Alterations on hepatic glycogen and lipid metabolism following the induction of diabetes in the rat

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Introduction: The liver plays a key role in controlling glucose homeostasis since it is responsible for both glucose production during fasting, and storage of both carbohydrate and lipid during the fed state. After a meal, the two major hepatic anabolic processes that are potentiated by insulin are glycogen synthesis and *de novo* lipogenesis (Figure 1). Insulin regulates the activity of glycogen synthase and ATP-citrate lyase through changes in the phosphorylation state; promotes the expression of genes encoding for glycolytic and lipogenic enzymes; and inhibits the expression of genes encoding for gluconeogenic enzymes [1]. In type 1 Diabetes insulin levels are highly reduced thus compromising hepatic glucose storage as glycogen and disrupting triglycerides (TG) metabolism. We developed a method for simultaneously assessing hepatic glycogen synthesis and *de novo* lipogenesis during the natural nocturnal feeding cycle of rats. Animals were administered deuterated water (^2H_2O) to assess the pathways to hepatic glycogen synthesis and *de novo* lipogenesis. This protocol was applied to healthy Wistar rats and streptozotocin (STZ)-induced diabetic rats at two stages of the disease.

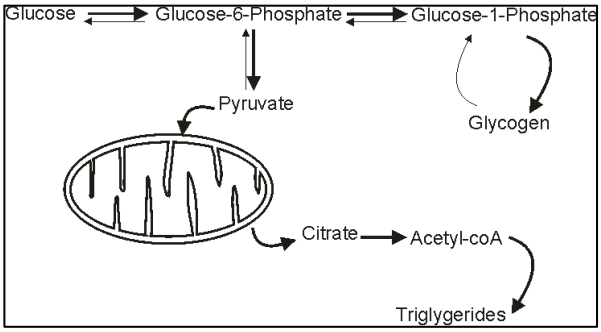

Figure 1. Schematic representation of the anabolic fates of glucose in the hepatocyte. Bold arrows indicate pathways activated by insulin.

Methods: Male Wistar rats weighing 250 ± 5 grams were subjected to a 12 hour light/12 hour dark cycle (lights on from 7 am to 7 pm) with free access to a standard chow diet. An intraperitoneal injection of STZ (65 mg/kg) dissolved in 10mM citrate buffer pH 4.5 was administered to induce diabetes. On experiment day at 7 pm all animals, STZ and control, received a loading dose of 99 % ^2H_2O in saline by injection into the intraperitoneal cavity, equivalent to 2% of body water. To maintain body water enrichment throughout the experiment, their drinking water was enriched to 5 % with ^2H_2O. At 8 am the next morning, animals were sacrificed, blood was collected to determine body water ^2H enrichment and the liver was excised and immediately freeze-clamped in liquid nitrogen. In two thirds of the liver, glycogen was extracted by KOH-ethanol treatment, enzymatically hydrolysed to glucose by amyloglucosidase incubation and converted to MAG by acetonation [2]. ^2H-NMR analysis of this derivative was performed; percent indirect pathway contribution to glycogen was calculated as the ^2H-enrichment in position 5 relative to the enrichment in position 2 ($^2H5/^2H2$) x 100 and direct pathway as 100 - % indirect. The remaider third of the liver was subjected to Folch extraction to recover the lipid content, which was also analyzed by ^2H-NMR. An external pyrazine standard was used to calculate the absolute ^2H-enrichment in the TG-methyl groups. The fractional contribution of *de novo* lipogenesis to hepatic TG was estimated as that enrichment relative to the ^2H-enrichment of the body water [3].

Results and Discussion: Figure 2 shows representative ^2H-NMR spectra for MAG glycogen obtained from the liver of healthy (A) and STZ-treated (B) rats. In the healthy group of animals, during the overnight feeding, the direct and indirect pathway contributions to hepatic glycogen were approximately equal (46 ± 4% and 54 ± 4%, respectively). Four days after induction of diabetes, the indirect pathway contribution was significantly increased and dominated glycogen synthesis (68 ± 4 %, $P < 0.01$ vs. control). Twenty days post induction, virtually all hepatic glycogen was synthesized via the indirect pathway (95 ± 3%, $P < 0.005$ vs. control). This observation suggests a progressive loss of direct pathway capacity, which is more dependent on insulin compared to the indirect pathway. The insulin deficiency induced by STZ-treatment also resulted in a significantly reduced contribution of *de novo* lipogenesis to hepatic triglyceride from 16 ± 2 % (healthy animals) to 7 ± 2 % at day 4 and 2.0 ± 0.2 % at day 20 following STZ administration.

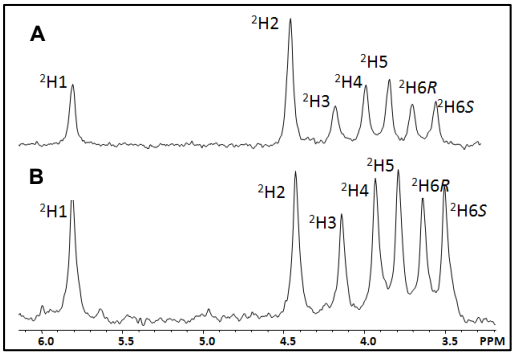

Figure 2. Inset of ^2H-NMR spectra of MAG glycogen samples obtained from the liver of a healthy (A) and a STZ-treated (B) rat, ^2H resonances from positions 1 to 6*S* are shown.

Conclusions

In this experiment we characterized the metabolic profile of healthy and STZ-treated animals during their natural feeding cycle. Our results demonstrate that the glycogenic and lipogenic pathways are altered for the diabetic animals and that these changes are more pronounced for longer periods following STZ administration, at least in the first weeks post-treatment. These observations may serve as valuable markers for assessing alterations in hepatic glucose and lipid metabolism during the progress of STZ-induced Diabetes.

References:

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3. Delgado, T.C., et al., *Sources of hepatic triglyceride accumulation during high-fat feeding in the healthy rat.* NMR in Biomedicine, 2009. **22**(3): p. 310-317.