

Postprandial ectopic lipid storage observed after a single meal: no influence of additional protein content

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Fat accumulation in non-adipose tissue, such as liver and muscle, results from an imbalance between lean tissue fatty acid uptake and/or synthesis and reduced catabolism. Although little is known about the dietary factors that determine excessive ectopic lipid retention, lipid accumulation in liver induced by 4-day fat or 7-day high fructose overfeeding can be blunted by ingestion of additional proteins [1,2]. In this study we 1) explored the feasibility to detect postprandial changes in hepatic and skeletal muscle lipid content after a *single meal* using *in vivo* ¹H-MRS and 2) investigated whether the beneficial effect of additional protein intake can already be observed after a single meal.

Methods: Intrahepatic (IHL) and intramyocellular (IMCL) lipid levels were determined before and after a high fat breakfast (62% fat, 31% carb, 7% prot, 50% of daily recommended energy intake) without (HF) or with (HFP) an additional protein drink (Protifar, Nutricia BV, NL, 20% of total energy content) in a randomized cross-over study. MRS experiments were performed on a 3T MR system (Achieva 3T-X, Philips Healthcare, Best, NL) using a 32-element sense cardiac/torso coil for determination of IHL (PRESS [3], TR 4s, TE 32.5ms, 40x40x40mm³, NSA 192, CHESS water suppression) and a two-element flex coil (14x17cm) for IMCL levels in the tibialis anterior muscle (PRESS, TR 2s, TE 38ms, 14x14x40mm³, NSA 128, water suppression by excitation, 2 fat-selective saturation bands). Non-water suppressed reference spectra were acquired from the same volumes (NSA 16). Care was taken to a) avoid contamination of subcutaneous fat or large blood vessels in the liver and b) reproduce the same positioning of the coils and the voxel location at t=3h and t=5h based on T2 weighted MR images. The ¹H MR spectra from liver were analyzed using a MATLAB routine, which allowed for automatic exclusion of spectra with motion artifacts, aligning, phasing and fitting of the CH₂ resonance in the spectral domain. Fitting of the IMCL signals was done in the time domain using AMARES (jMRUI v4.0) and corrected for T1 and T2 relaxation. Lipid levels were converted to absolute concentrations (g/kg wet weight), using water as an internal reference.

Results and Discussion: Nine lean healthy subjects (6m, 3f, age 22.7 ± 3.0 years, BMI of 21.8 ± 1.8 kg/m², and body fat percentage of 15.1 ± 8.4 %) were included in this study. IHL levels increased significantly in the postprandial period by ~20% (P < 0.001, ANOVA grouped analysis HF+HFD), which occurred mainly between 0-3hs as 3-5hs IHL levels were not significantly different (P = 0.99). IMCL levels were not altered during the postprandial period (P = 0.74 for HF+HFP). Addition of protein to a single high-fat meal did not change the accumulation of fat in the liver (P = 0.93) or in skeletal muscle (P = 0.84).

Conclusion: Here we demonstrate that net lipid accumulation after a single HF meal can be detected in the liver using *in vivo* ¹H MRS. This non-invasive approach, brings new opportunities for nutritional intervention studies. The addition of proteins to the diet did not change ectopic lipid retention after a single high-fat meal.

References: [1]: Bartolotti et al. Am J Clin Nutr 2009, [2] Theytaz et al. Am J Clin Nutr 2012, [3] Bottomly PA. Ann NY Acad Sci 1987. **Acknowledgements:** Funded by Top Institute Food and Nutrition (WM003).

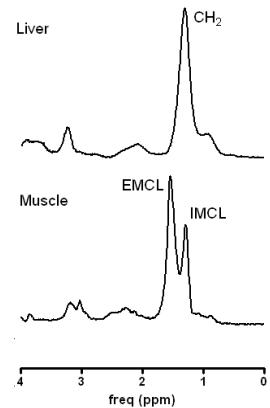


Figure 1: Baseline ¹H-MRS spectra in liver (top) and muscle (bottom), acquired

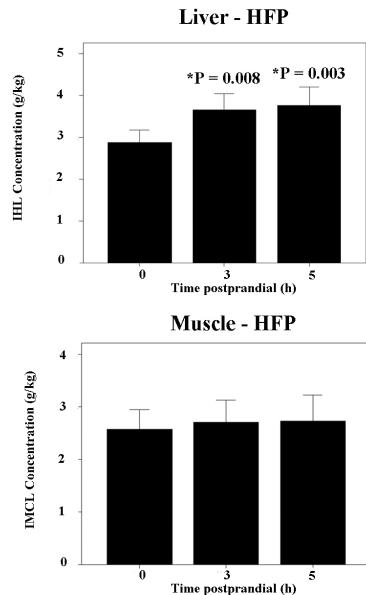


Figure 2: IHL and IMCL concentrations determined before and 3h and 5h postprandially, either after a HF or a HFP Breakfast. P-values represent differences compared to baseline (Post Hoc Bonferroni correction applied)