Assessment of hepatic glycogen turnover in mice by *in vivo* ^{13}C-MRS

Andreas Boss[1], Andor Veltien[1], and Arend Heerschap[1]
[1]*Radiology and Nuclear Medicine, Radboudumc, Nijmegen, Gelderland, Netherlands*

Introduction and Aim: One of the important roles of the liver is the regulation of blood glucose levels. While in the postprandial state, excess carbohydrates are stored as glycogen, glucose is released from liver glycogen in the postabsorptive state. Studies using ^{13}C-MRS have, however, contributed to the notion that even during periods of net glycogen synthesis, a substantial amount of glycogen is degraded simultaneously (1,2). This was concluded from pulse-chase experiments in which the ^{13}C-glycogen signal rose during the infusion of [1-^{13}C]-labeled glucose, and subsequently decreased upon switching to unlabeled glucose. These experiments were performed in both, humans (1) and rats (2), but to our knowledge glycogen turnover has not been assessed in mouse livers using *in vivo* ^{13}C-MRS. Next to glucose we evaluated galactose as tracer substance. This hexose is part of the dissacharide lactose found in milk, and is, in contrast to glucose, mainly metabolized in the liver. Furthermore, during the course of its hepatic metabolism UDP-glucose is produced, which is a direct precursor in the synthesis of glycogen. It can thus be speculated that turnover measurements can be achieved with lower amounts of tracer when galactose is used as a substrate. **Aim:** To test the feasibility of glycogen turnover assessment by *in vivo* ^{13}C-MRS in the liver of mice with glucose and galactose as tracer substrates.

Methods: Experiments were performed in 5 male, overnight-fasted **C57Bl6j mice** (weight: 22.6g ±2.0g [SD]). At 8 .a.m. mice were anesthetized using Isoflurane and subsequently a catheter was inserted in the jugular vein. **Infusion protocol:** [1-^{13}C]-labeled galactose or glucose was infused at a constant rate (1.2 and 2.4 µmol/min for galactose and glucose respectively) for a duration of 120min. Thereafter, the respective unlabeled substrate was infused for an additional 120 min. **MR acquisition:** The experiments were performed on a preclinical 11.7T MR scanner (Bruker BioSpin, Ettlingen). Mice were anesthetized with 1-2% Isoflurane in a gas mixture (O_2 to air ratio: 1 : 2) Respiration was monitored and kept constant at 60-100 breaths/min, temperature was maintained at 37°. A homebuilt ^{13}C surface coil (17x20 mm diameter) shaped and optimized for the liver was used for acquisition of ^{13}C-MR spectra, while an ^{1}H Alderman-Grant coil (43 mm diameter, 60 mm length) was used for imaging and ^{1}H-decoupling. First, the correct placement of the mouse liver on the ^{13}C surface coil was ensured (with help of a tube filled with a [1-^{13}C]-labeled acetate solution, surrounding the surface coil) and the mouse was repositioned if necessary. **^{13}C-MRS:** A simple pulse-acquire scheme was applied; pulse calibration was performed on the tube with [1-^{13}C]-labeled acetate surrounding the coil, using a block pulse. The pulse-chase experiments were then performed using a 180° pulse at the surface of the coil to minimize glycogen-signal contamination from the abdominal muscles close to the coil, while still reaching a ~90° pulse inside the liver. Further parameters were as follows: block pulse for excitation (duration: 43µs, center frequency on glycogen), ^{1}H-decoupling during acquisition (4k data points, spectral BW: 39000Hz), TR: 1260ms (3 times the estimated T1 of glycogen at 11.7T, extrapolated from (3)), 4 dummy scans, 471 averages, corresponding to a time resolution of 10 min per spectrum. **Post-processing:** MR spectra were post-processed using jMRUI. AMARES with prior knowledge was used for quantification. A two-step approach was used for fitting: For each experiment, line widths of the glycogen- and alpha-hexose peak were restricted to values found in spectra obtained at the end of the chase-period with high SNR. The decay of the alpha-hexose signals during ^{12}C-infusion was fitted with a monoexponential function: $A(t)=A_0*e^{-t/tau}+b$. **Estimation of turnover during galactose infusion:** Turnover was estimated for galactose based on differential equations, solved recursively with a finite time step using the solver function of Excel (c.f. **Fig.3A** for details).

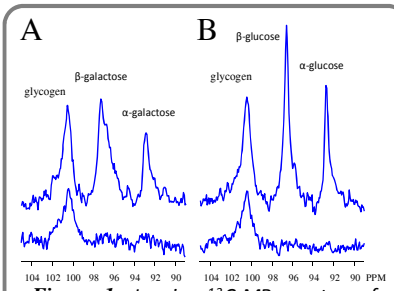

Figure 1: *In vivo ^{13}C-MR spectra of mice liver. **A:** Galactose-infusion; **B:** Glucose-infusion. The top spectra were obtained at the end of the 120min of ^{13}C-labeled substrate infusion. The bottom spectra were obtained at the end of the experiment.*

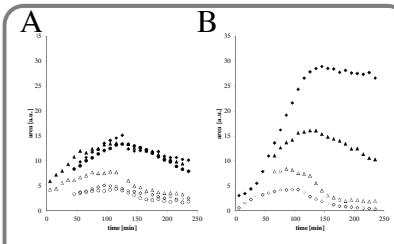

Figure 2: *The kinetics of glycogen and alpha-hexose signals .**A:**galactose-infusion (n=3), **B:**glucose-infusion (n=2). ^{13}C-labeled substrates were infused for 120min, followed by infusion of unlabeled substrates for the next 120min. Filled symbols: glycogen, corresponding open symbols: alpha-hexose.*

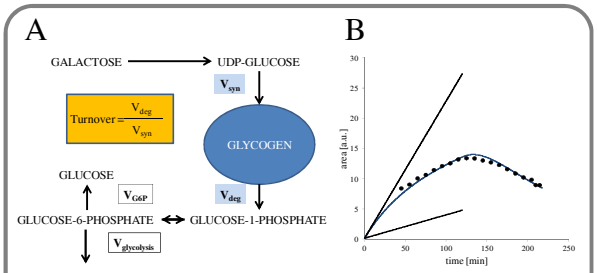

Figure 3A: *Metabolic model used to fit the time course of the glycogen curve during the galactose infusion. The rates of glycogen synthesis (V_{syn}) and degradation (V_{deg}) were assumed to be constant throughout the experiment. ^{13}C-enrichment of galactose as input-function for the model was assumed to be 100% during ^{13}C-infusion, and to be decreasing with the same time constant as the ^{13}C-galactose signals. Enrichment of UDP-glucose was estimated to be 30% lower than that of galactose, accounting for dilution by gluconeogenesis (2). Fitted parameters include V_{syn}, V_{deg} and initial glycogen concentration. Turnover is the ratio of V_{deg} over V_{syn}. The model further assumes that true cycling (glycogen→ glucose-1-phosphate → UDP-glucose → glycogen) is negligible. i.e. most of the glucose-1-phosphate derived from glycogen is converted to glucose-6-phosphate and from there either released as glucose (V_{G6P}) or undergoing glycolysis ($V_{glycolysis}$). V_{deg} is therefore the sum of net V_{G6P} and $V_{glycolysis}$.*
B: *Result of the fit (blue line) to the data points in one experiment with galactose infusion. The effect of initial glycogen levels is further illustrated: if they were very big, the signal would increase with V_{syn} (upper line), if initial glycogen was very low, the ^{13}C-glycogen signal is expected to increase with $V_{net}=V_{syn}-V_{deg}$ (lower line). Thus, in most cases, the apparent ^{13}C-signal increase will remain somewhere in between.*

Results/Discussion: ^{13}C-liver spectra obtained at the end of the 120 min infusion of ^{13}C-labeled substrates and corresponding spectra at the end of the experiment show signals reflecting the uptake and clearance of labeled galactose and glucose (**Fig.1**). The kinetics of glycogen and alpha-hexose ^{13}C labeling are shown in **Fig.2**. During the chase-period, the intensity of the alpha-hexose decays with a similar time constant for galactose (tau=28, 33, 88 min) and glucose (tau=22, 40 min), despite the fact that glucose was infused at double the concentration. Hepatic turnover was estimated to be 81, 83, and 87% for the galactose-infusion experiments (**Fig.3B**). A higher number of experiments is, however, needed to determine if the turnover of glycogen depends on the substrate infused. Furthermore, for a better kinetic modeling, blood enrichments of glucose and galactose need to be obtained.

Conclusion: We conclude that the assessment of glycogen turnover by ^{13}C-MRS is feasible in the livers of mice and can be used to study (transgenic) mouse models of diabetes or obesity.

References:

1. David, *The Journal of clinical investigation* 86, 612 (1990).
2. Magnusson, *The American journal of physiology* 266, E796 (1994)
3. Zang, *Biochemistry* 29, 6815 (1990)