Effect of Fasting on $^2$H-Enrichment Levels of Rat Plasma Glucose as measured by $^2$H NMR Spectroscopy Following Infusion of $^2$H$_2$O.

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**Introduction**

Under fasting conditions, the liver is the principal source of systemic glucose. Glucose is produced from the breakdown of hepatic glycogen (glycogenolysis) and by synthesis from non-glucose precursors (gluconeogenesis). The rate of glycogenolysis is proportional to the amount of glycogen stored in the liver and decreases as glycogen levels are depleted during a fast. In cirrhosis, glycogen synthesis during a meal is impaired, hence the contribution of glycogenolysis to postabsorptive hepatic glucose output is low even after a brief fast. Recent studies suggest this change occurs in the early stages of cirrhosis (1,2), hence a measurement of the contribution of glycogenolysis to hepatic glucose output could be a valuable aid in the prompt diagnosis of impaired hepatic glucose metabolism in cirrhosis. The relative contribution of glycogenolysis and gluconeogenesis to hepatic glucose output can be evaluated by analysis of $^2$H-enrichment in positions 2, 5 and 6 of plasma glucose following ingestion of $^2$H$_2$O (3). This inexpensive procedure has been shown to be safe and well tolerated by patients, including those with cirrhosis (1,2). $^2$H$_2$O has an advantage over traditional gluconeogenic carbon tracers in that it is less expensive and can be given orally. Furthermore, following its rapid equilibration with body water, the $^2$H-tracer represents all precursors of glucose and the metabolic flux analysis does not depend on the difficult and error-prone measurement of true precursor enrichment. We recently demonstrated that $^2$H NMR analysis of a monoacetone derivative of plasma glucose provides fully-resolved $^2$H-enrichment information at all positions in a simple and direct manner (4). In this report, we demonstrate that the $^2$H-enrichment levels of glucose are sensitive to the duration of fasting in the rat. $^2$H NMR analysis of relative $^2$H-enrichments at positions 2, 5 and 6 of plasma glucose yielded appropriate relative rates of glycogenolysis and gluconeogenesis at 4-6.5 hours and 24-26.5 hours of fasting.

**Methods**

Twelve Rats weighing 180-220g, after fasting for 4 hours (n=4) or 24 hours (n=8), were anesthetized via ketamine/xylazine and infused with 70% $^2$H$_2$O at a rate of 1 ml/hour over 2.5 hours. Following the infusion, 4 ml of blood was drawn from the carotid artery and the animal was sacrificed. Blood was immediately centrifuged and the plasma was deproteinized with perchloric acid and the protein removed by centrifugation. The supernatant was neutralized with KOH, centrifuged to remove KClO$_4$, and lyophilized to complete dryness. Glucose was converted to diacetone glucose using acetone/H$_2$SO$_4$. Monoacetone glucose was formed by hydrolysis of diacetone glucose in aqueous solution at pH 2.1(4). Monoacetone glucose extracts were dissolved in 150 microliters of 80% acetonitrile-20% water containing a few grains of sodium bicarbonate. $^2$H NMR spectra were gathered at 50°C using a 90° pulse and a 1-second acquisition time. Spectra were obtained with a 3-mm $^2$H-dedicated probe with a $^1$H-decoupling coil (Varian Inova 14.1T spectrometer). Peak areas were measured using the NUTS curve-fitting program. The relative contribution of glycogen to total glucose output was estimated from (1-$^5$H$_2$/H$_2$), the relative contribution of glycerol estimated from (H$_5$-H$_6^5$/H$_2$) and the relative contribution of PEP estimated from (H$_5$-H$_6^5$/H$_2$). For the 4-6.5-hour fasted group, the $^5$H$_2$/H$_2$ enrichment ratio was 0.62 ± 0.08 and the H$_6^5$/H$_2$ ratio was 0.45 ± 0.07. From these data, the relative contribution of glycogen was 38 ± 8%, glycerol, 17 ± 2% and PEP 45 ± 7%. The contribution of glycogen to hepatic glucose output was significantly higher in comparison to the 24-26.5 hour fasted group (p < 0.001) indicating that glycogenolysis was significant after 4-6.5 hours of fasting. The relative contribution of PEP was significantly lower in the 4-6.5 hour compared to 24-26.5 hour fasted group (p < 0.005) while the contribution from glycerol was not significantly different between the 4-6.5 hour and 24-26.5 hour of fasting in rat. These results are consistent with other studies that showed diminished rates of glycogenolysis and glycogen levels after 20-24 hours of fasting in rats (5,6). Our data indicates that the majority of gluconeogenic carbons were derived from PEP with only minor contributions from glycerol. For the 4-6.5-hour fasted group, the $^5$H$_2$/H$_2$ enrichment ratio was 0.62 ± 0.08 and the H$_6^5$/H$_2$ ratio was 0.45 ± 0.07. From this data, the relative contribution of glycogen was 38 ± 8%, glycerol, 17 ± 2% and PEP 45 ± 7%. The contribution of glycogen to hepatic glucose output was significantly higher in comparison to the 24-26.5 hour fasted group (p < 0.001) indicating that glycogenolysis was significant after 4-6.5 hours of fasting. The relative contribution of PEP was significantly lower in the 4-6.5 hour compared to 24-26.5 hour fasted group (p < 0.005) while the contribution from glycerol was not significantly different between the 4-6.5 hour and 24-26.5 hour fasted groups.

**Discussion**

In conclusion, the relative rates of glycogenolysis and gluconeogenesis in the rat, as measured by $^2$H NMR spectroscopy of monoacetone glucose, are significantly altered by physiologically-relevant changes in nutritional state. We anticipate that these measurements will also be sensitive to other interventions or disease models that alter hepatic glycogen metabolism in the rat.

**References**