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 (2H2O) 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.

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 % 2H2O 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 2H2O. 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 remainder 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.

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 glycolytic 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:

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

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 6S are shown.