# Modulation of ectopic fat and SCD activity during weight loss interventions in high saturated fat diet induced obese rats by in-vivo MRS and LC-MS

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**Target Audience:** Researchers interested in obesity and diabetes.

**Purpose**: High-fat diet is known to induce weight gain resulting in obesity in both humans and rodents. Saturated fatty acids (SFA) in particular promote increased accumulation of ectopic fat in liver and skeletal muscle. Ectopic fat has been implicated in the pathogenesis of cardio-metabolic diseases [1]. A high SFA diet is also associated with significantly higher stearoyl coenzyme desaturase (SCD) activity [2]. SCD is involved in the desaturation of saturated fatty acids to monounsaturated fatty acids, which are the preferred substrates for esterification. Increased SCD activity is associated with reduced fat oxidation and increased fat storage [3]. The goal of the current study is to evaluate the relative effectiveness of exercise and calorie restriction interventions at reversing the alterations in body weight, ectopic fat and SCD activity induced by a high SFA diet in diet-induced obese rats.

**Animal model and Methods:** The in-vivo animal studies are in compliance with the policies of the local institution. Rats (male Fischer 344, Clea Japan) were fed with high SFA diet (Research Diet, D12079B) from 5 to 18 weeks of age. The rats were split into the following cohorts: a high SFA diet control group (HFD), exercise once a day (Ex1), exercise twice a day (Ex2), calorie intake restricted by 15% (CR1) and calorie intake restricted by 30% (CR2). Interventions were performed for 4 weeks. Exercise interventions were performed once a day for 30 min (20m/min) Ex1, and twice a day for 30 min (20m/min) Ex2 using animal treadmill (Columbus-1055SRM-E54 Exer-3/6-Dual). In vivo $^1$H MRS were performed on a 7T Bruker ClinScan MR System equipped with 72 mm body coil (transmit) and 20 mm receive only coil. Localized PRESS based MR spectroscopy was performed on liver (voxel size of 4x4x4 mm$^3$) and muscle (voxel size of 3x3x3 mm$^3$) with TR = 4.0 sec, TE = 14 msec. Liver MRS scans were performed with motion compensated respiratory gating. LCModel was utilized to quantify liver fat fraction and intramyocellular lipids [4]. Estimates of liver and muscle SCD activity (SCD-16 index) at the terminal time-point were measured by product-to-precursor ratios (C16:1/C16:0) in the free fatty acid pool in liver and muscle tissue using liquid chromatography-mass spectrometry (LC-MS) [5]. Body weight, liver fat and IMCL were adjusted for pre-intervention values. Statistically significant differences from the HFD have been marked with '*' (P<0.05), '**' (P<0.01), or '***' (P<0.001).

**Results:** All the four (Ex1,Ex2,CR1 and CR2) intervention cohorts showed a statistically significant reduction in body weight (Fig. 1) and liver fat fraction (Fig. 2) with respect to the HFD control group, with CR2 showing the greatest reduction. There were no statistically significant changes in IMCL in any of the intervention cohortzs (Fig. 3). Post-intervention trends in the liver desaturase indices were also not identical to those seen in the muscle. Liver SCD16 index (Fig. 4) was significantly reduced only in the CR2 and Ex2 cohorts, while muscle SCD16 activity (Fig. 5) was significantly reduced in all the intervention cohorts, with the greatest reduction in Ex2 (P<0.001). The effect sizes (partial $\eta^2$) of the different intervention with respect to the HFD control group have been shown in Table 1. The tibialis muscle wet weight was significantly increased in the Ex1 ($\eta^2$ = 0.28, P <0.01) and Ex2 ($\eta^2$ = 0.33, P<0.001) cohorts.

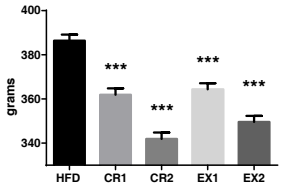

Fig.1 Body Weight

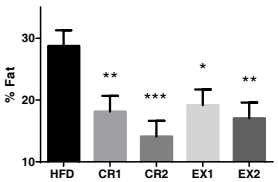

Fig.2 Liver Fat

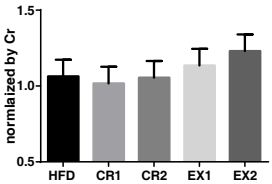

Fig.3 IMCL

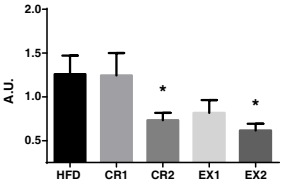

Fig.4 Liver SCD-16 index

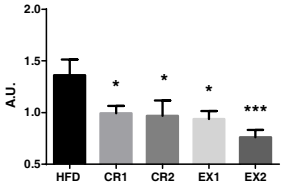

Fig.5 Muscle SCD-16 index

**Discussion:** Insufficient increase in fat oxidation [6] to accommodate the excess delivery of saturated fatty acids, associated with a high SFA diet results in increased esterification of fatty acids to triglycerides to protect the cells from FFA-induced lipotoxicity [7]. Fat oxidation in the liver and muscle, as measured indirectly by the SCD16 index, seems to respond differently to increased levels of exercise and calorie restriction. We obeserved marked increase in the effect size of the liver SCD16 index with a doubling of the amount of calories restricted and doubling of the exercise duration. However, increasing the degree of calorie restriction seems to be not as beneficial as increasing the exercise duration for improving the muscle fat oxidative capacity. The reduced liver fat content may result from a combination of increased fat oxidation and reduced influx of free fatty acids at lower levels of adiposity. The small increase (not statistically significant) in IMCL levels in the Ex1 ($\eta^2$ = 0.01) and Ex2 ($\eta^2$ = 0.04) when compared to the HFD cohort may be an attempt to optimally match the improved fat oxidative capacity and to act as a readily available fuel source during exercise.

**Conclusion:** Four weeks of exercise and calorie restriction were effective at reducing body weight and liver fat, but not the IMCL content in high SFA diet induced obese rats. The measured SCD-16 index post intervention, suggests that a large negative energy balance induced by increasing the exercise duration or calorie restriction may be required to increase the liver fat oxidation, while the muscle fat oxidation is improved even at lower levels of calorie deficit. Increasing the calorie restriction does not seem to be effective as increasing the exercise duration at improving the muscle fat oxidation.

Table. 1. Effect size (partial $\eta^2$)

| Parameters | CR1 | CR2 | Ex1 | Ex2 |
|---|---|---|---|---|
| **Body Weight** | 0.57 | 0.8 | 0.52 | 0.74 |
| **Liver Fat** | 0.24 | 0.37 | 0.19 | 0.27 |
| **IMCL/Cr** | 0.00 | 0.00 | 0.01 | 0.04 |
| **Liver SCD-16** | 0.00 | 0.24 | 0.18 | 0.33 |
| **MuscleSCD-16** | 0.15 | 0.17 | 0.19 | 0.32 |

**References:** [1] Kathryn A et al. Circulation 2011, 124:e837-841. [2] Vessby B, et al. British Journal of Nutrition 2013, 110:871–879. [3] Hodson L et al. Prog Lipid Res. 2013, 52(1):15-42. [4] Provencher et al. NMR Biomed. 2001, 14:260–264. [5] Kröger J, et al. Curr Opin Lipidol. 2012, 23(1):4-10. [6] Schrauwen P, et al. Am J Clin Nutr 1997, 66:276–282. [7] Listenberger LL, et al. Proc Natl Acad Sci USA 2003,100(6):3077-82.