수의학석사 학위논문

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지도교수 성제경

이 논문을 수의학석사 학위논문으로 제출함

2016년 4월

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수의학과 수의생명과학 전공

María Raquel Rojas Jiménez

María Raquel Rojas Jiménez의 석사학위논문을 인준함

2016년 6월

위 원 장	**윤 여 성**	**(인)**
부 위 원 장	**성 제 경**	**(인)**
위 원	**이 미 옥**	**(인)**

Abstract

Differential effects of a high fat or high carbohydrate diet on the onset of liver cancer in HBx TG mice

María Raquel Rojas Jiménez

Department of Veterinary Biomedical Sciences,

The Graduate School

Seoul National University

Little is known about the impact of dietary factors on the progression of liver cancer. Thus, the goal of this study was to determine the liver-related consequences of long-term diet-induced obesity on the onset of hepatocellular carcinoma among different disease phases in the HBx TG mouse; model of hepatocellular carcinoma. To investigate the tumorigenic effect of a high fat (HFD) or high carbohydrate diet (HCD) in HBx TG mouse livers, mice were fed a high fat or high carbohydrate diet for 28, 36, or 40 weeks and age-matched mice were sacrificed at 12 months old, after the diet challenge. Hepatic steatosis was apparent and serum alanine aminotransferase and aspartate aminotransferase levels were significantly increased in HFD or HCD-fed HBx TG mice compared

to mice fed regular chow. AKT and ERK showed greater activation in the HCD group compared to HFD and regular chow-fed groups. Together, these data indicated that chronic HCD consumption can produce more severe liver damage in HBx TG mice, which can potentially accelerate the onset of tumorigenesis by upregulating pERK-mediated ER stress. Consumption of excess fats or carbohydrates induced biochemical and pathological changes that could worsen the HCC. Food intake imbalance alone may not be sufficient to cause HCC, but it may be a risk factor that impairs liver cells functions and fosters tumor occurrence. Thus, diet may be an important and potentially modifiable determinant of liver cancer.

Keywords: Hepatocellular carcinoma; HBx TG mouse; High fat diet; High carbohydrate diet; pERK

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HCC	**Hepatocellular carcinoma**
HFD	**High fat diet**
HCD	**High carbohydrate diet**
RC	**Regular chow**
HBV	**Hepatitis B virus**
HCV	**Hepatitis C virus**
HBx	**Hepatitis B virus X protein**
HBx TG	**Hepatitis B virus X protein transgenic mouse**
PPARγ	**Peroxisome proliferator-activated receptor gamma**
AST	**Aspartate aminotransferase**
ALT	**Alanine aminotransferase**
TCHO	**Total cholesterol**
IPGTT	**Intraperitoneal glucose tolerance test**
eWAT	**Epididymal white adipose tissue**

PCNA	**Proliferating cell nuclear antigen**
ERK	**Extracellular signal-regulated kinases**
pERK	**Phospho extracellular signal-regulated kinases**
AKT	**Protein kinase B**
NAFLD	**Nonalcoholic fatty liver disease**
ER	**Endoplasmic reticulum**
ER^{UPR}	**Endoplasmic reticulum induced unfolded protein response**

Introduction

Accumulating evidence from epidemiological and experimental studies has indicated that liver cancer is the third leading cause of cancer-related deaths worldwide, and hepatocellular carcinoma (HCC) is the most frequent and aggressive type of primary liver tumor (Jemal et al., 2011).

Dietary factors are likely to be important determinants in the development of hepatic steatosis and its progression to hepatocellular carcinoma for several reasons. The first issue is that dietary factors are key, and probably causative, risk factors for obesity, insulin resistance, and diabetes, which are the most important, known risk factors for hepatic steatosis. The second issue is that dietary lipid composition influences both the quantity and composition of lipids that are delivered to the liver and incorporated into hepatocyte lipid droplets (Ioannou et al., 2009).

Dietary nutrients may also cause hepatic injury through pathways that do not involve the development or progression of hepatic steatosis. Carbohydrates, proteins, and lipids are all extensively metabolized in the liver and it is conceivable that they may influence the progression of chronic liver disease either positively or negatively. In hepatitis B virus (HBV) transgenic mice, a diet low in animal protein was associated with decreased liver injury and decreased incidence of hepatocellular carcinoma (Cheng et al., 1997; Hu et al., 1997).

Chronic hepatitis B virus (HBV) infection is one of the major causes of liver diseases like hepatitis, cirrhosis and hepatocellular carcinoma (Lee, 1997). Nowadays, emerging evidence from epidemiological and experimental studies suggests that chronic infection with HBV infection or HCV is associated with hepatic steatosis (Gordon et al., 2005; Yoon and Hu, 2006). The X protein of HBV (HBx) is a multifunctional regulator that modulates transcription, signal transduction, cell-cycle progression, protein degradation, apoptosis, and genetic stability by directly or indirectly interacting with host factors (Tang et al., 2006). Furthermore, HBx causes hepatic lipid deposition by inhibiting apolipoprotein B secretion (Kang et al., 2004). A previous study showed that increased HBx expression can cause lipid accumulation in hepatocytes, likely mediated by sterol regulatory element binding protein 1 and peroxisome proliferator-activated receptor (PPAR) γ (Kim et al., 2007).

Our rationale for testing the HFD was to challenge HBx TG mice with excess substrate to promote fatty acid build-ups, because previous studies reported a correlation between HBx and lipid accumulation or insulin resistance (Kim et al., 2007), and HBx promotes hepatic inflammation induced by fatty acids (Cho et al., 2014).

The deleterious effect of a high saturated fat diet is well documented. However, the influence of distinct types of hypercaloric diets, such as high carbohydrate diets on the development of liver cancer in HBx TG mice has not been investigated to our knowledge. Thus, the present study was designed to examine

the liver-related consequences of long-term diet-induced obesity and the effects of these liver changes on the onset of hepatocellular carcinoma among different disease phases in the HBx TG mouse model of hepatocellular carcinoma.

Materials and Methods

Animal models

Forty-three male HBx TG mice were utilized in this study, weighing 15-34 g. The production of the HBx transgenic mice used in this study has been described previously (Yu et al., 1999). All mice were housed in a specific pathogen-free animal facility under appropiate temperature (24±2°C) and humidity (60%), maintained on a 12 h light/dark cycle, and allowed *ad libitum* access to a balanced laboratory diet (LabDiet, Richmond, IN, USA) and water. All animal procedures were conducted in accordance with the "Guide for Animal Experiments" (edited by Korean Academy of Medical Sciences) and were approved by the Institutional Animal Care and Use Committee (IACUC) of Seoul National University (Permit Number: SNU-131028-2)

Experimental design and diets composition

HBx TG male mice at five, three, and two months of age were fed either RC (LabDiet), HFD (60% kcal fat), or HCD (70% kcal carbohydrate) for 28, 36, and 40 weeks respectively. The composition of each diet is shown in (Table 1). Mice body weights were determined weekly. Mice were age-matched and sacrificed at 12 months old, after the diet challenge. The experimental design is shown in (Fig. 1).

Table 1. Composition of experimental diets

Ingredients		High fat[a]		Ingredients		High CHO[b]	
		g%	kcal %			g%	kcal %
Protein		19.2	20	Protein		17.7	17.8
Carbohydrate		67.3	70	Carbohydrate		70	70.4
Fat		4.3	10	Fat		5.2	11.8
	Total		**100**		**Total**		**100**
	kcal/g	**3.85**			**kcal/g**	**4**	
		g	kcal				g
Casein, 30 Mesh		200	800	Casein			200
L-Cystine		3	12	DL-Methionine			3
Corn Starch		0	0	Sucrose			645.6
Maltodextrin 10		125	500	Corn Starch			20
Sucrose		68.8	275.2	Maltodextrin			20
Cellulose, BW200		50	0	Soybean oil			50
Soybean Oil		25	225	Cellulose			9.89
Lard*		245	2205	Vitamin Mix, AIN-93-VX (94047)			10
Mineral Mix S10026		10	0	Choline Bitartrate			2.5
DiCalcium Phosphate		13	0	TBHQ, antioxidant			0.01
Calcium Carbonate		5.5	0	Mineral Mix, AIN-93G-MX (94046)			35
Potassium Citrate, 1 H2O		16.5	0	Calcium Phosphate, dibasic			4
Vitamin Mix V10001		10	40				
Choline Bitartrate		2	0				
FD&C Blue Dye #1		0.05	0				
Total		**774**	**4,057**	**Total**			**1,000**

[a]Formulated by E. A. Ulman, Ph.D., Research Diets, Inc., 8/26/98 and 3/11/99

*Typical analysis of cholesterol in Lar = 0.72 mg/gram. Cholesterol (mg)/4057 kcal = 216.4; Cholesterol (mg)/kg = 279.6; [b]Formulated by Teklad Diet (Harlan, Madison, USA) Sucrose makes up approximately 66% of the diet.

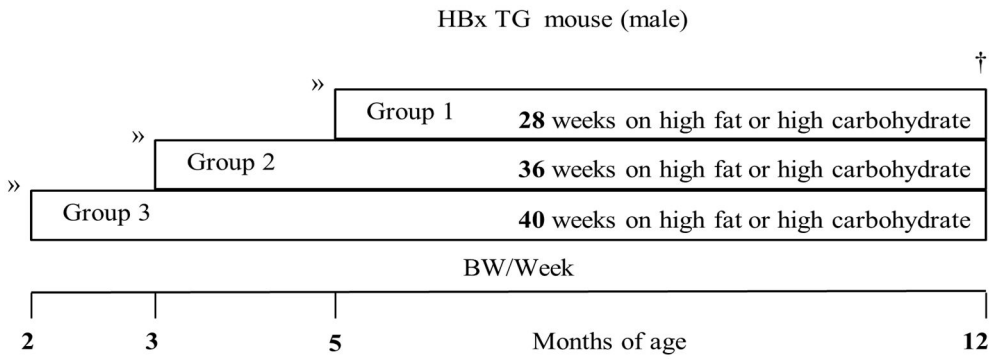


Fig. 1. Experimental design. Five, three, and two month old male HBx TG mice were fed either RC (LabDiet, Richmond, IN, USA), HFD (60% kcal fat, Research diet, #D12492) or HCD (70% kcal carbohydrate, Harlan Teklad, #TD.98090) for either 28, 36, or 40 weeks . Body weight was determined weekly. Mice were age matched and sacrificed at 12 months old after the diet challenge**.** » Diet challenge start point, † sacrifice. RC, regular chow; HFD, high fat diet; HCD, high carbohydrate diet.

Genotyping

Genotypes of the HBx (+/+) transgenic mice were verified by polymerase chain reaction (PCR) analysis using the primer sets mentioned below. DNA was isolated from mouse tails as follows. Direct PCR reagent (VIAGEN Biotech Inc., Los Angeles, CA) with proteinase K solution (Sigma, St Louis, MO) was added to approximately 5 mm mouse tail sections and incubated overnight at 55°C in a dry oven. The tail lysate was then centrifuged for 2 min at 13000rpm and 4°C; 2 µl of supernatant was used for PCR analysis. PCR was performed with ExTaq polymerase (TAKARA Co., Japan) using the following primers: the first primer set was sense primer 5′-TTC TCA TCT GCC GGT CCG TG-3′, and antisense primer 5′-GGG TCA ATG TCC ATG CCC CA-3′, and the second set was sense primer 5′-GAA AAC ACA CTC ACT GTT CAG AG-3′ and antisense primer 5′-GTA AGC CGC TTT CTC TTA TGC AG-3′-.

Sample collection

At the end of the experiment (12 months), mice were weighed, sacrificed under ether anesthesia, and blood was collected by cardiac puncture. Blood samples were centrifuged for 15 min at 3000 rpm and, 4ºC in a cooling centrifuge (Eppendorf 5424R, Germany). The serum was divided into two aliquots; one portion was used for immediate determination of biochemical parameters (ALT, AST, and TCHO) and the other portion was stored at -80ºC for further analysis. Livers were dissected and weighed. Relative liver weight was calculated as liver weight (g)/final body weight (g). Liver tissue was minced quickly and stored at -80ºC.

Histopathological evaluation and immunohistochemistry

To evaluate histopathological changes in liver tissue, mice were euthanized and their liver tissues were dissected, fixed by immersion in 4% paraformaldehyde, and embedded in paraffin. The tissue blocks were cut into 3.5-μm sections and subjected to hematoxylin and eosin (H&E) staining using a commercial kit (HHS123, Sigma-Aldrich, St Louis, MO).

Immunohistochemistry (IHC) was performed to determine cell proliferation. Slides were incubated with the following primary antibodies: proliferating cell nuclear antigen (PCNA, Abcam; Cambridge, UK) and Ki-67 (Chemicon Merck Millipore, Darmstadt, Germany). Slides were incubated with secondary antibody; Dako REAL™ Envision™ anti-rabbit/Mouse HRP (Dako, Denmark) for 30 min, and then slides were incubated in DAB+ CHROMOGEN substrate. The staining protocols were performed according to standard methods.

Western blot analysis

For immunoblot analysis, tissue was lysed in PRO-PREP buffer (iNtRON Biotechnology Inc., Seoul, Korea) containing a phosphatase-inhibitor cocktail (GenDEPOT, Barker, TX, USA). Protein extracts were separated by SDS-PAGE gel electrophoresis, transferred to PVDF membranes (Millipore, Billerica, Massachusetts, USA), and subjected to immunoblot analysis. Proteins were detected with antibodies against pAKT (Ser473), AKT, pERK1/2, ERK1/2, CYCLIN D1, and GAPDH (Cell signaling Technologies, Beverly, MA) followed by the appropriate HRP-conjugated secondary antibodies (Cell Signaling Technologies, Beverly, MA). Proteins were visualized with ECL

chemiluminescence (AbClon, Seoul, Korea). GAPDH was detected as a loading control. Immunoreactive signals were detected through their enhanced chemiluminescence, recorded using the MicroChemi 4.2 system (DNR Bio-Imaging Systems, Jerusalem, Israel) and quantitated using Image J software.

Glucose tolerance test

The intraperitoneal (IP) glucose tolerance test (IPGTT) outcomes of interest included basal (fasted) serum glucose concentration. At 14 h prior to performing the IPGTT, animals were fasted with access to water overnight. After the mice were weighed, a baseline blood sample was obtained by tail bleed, and additional blood samples were obtained 15, 30, 60, 90, and 120 min after injection with intraperitoneal glucose at 1.5 g/kg body weight. Plasma glucose concentrations were measured using a blood glucose meter (Accu-check, Roche Diagnostics; Indianapolis, USA).

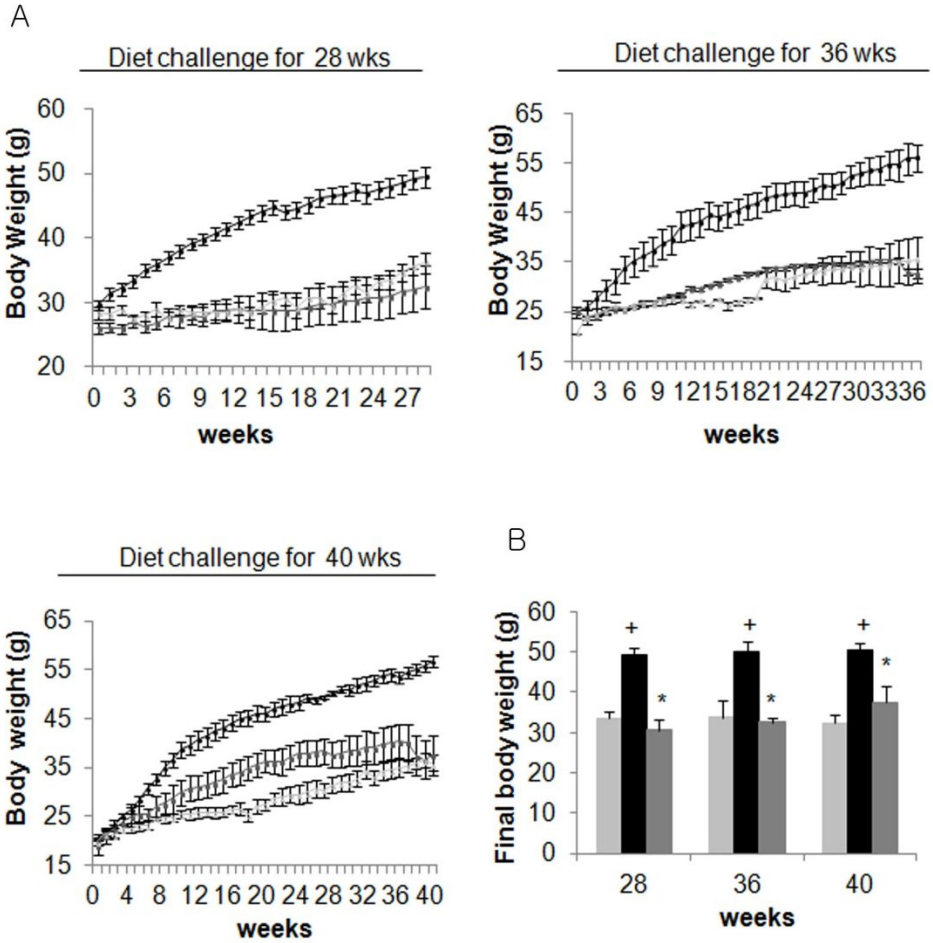
Statistical analysis

All values are expressed as the mean±SEM. Statistical significance was calculated with a pair-wise t test. To correct for false positives from multiple tests, the p-value was adjusted by Benjamini & Hochberg's method. P-values of <0.05 were considered statistically significant.

Results

Differential effects of high fat and high carbohydrate diet in HBx TG mice

To evaluate clinical markers of obesity and liver injury; five, three, and two month old HBx TG male mice were fed either RC, HFD (60% kcal fat), or HCD (70% kcal carbohydrate) for 28, 36, and 40 weeks respectively. The composition of the different diets and the experimental design are illustrated in Table 1; and Fig. 1, respectively. Body weight increased significantly in mice fed a HFD for 28, 36, and 40 weeks relative to mice fed the RC, although there were no significant differences in body weight of mice fed a HCD compared to mice fed RC, until 40 weeks when; the body weight of HCD feed mice was significantly greater (Fig. 2A-B). The weight of epididymal white adipose tissue (eWAT) was significantly greater in mice fed a HFD than for mice fed a HCD or RC, for which the eWAT values showed no significant difference. In contrast to previous studies that demonstrated similar body weight and adiposity between mice fed HCD or HFD (Ferreira et al., 2011; Swinburn et al., 2004), we observed obesity especially during HFD challenge, as indicated by higher body and eWAT weights, with a higher eWAT/body weight ratio (Fig. 2C-D). However, the plasma total cholesterol of mice fed HFD or HCD was increased compared to mice fed the RC, with the exception of mice that were challenged with HCD for 28 weeks, in which the level of total cholesterol was less than that in mice fed RC (Fig. 2E).


A
Diet challenge for 28 wks
Body Weight (g)
60
50
40
30
20
0 3 6 9 12 15 18 21 24 27
weeks
Diet challenge for 36 wks
Body Weight (g)
65
55
45
35
25
15
0 3 6 9 12151821242730336
weeks
Diet challenge for 40 wks
Body weight (g)
65
55
45
35
25
15
0 4 8 12 16 20 24 28 32 36 40
weeks
B
Final body weight (g)
60
50
40
30
20
10
0
+
*
28
36
40
weeks

(Continued Fig. 2.)

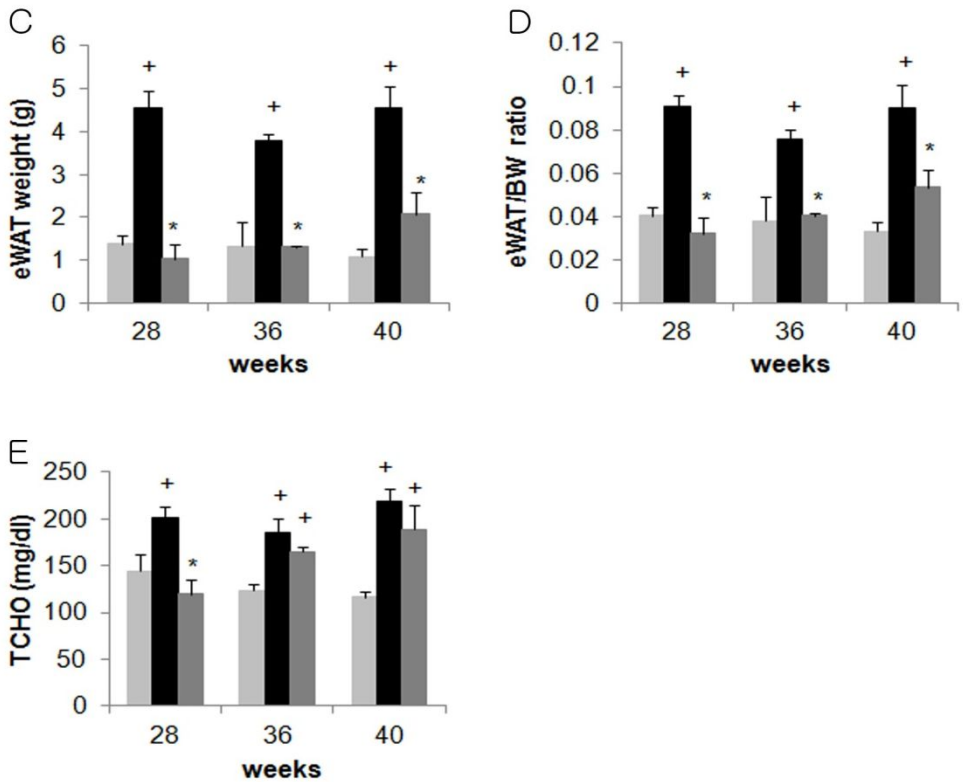


Fig. 2. Differential effects of high fat and high carbohydrate diet on HBx TG mouse body weight and fat accumulation. (A) Body weight changes; (B) final body weight; (C) eWAT weight; (D) eWAT weight per body weight ratio; (E) serum total cholesterol of HBx TG mice during HFD and HCD challenge for 28, 36, or 40 weeks. Values are expressed as mean ±SEM (n=4). + P< 0.05 vs. RC; * P< 0.05 vs. HF. ■ Regular chow, ■ High fat diet, ■ High carbohydrate diet.

Effects of diet intake imbalance on glucose tolerance in HBx TG mice

The basal blood glucose concentration of mice fed a HFD was increased compared to levels in mice fed RC and HCD for 28 weeks; however, there were no other significant differences in mice challenged with HFD or HCD for 36 and 40 weeks. In the intraperitoneal glucose tolerance test, the blood glucose concentration of mice fed the HCD for 36 and 40 weeks increased to a maximum 15 min after intraperitoneal injection of glucose and remained elevated at 120 min. The glucose levels of mice fed HFD were elevated at 30, 60, and 120 min compared to the control group (Fig. 3).

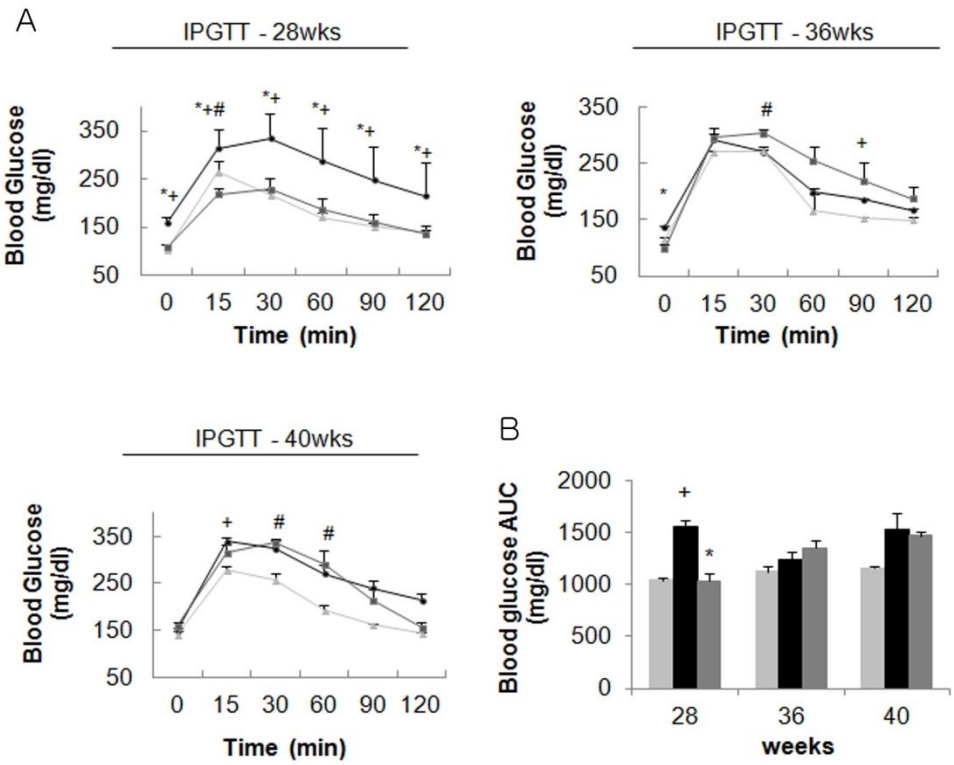


Fig. 3. Effects of diet intake imbalance on glucose tolerance in HBx TG mice. (A) Glucose tolerance test; (B) area below the curve of HBx TG mice during HFD or HCD challenge for 28, 36, or 40 weeks. Values are expressed as mean ±SEM (n=4). IPGTT: P < 0.05 + HFD vs. RC, * HFD vs. HCD, # HCD vs. RC; AUC: + P< 0.05 vs. RC; * P< 0.05 vs. HF. ■Regular chow, ■High fat diet, ■High carbohydrate diet.

Long-term effects of high fat and high carbohydrate feeding on liver changes in HBx TG mouse

We next examined the effect of the experimental diets in livers of HBx TG mice. The liver weights of mice challenged with HFD for 28 weeks were significantly higher than mice eating either RC or HCD. However, at 36 weeks, there were no significant differences in liver weight between RC, HFD, and HCD groups. In addition, at 40 weeks, there was a trend toward higher liver weight in HFD and HCD groups; however, this difference did not reach significance (Fig. 4A). This data was complemented with a higher liver weight/body weight ratio in mice fed a HCD for 36 and 40 weeks (Fig. 4B).

We next evaluated the liver damage by analyzing serum AST and ALT levels. The levels of these enzymes were significantly increased in mice fed HFD for 28 and 40 weeks compared to mice fed RC, reflecting liver tissue damage. Moreover, in mice challenged with a HFD for 36 weeks, there was a trend toward higher average AST and ALT enzymes activities; however, this difference did not reach significance for ALT (Fig. 4C-D).

In this study, histopathological analysis of liver tissues was performed to further support the biochemical analysis data. Using hematoxylin and eosin staining, we showed that the histology of the liver sections of RC group exhibited normal hepatic cells with well-preserved cytoplasm, prominent nucleus and nucleolus and visible central veins. In contrast, the HFD group exhibited the most severe damage of all the groups; liver sections showed massive fatty changes with some

inflammatory cells close to the peripheral zone, ballooning degeneration and the loss of cellular boundaries. The liver sections of mice from the HCD group revealed similar pathomorphological changes; but to a lesser degree than the HFD group (Fig. 5). The histopathology was in good correlation with the biochemical results of serum liver damage markers and other biochemical parameters.

We further determined the level of hepatocyte proliferation after diet induced liver injury using PCNA and Ki-67 analysis. Our data indicate increased liver cell proliferation in HFD fed mice. HCD fed mice also showed an increase in liver cell proliferation compared to the control, but it was significantly at a lower than HFD fed mice (Fig. 6-7).

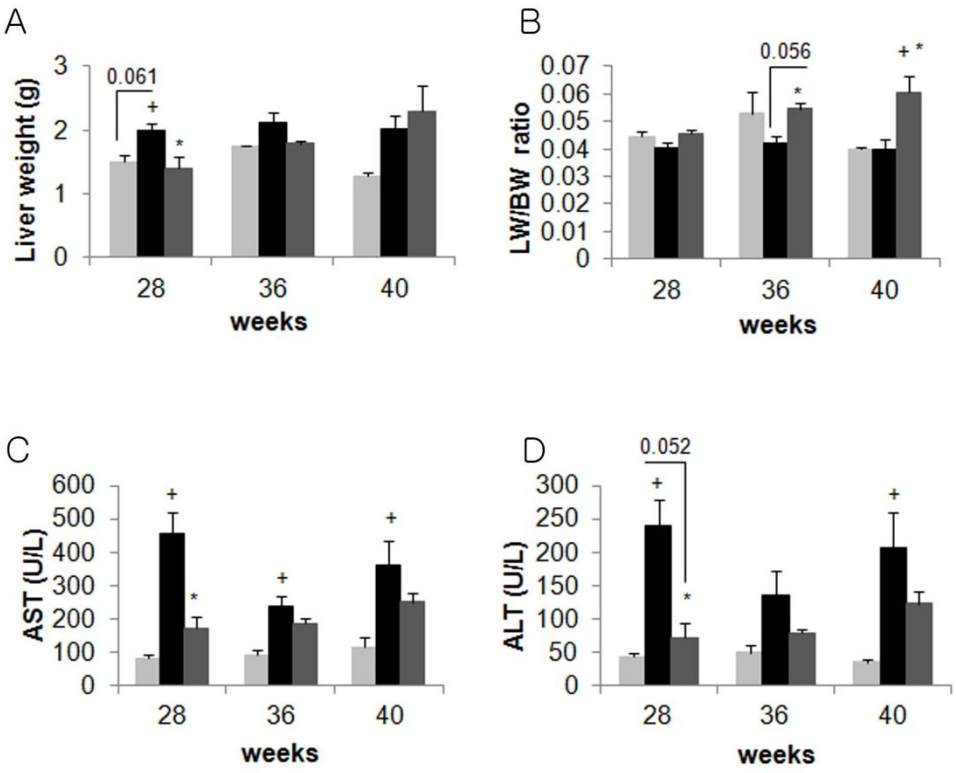


Fig. 4. Differential effects of high fat and high carbohydrate diets on HBx TG mouse livers. (A) Liver weight; (B) liver weight per body weight ratio; (C) aspartate aminotransferase (AST) levels; (D) alanine transaminase (ALT) levels of HBx TG mice during HFD or HCD challenge for 28, 36, or 40 weeks. Values are expressed as mean ±SEM (n=4). + P< 0.05 vs. RC; * P< 0.05 vs. HF. ▪Regular chow, ■ High fat diet, ■ High carbohydrate diet.

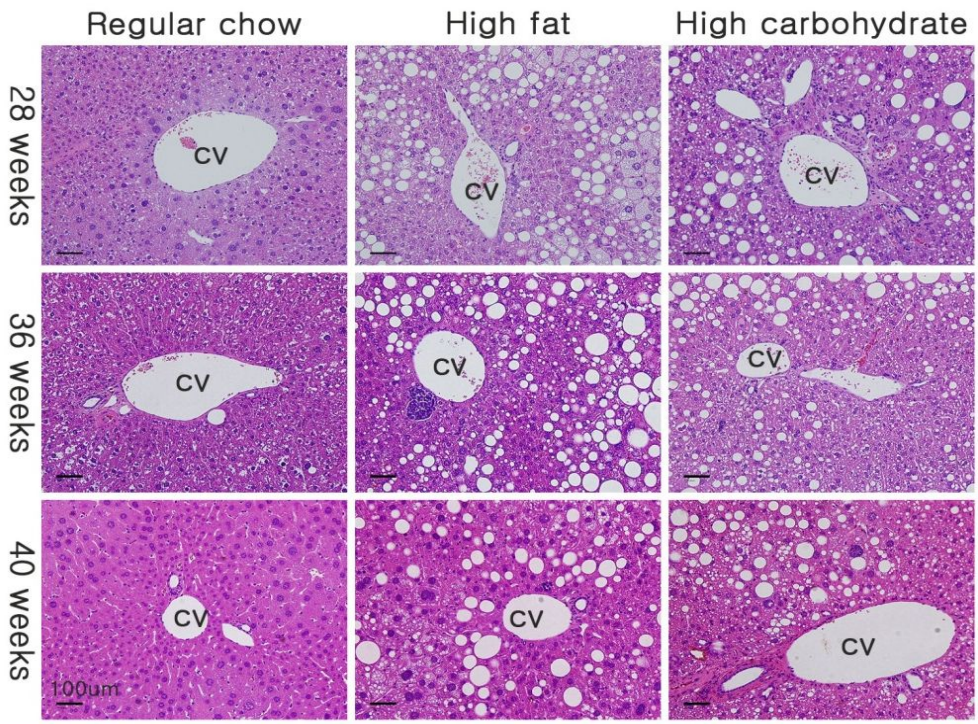
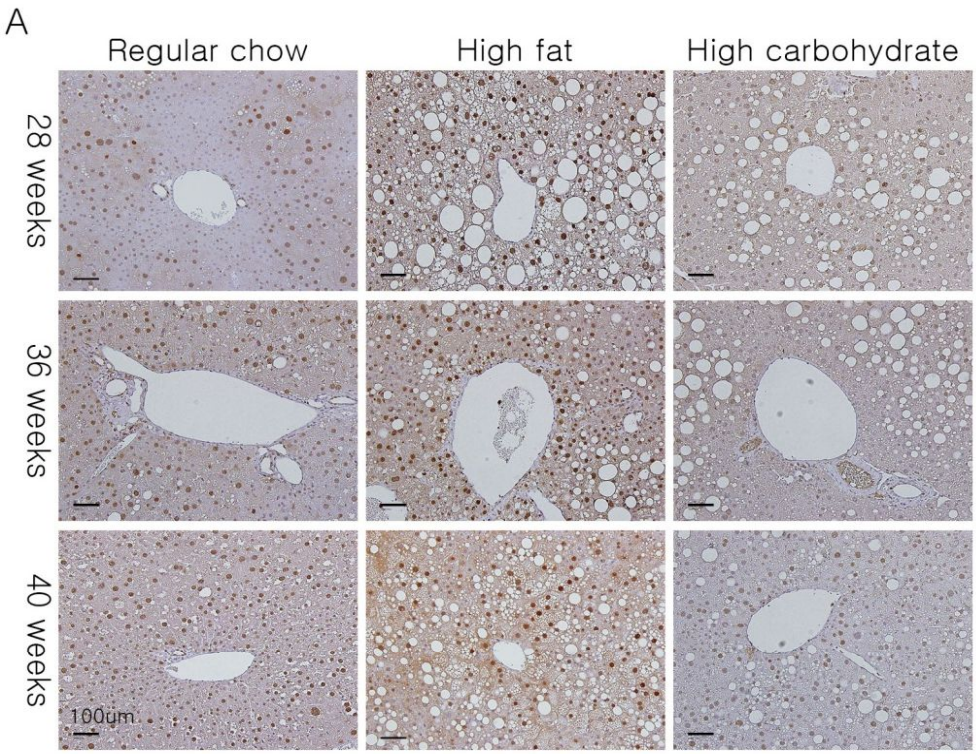


Fig. 5. Histopathology of HBx TG mouse liver sections after high fat and high carbohydrate feeding. Liver sections were stained with hematoxylin and eosin stain. Magnification: 200X. Scale bar represents 100 μm. CV: central vein.


A
Regular chow
High fat
High carbohydrate
28 weeks
36 weeks
40 weeks
100um

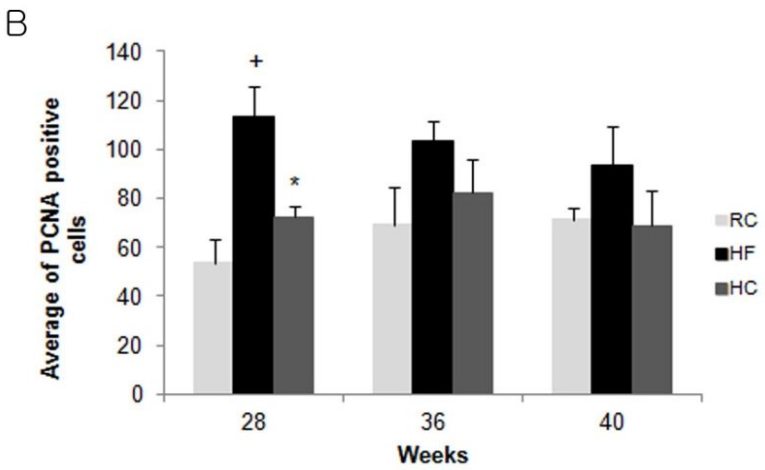
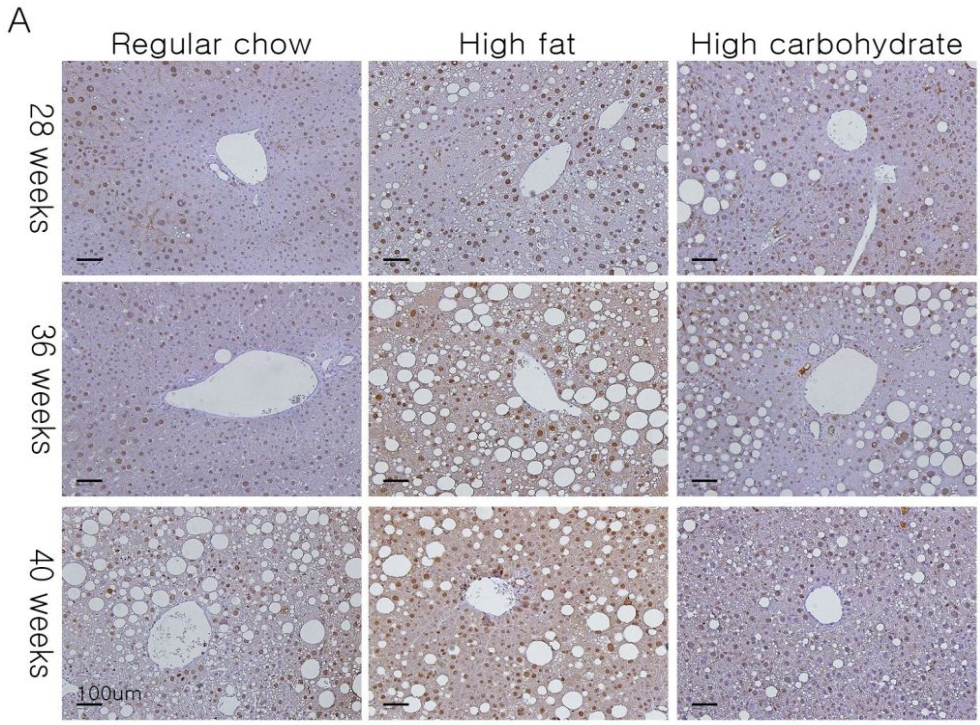

B
Average of PCNA positive cells
140
120
100
80
60
40
20
0
+
*
28
36
40
Weeks
RC
HF
HC

Fig. 6. PCNA expression in HBx TG mouse liver sections after high fat or high carbohydrate feeding. (A) Representative photomicrographs of PCNA IHC staining; (B) average number of PCNA positive cells in liver sections of HBx TG mice fed RC, HFD or HCD for 28, 36, and 40 weeks; . Magnification: 200X. Scale bar represents 100 μm. IHC: immunohistochemistry. Values are expressed as mean ±SEM (n=4). + $P< 0.05$ vs. RC; * $P< 0.05$ vs. HF.


A
Regular chow
High fat
High carbohydrate
28 weeks
36 weeks
40 weeks
100um

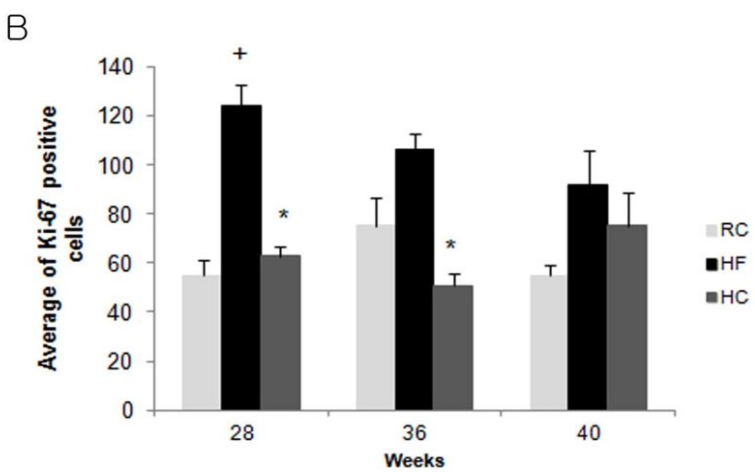

B
Average of Ki-67 positive cells
140
120
100
80
60
40
20
0
+
*
*
28
36
40
Weeks
RC
HF
HC

Fig. 7. Ki-67 expression in HBx TG mouse liver sections after high fat or high carbohydrate feeding. (A) Representative photomicrographs of Ki-67 IHC staining; (B) average number of Ki-67 positive cells in liver sections of HBx TG mice fed RC, HFD, or HCD for 28, 36, or 40 weeks; . Magnification: 200X. Scale bar represents 100 μm. IHC: immunohistochemistry. Values are expressed as mean ±SEM (n=4). + $P< 0.05$ vs. RC; * $P< 0.05$ vs. HF.

HCD feeding activates tumor-related genes AKT and MAPK-ERK in HBx TG mouse livers

To further explore the potential mechanisms responsible for above changes in cell growth, we examined the response of AKT and MAPK signaling pathway in this model. The phosphorylated ERK1/2 levels were significantly increased in HCD fed mice. Activated ERK acts as a survival factor to promote PCNA and cyclinD1 expression. Interestingly, replacing dietary fat with carbohydrates induced AKT phosphorylation in liver tissue. HCD-mediated AKT activation was associated with ERK1/2 phosphorylation. (Figs. 8-9)

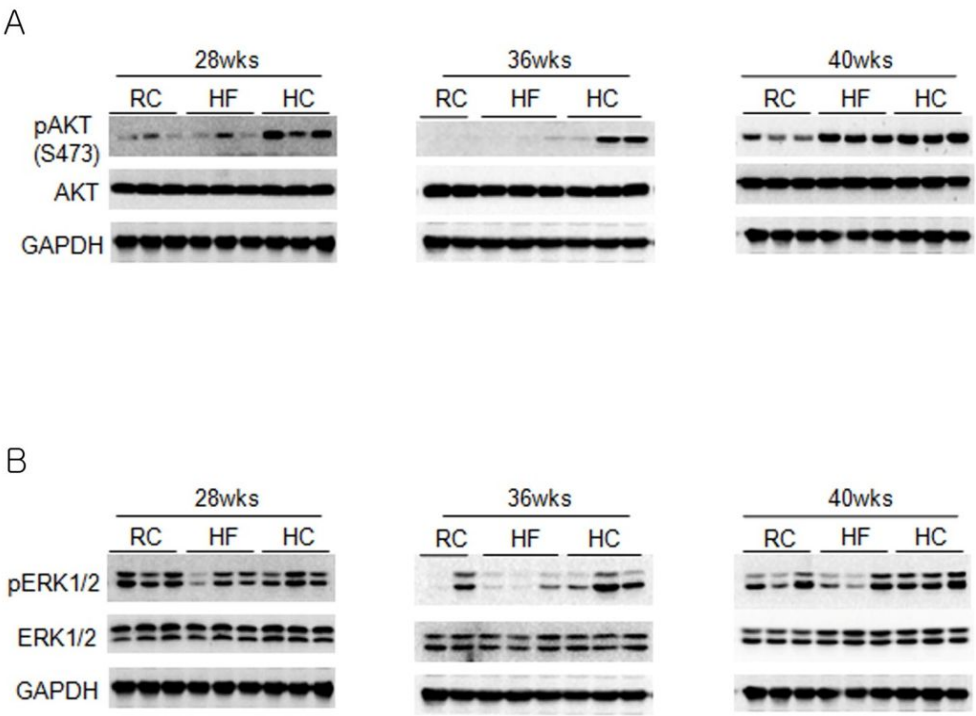


Fig. 8. Expression of tumor-related genes in HBx TG mouse livers after high fat or high carbohydrate diet feeding. Representative western blots with 3 samples per group are shown. (A) pAKT (S473), (total AKT as control). (B) pERK1/2, (total ERK as control). pAKT and pERK1/2 were highly up-regulated in livers of high carbohydrate fed mice.

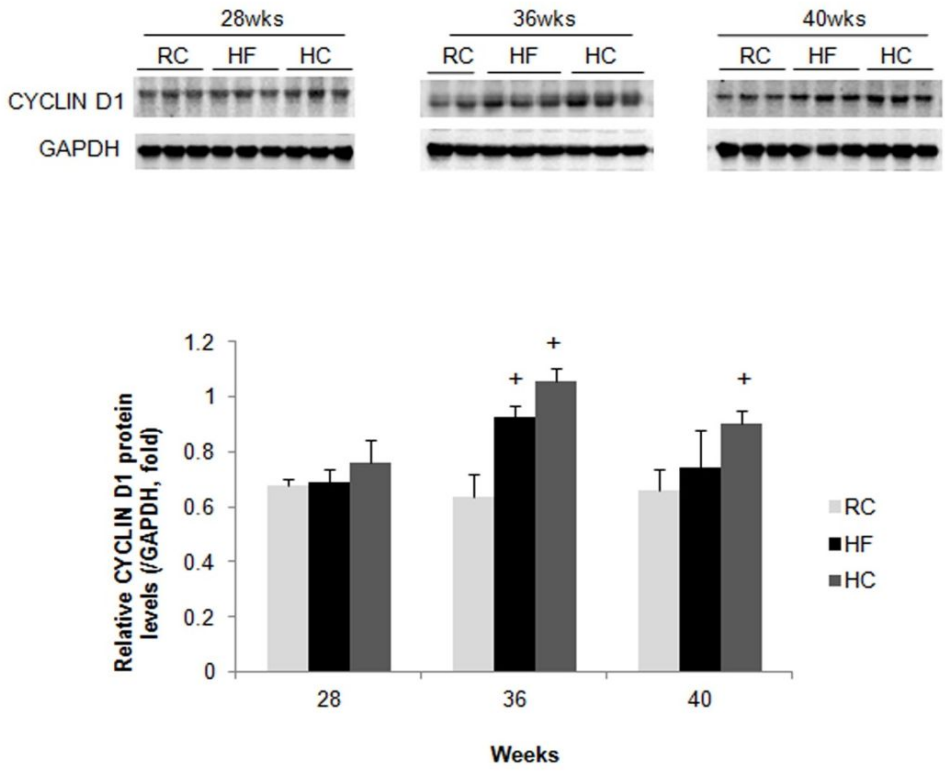


Fig. 9. Levels of CYCLIN D1 assessed by immunoblotting in liver tissue of HBx TG mice after high fat or high carbohydrate feeding; GAPDH was used as loading control. The data are shown as the mean ±SEM (n=3 per group). + P< 0.05 vs. RC; * P< 0.05 vs. HF.

Discussion

We hypothesized that the long-term effects of a high-calorie diet coupled with hepatitis B virus protein x expression (HBx TG mouse) would lead to both obesity and hepatic steatosis, pivotal features in development of hepatocellular carcinoma. This initial hypothesis was based on previous studies using HBx TG mice fed an excessive caloric diet (Cho et al., 2014; Ferreira et al., 2011; Kim et al., 2015; Kim et al., 2007; Park et al., 2010; Wang et al., 2016; Wang et al., 2009). Our results indicated that weight gain in the HFD group was significantly greater than in the HCD group. The high energy density of fats (9 kcal/g), compared with the low energy density of carbohydrates (4 kcal/g), results in a less satiating effect for the fatty food, thus, promoting passive over-consumption and subsequent weight gain (Swinburn et al., 2004).

The liver plays an important role in glucose and lipid metabolism as well as in the control of energy balance and body weight. Hepatic carbohydrate and lipid metabolism influence satiety signaling. After food intake, glucose concentrations increase in the hepatic portal vein. It is hypothesized that glucose itself, increased glucose utilization, or a secondary regulatory peptide activated by glucose influences the fat mass set point to promote increased food intake, decreased energy expenditure, and increased fat storage (Langhans et al., 2001; Mithieux et al., 2005). Studies showed that HCD fed mice had a comparable degree of fat accumulation in the liver as HFD feed mice, despite having significantly reduced body weights, presumably because of the differential energy densities of the diets.

Therefore, HCD feeding may use alternative pathways to promote liver tumorigenesis than those that are obesity and/or weight-dependent.

The endoplasmic reticulum (ER)-induced unfolded protein response (ER^{UPR}) plays an important role in non-alcoholic fatty liver disease (NAFLD) development and progression (Fu et al., 2012; Malhi and Kaufman, 2011); previous studies demonstrated that ER^{UPR} could be induced by HCD or HFD feeding. ER^{UPR} is characterized by the activation of three distinct signal transduction pathways originating from the ER membrane that are mediated by the inositol requiring enzyme 1a/X-box binding protein 1 system, PERK/eIF2a signaling, and the activation of transcription factor 6a (Fu et al., 2012; Malhi and Kaufman, 2011). We observed that replacing dietary fat with carbohydrates induced enhanced pERK1/2 signaling in the liver. It has been reported that pERK/ eIF2a activation was important for cancer cell survival (Bi et al., 2005; Koumenis et al., 2002). Because others reported that tumors in pERK knockout mice grew much slower and exhibited a greater degree of apoptosis than in the respective wild-type mice (Bi et al., 2005), our results suggested that high carbohydrate diet may promote liver tumorigenesis through eliciting pERK signaling.

Interestingly, increased pERK activation induced by HCD in the current study was associated with elevated Akt-Erk1/2 signaling activation. This observation was accompanied by HCD-induced increases in cyclin D1 protein expression.

The induction of the phosphatidyl inositol 3-kinase–Akt pathway is important to

facilitate cancer cell survival, which requires pERK/eIF2a signaling (Hamanaka et al., 2009; Mounir et al., 2011). Akt activation in transformed cells of human liver tumors is a risk factor for early disease recurrence (Calvisi et al., 2011; Nakanishi et al., 2005) and correlates with increased expression of the cell cycle promoter cyclin D1 (Alexia et al., 2006; Kazemi et al., 2007). Our data indicated that chronic HCD consumption in HBx TG mice can produce increased severity of liver damage and accelerate the onset of tumorigenesis potentially by upregulating pERK-mediated ER stress. Therefore, further studies are needed to verify the effects of long-term hypercaloric diets in earlier disease stages, and how these diets affect oncogenesis along with other host factors such as genotype, age and gender. Many determinants of liver disease progression are currently unknown, as evidenced by the fact that we cannot accurately predict which patients with any of the major liver diseases (HBV, HCV, NAFLD, and alcoholic liver disease) will progress to cirrhosis and which ones will have a relatively benign course. The present study raises the possibility that diet may be an important and potentially modifiable determinant of HCC progression and the animal model used in this research can provide us also key insights into the development of HCC as a result of high calorie intake.

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국문초록

식이 요인이 간암의 진행에 미치는 영향에 대해서는 알려진 바가 거의 없다. 따라서, 이 논문에서는 간암, HCC (Hepatocellular carcinoma) 모델인 HBx TG 마우스 모델에서 장기간의 식이 유도 비만에 의한 간의 변화와, HCC 발암 정도를 확인하고 각 발암 단계에서 간의 변화를 확인하였다. 먼저, 고지방식이(HFD)와 고탄수화물식이(HCD)가 HBx TG 마우스의 간에 미치는 발암 효과를 조사하기 위해 HBx TG 마우스에 각 28주, 36주, 40주 동안 고지방 혹은 고탄수화물 식이를 주었다. 일반 사료를 먹인 그룹과 비교했을 때 HFD 혹은 HCD식이를 먹인 HBx TG 마우스에서 간지방증이 뚜렷하게 나타났고 alanine aminotransferase와 aspartate aminotransferase 의 혈청 농도가 크게 증가하였다. 그러나 HFD 식이과 일반 사료를 먹인 마우스에 비해 HCD식이를 먹인 그룹에서 AKT와 ERK가 크게 활성화되었다. 결론적으로, 만성적인 HCD 식이는 HBx TG 마우스에 심한 간 손상을 가져오고 pERK에 의한 ER-스트레스의 증가를 통해 발암 시기를 가속화시켰으며, 지방 혹은 탄수화물의 과다 섭취에 의한 생화학 및 병리학적 변화는 간지방증을 악화시킨다고 볼 수 있다. 또한, 음식물 섭취의 불균형은 그 자체가 HCC유발 인자는 아닐 수 있으나 간세포의 능력을 저해하고 종양의 발생을 유발하는 위험 인자일 가능성이 있다.

따라서, 고칼로리 섭취는 간암 발생에 중요한 영향을 미치는 잠재적인 요소라고 할 수 있다.

주요단어: 간암; HBx TG 마우스; 고지방식이; 고탄수화물 식이; pERK

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