition will help in understanding the mechanism of action of anti-diabetic drugs.

METHODS

Studies were performed on male Han Wistar rats (232±23g), following a fast of either 8 hours (group 1, n=5) or 20 hours (group 2, n=2; & group 3, n=5). Isofluorane anaesthesia was maintained throughout the experiment and the animals' temperature and breathing rate were monitored throughout. A bolus of either 0.5g/kg (groups 1&2) or 1g/kg (group 3) of 1-^{13}C glucose was injected into the anaesthetised animal via a tail vein catheter, followed by a continuous 3 hour infusion of either 20 (groups 1&2) or 40 (group 3) mg/kg/min.

All MR measurements were performed using a Varian Inova 9.4T horizontal bore magnet and a concentric $^{1}H/^{13}C$ surface coil. The animal was placed supine in the cradle and correct positioning of the coil was verified by acquisition of axial gradient echo images. ^{13}C NMR spectra (np=1000, sw=20000Hz, tr=1sec), with WALTZ decoupling applied at the proton resonance frequency during acquisition, were acquired in blocks of 600 averages. A baseline spectrum was acquired before administration of the glucose, and continuously every 10 minutes thereafter.

At the end of the experiment, animals were euthanised. The liver was removed and frozen in liquid N_2 for subsequent measurement of glycogen content by assay[6], to allow quantitation of the NMR signal. Blood samples were taken before and after induction of anaesthesia, and at the end of the experiment, for determination of blood glucose and plasma insulin concentrations.

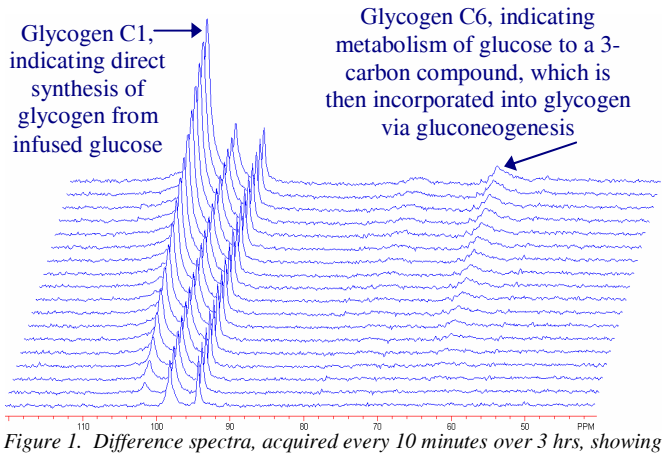

Figure 1. Difference spectra, acquired every 10 minutes over 3 hrs, showing the pattern of label incorporation into liver glycogen in a subject from gp 3.

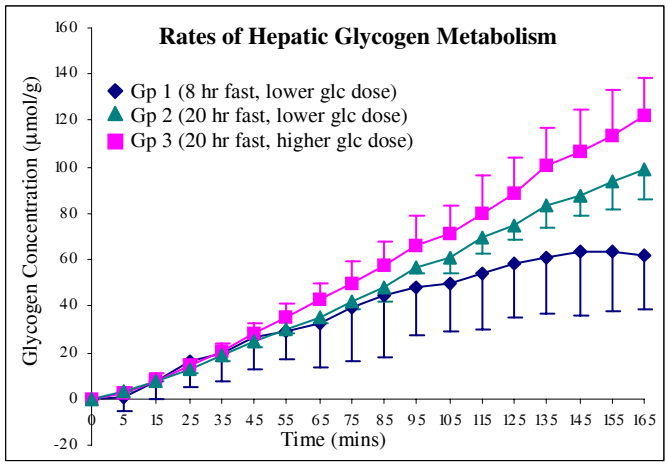

Figure 2. Rate of label incorporation into glycogen C1.

RESULTS

Figure 1 shows the spectral time course of label incorporation into both the C1 and C6 positions of liver glycogen. Figure 2 shows the rate of label incorporation into the C1 position of liver glycogen in the different groups. Table 1 lists the rates of label incorporation into both the C1 and C6 positions of liver glycogen calculated over the whole time course of the experiment, and the ratios of these rates in the three groups.

	Rate of label incorporation into glycogen C1 (µmol/g/min)	Rate of label incorporation into glycogen C6 (µmol/g/min)	Ratio of C6:C1 label incorporation
Group 1	0.4 ± 0.2	0.16 ± 0.08	0.4 ± 0.1
Group 2	0.59 ± 0.08	0.05 ± 0.03	0.09 ± 0.06*
Group 3	0.7 ± 0.1*	0.09 ± 0.02	0.12 ± 0.03*

*Table I. Rate of label incorporation into both the C1 and C6 positions of liver glycogen. Data are means ±SD. * p<0.05 compared to group 1*

DISCUSSION

The relative contribution to the indirect pathway for hepatic glycogen synthesis was found to be increased when the initial basal hepatic glycogen would be expected to be higher. This was despite the fact no significant effect on the absolute rate of label incorporation into either the C1 or C6 positions of hepatic glycogen was observed. The animals that had been fasted for longer, and hence had a lower starting glycogen concentration, showed a decreased contribution from the indirect pathway, although this does not take into account endogenous sources of carbohydrate. It is possible that the direct pathway dominates until a required certain threshold of glycogen is reached. This observation appears to be independent of the glucose load, despite a notably higher plasma glucose concentration in the rats that were given the higher glucose dose (20±5 vs. 12±2 mmol/l). This is in contrast to observations from previous studies[1,3,4], however those studies were performed in a different strain of rat using sufficiently different experimental protocols. It is possible that the rate of glucose deposition is limited by the uptake rate following the higher glucose dose. To challenge this hypothesis, preliminary studies will be performed in which the rate of glucose uptake is increased pharmacologically by use of a glucokinase activator[7-9].

REFERENCES

1. Newgard et al, J. Biol Chem, 259, pp. 6958-6963, 1984. 2. Siegfried et al, J. Biol Chem, 260, pp. 16137-16142, 1985. 3. Shulman et al, J. Clin. Invest, 76, pp. 1229-1236. 4. Shulman et al, Am. J. Physiol, 263, pp. E731-E735, 1991. 5. Bergans et al, NMR in Biomed, 16, pp. 36-46, 2003. 6. Keppler & Decker in Methods of Enzyme Analysis, pp. 1127-1131 (1974). 7. Grimsby et al, Science, 301, pp. 370-373, 2003. 8. Leighton et al, Biochem Soc Trans, 33, pp. 371-374, 2005. 9. Coope et al, Brit J. Pharmacol, 149, pp. 328-335, 2006.