An animal model for the study of developmental origins of adult disease associated to dietary fetal fatty acids: MRI assessment

K. Hollander[1], C. Tempel-Brami[2], F. M. Konikoff[1], M. Fainaru[3], and A. Leikin-Frenkel[1]
[1]Minerva Center for Lipid Metabolism in the Liver, Sackler School of Medicine, Tel Aviv University, Israel, [2]Alfredo Federico Strauss Center for Computational Neuro-Imaging, Tel Aviv University, Tel Aviv, Israel, [3]Department of Physiology, Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel

Introduction: The mammalian fetus is completely dependent on the fatty acids supplied by its mother inside the uterus. Disturbances in nutrient supply can modify fetal development, with lasting consequences for growth and metabolism of the offspring throughout life [1]. However, the impact of fatty acids and, in particular essential fatty acids (EFA) on developmental origins of adult disease is still unknown. The present study was designed to analyze the impact of EFA in the diet of pregnant mothers on obesity and insulin resistance in adult offspring. MRI was used as tool for the quantitation of body fat in pregnant mothers and offspring and for the subsequent comparison with lipid content of tissues and insulin resistance in adult offspring.

Methods:

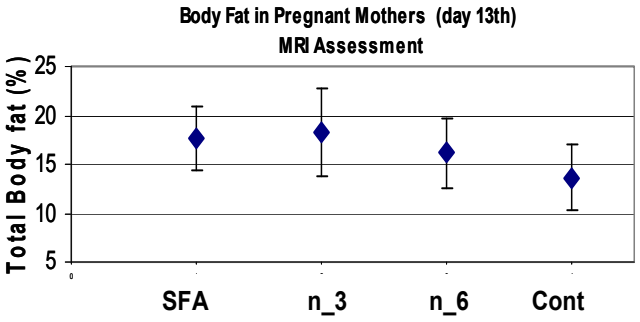
Animal set up: C57Bl6/J female mice were given experimental diet two weeks before mating and during pregnancy. Isocaloric diets included 6% fat differing in the predominant fatty acids: control (C-PRD), saturated (SFA-PRD), n-3 (n-3PRD) or n-6 (n-6PRD) respectively. After weaning, pups were fed regular chow diet (RD) for two month and subsequently a high fat diet (HFD: 18% fat w/w) for additional two months. At pregnancy, glucose tolerance test (GTT) and MRI analysis of body fat were performed in the mothers. During HFD feeding, GTT and MRI were performed in the offspring. After sacrifice, liver, pancreas, muscle and epididimal fat lipid content and fatty acid composition were analyzed. Glucose, insulin levels and their relationship were quantified as HOMA index.

Lipid extraction and quantitation: Lipids were extracted from selected tissues by the procedure of Folch [2]. Fatty acids were analyzed, as methyl esters, by GC [3]. Biochemical determinations: Blood glucose levels and GTT were measured by Sugar AccuCheck Go,sensor (Roche Mainheim, Germany). Plasma insulin was determined using an insulin immunoassay kit (MRC Mouse Insulin, Elisa 96T, Mercodia, Sweden). The HOMA index, as an expression of insulin resistance, was calculated as Plasma glucose [uU/ml] X Plasma Insulin [mM] / 22.

MRI: MRI experiments were performed on 7T/30 system (Bruker, Rheinstetten, Germany) using a 7.5 cm volume coil. The mice were anesthetized with isoflurane (1–3%) in 1liter/min oxygen and the respiration rate was monitored and maintained 30–40 breaths min^{-1} throughout the experimental period. Coronal T_1w images were acquired with Spin echo TR 600ms, TE 12ms, ns 2, 256x128 with zero filling to 256x256, slice thickness 1 mm, FOV 8 cm, no fat suppression. Total fat volume was measured by counting the fat pixels by automatic segmentation of the fat using imageJ 1.4 software and multiplying it by the pixel volume. Total animal volume was also measured permitting to extract the percentage of fat for each animal.

Results and Discussion: Body fat (BF) accumulation in pregnant mothers, measured by MRI, was not influenced by the different fatty acid composition in their diet (fig). GTT was mildly and insignificantly lower in those receiving n-3 or n-6 fatty acids. Conversely, body fat accumulation in adult offspring after HFD was affected by fatty acid composition in pregnant mother's diet. After HFD, in SFA-PRD, body weight, and HOMA index were 10% higher than in C-PRD. Remarkably, body fat accumulation in n-3 PRD was 50% lower than C-PRD. Significantly, HOMA index in n-3 PRD was 65% lower than C-PRD and in n-6PRD 35% lower, than in C-PRD. Fat accumulation in adult offspring tissues, after HFD, was also influenced by fetal dietary fatty acids. In liver, the lipid content in n3-PRD and n-6 PRD was 20 % and 40% lower than in C-PRD whereas in SFA-PRD it was 36% higher. In pancreas, the lipid content in n-3PRD and n-6PRD was 48% lower than in C-PRD. In muscle, the lipid content in n-3PRD and n-6PRD was 78% and 44% lower than in C-PRD whereas in SFA-PRD it was 18% higher. White adipose tissue measured as epididimal fat / body weight ratio in males n-3PRD and n-6PRD was 75% lower than in C-PDR. The monounsaturated/saturated fatty (MFA/SFA) acid ratio, surrogate marker of Stearoyl-CoA desaturase (SCD) activity in n3-PRD and n-6 PRD was 20% and 10% lower than in C-PRD. MRI of BF in adult offspring directly correlated with HOMA index as well as with tissues lipid content, and SCD activity.

Conclusions: (1) Altogether the results indicate that dietary fatty acids in fetal life set off the response of adult offspring to HFD leading to obesity and insulin resistance. **(2)** A developing fetus is highly vulnerable to the quality of dietary fatty acids quality, independent of maternal diabetes and/or obesity. **(3)** EFA prevent risk of adult offspring obesity and insulin resistance whereas saturated fatty acids promote it. **(4)** Among EFA, n-3 has a stronger preventive effect than n-6 fatty acids. **(5)** MRI correlates with HOMA index, with lipid content in liver, pancreas and muscle, and with SCD activity in white adipose tissue. This strong correlation points to MRI as a reliable, noninvasive tool to assess obesity and insulin resistance in small animal models, without sacrifice, during long periods, for different biological analysis throughout treatment up to longevity.



References

[1] D.J.P. Barker. The developmental origins of adult disease. *European Journal of Epidemiology* 2003.18: 733–736.

[2] Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry* 1957;226:497-509

[3] Leikin A, Brenner RR. Cholesterol induced microsomal changes modulate desaturase activities. *Biochim.Biophys.Acta* 1987;922:294-303.