Fat storage in the liver during feast or famine

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Introduction - In early days, primitive man as a hunter and gatherer was exposed occasionally to long periods of food deprivation and starvation. As a survival strategy, adipose tissue elsewhere in the body, deliveres the lipid stores to the liver where they are stored as triglycerides (TG), which can be mobilized to generate ketone bodies to drive vital organs like the brain, cardiac and skeletal muscle during starvation. Nowadays obesity is becoming a huge problem because food is mostly available on demand. Because the dynamics of lipid accumulation in the liver of a mammal is largely unknown, we qualified and quantified the process of triglyceride (TG) accumulation in a mouse model after 24 hours of starvation and 40 days of a fatty diet using 1H-MRS. We also validated the *in vivo* MRS protocol using High Performance Thin Layer Chromatography (HPTLC).

Methods - Male, 12-16 weeks old C57Bl/6 mice were used in all experiments. For the starvation experiment animals were fasted for 5, 12, 24 h. For the fatty diet, animals had free access to a high fat diet + 0.25% cholesterol and 45% energy from bovine lard, Hope Farms, USA. In vivo ^{1}H MRS localized spectroscopy was carried out on a 9.4 T vertical bore imaging system equipped with an Avance console controlled with paravision 3.01 software Bruker Biospin (Karlsruhe, Germany). A Bruker Micro gradient system of 1 T/m was used with a solenoidal 30-mm-I.D. birdcage coiltransmit/receive mode. A (2.5 mm)^3 voxel was positioned in the liver and a water/fat spectrum was obtained with a PRESS protocol: TE=50 ms, TR=3 s, Nav=256.

Three types of experiments were performed:

A. Validation of the 1H MRS method: 33 mice with different grades of liver fattening were measured with MRI followed by a liver biopsy for validation with bligh and dyer extraction followed by HPTLC.

B. Dynamics of TG accumulation during starvation: For the starvation group not the same animals were used because it was expected three times handling and anaesthetizing the animals within 24 hours would have a too large impact on the animal. So for t=0, 5, 12, 24 hours for each sample point 6 mice were used (total 24 mice).

C. Dynamics of TG accumulation during a fatty diet: The fatty diet group were provided unrestricted amounts of 'fatty' food and water. On day 0 a baseline spectrum of 6 animals was measured. On day 4,12,18, 28 and 40 the MRS measurements were repeated for the same animals.

D. Analysis of lipid components: The spectral resolution obtained allows the identification of resonances arising from saturated and unsaturated fatty acid moieties which were assigned according to the spectrum given by Corbin et al. (see Fig. 2). At the end of experiments B and C, the mice were sacrificed and liver biopsies were taken for mass spectrometry.

Results

A. Validation of ^{1}H MRS using HPTLC: TG content of the liver measured by *in vivo* ^{1}H MRS correlated linearly with HPTLC quantification of TG in liver biopsies ($r = 0.7$, $p \leq 0.001$).

B/C. Dynamics of triglyceride accumulation in liver tissue during starvation and during a 40-day-period of fatty diet: Figure 1 shows the accumulation of triglycerides due to fasting or feasting. Though the timescales are different, both protocols result in similar amounts and patterns of TG storage.

D. Analysis of lipid components using ^{1}H MRS and mass spectrometry

Both fasting and feasting result in a fatty liver (accumulation of triglycerides (TG)). However, there are distinct differences between the triglyceride composition of 'fasting' and 'feasting livers' with respect to their fatty acid composition (chain length and number of double bonds). The composition of TG in fasting livers and control livers is very similar, whereas the composition of TG in feasting liver appears to reflect the diet. The intake of saturated and single-unsaturated fatty acids results in lower levels of triglycerides containing poly-unsaturated fatty acids. Furthermore significant differences in content and composition of lyso-phosphatidylcholines (LPC), phosphatidylcholines (PC), cholesterolesters (ChE) and sphingomyelin (SPM) between 'fasting' and 'feasting' livers where observed.

Discussion - The results show that ^{1}H MRS is a suitable method for frequent, repetitive estimation of hepatic fat *in vivo* as a non-invasive alternative to biopsy.

Although the dynamics of hepatic steatosis in a mouse model during 24 hours of starvation and a 40 days fatty diet showes a similar pattern, we conclude based on the formation of different lipid end products that these are different processes regulated via different metabolic pathways.

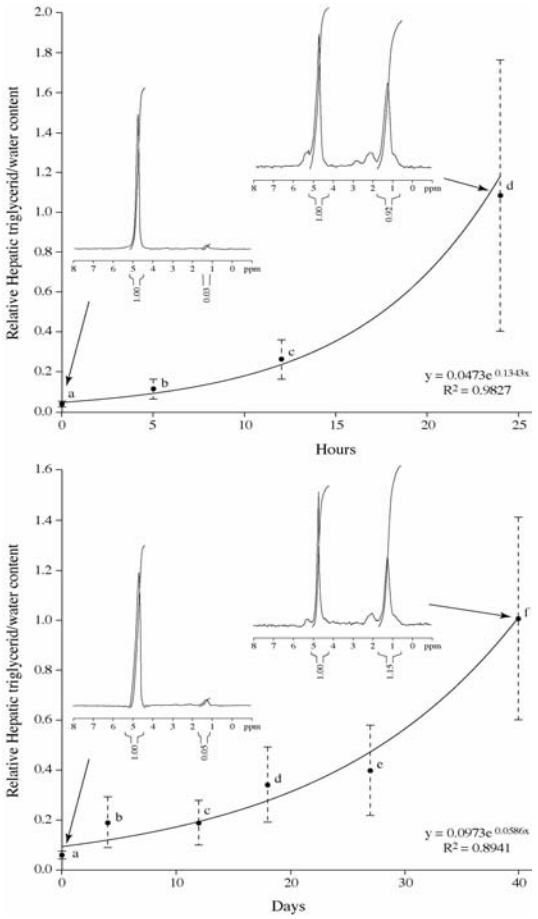
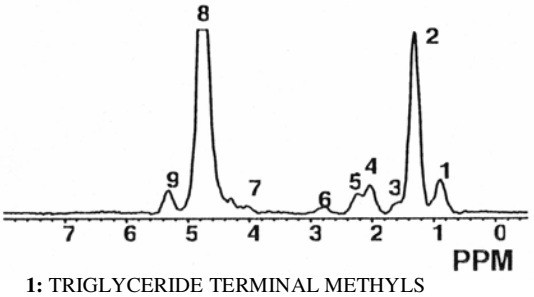


Fig. 1 Dynamics of TG accumulation after 24 hrs starvation (A) or 40 days of fatty diet (B).



1: TRIGLYCERIDE TERMINAL METHYLS
2: METHYLENE $(CH_2)_n$
3: CH_2CH_2CO
4: $CH_2C{=}C$
5: CH_2CO
6: $C{=}CCH_2C{=}C$
7: CH_2OCOR
8: WATER
9: $CH{=}CH$

Fig. 2 Neutral lipid resonances from fat (Corbin et al. 2004, Proc.ISMRM 11: 896).

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