

Evaluation of Hepatic Fat Fraction Measured by MRI and Plasma Lipoprotein Levels in High-Fat Diet Fed Non-Human Primate

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Target Audience: Researchers involved in the development of animal models for cardio-metabolic disease and liver fat imaging.

Purpose: Excess fat accumulation in liver has been associated with cardiovascular diseases or metabolic disorders, such as dyslipidemia, atherosclerosis, or diabetes mellitus¹. Therefore, significant efforts have been devoted to determine hepatic fat fraction (FF), where liver biopsy along with histological assessment has been often used. Alternatively, non-invasive MRI techniques have been developed and validated to derive hepatic FF with higher sensitivity for the detection of lower FF and the feasibility of longitudinal monitoring²⁻⁴. To better delineate cardio-metabolic diseases and develop potential therapeutics, non-human primate (NHP) models of human diseases have been often pursued to capitalize on the potential to translate pathological characteristics to humans. Whilst each animal model often can only recapitulate specific perspectives of the targeted disease, comprehensive characterization of the disease processes model remains essential. In this work, using a high-fat diet (HFD) induced NHP model of dyslipidemia, we aimed to investigate the correlation between hepatic FF measured by MRI and plasma lipid levels, such as triglyceride (TG), total cholesterol (TC), low-density lipoprotein-cholesterol (LDL-c) and high-density lipoprotein-cholesterol (HDL-c), in order to gain insights into the dysfunction of lipid metabolism in a NHP model with high dietary cholesterol intake.

Methods: Animals and Clinical Chemistry: Male cynomolgus macaques (6-7 yrs, 5-7 kg, n=28) were housed at Maccine's facility (Maccine Pte Ltd, Singapore) in accordance with IACUC guidelines. Two monkey chows free of animal protein (LabDiet, cholesterol content: 0.28 mg/kcal (n=8, HFD#1) or 0.61 mg/kcal (n=20, HFD#2) St. Louis, MO) were provided twice daily (over 9 mo.) and water was offered *ad libitum*. Fresh plasma samples from each cynomolgus monkey were analyzed for TC, HDL-c, LDL-c, and TG using Roche Cobas 6000 clinical analyzer (Roche Diagnostics, Indianapolis). **Liver Fat MRI:** All imaging experiments were conducted on a Siemens 3T MRI scanner with a 4-channel flex coil. Animals were anesthetized using ketamine (10 mg/kg IM, induction) and then isoflurane (~1.5%, maintenance) and vital signs were monitored. Images were collected using a multi-echo gradient echo sequence with a total of six echoes⁴ with the following imaging parameters: FOV=263×197 mm²; TR=192 ms; TEs=1.29/2.46/3.69/4.92/6.15/7.38 ms; matrix size=192×144, slice thickness=5 mm, and a flip angle=5° to minimize T₁ bias⁵. **Data Analysis:** Post-processing of imaging data to generate separate fat fraction and R₂^{*} maps were performed using software programs written in a plug-in in Osirix (LIG Processing Tool), which allows correction of confounding factors, such as T₂^{*} decay and multi-peak spectra modeling of fat⁶. FF maps of the whole liver were then derived on a pixel-pixel basis⁴. Subsequently, region-of-interest (ROI) analysis was performed on liver FF maps by manually drawing eight individual ROIs with reference to the Couinaud classification of liver anatomy that divides the liver into eight functionally independent segments, where great similarity was found between monkeys and human⁷. Finally, the mean value of FF obtained from eight ROIs across the entire liver for each animal was reported. The correlation between plasma lipoprotein levels and liver fat fraction was assessed using multivariate correlation analysis.

Results and Discussion: Previously, we found NHPs fed with a normal diet (<0.1 mg/kcal) have average TC =110 mg/dL, HDL-c=55 mg/dL, LDL-c=50 mg/dL and TG=50 mg/dL. In this work, elevated lipid levels, except TG, are observed in these HFD-fed animals. Specifically, animals fed with HFD#1 show lipid levels (mean ± SD) at TC =177 ± 71 mg/dL, HDL-c=102 ± 45 mg/dL, LDL-c=88 ± 83 mg/dL and TG=36 ± 20 mg/dL, while those of obtained from ones fed with HFD#2 are TC=215 ± 23 mg/dL, HDL-c=113 ± 30 mg/dL, LDL-c=115 ± 30 mg/dL and TG=32 ± 11 mg/dL. Figure 1 shows the representative liver FF maps and the anatomical locations of 8 ROIs manually outlined to cover all the liver segments. Our results indicate that calculated FF (mean ± SD) from animals fed with HFD#1 and HFD#2 are 6.3 ± 2.4% and 8.2 ± 5.0%, respectively. There is no significant difference in lipid levels and liver FF values measured from these two high fat diet groups. Additionally, it was found that FF does not correlate with any lipoprotein levels or their ratios in these animals; regardless both diet groups are analyzed collectively or separately; however, compared to previous results with NHPs fed with a normal diet, both TC and liver FF are significantly higher (Fig. 2). The development of fatty liver can result from a complex interplay between the production of fatty acid and its use for oxidation or secretion, which encompasses peripheral fats stored in adipose tissue that transfer into the liver via the plasma non-esterified fatty acid pool, as well as fatty acid recently synthesized within the liver via de novo lipogenesis and through dietary fatty acid uptake⁸. Thus, it is unlikely FF can be solely attributed to the cholesterol content of the diet, although both groups of animals on HFD have elevated FF and cholesterol levels. Additionally, the insignificant correlation might be reconciled with limited ranges of liver FF and lipid levels examined in the current study and therefore further studies using a larger animal cohort with wider spreads of both lipid and FF values are needed. Furthermore, correlations of liver FF with other circulating biomarkers (e.g. miRNA) also remain to be explored.

Conclusion: we have characterized this NHP model of dyslipidemia by determining the disease phenotypes (i.e. elevated lipoprotein levels and hepatic FF) and demonstrated the dysfunction of lipid metabolism in dyslipidemic animals.

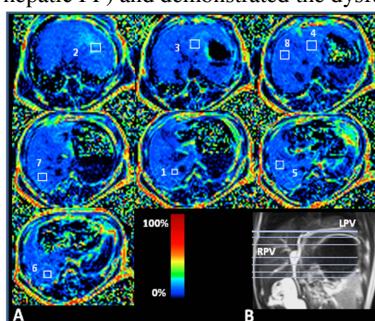
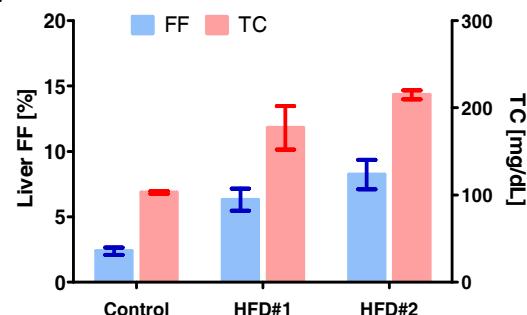


Figure 1 (left) (A) Representative liver fat fraction maps obtained from the animal fed with high-fat-diets. (B) MRI sagittal image highlighting the anatomical locations of liver fat imaging slices. ROIs (n=8) sampling was covered across the entire liver, where each ROI has been placed at every liver segment according to the Couinaud liver segmentation: segment 1 located at the caudate lobe and segment 7 and 6 are posterior to the segment 8 and 5, respectively (RPV=right portal vein; LPV= left portal vein). **Figure 2 (right)** Liver fat fraction (FF) and plasma total cholesterol (TC) (mean ± SD measured from control diet- and high fat-diet (HFD)-fed animals.



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