The effects of a high fat diet on hepatic lipid levels and sources in the adult rat

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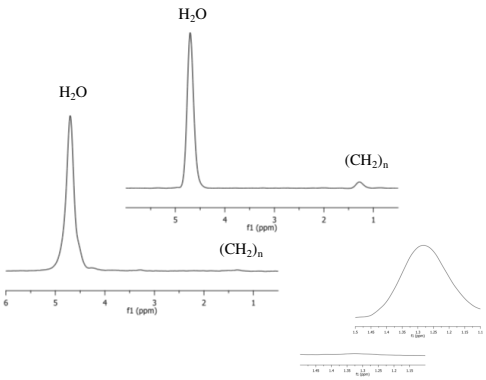
Introduction: High fat diet (HFD) is associated with the development of hepatic insulin resistance, impaired insulin action and decreased whole body glucose disposal. High fat diet feeding also promotes the accumulation of hepatic triglyceride (HTG) content leading to steatosis. HTG can originate from dietary lipid and also by *de novo* lipogenesis from acetyl-CoA. To determine the effects of HFD on the overall levels of HTG we used *in vivo* ^{1}H MRS to quantify hepatic triglyceride levels. To determine the effects of HFD on the contributions of dietary lipid and de novo lipogenesis to HTG, the lipogenic fraction was quantified by a novel ^{2}H NMR analysis of HTG ^{2}H-enrichment from ^2H_2O.

Methodology: 180-220 g male Sprague Dawley rats were housed during 35 days at 20ºC and on a 12-h light-dark cycle with *ad libitum* access to water. One group of animals (n=4) was fed with a standard chow rat diet, with 2.7% of fat content, and the other (n=5) was given a HFD where 40% of calories are derived from fat. Hepatic ^{1}H MR spectra were acquired on a 7.0 T Bruker Pharmascan system using a PRESS sequence (TR 1100 ms/ TE 28 ms) without water saturation and with 128 signal averages. T1-weighted transverse images of the liver were used to ensure accurate positioning of the (5x5x5 mm) voxel in the liver. Spectra were analysed using an NMR data processing program, Nuts (Acorn NMR). Peak areas for all resonances were obtained and lipid resonances were quantified with reference to water resonance. On day 35, rats were given a bolus of deuterated water (^2H_2O) (99.9%) and kept during 48h with *ad libitum* access to food and water enriched with 3% ^2H_2O. Afterwards, animals were sacrificed after 6h fasting, blood was withdrawn and the liver freeze-clamped. Hepatic lipids were extracted from frozen livers by a Folch extraction and analyzed by ^{1}H and ^{2}H NMR on a Varian 500 MHz System. ^{2}H enrichment of the aggregate triglyceride methyl hydrogens were quantified by comparing the composite HTG methyl ^{2}H signal with that of an internal pyrazine-d_4 standard. The fraction of HTG derived from lipogenesis was estimated as HTG ^{2}H-methyl/body water ^{2}H-enrichment. Statistical results were analyzed using the *t*-student's test where $p<0.05$ was considered significant, and are displayed as average ± SEM.



Results: After 35 days, HFD animals showed normal fasting blood glycemia (175 ± 6 *vs.* 150 ± 10 mg/dL in the control animals), insulinemia is not altered (0.34 ± 0.11 *vs.* 0.27 ± 0.06 ng/mL in the control animals) but the rate of gain weight was lower (191 ± 7 *vs.* 138 ± 8* g in the control animals, *p<0.05). HTG content was significantly increased in HFD fed animals (33.0 ± 6.0* mg/gram wet weight of liver *vs.* 11.2 ± 3.0 mg/gram wet weight of liver in controls, *p<0.05).

Figure 1. Rat hepatic ^{1}H Magnetic Resonance Spectra of an animal with elevated hepatic triglyceride content (upper) *vs.* animal with low HTGC (lower). H_2O is the water signal whereas $(CH_2)_n$ corresponds to the methylene protons of triglyceride acyl chains that are shown expanded in the insight.

With the standard chow diet, 10.9 ± 1.0 % of HTG was derived from *de novo* lipogenesis over 48 hours while 89.1 ± 1.0 % was obtained from dietary sources. With HFD, the contribution of *de novo* lipogenesis to HTG was reduced approximately 10 fold, with only 1.0 ± 0.2%* of HTG being accounted for by lipogenesis and 99 ± 0.2% derived from dietary sources. (*p < 0.01 compared to the lipogenic contribution in standard chow fed animals). Absolute lipogenic HTG production over 48 hours, estimated as the product of HTG and lipogenic fraction, was 1.27 ± 0.40 mg/g liver wet weight in Chow fed animals and 0.34 ± 0.10 mg/g liver wet weight in HFD animals, (p = 0.10) indicating a tendency for absolute lipogenic rates to be reduced by HFD.

Discussion and Conclusions: In healthy rats fed on a normal chow diet, HTG are largely derived from outside the liver with *de novo* lipogenesis accounting for about 5% of daily HTG turnover. Exposure to HFD tended to reduce the rate of *de novo* lipogenesis but this change was not sufficient to prevent an overall elevation of HTG. There were no significant alterations in fasting plasma glucose and insulin levels suggesting that insulin resistance and glucose intolerance had not yet been established. These results suggest that under these conditions of lipid overload, HTG accumulation precedes the loss of glucose homeostasis. Therefore, a non-invasive assay of HTG using ^{1}H MRS could provide a valuable early marker of dietary lipid overload and hepatic steatosis which in turn are significant risk factors for type 2 diabetes.