Dietary Models of Nonalcoholic Fatty Liver Disease in Normal and Diabetic Nonhuman Primates

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is the hepatic manifestation of metabolic syndrome and is a rising global health issue. Obesity and diabetes appear to be correlated with high NAFLD occurrence. Currently, the underlying mechanism causing NAFLD and disease progression remains unclear and effective therapies are unavailable. Huge investment in studying NAFLD pathogenesis and into innovative therapies is underway. As mechanistic studies are more difficult or unethical to perform in humans, animal models recapitulating human disease are urgently needed. Nonhuman primates (NHPs) with NAFLD can highly mimic the human disease. This study aimed to induce NAFLD by various diets (Table 1) in normoglycemic and diabetic NHPs with biomarker and histopathology assays to quantify NAFLD occurrence. Our data show that diabetic NHPs fed a high-fat high-fructose (HFHF) diet developed NAFLD which could resemble clinical disease and be highly valuable for NAFLD research. However, low choline (LC), LC + 10% fructose, LC + low methionine (LCM) + 10% fructose diets for 31 weeks had no significant effects on liver function, except a significant decrease in body weight and food intake in the LCM + 10% F group and some increase in liver histological scores in LC + 10% F group (Table 2). In summary, normal diet with LC or LCM ± high fructose barely induces NAFLD in normoglycemic NHPs, at least over 31 weeks. However, HFHF could induce or enhance NAFLD in diabetic NHPs.

METHODS

Animals: Normoglycemic and diabetic cynomolgus NHPs (Table 1) were enrolled in the study and were maintained on specific diets for 16 or 31 weeks. The study was approved by the IACUC committee and performed in accordance with AAALAC regulations.

Diets (SYSE Biotech Inc., China): Normal, LC (low choline), LC + 10% F (fructose in drinking water), LC + LM (low methionine) + 10% F (fructose in drinking water). LC and LC + LM diets indicate no additional choline (C) or methionine (M) being added to the normal diet. High-fat high-fructose diet (HFHF) with calorie source (%): fat 40, carbohydrate 43.8 (fructose 10) and protein 16.3 with cholesterol 0.55% (w/w). Normal diet with calorie source (%): fat 18, carbohydrate 55 (fructose < 0.5), and protein 27.

Table 1. Animal grouping and diets

NHPs	Groups	n	Age (years)	Study Duration
Normoglycemic	Control	9	16.7 ± 0.9	31 weeks
	LC	10	17.8 ± 1.1	31 weeks
	LC + 10% F	9	16.4 ± 1.2	31 weeks
	LC + LM + 10% F	10	14.4 ± 1.1	31 weeks
Diabetic	HFHF	12	17.0 ± 1.0	16 weeks

Data collection and analysis: Body weight and food intake were measured and blood samples were collected after approximately 16-hrs fasting. Data are presented as mean ± SEM. The results were compared using ANOVA and statistical significance was considered as p<0.05.

RESULTS

Fig 1. Changes in body weight (left upper panel), food intake (left lower panel), plasma glucose (right upper panel), and insulin (right low panel)

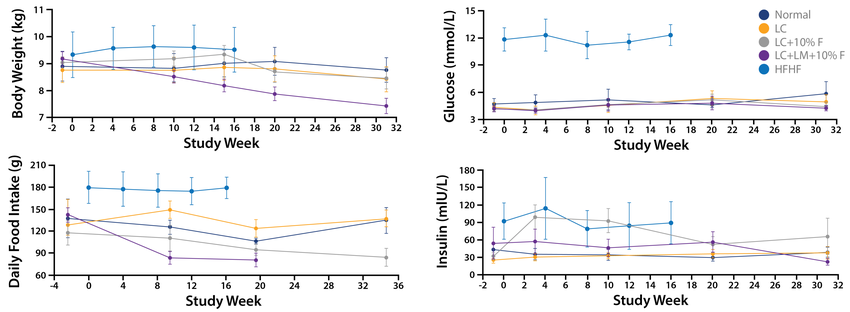


Fig 2. Changes in plasma lipids, such as TG (left upper panel), TC (left low panel), LDL (right upper panel), and HDL (right low panel)

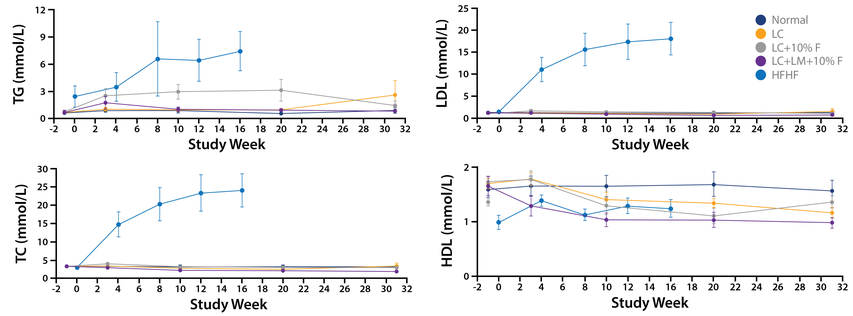
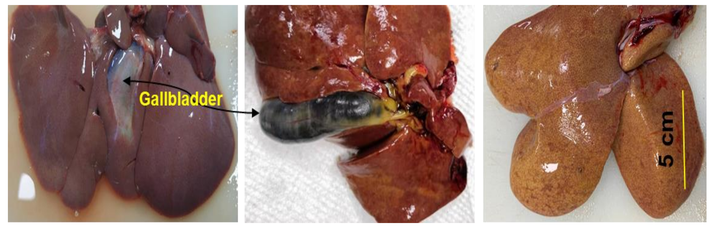


Table 2. Diet-induced delta changes of blood biochemistry and liver function in normoglycemic and diabetic cynomolgus NHPs

NHP	Dietary Group	Delta Changes after New Diets												Liver Biopsy Histology (score)		
		BW (kg)	FI (g)	Glucose (mmol/L)	Insulin (mIU/L)	C-peptide (nmol/L)	HbA1c (%)	TG (mmol/L)	TC (mmol/L)	HDL (mmol/L)	LDL (mmol/L)	ALT (U/L)	AST (U/L)	Steatosis	Cellular Ballooning	NAS
Normoglycemic	Control (n=9)	-0.1	3	1.1	-4.5	-1.4	0.4	0.3	0.1	0	-0.1	-10*	0.2	0.3	-0.2	0.2
	LC (n=10)	-0.3	-3	0.6	12.4	0.3	0.5	1.9	0.1	-0.5*	0.4	-14	11	0.7	0.3	0.7
	LC+10% F (n=9)	-0.4	-25	0.2	34.6	0.7	0.6	0.7	-0.3*	-0.25*	0.0	-13	-1	1.5	1.0	2.3
	LCM+10% F (n=10)	-1.7*#	-74*#	0.0	-31.7	-0.3	0.5	0.1	-1.3*#	-0.7*#	-0.5*#	2	6	0.3	0.3	0.7
Diabetic	HFHF (n=12)	0.0	0.0	0.6	-2.7	1.8	-0.5	3.5*	17.5*	0.3	13.4*	33*	1	2.3*	1.3*	3.7*

Control: NHPs fed with normal diet containing normal additions of choline and methionine for 31 weeks; LC: low choline, no addition of choline in the control diet; 10%F: 10% fructose in the drinking solution; LCM: no choline and methionine additions in the diet; HFHF: high fat (40%) high fructose (10%) diet for 16 weeks; NAS: NAFLD activity score. *p<0.05 or 0.01; vs pre-diet; #p<0.05 or 0.01; vs control group; no comparison between HFHF and all other normal dietary groups.

Fig 3. Liver gross anatomy collected from cynomolgus NHPs, normal liver (left panel), enlarged gallbladder (middle panel), and fatty liver (right panel)



SUMMARY

- Compared to normal diet, LCM + 10% fructose could significantly reduce food intake and body weight (p< 0.01, Fig. 1)
- Plasma insulin significantly increased in the NHPs fed LC + 10% F, especially in the first 10 weeks (p< 0.05 or 0.01, Fig. 1)
- Compared to the normoglycemic NHPs fed with other diets, the diabetic NHPs fed HFHF showed significant increases in TG, TC, and LDL (p< 0.05 or 0.01, Fig. 2)
- Liver ALT and AST levels increased significantly in diabetic NHPs fed HFHF (Table 2)
- Echo imaging showed marked enlargement of liver gallbladder in LCM + 10% F NHPs, similar to that in Fig. 3 (middle panel). Gross anatomy (Fig. 3, left panel) and histological data showed steatosis, inflammation, ballooning, and high NAS in HFHF diabetic NHPs (Table 2)

In conclusion, normal diet or normal diet with LC, LC + 10% F or LCM + 10% F barely (or was not enough to significantly induce) induced NAFLD in normoglycemic NHPs, at least in 31 weeks. However, HFHF could induce or enhance NAFLD/NASH in diabetic NHPs in 16 weeks

REFERENCES

Liu *et al*. Hepatic Steatosis and Fibrosis in Obese, Dysmetabolic and Diabetic Nonhuman Primates Quantified by Noninvasive Echography. 2017, *J Diabetes Metab* 8: 767. doi:10.4172/2155-6156.1000767

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