Research Article

ผลกระทบของอาหารที่มีไขมันและฟรุคโตสปริมาณสูงต่อจุลกายวิภาคศาสตร์ของตับและโปรไฟล์ของไซโตโครมพี 450 3 เอ 11 ในตับหนูเมาส์

ณัฐรัตน์ เจียระพงษ์[1,2], วรัญญา จตุพรประเสริฐ[1,3], กนกวรรณ จารุกำจร[1,2]*

[1] กลุ่มวิจัยฤทธิ์ทางยาของผลิตภัณฑ์ธรรมชาติโดยเทคโนโลยีชีวภาพทางเภสัชศาสตร์ (PANPB)
คณะเภสัชศาสตร์ มหาวิทยาลัยขอนแก่น จังหวัดขอนแก่น 40002
[2] คณะเภสัชศาสตร์ มหาวิทยาลัยขอนแก่น จังหวัดขอนแก่น 40002
[3] คณะแพทยศาสตร์ มหาวิทยาลัยมหาสารคาม จังหวัดมหาสารคาม 44000
*** ติดต่อผู้นิพนธ์:** กนกวรรณ จารุกำจร คณะเภสัชศาสตร์ มหาวิทยาลัยขอนแก่น อำเภอเมือง จังหวัดขอนแก่น 40002
โทรศัพท์: 043-202305, โทรสาร: 043-202379, อีเมล์: kanok_ja@kku.ac.th

บทคัดย่อ

ผลกระทบของอาหารที่มีไขมันและฟรุคโตสปริมาณสูงต่อจุลกายวิภาคศาสตร์ของตับและโปรไฟล์ของไซโตโครมพี 450 3 เอ 11 ในตับหนูเมาส์

ณัฐรัตน์ เจียระพงษ์[1,2], วรัญญา จตุพรประเสริฐ[1,3], กนกวรรณ จารุกำจร[1,2]*

ว. เภสัชศาสตร์อีสาน 2560; 13(1) : 71-80

รับบทความ : 12 มกราคม 2560 **ตอบรับ :** 28 มีนาคม 2560

ฟรุคโตสและน้ำมันไฮโดรจิเนชันที่เป็นส่วนประกอบในอาหารแปรรูปหลายชนิดเป็นปัจจัยเสี่ยงของการพัฒนาเป็นโรคทางเมแทบอลิก ตับเป็นอวัยวะสำคัญหนึ่งที่ได้รับผลกระทบดังกล่าว รวมถึงไซโตโครมพี 450 3 เอ (CYP3A) ซึ่งเป็นเอนไซม์ที่มีมากในตับและทำหน้าที่หลักในการเมแทบอลิซึมยาทางคลินิก การศึกษานี้มีวัตถุประสงค์เพื่อประเมินลักษณะทางจุลกายวิภาคศาสตร์เนื้อเยื่อตับและรูปแบบของไซโตโครมพี 450 3เอ 11 (CYP3A11) ในตับของหนูถีบจักรที่ได้รับไขมันและฟรุคโตสปริมาณสูง **วิธีการทดลอง**: หนูถีบจักรสายพันธุ์ ICR เพศผู้ อายุ 7 สัปดาห์ (n=5) ได้รับการป้อนน้ำมันถั่วเหลืองไฮโดจิเนชัน (1 มล./วัน) ร่วมกับสารละลายฟรุคโตส (20%) ในน้ำดื่มเป็นเวลา 2, 4 และ 8 สัปดาห์ การแสดงออกของโปรตีนและสมรรถนะของเอนไซม์ CYP3A11 ทำการศึกษาด้วยเทคนิคทางภูมิคุ้มกันและปฏิกิริยาเอ็น-ดีเมธิเลชั่นของอีรีโธรมัยซิน ตามลำดับ **ผลการทดลอง**: นิวเคลียสของเซลล์ตับมีการเหี่ยวและย้อมติดสีเข้ม รวมถึงแตกออกเป็นชิ้นเล็กๆ ภายหลังได้รับไขมันและฟรุคโตสปริมาณสูงนาน 4 สัปดาห์ ขณะที่พบเซลล์คัฟเฟอร์จำนวนมากภายในไซนูซอยด์และการเกิดภาวะที่มีช่องว่างขนาดเล็กของเซลล์ตับในสัปดาห์ที่ 8 ของการเหนี่ยวนำ การเปลี่ยนแปลงของลักษณะทางจุลกายวิภาคศาสตร์เนื้อเยื่อตับสอดคล้องกับการเพิ่มขึ้นของระดับโปรตีนและสมรรถนะของเอนไซม์ CYP3A11 นอกจากนี้ยังพบการเพิ่มขึ้นของค่าระดับน้ำตาลในเลือดภายหลังการอดอาหารและค่าพื้นที่ใต้กราฟ (AUC) ของการทดสอบความทนทานต่อน้ำตาลในหนูที่ได้รับไขมันและฟรุคโตสปริมาณสูงในวันสุดท้ายของการศึกษาด้วย **สรุปผลการทดลอง:** การบริโภคไขมันและฟรุตโตสปริมาณสูงส่งผลกระทบเชิงลบโดยก่อให้เกิดจุลพยาธิวิทยาของเนื้อเยื่อตับร่วมกับการเหนี่ยวนำโปรไฟล์ของ CYP3A11 แม้ว่าจะไม่ส่งผลต่อการเพิ่มขึ้นของน้ำหนักตัวของหนูถีบจักร ดังนั้นผู้บริโภคอาหารที่มีไขมันและฟรุคโตสปริมาณสูงติดต่อกันเป็นเวลานานจึงอาจมีความเสี่ยงของการเกิดพยาธิสภาพของตับและภาวะการเหนี่ยวนำเอนไซม์ CYP3A11 ที่อาจส่งผลเปลี่ยนแปลงการเมแทบอลิซึมยาและเพิ่มความเสี่ยงของการเกิดอันตรกิริยาระหว่างยา

คำสำคัญ: ไซโตโครมพี 450 3 เอ 11, จุลกายวิภาคศาสตร์เนื้อเยื่อ, อาหารไขมันปริมาณสูง, อาหารฟรุคโตสปริมาณสูง

Impact of High Fat- and High Fructose-Diet on Murine Hepatic Histological Feature and CYP3A11 Profile

Nattharat Jearapong[1,2], Waranya Chatuphonprasert[1,3], Kanokwan Jarukamjorn[1,2]*

[1] Research Group for Pharmaceutical Activities of Natural Products using Pharmaceutical Biotechnology (PANPB), Khon Kaen University, Khon Kaen 40002 Thailand

[2] Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen 40002 Thailand

[3] Faculty of Medicine, Mahasarakham University, Mahasarakham 44000 Thailand

***Corresponding author**: Kanokwan Jarukamjorn, Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen 40002 Thailand.
Tel: 043-202305, Fax: 043-202379, Email: kanok_ja@kku.ac.th

Abstract

Impact of High Fat- and High Fructose-Diet on Murine Hepatic Histological Feature and CYP3A11 Profile

Nattharat Jearapong[1,2], Waranya Chatuphonprasert[1,3], Kanokwan Jarukamjorn[1,2]*

IJPS, 2017; 13(1) : 71-80

Received : 12 January 2017 **Accepted :** 28 March 2017

Fructose and hydrogenated oil appeared as ingredients in several processed food are risk factors to develop metabolic diseases. Liver is a major organ which is impacted by these diets. Cytochrome P450 3A (CYP3A) is a major hepatic cytochrome P450 enzyme that takes responsible for metabolism of almost clinical drugs. The present study aimed to investigate hepatic histology and CYP3A11 pattern in mice fed with high fat- and high fructose-diet (HFFD). **Methods:** Seven-week-old male ICR mice (n= 5) were intragastrically administered hydrogenated soybean oil (1 mL/ day) with free access to 20% (w/ v) aqueous fructose solution for 2, 4, and 8 weeks. The protein expression and catalytic activity of CYP3A11 were determined employing immunoblotting techniques and a specific reaction of erythromycin *N*-demethylation (ENDM), respectively. **Results:** Karyorrhexis and pyknosis of nuclei were detected after the HFFD feeding for 4 weeks while Kupffer cells in sinusoid and microvesicular hepatocytes were observed after 8 weeks of the induction. The change of hepatic histological features was correlated with an increase in the protein level and catalytic activity of CYP3A11. Moreover, the fasting blood glucose level and AUC in the oral glucose tolerance test were increased in the HFFD-fed mice at the end of the study. **Conclusion:** High fat- and high fructose-consumption worsen the physiological feature of hepatic tissue along with induction of murine CYP3A11 profile, though it did not affect the weight gaining. Hence, a person with long-term consumption of high fat- and/or high fructose-diet could have a risk of hepatic pathology and induction of CYP3A11 expression, leading to alter drug metabolism and extensive risk of drug interaction.

Keywords: CYP3A11, Histology, High fat diet, High fructose diet

Introduction

Refined carbohydrate sources such as high fructose syrup and hydrogenated vegetable oil are now generally appeared in modern supermarket stores as a component in many processed foods and sugar-sweetened beverages. This type of fat is added to increase shelf life, and maintain stability and taste of the processed foods and baking products (e.g., pastries, doughnuts, biscuits, chips, candy, and cakes) (Akoh, 1998). In addition, high fructose syrup was gained interested from the food and beverage industries as an alternative to refined sugar from sugarcanes (White *et al.*, 2015). These syrups type are made by conversion of starch (e.g. corn and tapioca root) to monosaccharide

sugars, which have a higher content of fructose than the normal refine sugar does. The increasing consumption of sugar-added beverage is problems in many countries include Thailand. The high amounts of total sugar in non-alcohol beverages (e.g. energy drinks, beauty drinks, fruit juice, carbonated beverages) in Thailand was reported, which were found 4-75% higher than the labels (Weerawatanakorn, 2013). Excessive consumption of fat and carbohydrate diet proved to develop metabolic risk factors such as dyslipidemia, obesity, diabetes mellitus (Yang *et al.*, 2012; de Castro *et al.*, 2013), and hepatic pathology includes non-alcoholic fatty liver disease (NAFLD) (Ouyang *et al.*, 2008).

Liver is a crucial organ for metabolism of compounds before distribution to other organs. Among the other enzymes, microsomal cytochrome P450s (CYP450s) are superfamily of monooxygenase enzymes that play major roles in oxidative biotransformation of endogenous compounds and xenobiotic substances such as drugs and environmental chemicals. CYP450s are arranged into families and subfamilies depending on amino acid sequence identity (Nebert and Russell, 2002). Human CYP3A4 is a CYP450 isoform that highly expressed in liver and known as a major metabolizing enzyme of several clinical drugs. Since CYP3A takes responsible for metabolism of several drugs or herbs, alteration of CYP450 expression might affect their efficacy or toxicity (Zanger and Schwab, 2013). In addition, pathology of the liver can lead to modification of the CYP3A activity (Lu and Cederbaum, 2008; Fisher *et al.*, 2009).

Some liver diseases related with dietary habits such as NAFLD, which is a condition characterized by excessive accumulation of lipids, especially triglycerides in hepatocytes. NAFLD is associated with metabolic risk factors including obesity and diabetes mellitus. Clinical manifestations of NAFLD range from simple steatosis to nonalcoholic steatohepatitis (NASH) with the presence of inflammation, hepatocyte injury, ballooning degeneration, sinusoidal fibrosis, and/or Mallory bodies insulin resistance, oxidative stress, and mitochondrial dysfunction. The latter event leads to decrease of hepatic ATP production and the triggering of necro-inflammation by inflammatory cytokines (Rolo *et al.*, 2012). Since liver is an important organ that related to the first-pass metabolism of food and drugs. Therefore, the present study aims to investigate the effect of high fat- and high fructose-diet (HFFD) on the biochemical parameters including the blood glucose, cholesterol, and triglyceride levels, the body weight profile, histological features of the livers, and alteration of the hepatic CYP3A11 expression profile and the specific CYP3A11 enzyme activity, erythromycin *N*-demethylase, in the HFFD-fed mouse model.

Materials and Methods

Chemicals

Bradford solution was purchased from BioRad (Hercules, CA, USA). Rabbit antibody to CYP3A (299203) was obtained from Daiichi Pure Chemicals (Tokyo, Japan). Vectastain® Elite ABC Kit and 3, 3'-diaminobenzidine (DAB) substrate kit were supplied by Vector Laboratories (Burlingame, CA). Erythromycin and NADPH were purchased from Sigma-Aldrich Chemical (St. Louis, MO). Xylene (Fisher Scientific, UK), Permount® (Fisher Scientific, UK), Paramat extra pastillated Gurr® (BDH Lab Supplies, Poole, UK), Eosin Y 1% aqueous solution (Bio Optica, Italy), and Mayer's hematoxylin (Bio Optica, Italy) were purchased for tissue fixation and staining. All other laboratory chemicals were of the highest purity available from commercial suppliers.

Animals

Seven-week-old ICR mice were supplied by the National Laboratory Animal Center (Mahidol University, Nakhon Pathom, Thailand) and were housed in the Northeast Laboratory Animal Center (Khon Kaen University, Khon Kaen, Thailand) under the supervision of a certified laboratory veterinarian. The animal handling and research protocols were approved by the Animal Ethics Committee for Use and Care of Khon Kaen University, Khon Kaen, Thailand (Approval No. AEKKU 92/2555). At all times, the animals were housed on wood chip bedding in polysulfone cages with a 12-h dark/light cycle under a controlled temperature (23±2°C) and humidity (45±2%). All mice were acclimated for 1 week before separating into 4 groups and starting the intervention. The regular diet group (RD) was freely accessed to commercial mouse diet and drinking water while the high fat- and high fructose-diet groups

(HFFD) were additionally daily given hydrogenated soybean oil (1 mL/day, i.g.; 44.1% (w/w) saturated fat and 0.2% (w/w) *tran*-fatty acid, certified by Institute of Nutrition, Mahidol University, Thailand) with free access to 20% (w/v) aqueous fructose solution for 2, 4, and 8 weeks (n=5 for each group). The mouse body weight was recorded 3 times/week. All mice were euthanized 24 h after the last treatments for collecting fresh blood and livers. The organs were kept at -80°C prior to further analysis.

Oral glucose tolerance test (OGTT)

The protocol of OGTT was based on the method described previously (Chatuphonprasert *et al.*, 2013). After 8 weeks of the continuous diet feeding, both of the RD and the HFFD mice were tested for oral glucose tolerance. Briefly, the mice were fasted overnight and then they were intragastrically administered glucose solution in the dose of 2 g/kg body weight. The tail-vein-blood was collected at the time point of -30, 0, 30, 60 and 120 min after gavage. Glucose concentration was monitored using the ACCU-CHECK® Performa with a specific test strip. The area under the curve (AUC) was determined using the plots between glucose concentration and the blood sampling time.

Tissue fixation, processing, and staining

A fragment of liver tissue was fixed by immersion in 10% (v/v) neutral-buffered-formalin overnight, followed by dehydration and paraffin-embedding (Jearapong *et al.*, 2015). After section, the embedded tissue was stained with hematoxylin and eosin (H&E) for evaluation of histologic features using an inverted light microscope (Nikon Eclipse TS100, Japan) at 200× and 400× magnification.

Preparation of hepatic microsomal fraction

Hepatic microsomal fraction was prepared by ultracentrifugation technique (Chatuphonprasert and Jarukamjorn, 2012). Briefly, the homogenized liver was centrifuged at 10,000×*g* at 4°C for 10 min followed by ultracentrifugation at 104,000×*g* at 4°C for 60 min. The pellet was collected and reconstituted with ice-cold distilled water and kept at -80°C until being analyzed. The method of Bradford was employed for determination of protein content by measuring the protein-dye complex at a wavelength of 595 nm using bovine serum albumin (BSA) as a standard (Bradford, 1976).

Assessment of CYP3A11 protein expression by immunoblotting

The expression of CYP3A11 protein was accessed by Western blot analysis (Jearapong *et al.*, 2015). Briefly, twenty micrograms of microsomal protein were resolved by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis before transferred to a nitrocellulose membrane. After blocking at 4°C overnight in phosphate buffered saline (PBS) containing 0.03% (v/v) Tween-20 and 1% (w/v) BSA, CYP3A11 was detected using rabbit polyclonal antibody against CYP3A11. Then, the blot was incubated with biotinylated goat anti-rabbit antibody followed by biotinylated horseradish H avidin complex. The antigen-antibody complex was visualized with DAB and hydrogen peroxide. The specific CYP3A11 bands were densitometrically quantified and expressed as arbitrary units corresponding to the signal intensity using ImageJ 1.47V program (NIH, Bethesda, Maryland, USA).

Determination of erythromycin *N*-demethylase activity

The catalytic activity of CYP3A enzyme was determined using erythromycin *N*-demethylation (ENDM) as described previously (Chatuphonprasert and Jarukamjorn, 2012). Briefly,15 mM $MgCl_2$, 1 mM erythromycin, 70 mM PBS (pH7.4), and 1 mM NADPH were mixed and incubated at 37°C for 20 min with microsomal protein. The reaction was terminated by equal volume of 12.5% TCA and followed by adding Nash reagent. The rate of formaldehyde formation was measured spectrophotometrically at a wavelength of 405 nm compared with the formaldehyde standard.

Statistical analysis

For statistical analysis, the results comparison between 2, 4, 8 weeks of the HFFD against the RD were analyzed using one-way analysis of variance (ANOVA) followed by Tukey *post hoc* test (version 11.5; SPSS Inc., Chicago, IL) while the comparison between the RD and the HFFD at 8 weeks were employing Independent Sample T-Test. *P*-values less than 0.05 were considered statistically significant.

Results

Effect of high fat and high fructose diet on body weight and biochemical parameters

The body weight of mice was recorded compared between the regular diet (RD) and the high fat- and high fructose-diet (HFFD) groups as shown in Fig.1 and Table 1. There was no significant difference of the weight gaining between the RD- and the HFFD-fed mice through 8 weeks of the study. The blood biochemical parameters were measured at the end of the study. The blood glucose level (112±7 mg/dL) of the HFFD mice was significantly elevated compared to the RD mice (91±2 mg/dL). The lipid profiles including cholesterol and triglyceride levels were increased from the baseline. The blood cholesterol levels between the HFFD and the RD group did not significantly different. Unexpectedly, the HFFD group demonstrated lower triglyceride level (98±7 mg/dL) than those of the RD (155±12 mg/dL).

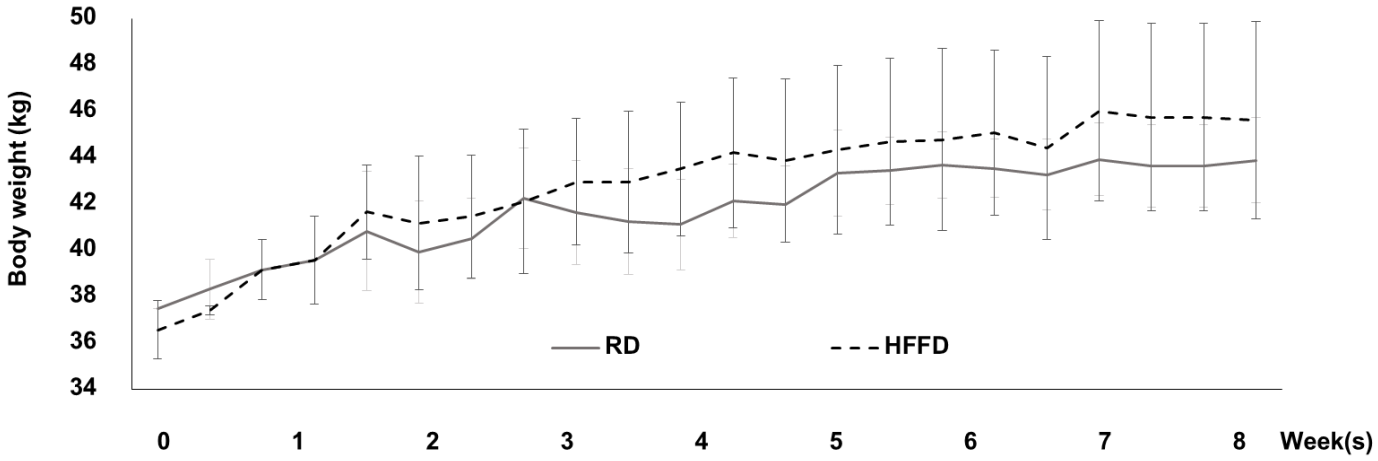

Figure. 1 Body weight profile of the male ICR mice fed with the regular diet (RD) and the high fat and high fructose diet (HFFD) during 8 weeks of study period.

Table 1 Body weight and blood biochemical parameters of the male ICR mice fed with the regular diet (RD) and the high fat and high fructose diet (HFFD) for 8 weeks

	Baseline	RD	HFFD
Body weight (kg)	38 ± 2	44 ± 2	46 ± 4
Glucose (mg/dL)	91 ± 11	91 ± 2	112 ± 7*
Cholesterol (mg/dL)	< 150	175 ± 3	168 ± 3
Triglyceride (mg/dL)	< 70	155 ± 12	98 ± 7*

Note. The data is expressed as mean ± SD. A significant difference was determined by Independent T-Test. *p<0.05.

Effect of high fat- and high fructose-diet on the oral glucose tolerance test

The oral glucose tolerance test (OGTT) profiles of the RD- and the HFFD-fed mice were shown (Fig. 2A), which were plotted between the level of tail-tip blood glucose toward the time of glucose administration. The HFFD affected the fasting blood glucose level which shown at the beginning of the plot. The blood glucose levels of both groups were similarly raised to a maximum level approximately 200-220 mg/dL at 30 min. However, the HFFD-fed mice had a tendency of higher blood glucose level after 60 min of the glucose administration and significantly did at the last sampling time point of 120 min compared to the RD. Corresponding to the OGTT, the HFFD significantly increased the glucose AUC value compared to the RD (Fig. 2B).

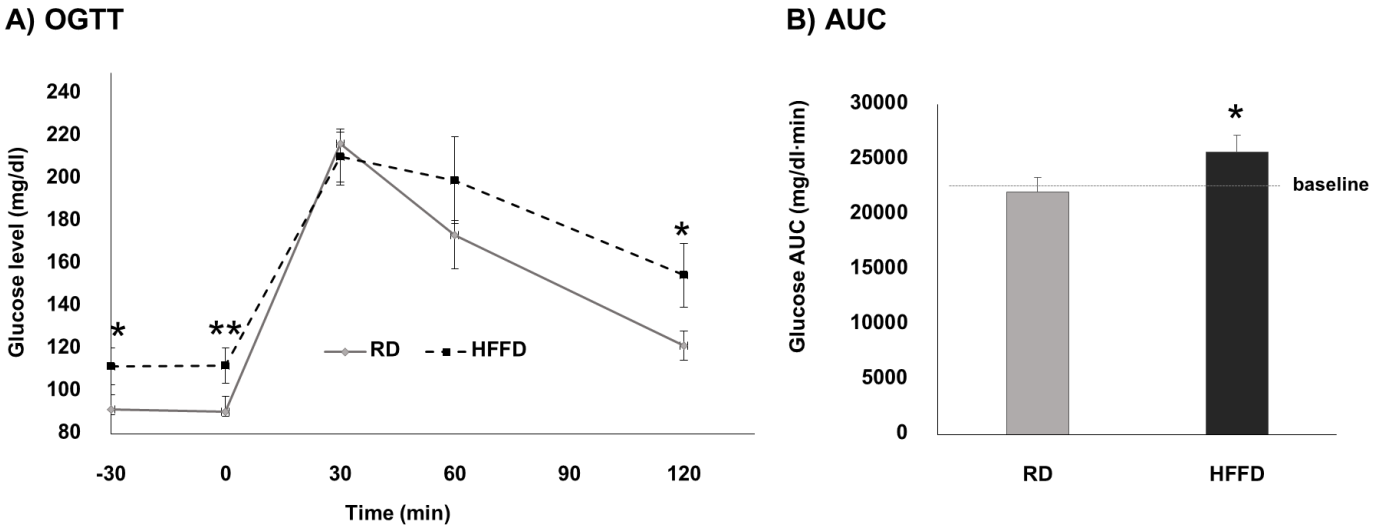

Figure. 2 Effect of the high fat and high fructose diet (HFFD) on the oral glucose tolerance test (OGTT) in the male ICR mice after 8 weeks of the study. The OGTT was compared between the HFFD- and the regular diet-fed mice (RD) as shown in A) the tail tip-blood glucose level (mg/dL) against the sampling times at -30, 0, 30, 60, and 120 minutes of the glucose administration time and B) the area under the curve (AUC) value of glucose (mg/dL·min). A significant difference was determined by Independent T-Test. *p<0.05, **p<0.01.

Effect of high fat and high fructose diet on the hepatic histopathological features

Micrographs of the H&E stained mouse hepatic tissues are shown in Fig. 3. Comparison to the RD-fed mice (Fig. 3A and 3B), the hepatic tissue of the 2-week-HFFD-fed mice were noted normal histomorphology (Fig. 3C and 3D). After the 4-week-HFFD-feeding, additional minor changes of hepatocytes were detected including karyorrhexis of nuclei (Fig. 3E and 3F). And after the 8-week-HFFD-feeding, the H&E stained of hepatic tissues exhibited microvesicular changes with centrally located nuclei, which some were pyknosis or karyorrhexis (Fig. 3G and 3H), and the hepatocytes exhibited more vacuolated, which were shown in clear non-stained vacuoles compared to the hepatic tissue of the RD-fed mice (Fig. 3A and 3B). At the end of the study, the loss of hepatic architecture and swollen hepatocytes were also detected, which sinusoids appeared as narrow spaces lined from enlarging hepatocytes and few Kupffer cells in hepatic sinusoids.

Effect of high fat and high fructose diet on the CYP3A11 protein expression and the erythromycin *N*-demethylase activity

The effect of continuous HFFD consumption for 8 weeks on the levels of CYP3A11 protein expression was investigated in the mouse livers (Fig. 4A). Though no significant change was detected at the first 2 weeks of HFFD consumption, the expression of CYP3A11 protein was significantly up-regulated after the 4 and 8 weeks of the HFFD intervention. A dose- and duration-dependent pattern was noted. The CYP3A activity, as assessed by erythromycin *N*-demethylation, was remained unchanged till 4 weeks of the HFFD intervention (Fig. 4B), and the extensive increase of the CYP3A activity was observed after the 8 weeks of the HFFD consumption, in accord with the levels of CYP3A11 protein.

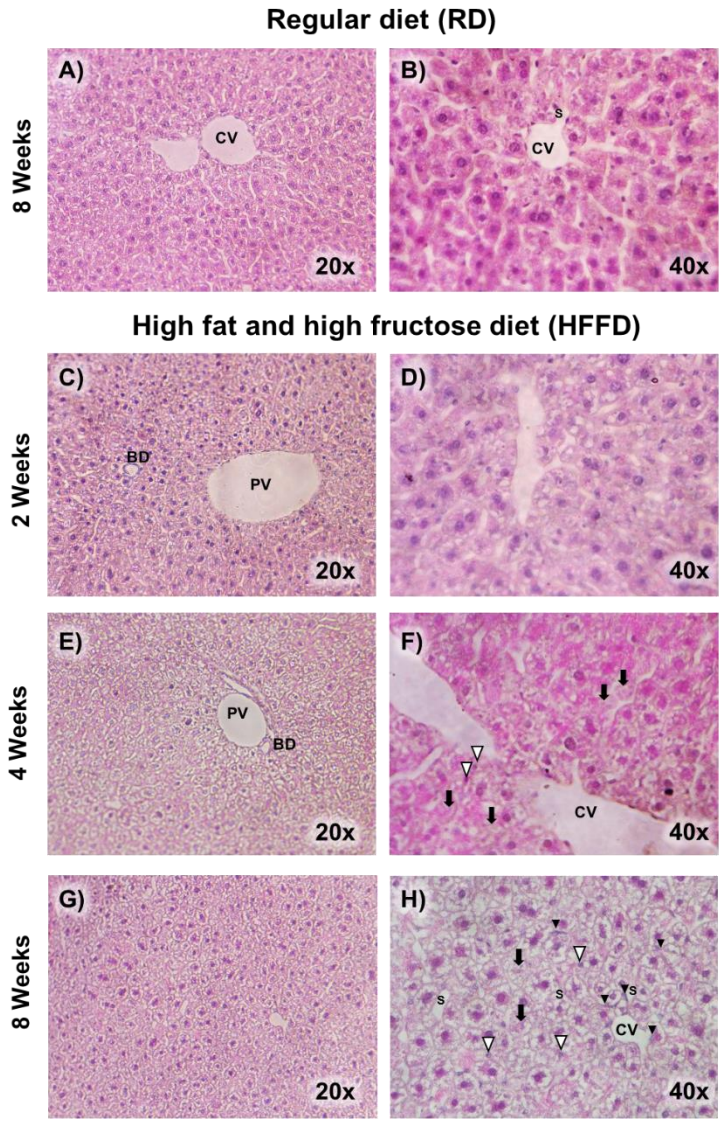

Figure. 3 Histological examination of hematoxylin and eosin (H&E) stained liver tissues of the male ICR mice. The representative images include livers from the mice fed with regular diet (RD) (A and B), and livers from the mice fed with high fat and high fructose diet (HFFD) for 2 (C and D), 4 (E and F), and 8 weeks (G and H), respectively. Micrographs are shown at a final magnification of 200 (Left side) and 400 (Right side), respectively. The black arrow (⬇) indicates karyorrhexis of nuclei; The black arrow head (▼) indicates Kupffer cell; The white arrow head (▽) indicates pyknosis of nuclei. BD, bile duct; CV, central vein; PV, portal vein; S, sinusoid.

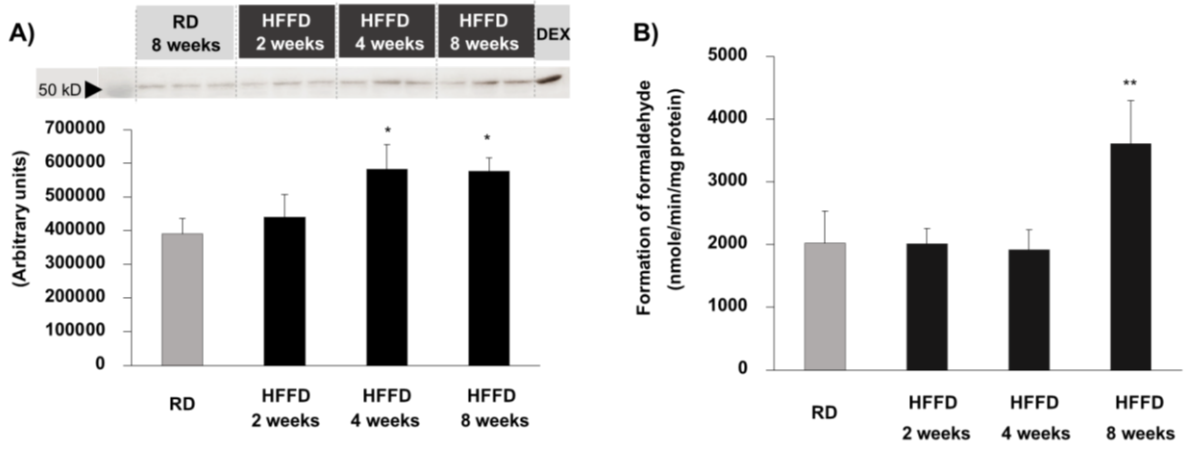

Figure. 4 Effect of high fat and high fructose diet (HFFD) on the protein expression and catalytic activity of CYP3A11 in the male ICR mice. (A) The expression of CYP3A11 protein was detected employing a rabbit polyclonal antibody against CYP3A11 (n=3) and (B) The catalytic activity of CYP3A was assessed using the reaction of erythromycin *N*-demethylation (n=6-10) from 3 independent experiments. A significant difference was determined by one-way analysis of variance (ANOVA) followed by Tukey *post hoc* test. *p<0.05, **p<0.001.

Discussion

The present study demonstrated the impacts of continuous consumption of the diet contained high amount of fat and fructose on the blood glucose level in the OGTT test, histopathological features of the livers, and alteration of the hepatic CYP3A11 profile. Though body weight of the mice with continuous feeding of HFFD for 8 weeks was not different from those of the RD mice, the HFFD allowed development of prediabetes, such as significant increases in the fasting blood glucose level and the blood glucose AUC value in the OGTT. Correspondingly, 2-month-fructose-rich diet fed- C57Bl/ 6 mice developed transient metabolic disorders and reduction of insulin sensitivity with normal weight gain (Tillman *et al.*, 2014). The evidence resulted from expansion of adipose cells, differently from mice fed with high fat which developed long term weight gains (Podrini *et al.*, 2013). Form our results, more than 50% of the caloric/ energy intake came from fructose source, which might be the reason of not develop obesity in the HFFD mice.

The HFFD fed-mice in the present study revealed an early signs of liver injury after 8 weeks of induction by showing ballooning degeneration including cell enlargement, cytoplasmic clearing and microvesicular hepatocytes. The H&E stained liver tissues showed the liver injury associated inflammation by observing more Kupffer cells in the hepatic sinusoids compared to the RD-fed mice. Kupffer cell plays an important role to initiate a response to liver injury by rapidly producing and releasing cytokines and chemokines (e.g. interleukin (IL)-1β, tumor necrosis factor (TNF)-α, CCL2, and CCL5), and a variety of biologically active mediators such as eicosanoids, proteolytic enzymes, reactive oxygen species (ROS), and nitric oxide, which trigger and promote the progression of liver injury. These lead to collagen synthesis and fibrosis, which further cause the loss of liver functions (Baffy, 2009; Ju and Tacke, 2016). Moreover, the signs of hepatocytes undergone necrosis with the changes of nucleus included karyorrhexis and pyknosis were found, correlated with the duration of HFFD feeding. The changes of nucleus were observed after 4 weeks of the HFFD induction while these features were not seen in the livers of RD-fed mice. However, the hepatocytes have not demonstrated the pattern of macrovesicular steatosis yet. These observations did not correspond with a previous study employed C57BL/6 mice (Tetri *et al.*, 2008) which was more susceptible strain to induce steatosis but not optimal represented normal population. Besides the different mouse strain, the previous study employed a commercial trans-fat customized diet with high fructose corn syrup in gel water

with a longer administered duration. The theory of dietary imbalance including high fat and/or high fructose intake has been extensively reviewed as a contributing factor in NAFLD and NASH (Zivkovic *et al.*, 2007; Lim *et al.*, 2010; Nomura and Yamanouchi, 2012). The pathogenesis of NAFLD and NASH involves inflammation and oxidative stress associated with lipid peroxidation and increasing numbers of cytokines (Jarrar *et al.*, 2008; Liu *et al.*, 2016)

In addition to hepatic histology, the expression of CYP3A11 protein and its catalytic activity were evaluated. The overexpression of microsomal CYP3A11 protein and its catalytic activity were correlated to the hepatic histological features and the duration of HFFD intake. Mouse CYP3A11 is human CYP3A4 homologous, and both are highly abundant in the livers and responsible for biotransformation of wide ranges of substances (Hart *et al.*, 2009). CYP450s were claimed as a source of ROS form oxidative reaction. Induction of CYP450s by phenobarbital, a CYP3A inducer, in rodents caused oxidative stress and worsen liver injury by increasing of ROS formation while attenuation of protective systems (Minamiyama *et al.*, 2004; Dostalek *et al.*, 2008). Therefore, the up-regulation of CYP3A expression and its catalytic activity might bring the extra-formation of ROS, which further worsen the liver pathology. However, the HFFD-feeding for 8 weeks was not enough to induce the development of dyslipidemia. No significant change of the total blood cholesterol with a lower level of triglyceride in the HFFD-fed mice was noted compared to the RD-fed mice.

In conclusion, the prediabetes state and hepatic histological changes including narrowing sinusoids and microvesicular, clear cytoplasm, and enlarge hepatocytes were time-dependently occurred in our *in vivo* HFFD-fed mouse model, followed by alteration of hepatic CYP3A11 profile at both the protein level and the catalytic activity. Therefore, the excessive high fat and/or high fructose intake for long period might be of concern for a risk of drug metabolism and interaction.

Acknowledgements

NJ expresses sincere gratitude to the Royal Golden Jubilee Ph.D. program, Thailand Research Fund, Thailand, for the scholarship (5.RD.KK/53/F.1) National Research Council of Thailand (KKU-NRCT 001/2560), and the Research Group for Pharmaceutical Activities of Natural Products using Pharmaceutical Biotechnology (PANPB), Faculty of Pharmaceutical Sciences, Khon Kaen University, Thailand, for the research grant and facilities.

Conflict of interest

The authors declare no conflict of interest.

References

Akoh CC. Fat Replacers. *Food Technol* 1998; 52(3): 47-53.

Baffy G. Kupffer cells in non-alcoholic fatty liver disease: the emerging view. *J Hepatol* 2009; 51(1): 212-223.

Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976; 72: 248-254.

Chatuphonprasert W, Jarukamjorn K. Impact of six fruits--banana, guava, mangosteen, pineapple, ripe mango and ripe papaya--on murine hepatic cytochrome P450 activities. *J Appl Toxicol* 2012; 32(12): 994-1001.

Chatuphonprasert W, Lao-Ong T, Jarukamjorn K. Improvement of superoxide dismutase and catalase in streptozotocin-nicotinamide-induced type 2-diabetes in mice by berberine and glibenclamide. *Pharm Biol* 2013; 52(4): 419-427.

de Castro UG, dos Santos RA, Silva ME*, et al.* Age-dependent effect of high-fructose and high-fat diets on lipid metabolism and lipid accumulation in liver and kidney of rats. *Lipids Health Dis* 2013; 12: 136.

Dostalek M, Hardy KD, Milne GL*, et al.* Development of oxidative stress by cytochrome P450 induction in rodents is selective for barbiturates and related to loss of pyridine nucleotide-dependent protective systems. *J Biol Chem* 2008; 283(25): 17147-17157.

Fisher CD, Lickteig AJ, Augustine LM*, et al.* Hepatic cytochrome P450 enzyme alterations in humans with progressive stages of nonalcoholic fatty liver disease. *Drug Metab Dispos* 2009; 37(10): 2087-2094.

Hart SN, Cui Y, Klaassen CD, Zhong XB. Three patterns of cytochrome P450 gene expression during liver maturation in mice. *Drug Metab. Dispos.* 2009; 37(1): 116-121.

Jarrar MH, Baranova A, Collantes R*, et al.* Adipokines and cytokines in non-alcoholic fatty liver disease. *Aliment Pharmacol Ther* 2008; 27(5): 412-421.

Jearapong N, Chatuphonprasert W, Jarukamjorn K. Effect of tetrahydrocurcumin on the profiles of drug-metabolizing enzymes induced by a high fat and high fructose diet in mice. *Chem Biol Interact* 2015; 239: 67-75.

Ju C, Tacke F. Hepatic macrophages in homeostasis and liver diseases: from pathogenesis to novel therapeutic strategies. *Cell Mol Immunol* 2016;13(3): 316-327.

Lim JS, Mietus-Snyder M, Valente A, Schwarz JM, Lustig RH. The role of fructose in the pathogenesis of NAFLD and the metabolic syndrome. *Nat Rev Gastroenterol Hepatol* 2010; 7(5): 251-264.

Liu W, Baker RD, Bhatia T, Zhu L, Baker SS. Pathogenesis of nonalcoholic steatohepatitis. *Cell Mol Life Sci* 2016; 73(10): 1969-1987.

Lu Y, Cederbaum AI. CYP2E1 and oxidative liver injury by alcohol. *Free Radic Biol Med* 2008; 44(5): 723-738.

Minamiyama Y, Takemura S, Toyokuni S*, et al.* CYP3A induction aggravates endotoxemic liver injury via reactive oxygen species in male rats. *Free Radic Biol Med* 2004; 37(5): 703-712.

Nebert DW, Russell DW. Clinical importance of the cytochromes P450. *Lancet* 2002; 360(9340): 1155-1162.

Nomura K, Yamanouchi T. The role of fructose-enriched diets in mechanisms of nonalcoholic fatty liver disease. *J Nutr Biochem* 2012; 23(3): 203-208.

Ouyang X, Cirillo P, Sautin Y*, et al.* Fructose consumption as a risk factor for non-alcoholic fatty liver disease. *J Hepatol* 2008; 48(6): 993-999.

Podrini C, Cambridge EL, Lelliott CJ*, et al.* High-fat feeding rapidly induces obesity and lipid derangements in C57BL/6N mice. *Mamm Genome* 2013; 24(5-6): 240-251.

Rolo AP, Teodoro JS, Palmeira CM. Role of oxidative stress in the pathogenesis of nonalcoholic steatohepatitis. *Free Radic Biol Med* 2012; 52(1): 59-69.

Tetri LH, Basaranoglu M, Brunt EM, Yerian LM, Neuschwander-Tetri BA. Severe NAFLD with hepatic necroinflammatory changes in mice fed trans fats and a high-fructose corn syrup equivalent. *Am J Physiol Gastrointest Liver Physiol* 2008; 295(5): G987-995.

Tillman EJ, Morgan DA, Rahmouni K, Swoap SJ. Three Months of High-Fructose Feeding Fails to Induce Excessive Weight Gain or Leptin Resistance in Mice. *PLoS One* 2014; 9(9) : e107206. doi: 10. 1371/ journal.pone.0107206

Weerawatanakorn M. Dicarbonyl compounds and sugar contents of Thai commercial beverages. *Songklanakarin J Sci Technol* 2013; 35(6): 631-639.

White JS, Hobbs LJ, Fernandez S. Fructose content and composition of commercial HFCS- sweetened carbonated beverages. *Int J Obes (Lond)* 2015; 39(1): 176-182.

Yang ZH, Miyahara H, Takeo J, Katayama M. Diet high in fat and sucrose induces rapid onset of obesity-related metabolic syndrome partly through rapid response of genes involved in lipogenesis, insulin signalling and inflammation in mice. *Diabetol Metab Syndr* 2012; 4(1): 32.

Zanger UM, Schwab M. Cytochrome P450 enzymes in drug metabolism: regulation of gene expression, enzyme activities, and impact of genetic variation. *Pharmacol Ther* 2013; 138(1): 103-141.

Zivkovic AM, German JB, Sanyal AJ. Comparative review of diets for the metabolic syndrome: implications for nonalcoholic fatty liver disease. *Am J Clin Nutr* 2007; 86(2): 285-300.