

***Garcinia Cambogia* attenuates diet-induced adiposity but exacerbates hepatic collagen accumulation and inflammation**

Young-Je Kim, Myung-Sook Choi, Yong Bok Park, Sang Ryong Kim, Mi-Kyung Lee, Un Ju Jung

Young-Je Kim, Myung-Sook Choi, Department of Food Science and Nutrition, Kyungpook National University, Daegu 702-701, South Korea

Myung-Sook Choi, Un Ju Jung, Center for Food and Nutritional Genomics Research, Kyungpook National University, Daegu 702-701, South Korea

Yong Bok Park, Sang Ryong Kim, School of Life Sciences and Biotechnology, Kyungpook National University, Daegu 702-701, South Korea

Sang Ryong Kim, Brain Science and Engineering Institute, Kyungpook National University, Daegu 702-701, South Korea

Mi-Kyung Lee, Department of Food and Nutrition, Sunchon National University, Jeonnam 540-742, South Korea

Author contributions: Kim YJ and Choi MS contributed equally to this work; Kim YJ performed experiments and analyzed the data; Choi MS designed the study and reviewed and revised the manuscript; Park YB, Kim SR and Lee MK contributed to the critical edition of the manuscript; Jung UJ designed the study, performed experiments, analyzed the data and wrote the manuscript.

Supported by The Basic Science Research Program, No. 2011-0022387; and the SRC program, Center for Food and Nutritional Genomics: No. 2012-0000644 through the National Research Foundation of Korea funded by the Ministry of Education, Science and Technology

Correspondence to: Un Ju Jung, PhD, Center for Food and Nutritional Genomics Research, Kyungpook National University, 1370 San-Kyuk Dong, Puk-Ku, Daegu 702-701, South Korea. jungunju@naver.com

Telephone: +82-53-9507937 Fax: +82-53-9581230

Received: March 22, 2013 Revised: May 15, 2013

Accepted: June 1, 2013

Published online: August 7, 2013

Abstract

AIM: To investigate long-term effects of *Garcinia Cambogia* (GC), weight-loss supplement, on adiposity and non-alcoholic fatty liver disease in obese mice.

METHODS: Obesity-prone C57BL/6J mice were fed a high-fat diet (HFD, 45 kcal% fat) with or without GC

(1%, w/w) for 16 wk. The HFD contained 45 kcal% fat, 20 kcal% protein and 35 kcal% carbohydrate. They were given free access to food and distilled water, and food consumption and body weight were measured daily and weekly, respectively. Data were expressed as the mean \pm SE. Statistical analyses were performed using the statistical package for the social science software program. Student's *t* test was used to assess the differences between the groups. Statistical significance was considered at $P < 0.05$.

RESULTS: There were no significant changes in body weight and food intake between the groups. However, the supplementation of GC significantly lowered visceral fat accumulation and adipocyte size *via* inhibition of fatty acid synthase activity and its mRNA expression in visceral adipose tissue, along with enhanced enzymatic activity and gene expression involved in adipose fatty acid β -oxidation. Moreover, GC supplementation resulted in significant reductions in glucose intolerance and the plasma resistin level in the HFD-fed mice. However, we first demonstrated that it increased hepatic collagen accumulation, lipid peroxidation and mRNA levels of genes related to oxidative stress (superoxide dismutase and glutathione peroxidase) and inflammatory responses (tumor necrosis factor- α and monocyte chemoattractant protein-1) as well as plasma alanine transaminase and aspartate transaminase levels, although HFD-induced hepatic steatosis was not altered.

CONCLUSION: GC protects against HFD-induced obesity by modulating adipose fatty acid synthesis and β -oxidation but induces hepatic fibrosis, inflammation and oxidative stress.

© 2013 Baishideng. All rights reserved.

Key words: *Garcinia Cambogia*; Anti-adiposity; Metabolic changes; Hepatic collagen accumulation; Hepatic inflammation; Hepatic oxidative stress

Core tip: *Garcinia Cambogia* (GC) is a popular dietary supplement for weight loss. However, little is known about the efficacy and hepatotoxicity of long-term GC supplementation in mice fed a high-fat diet (HFD). We observed that GC ameliorated HFD-induced adiposity by modulating enzymatic activity and gene expression involved in fatty acid metabolism. GC also reduced the plasma resistin level and glucose intolerance. However, GC caused hepatic collagen accumulation, inflammation and oxidative stress without affecting hepatic steatosis.

Kim YJ, Choi MS, Park YB, Kim SR, Lee MK, Jung UJ. *Garcinia Cambogia* attenuates diet-induced adiposity but exacerbates hepatic collagen accumulation and inflammation. *World J Gastroenterol* 2013; 19(29): 4689-4701 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v19/i29/4689.htm> DOI: <http://dx.doi.org/10.3748/wjg.v19.i29.4689>

INTRODUCTION

Obesity is one of the global public health problems commonly associated with metabolic diseases including insulin resistance, type 2 diabetes, non-alcoholic fatty liver disease (NAFLD) and dyslipidemia^[1]. According to the World Health Organization global estimates from 2008, more than 1.4 billion adults are overweight and at least 500 million adults are obese^[2]. Although the use of dietary supplements for weight loss becomes common^[3], the optimal dose and safety profiles of many dietary supplements are poorly studied. The United States Food and Drug Administration (FDA) do not regulate dietary supplements in the same manner as pharmacological agents^[4,5]. While pharmaceutical companies are required to obtain FDA approval, which involves assessing the risks and benefits prior to their entry into the market, dietary supplements are not subject to the same scientific scrutiny and are not required to demonstrate any scientific safety and efficacy pertaining to the claims made by manufacturers.

Several studies have shown that *Garcinia Cambogia* (GC), a fruit native to southeastern Asia and Western Africa, has beneficial effects on body weight and fat loss in both experimental animals and human^[6-10]. Its main component hydroxycitric acid (HCA) not only inhibits ATP-citrate lyase, the enzyme response for *de novo* fatty acid synthesis, but also increases hepatic glycogen synthesis, reduces food intake by suppressing appetite and decreases body weight gain^[6-9]. Although extensive experiments reported the weight loss and body fat-lowering effects of GC, some animal and clinical studies have shown controversial findings^[6,10-13] and no studies have shown whether these effects persist beyond 13 wk of GC treatment. Furthermore, some studies have reported the potential for hepatotoxicity of hydroxycitric acid, a formulation that contains GC among other ingredients^[14,15].

The present study was therefore done to investigate

the effect of long-term GC supplementation on adipogenesis and obesity-related metabolic changes, such as glucose intolerance and hepatic steatosis, in mice fed a high fat diet (HFD). We also examined the effect of GC on liver dysfunction, collagen accumulation, inflammation and oxidative stress.

MATERIALS AND METHODS

Animals and diets

Male C57BL/6J mice (4-wk-old) were purchased from Jackson Laboratories (Bar Harbor, ME, United States). The mice were individually housed in polycarbonate cages, which were kept in a room maintained at a constant temperature (24 °C) with a 12-h light/dark cycle. The mice were fed a normal chow diet for acclimation for 1 wk after delivery. At 5 wk of age, they were randomly divided into two groups of 10 mice each and fed a HFD (D12451, Research Diets, New Brunswick, NJ, United States) with or without GC (1%, w/w, 60% hydroxyl citric acid; Newtree Inc., United States) for 16 wk. The HFD contained 45 kcal% fat, 20 kcal% protein and 35 kcal% carbohydrate. They were given free access to food and distilled water, and food consumption and body weight were measured daily and weekly, respectively. At the end of the experimental period, all the mice were anesthetized with isoflurane (5 mg/kg body weight, Baxter, United States) after a 12-h fast, and blood samples were collected from the inferior vena cava into heparin-coated tube for the measurement of plasma parameters. The blood was centrifuged at 1000 g for 15 min at 4 °C, and the plasma was separated.

Fasting blood glucose, intraperitoneal glucose tolerance test and homeostatic index of insulin resistance

The blood glucose concentration was measured with whole blood obtained from the tail veins after withholding food for 12 h using a glucose analyzer (OneTouch Ultra, Lifescan Inc., United States) based on the glucose oxidase method. The intraperitoneal glucose tolerance test was performed on the 15th week. After a 12-h fast, the mice were injected intraperitoneally with glucose (0.5 g/kg body weight). The blood glucose level was measured from the tail vein at 0, 30, 60 and 120 min after glucose injection. Area under the curve (AUC) was calculated for all glucose levels as an index of glucose tolerance. Homeostatic index of insulin resistance (HOMA-IR) was calculated according to the homeostasis of the assessment as follows: $HOMA-IR = [\text{fasting glucose (mmol/L)} \times \text{fasting insulin } (\mu\text{L U/mL})] / 22.51$.

Plasma biomarkers

Plasma adipokines were measured with a multiplex detection kit (171-F7001M, Bio-Rad, Hercules, CA, United States). Capture antibodies directed against the adipokines (resistin, leptin) were covalently coupled to the beads, and the coupled beads reacted with plasma. After a series of washes to remove unbound protein,

a biotinylated detection antibody was added to create a sandwich complex. The final detection complex was formed with the addition of streptavidin-phycoerythrin conjugate. Phycoerythrin served as a fluorescent indicator, or reporter. All samples were assayed in duplicate and analyzed with a Luminex 200 Labmap system (Luminex, Austin, TX, United States). Data analyses were done with Bio-Plex Manager software version 4.1.1 (Bio-Rad, Hercules, CA, United States).

Plasma cytokines were measured with a multiplex detection kit (M60-009RDPD, Bio-Rad, Hercules, CA, United States). Capture antibodies directed against the cytokines [insulin, tumor necrosis factor- α (TNF- α) and monocyte chemoattractant protein-1 (MCP-1)] were covalently coupled to the beads, and the same procedure for plasma adipokine analysis as described above was used to determine the plasma cytokines levels.

Plasma lipid and apolipoprotein concentrations were determined with commercially available kits: Plasma free fatty acid (01120301.HE98), phospholipid (01120251), apolipoprotein A (14535014) and apolipoprotein B (14537014) levels were measured using the Nittobo enzymatic kit (Nittobo medical Co., Tokyo, Japan), and triglyceride (AM157S-K), total cholesterol (AM202-K) and high-density lipoprotein (HDL)-cholesterol (AM203-K) levels were determined using Asan enzymatic kits (Asan, Seoul, South Korea).

Plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined using enzymatic kits (AM101-K, Asan, Seoul, South Korea).

Hepatic lipids contents

Hepatic lipids were extracted^[16], and then the dried lipid residues were dissolved in 1 mL of ethanol for triglyceride and cholesterol assays. Triton X-100 and a sodium cholate solution in distilled water were added to 200 μ L of the dissolved lipid solution for emulsification. The hepatic triglyceride and cholesterol contents were analyzed with the same enzymatic kit used for the plasma analysis.

Lipid-regulating enzyme activity

To measure the lipid-regulating enzymes activities in the epididymal white adipose tissue (WAT) and liver, samples were prepared and analyzed as previously described^[17]. Briefly, fatty acid synthase (FAS) activity was determined with a spectrophotometric assay according to the method by Carl *et al.*^[18]; one unit of FAS activity represented the oxidation of 1 nmol of NADPH per minute at 30 °C. Carnitine palmitoyltransferase (CPT) activity was determined according to the method by Markwell *et al.*^[19] and the results were expressed as nmol/min per milligram protein. Fatty acid β -oxidation was measured spectrophotometrically by monitoring the reduction of NAD to NADH in the presence of palmitoyl-CoA as described by Lazarow^[20], with slight modification. Protein concentration was measured by the Bradford method using BSA as the standard^[21].

Lipid peroxidation assay

The hepatic thiobarbituric acid-reactive substances (TBARS) concentration, as a marker of lipid peroxide production, was measured spectrophotometrically by the method of Ohkawa *et al.*^[22]. Hepatic homogenates containing 8.1% sodium dodecyl sulfate were mixed with 20% (w/v) acetic acid (pH 3.5), distilled water and 0.8% (w/v) TBA. The reaction mixture was heated at 95 °C for 60 min. After cooling the mixture, n-butanol: pyridine (15:1, v/v) was added and centrifuged at 3000 rpm for 15 min. The resulting colored layer was measured at 535 nm.

Analysis of gene expression

Epididymal WAT and liver were homogenized in TRIzol reagent (15596-026, Invitrogen Life Technologies, Grand Island, NY, United States) and total RNA was isolated according to the manufacturer's instructions. The total RNA was converted to cDNA using the QuantiTect Reverse Transcription Kit (205313, QIAGEN GmbH, Hilden, Germany). The RNA expression was quantified by quantitative real-time PCR using the QuantiTect SYBR green PCR kit (204143, QIAGEN GmbH, Hilden, Germany) and the SDS7000 sequence-detection system (Applied Biosystems, CA, United States). Each cDNA sample was amplified using primers labeled with SYBR Green dye for glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The amplification was performed as follows: 10 min at 90 °C, 15 s at 95 °C and 60 s at 60 °C for a total of 40 cycles. The cycle threshold values obtained were those cycles at which a statistically significant increase in the SYBR green emission intensity occurred. Ct data were normalized using GAPDH, which was stably expressed in mice. Relative gene expression was calculated with the $2^{-\Delta\Delta Ct}$ method^[23]. The following gene-specific primers were used: for catalase (CAT), 5'-GCGTCCGTCCT-GCTGTC-3' (forward), 5'-TGCTCCTTCCACT-GCTTCATCTG-3' (reverse); for cell death-inducing DNA fragmentation factor- α -like effector A (CIDEA), 5'-TTTCAAACCATGACCGAAGTAGCC-3' (forward), 5'-CCTCCAGCACCAGCGTAACC-3' (reverse); for CPT, 5'-ATCTGGATGGCTATGGTCAAGGTC-3' (forward), 5'-GTGCTGTCATGCGTTGGAAGTC-3' (reverse); for FAS, 5'-CGCTCCTCGCTTGTCTGTCGTCG-3' (forward), 5'-AGCCTTCCATCTCCTGTCAT-CATC-3' (reverse); for fatty acid translocase/cluster of differentiation 36 (FAT/CD36), 5'-ATTGGTCAAGC-CAGCT-3' (forward), 5'-TGTAGGCTCATCCACTAC-3' (reverse); for glyceraldehyde-3-phosphate dehydrogenase (GAPDH), 5'-ACAATGAATACGGCTACAGCAA-CAG-3' (forward), 5'-GGTGGTCCAGGGTTTCT-TACTCC-3' (reverse); for glutathione peroxidase (GHS-Px), 5'-TCGCAATGAGCCAAAACCTGACG-3' (forward), 5'-GCCAGGATTCGTAAACCACACTC-3' (reverse); for MCP-1, 5'-TTCTCCACCACCATG-CAG-3' (forward), 5'-CCAGCCGGCAACTGTGA-3' (reverse); for peroxisome proliferator-activated receptors (PPAR) α , 5'-CCTGAACATCGAGTGTCTCGAATAT (forward), 5'-GGTCTTCTTCTGAATCTTGCAGCT-3'

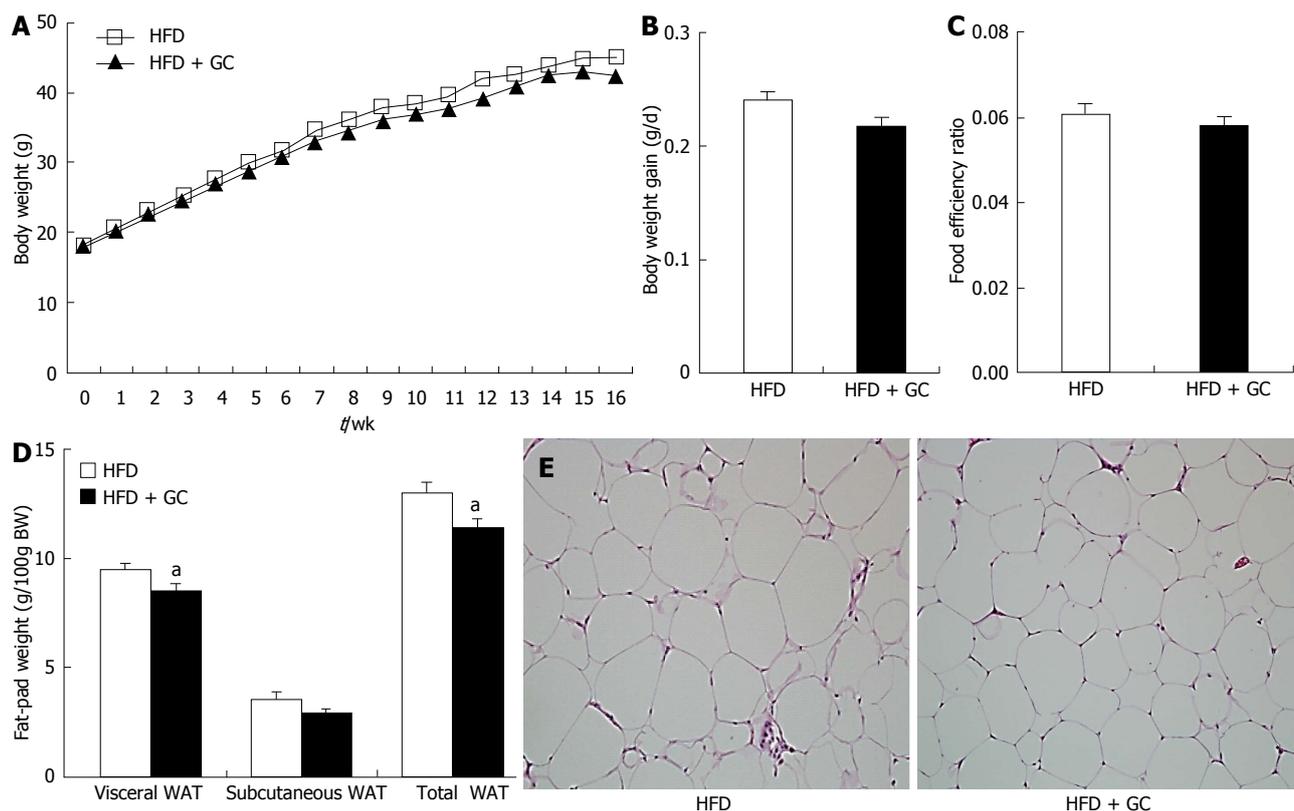


Figure 1 Effects of *Garcinia Cambogia* supplementation on body weight gain, food intake, fat-pad weight and adipocyte size in mice fed a high-fat diet for 16 wk. A-D: Data are expressed as the mean \pm SE ($n = 10$); E: HE staining is shown. Representative photographs of epididymal white adipose tissue (WAT) (original magnification $\times 200$). High-fat diet (HFD), mice fed a high-fat diet alone; HFD + *Garcinia Cambogia* (GC), mice fed a high-fat diet containing GC (1%, w/w). ^a $P < 0.05$ vs control group.

(reverse); for TNF- α , 5'-GCAGGTCTACTTTAGAGT-CATTGC-3' (forward), 5'-TCCCTTTGCAGAACTCAG GAATGG-3' (reverse); for stearoyl-CoA desaturase (SCD1), 5'-CCCCTGCGGATCTTCCTTAT-3' (forward), 5'-AGGGTCGGCGTGTGTTTCT-3' (reverse); for superoxide dismutase (SOD), 5'-TGGTTGAGAA-GATAGGCGACA-3' (forward), 5'-CATCTCG-GCAGCATCCACCTC-3' (reverse); and for sterol-regulatory-element-binding protein 1c (SREBP1c), 5'-GGAGCCATGGAT'TGCACAT'T-3' (forward), 5'-CCTGTCTCACCCCCAGCATA-3' (reverse).

Histological analysis

Epididymal WAT and liver were fixed in a buffer solution of 10% formalin and embedded in paraffin for staining with hematoxylin and eosin (HE) and Masson's trichrome. Stained areas were viewed using an optical microscope (Nikon, Tokyo, Japan) with a magnifying power of $\times 200$.

Ethics

After blood collection, epididymal WAT, perirenal WAT, retroperitoneal WAT, mesentery WAT, subcutaneous WAT and liver were promptly removed, rinsed with physiological saline and weighed. Among them, epididymal WAT and liver were snap-frozen in liquid nitrogen and stored at $-70\text{ }^{\circ}\text{C}$ until enzyme activity and RNA analyses. All experimental procedures were performed in accor-

dance with the protocols for animal studies approved by the Kyungpook National University Ethics Committee (Approval No. KNU-2011-49).

Statistical analysis

Data were expressed as the mean \pm SE. Statistical analyses were performed using the statistical package for the social science software (SPSS) program. Student's *t* test was used to assess the differences between the groups. Statistical significance was considered at $P < 0.05$.

RESULTS

Long-term GC supplementation did not alter body weight but significantly lowered body fat weight in HFD-induced obese mice

To investigate the effects of long-term GC supplementation in diet-induced obese mice, we provided 5-wk-old male C57BL/6J mice with HFD or 1% (w/w) GC supplemented HFD for 16 wk. During the experimental period, there was no significant difference in daily food intake between the groups (HFD, 3.96 ± 0.14 g; GC, 3.87 ± 0.07 g). The body weight gain was slightly lower in the GC-supplemented mice compared to the HFD control mice but the effects of GC were not significant (Figure 1A and B). Thus, the food efficiency ratio was not significantly different between the groups (Figure 1C). However, the weight of the visceral WAT including

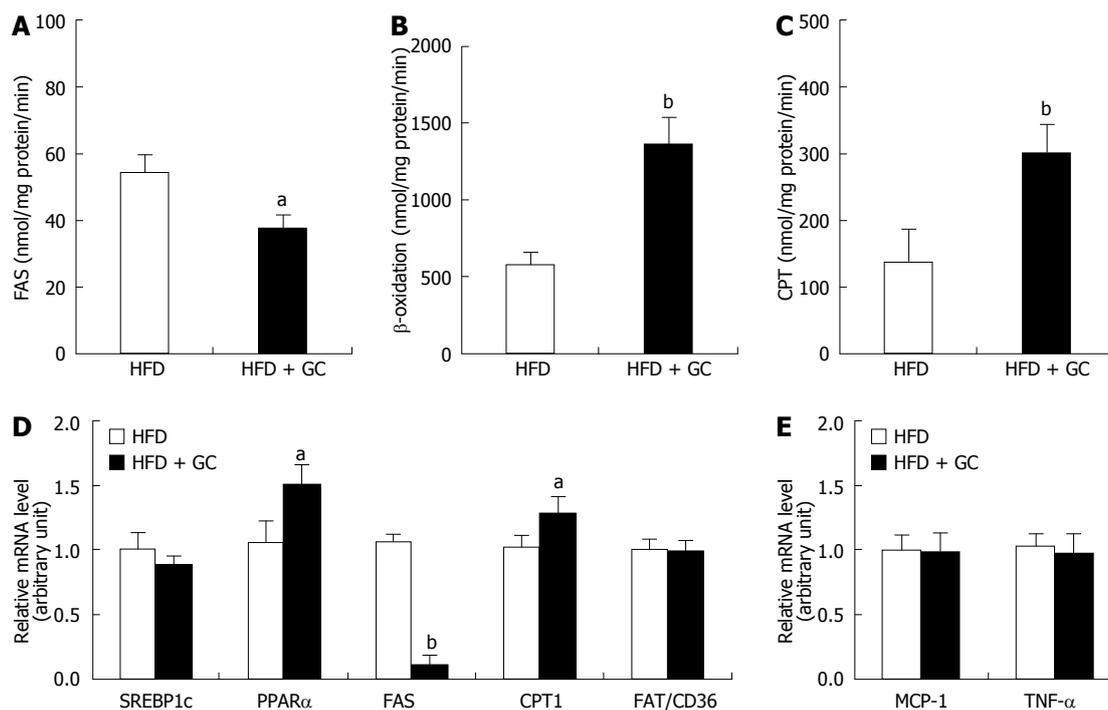


Figure 2 Effects of *Garcinia Cambogia* supplementation on fatty acid-regulating enzyme activity and gene expression in epididymal white adipose tissue of mice fed a high-fat diet for 16 wk. Data are expressed as the mean \pm SE ($n = 10$). A: Fatty acid synthase (FAS); B: β -oxidation; C: Carnitine palmitoyltransferase (CPT); D, E: Relative mRNA level. High-fat diet (HFD), mice fed a high-fat diet alone; HFD + *Garcinia Cambogia* (GC), mice fed a high-fat diet containing GC (1%, w/w). ^a $P < 0.05$, ^b $P < 0.01$ vs control group. WAT: White adipose tissue; PPAR α : Peroxisome proliferator-activated receptor α .

the epididymal, perirenal, retroperitoneal and mesentery WAT was significantly lower in the GC-supplemented mice than in the HFD control mice (Figure 1D). The GC supplementation also tended to lower the subcutaneous WAT weight compared to the HFD control group by 17% although it was not significantly different. Hence, the weight of the total WAT (visceral and subcutaneous WAT) was significantly lower in mice fed a GC supplemented HFD. Morphological observations also indicated the epididymal adipocyte size was smaller in the GC-supplemented mice than in the HFD control mice (Figure 1E). However, GC supplementation did not alter the extent and degree of fibrosis in the epididymal WAT of HFD-fed mice (data not shown).

Long-term GC supplementation alters the activity of enzymes and expression of genes related to fatty acid synthesis and fatty acid oxidation in visceral WAT

To examine the mechanism through which GC supplementation reduces the visceral WAT weight, we measured the activity of enzymes that regulate lipid accumulation in visceral WAT. The GC supplementation resulted in a significant decrease in the activity of FAS in the epididymal WAT of mice fed a HFD (Figure 2A). Furthermore, GC-supplemented mice showed a significant increase in the activity of CPT and β -oxidation in the epididymal WAT (Figure 2B and C).

We also examined the expression of genes that regulate adipogenesis and inflammation. Consistent with the activity of adipose enzymes, GC supplementation

significantly down-regulated FAS mRNA expression, whereas it markedly up-regulated CPT mRNA expression in the epididymal WAT of HFD-fed mice (Figure 2D). Moreover, GC-supplemented mice showed a significant increase in the mRNA expression of transcription factor PPAR α in the epididymal WAT compared to the control mice. However, there were no significant differences in the mRNA expression of SREBP1c, FAT/CD36, MCP-1 and TNF- α between the two groups (Figure 2D and E).

Long-term GC supplementation improved HFD-induced glucose intolerance but did not alter plasma lipid, apolipoprotein and pro-inflammatory cytokine levels

We next determined whether GC influenced HFD-induced glucose intolerance. The fasting blood glucose, plasma insulin and HOMA-IR levels were not significantly altered by GC supplementation (data not shown). However, GC supplementation significantly lowered the blood glucose level compared to the control group at 120 min after glucose loading (Figure 3A). The level of AUC was also markedly decreased in the GC-supplemented mice compared to the control obese mice.

No significant differences were observed in the levels of plasma lipids (triglycerides, total cholesterol, HDL-cholesterol, phospholipids and free fatty acids) and apolipoproteins (apolipoproteins A and B) between the two groups (Table 1). GC supplementation also did not affect the plasma leptin, TNF- α and MCP-1 levels in the HFD-fed mice; however, it significantly lowered the plasma resistin level (Figure 3B and C).

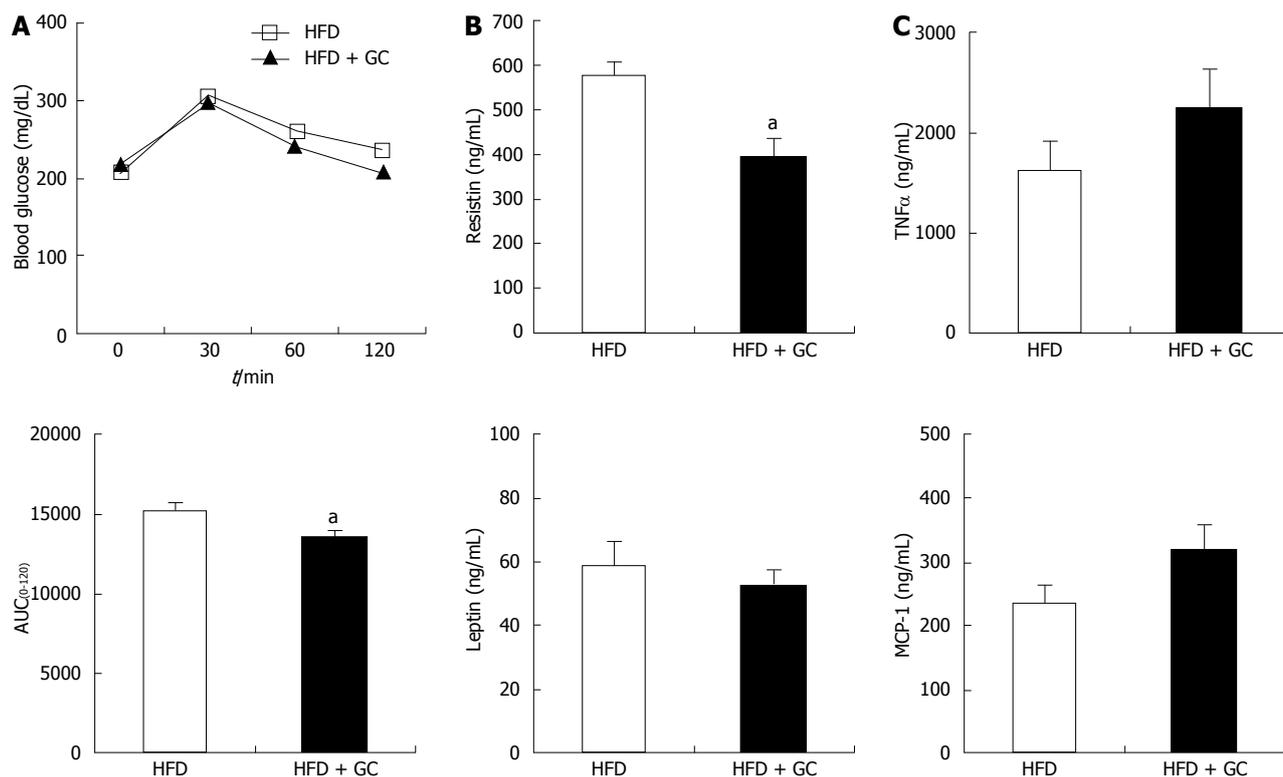


Figure 3 Effects of *Garcinia Cambogia* supplementation on glucose tolerance and plasma adipocytokine levels in mice fed a high-fat diet for 16 wk. Data are expressed as the mean ± SE (n = 10). A: The intraperitoneal glucose tolerance test was performed on the 15th week of *Garcinia Cambogia* (GC) supplementation in high-fat diet (HFD)-fed mice. Following a 12-h fast, the mice were injected intraperitoneally with glucose (0.5 g/kg body weight). Blood glucose was then measured via the tail vein at the indicated time [above: Blood glucose values; below: Areas under the curves (AUC)]; B, C: Plasma levels of leptin, resistin, monocyte chemoattractant protein-1 (MCP-1) and tumor necrosis factor-α (TNF-α) were assayed after 16 wk of GC supplementation in HFD-fed mice. HFD, mice fed a high-fat diet alone; HFD + GC, mice fed a high-fat diet containing GC (1%, w/w). ^aP < 0.05 vs control group.

Table 1 Effects of *Garcinia Cambogia* supplementation on plasma lipids and apolipoproteins levels in mice fed a high-fat diet for 16 wk

	HFD	HFD + GC
Triglyceride (mg/dL)	97.47 ± 5.62	83.56 ± 4.51
Total cholesterol (mg/dL)	162.65 ± 10.16	167.73 ± 14.40
HDL-cholesterol (mg/dL)	76.27 ± 6.05	76.06 ± 6.37
Phospholipid (mmol/L)	2.35 ± 0.15	2.05 ± 0.15
Free fatty acid (mmol/L)	0.95 ± 0.08	1.02 ± 0.17
Apolipoprotein B (mmol/L)	5.32 ± 0.59	5.97 ± 0.39
Apolipoprotein A (mmol/L)	50.87 ± 1.24	45.22 ± 1.35

Data are expressed as the mean ± SE (n = 10). HFD: High-fat diet; GC: *Garcinia Cambogia*; HDL: High-density lipoprotein.

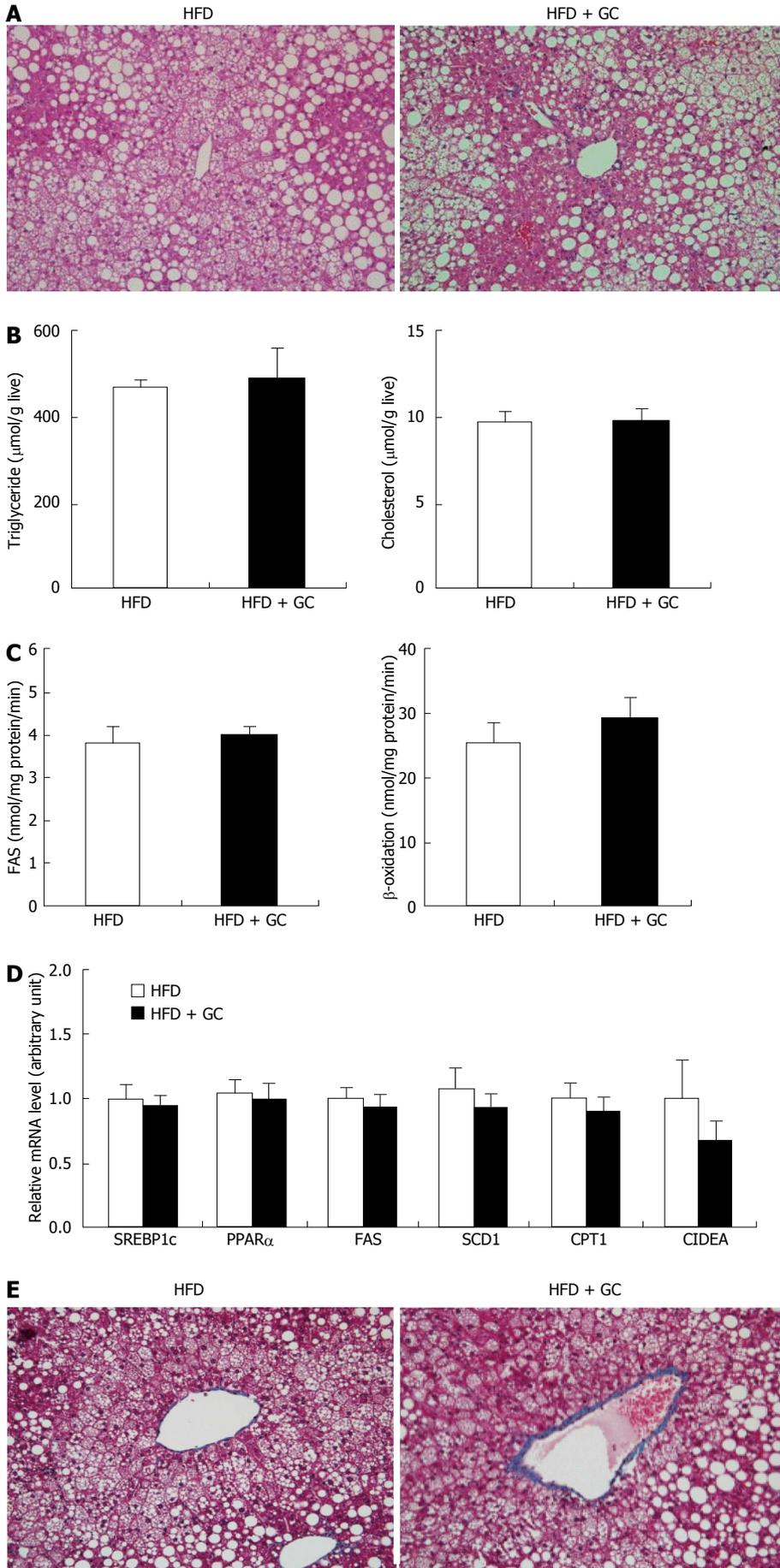
Long-term GC supplementation did not affect HFD-induced hepatic steatosis but increased hepatic collagen accumulation, inflammation and oxidative stress

Next, we examined the effect of GC supplementation on NAFLD induced by HFD. GC supplementation did not alter the hepatic triglyceride and cholesterol contents as well as the accumulation of hepatic lipid droplets in HFD-fed mice (Figure 4A and B). There were also no significant changes in the activities of hepatic FAS and β-oxidation and in the mRNA levels of the genes involved in lipogenesis and fatty acid oxidation, includ-

ing FAS, SCD1, CPT, CIDEA, SREBP1c and PPARα, between the two groups (Figure 4C and D). However, trichrome staining of the liver revealed GC supplementation increased collagen deposition (blue staining) compared to the control mice (Figure 4E). Furthermore, Plasma ALT and AST levels were significantly increased in the GC group compared to the control mice (Figure 4F). The mRNA levels of TNF-α and MCP-1, pro-inflammatory markers, were significantly increased in the liver of GC-supplemented mice compared to the control mice (Figure 4G). GC supplementation also caused significant increases of hepatic SOD and glutathione peroxidase (GSH-Px) mRNA levels as well as TBARS level compared to the control mice, although there was no significant difference in hepatic CAT mRNA level between the two groups (Figure 4H and I).

DISCUSSION

Since unhealthy eating habits combined with limited activity are a major contributor to obesity and its related metabolic disease, lifestyle changes may present a cost-effective first-line of intervention for obesity^[24-26]. Dietary supplements seemed to be an inefficient agent for dietary intervention in obese subjects^[26]. Such supplements are not recommended by the position papers/guidelines for management of NAFLD^[27-32], and have been associated



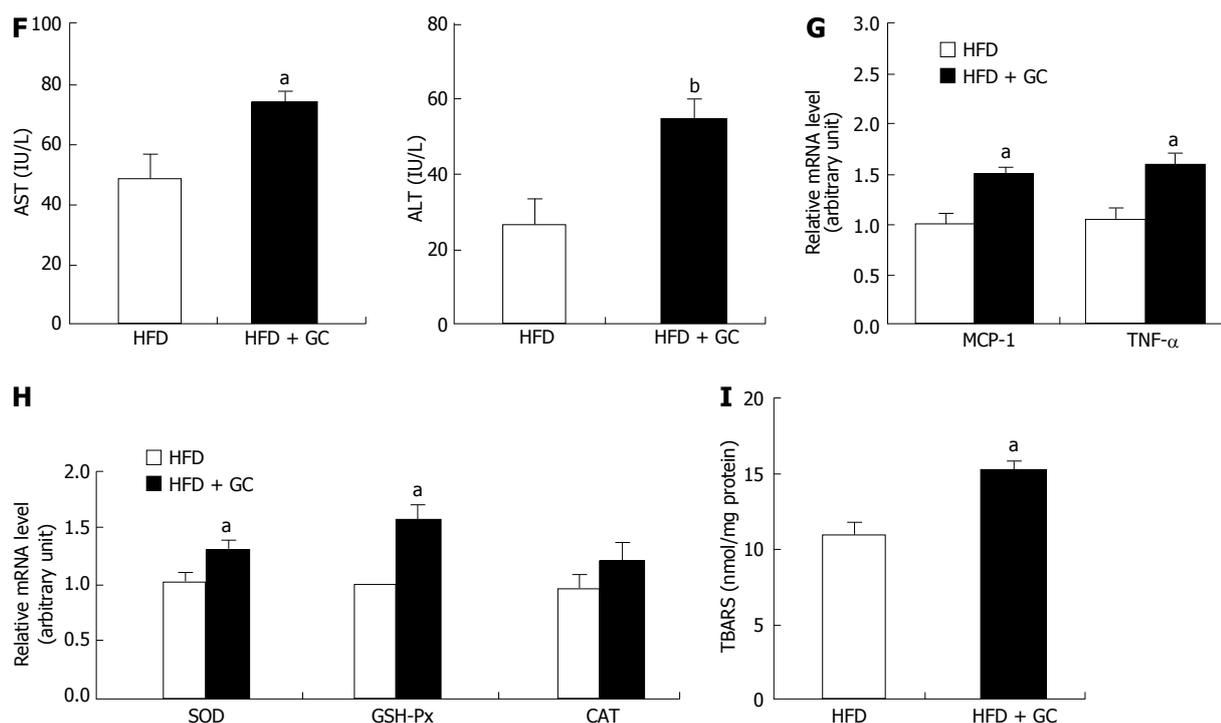


Figure 4 Long-term *Garcinia Cambogia* supplementation did not affect high-fat diet-induced hepatic steatosis but increased hepatic collagen accumulation, inflammation and oxidative stress. A: The liver tissue sections were stained with hematoxylin and eosin; B: Triglyceride and cholesterol level; C: Fatty acid synthase (FAS) and β -oxidation level; D: Relative mRNA level; E: The liver tissue sections were stained with Masson's trichrome; F: Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) level; G, H: Relative mRNA level; I: thiobarbituric acid-reactive substances (TBARS) concentration. Representative images are shown (original magnification $\times 200$). Data are expressed as the mean \pm SE ($n = 10$). High-fat diet (HFD), mice fed a high-fat diet alone; HFD + *Garcinia Cambogia* (GC), mice fed a high-fat diet containing GC (1%, w/w). ^a $P < 0.05$, ^b $P < 0.01$ vs control group. CAT: Catalase; CIDEA: Cell death-inducing DNA fragmentation factor- α -like effector A; CPT: Carnitine palmitoyltransferase; FAS: Fatty acid synthase; GSH-Px: Glutathione peroxidase; MCP-1: Monocyte chemoattractant protein-1; PPAR α : Peroxisome proliferator-activated receptor α ; SCD1: Stearoyl-CoA desaturase; SOD: Superoxide dismutase; SREBP1c: Sterol-regulatory-element-binding protein 1c; TNF- α : Tumor necrosis factor- α .

with increased mortality in some studies^[33]. However, there are a number of natural dietary supplements for weight management, including GC, guar gum and chitosan^[34]. Among them, HCA-containing GC has been shown to be efficacious in lowering body weight and body fat^[6,10-13]. But, some clinical trials and animal studies have shown conflicting results^[6,12], and a case series on hepatotoxicity has been reported in patients taking GC-containing hydroxycut, although the individual chemical component underlying liver injury remains poorly understood^[14,15]. Therefore, the aim of this study was first to determine the effects from long-term GC supplementation on obesity and related metabolic diseases as well as the hepatotoxicity in mice fed a HFD.

In the present study, GC supplementation (1%, w/w) in a HFD for 16 wk did not lead to significant changes in body weight and food intake in mice. However, it resulted in significant decreases in visceral WAT weight and adipocyte size in HFD-induced obese mice. The anti-adiposity effect of GC was partly associated with marked decreases in FAS activity and its gene expression in the epididymal WAT. FAS is a key enzyme involved in *de novo* fatty acid synthesis and WAT is a major site of fatty acid synthesis and storage. We also found that the activity of CPT as well as fatty acid oxidation in epididymal WAT was elevated by GC supplementation. Furthermore, the

enhanced adipose fatty acid oxidation in GC-supplemented mice was accompanied by the up-regulated mRNA expression of genes involved in fatty acid oxidation such as CPT and PPAR α in the epididymal WAT. The CPT is a major rate-limiting enzyme for fatty acid oxidation, and its gene expression is regulated by PPAR α in adipocytes^[35]. The PPAR α mRNA expression was decreased in the WAT of both genetic and HFD-induced obese mice, and the down-regulation of PPAR α in obese WAT was involved in obesity-induced mitochondrial dysfunction and metabolic disorders^[36]. Taken together, our findings suggest that in HFD-fed mice, a significant reduction in visceral fat accumulation by GC supplementation could be partly due to decreased fatty acid synthesis as well as increased fatty acid oxidation in adipose tissue. The results of this study are supported by the findings of a previous study which suggested GC supplementation significantly lowered body fat mass, but not body weight and food intake, by inhibiting adipose ATP-citrate lyase (ACL) activity in Zucker obese rats^[12]. The ACL is another lipogenic enzyme catalyzing the cleavage of citrate to oxaloacetate and acetyl-CoA for *de novo* fatty acid synthesis^[37]. The inhibitory action of HCA on ACL reduces the acetyl-CoA pool, which can lead to a decreased concentration of malonyl-CoA, a physiological inhibitor of CPT^[38], and thus results in the suppression of body

fat accumulation through stimulation of fatty acid oxidation^[39]. Kim *et al.*^[11] also reported that HCA-containing GC (1%, w/w) supplementation in HFD-fed mice for 12 wk significantly lowered body fat accumulation by modulating multiple genes associated with adipogenesis in mice fed a HFD.

Along with the anti-obesity effects of HCA, previous studies have reported on the beneficial effects of HCA on insulin resistance^[40,41]. HCA increases the cellular pool of citrate by inhibiting ACL, which in turn can increase glycogen production^[42]. Recently, HCA supplementation enhanced the glycogen synthesis rate in skeletal muscles and improved post-meal insulin sensitivity^[41]. Although we did not measure the level of glycogen in the liver, HCA-containing GC supplementation improved glucose tolerance in HFD-induced obese mice. Furthermore, the plasma resistin level was significantly lowered by GC supplementation in the current study. Resistin is one of the adipokines proposed to link obesity with insulin resistance. Circulating resistin level was elevated in obesity and insulin resistance^[43], and HFD significantly increased plasma resistin levels in mice compared to a standard low-fat/high-carbohydrate diet^[44]. Resistin deficiency ameliorated glucose homeostasis in mice^[45], whereas administration of resistin impaired glucose tolerance and insulin action^[43,46]. Thus, the beneficial effect of GC on glucose intolerance might be associated with the decreased resistin level in plasma. Another mechanism in which GC could contribute to improve glucose tolerance is the lowered body fat mass because excess adiposity, especially in visceral WAT, is considered to promote insulin resistance^[47]. However, there was no significant difference in HOMA-IR which estimates insulin sensitivity from fasting glucose and insulin concentrations. Tripathy *et al.*^[48] reported a significant relationship between hepatic insulin sensitivity and HOMA-IR regardless of the stage of glucose tolerance, suggesting that the HOMA-IR is dependent upon hepatic insulin sensitivity rather than peripheral insulin sensitivity. Another clinical study also demonstrated that HOMA-IR did not accurately predict insulin sensitivity^[49].

Increased adiposity with the consequences of inflammation and insulin resistance has been linked to the development of NAFLD, which refers to a wide spectrum of liver damage, ranging from simple steatosis to steatohepatitis and cirrhosis. Steatosis represents the accumulation of fat within the liver through multiple mechanisms including an altered balance in fatty acid uptake and triglycerides secretion, increased *de novo* lipogenesis, and decreased fatty acid oxidation^[50,51]. Steatohepatitis is the combination of steatosis with hepatic inflammation and fibrosis. Liver fibrosis is excess synthesis and deposition of extracellular matrix proteins including collagen^[52], and pro-inflammatory factors including MCP-1 and TNF- α that contribute to the second hit in the pathogenesis of steatohepatitis. Among the inflammatory mediators, MCP-1 is a pro-inflammatory chemokine which coordinates leukocyte recruitment to the liver by activation of

the CC chemokine receptor 2 (CCR2) on inflammatory cells including monocytes and macrophages promoting the inflammatory response^[53]. Several studies indicate that MCP-1 is an important mediator of liver fibrosis^[54-56]. MCP-1 mRNA expression was markedly increased in the livers of patients with steatohepatitis and in murine models of steatohepatitis such as mice fed a HFD or methionine-choline deficient diet^[57-63]. CCR2 inhibitor suppressed the early and late features of steatohepatitis including fibrosis^[64], and chronic exposure of HFD induced hepatic MCP-1 mRNA expression in mice before induction of other pro-inflammatory cytokine mRNAs including TNF- α and prior to the onset of steatohepatitis, suggesting that MCP-1 plays a major role in initiating the inflammatory process in steatohepatitis^[65]. Moreover, MCP-1 deficiency reduced liver fibrosis (collagen deposition) and pro-fibrogenic gene expression in mice fed a methionine-choline deficient diet although it did not affect liver steatosis in this model^[66].

Similarly, we found that GC supplementation did not affect hepatic lipogenesis and lipid droplet formation, but it markedly increased collagen deposition as well as pro-inflammatory MCP-1 and TNF- α mRNA expression in the liver of HFD-fed mice. Furthermore, GC-supplemented mice exhibited impaired liver function indicated by the elevations of plasma ALT and AST, suggesting that GC possibly promotes liver injury in HFD-fed mice. Several case reports have suggested HCA-containing hydroxycut has potential hepatotoxicity but the underlying mechanism remains unknown^[14,15,67,68]. Our experiments provide new information regarding hepatotoxicity after long-term GC supplementation in HFD-induced obese mice. Accordingly, GC supplementation contributes to steatohepatitis by increasing hepatic collagen accumulation and hepatic MCP-1 and TNF- α expression in mice fed a HFD, which are independent of its effects on HFD-induced hepatic steatosis.

On the other hand, oxidative stress is considered to play an important role in progression of nonalcoholic steatohepatitis and hepatocellular injury^[69]. Reactive oxygen species (ROS) can damage DNA, lipids and proteins, induce necrosis and apoptosis of hepatocytes and amplify the inflammatory response. The ROS also stimulate the production of pro-fibrogenic mediators from Kupffer cells and inflammatory cells and directly induce hepatic stellate cells proliferation, resulting in the initiation of fibrosis^[70]. Antioxidant enzymes such as SOD and GSH-Px ameliorate the damaging effects of ROS. SOD converts superoxide radicals into hydrogen peroxide, which is then further metabolized by GSH-Px, where it catalyzes the destruction of hydrogen peroxide and lipid hydroperoxide. We observed that the supplementation of GC significantly up-regulated hepatic SOD and GSH-Px mRNA expression with concomitant increase in lipid peroxidation in the liver, suggesting that the increases in antioxidant gene expression by GC seems to be a compensatory response of the liver to cope with oxidative stress.

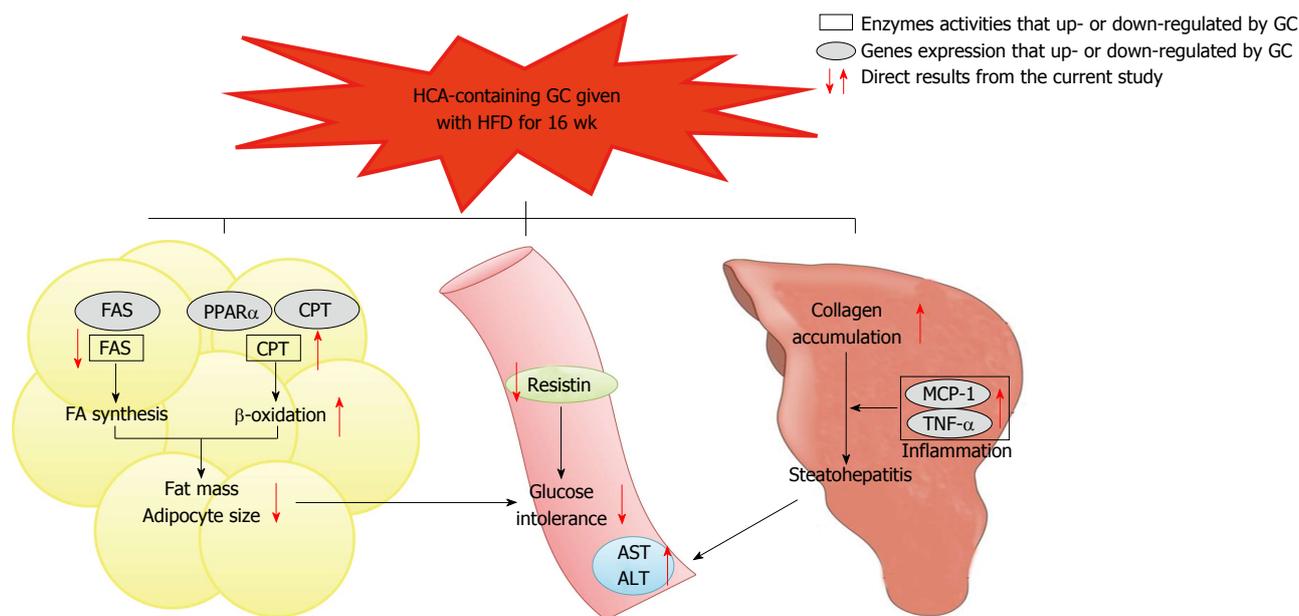


Figure 5 Summary of the long-term *Garcinia Cambogia* supplementation effects on adiposity, glucose tolerance and steatohepatitis in high-fat diet-induced obese mice. The *Garcinia Cambogia* (GC) supplementation decreased fatty acid synthase (FAS) mRNA expression and its activity, while peroxisome proliferator-activated receptor α (PPAR α) and carnitine palmitoyltransferase (CPT) mRNA expression along with the activities of CPT and β -oxidation were increased in the visceral adipose tissue, indicating that these changes may be potential mechanisms for reducing body fat accumulation and glucose intolerance induced by high-fat diet (HFD). Furthermore, GC supplementation decreased the plasma resistin level, which may be also related to improved glucose tolerance. There were no significant changes in hepatic lipid accumulation as well as in hepatic gene expression and enzymatic activity involved in fatty acid synthesis, oxidation and storage. However, GC increased pro-inflammatory monocyte chemoattractant protein-1 (MCP-1) and tumor necrosis factor- α (TNF- α) mRNA expression, lipid peroxidation and collagen accumulation in the liver. Plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were also increased by GC supplementation in HFD-induced obese mice, thus suggesting that GC may negatively affect liver function by increasing hepatic fibrosis, inflammation and oxidative stress without affecting hepatic fat accumulation. FA: Fatty acid; HCA: Hydroxycitric acid. Open square means enzymes activities that up- or down-regulated by GC. Solid circle means genes expression that up- or down-regulated by GC.

In conclusion, this study demonstrated that long-term GC supplementation ameliorated adipogenesis in mice fed a HFD by promoting fatty acid oxidation with a simultaneous decrease in fatty acid synthesis in visceral WAT. Furthermore, GC exhibited a protective role against glucose intolerance induced by HFD. Moreover, this study provides the first evidence that long-term GC supplementation significantly increased hepatic collagen accumulation, lipid peroxidation and MCP-1 and TNF- α mRNA expression as well as plasma AST and ALT levels, thereby contributing partly to the exacerbation of steatohepatitis in HFD-induced obese mice at the doses given. The observations described above are summarized in Figure 5. Although further research is required to elucidate the efficacy and safety of long-term use of GC in humans, caution is needed when using GC supplements for weight management.

COMMENTS

Background

Garcinia Cambogia (GC) is a popular dietary supplement for weight loss, but the efficacy and hepatotoxicity of long-term GC supplementation remain poorly understood. Thus, authors investigated the long-term supplementation effects of GC on adiposity and non-alcoholic fatty liver disease (NAFLD) in mice fed a high-fat diet (HFD).

Research frontiers

A number of experiments reported GC has beneficial effects on weight loss and body fat in some animals and human. However, there are controversial findings

and little studies have reported whether these effects persist beyond 13 wk of GC supplementation.

Innovations and breakthroughs

This is first report which shows the supplementation of GC increased hepatic collagen accumulation, inflammatory genes expression and lipid peroxidation as well as plasma alanine aminotransferase and aspartate aminotransferase levels, although HFD-induced hepatic steatosis did not change. Thus their findings suggest that long-term supplementation of GC induces hepatic fibrosis and inflammation, although it protects against HFD-induced adiposity and glucose intolerance in mice fed a high fat diet.

Applications

Extensive dietary supplements are popular and widely used for weight management. However, the optimal dose and safety profiles of many dietary supplements are poorly studied and they are not regulated by the Food and Drug Administration in a manner observed for pharmacological agents. The authors suggest that caution is needed when using GC supplements for weight management, although further research is required to elucidate the efficacy and safety of long-term use of GC in humans.

Peer review

The authors investigated long-term supplementation effects of GC on adiposity and NAFLD in diet-induced obese mice. They reported that long-term GC supplementation improved adipogenesis by promoting fatty acid oxidation along with a decrease in fatty acid synthesis in visceral white adipose tissue. They also reported a protective role of GC against glucose intolerance induced by HFD. However, they also showed that long-term GC supplementation increased hepatic collagen accumulation and cytokine expression, thereby exacerbating steatohepatitis. The manuscript has no structural flaws. The hypothesis is relevant and methods to test the hypothesis were up-to-date.

REFERENCES

- Gallagher EJ, Leroith D, Karnieli E. Insulin resistance in

- obesity as the underlying cause for the metabolic syndrome. *Mt Sinai J Med* 2010; **77**: 511-523 [PMID: 20960553 DOI: 10.1002/msj.20212]
- 2 **World Health Organization.** Global Strategy on Diet, Physical Activity and Health. Available from: URL: http://www.who.int/dietphysicalactivity/media/en/gsf_ obesity.pdf
 - 3 **Pillitteri JL**, Shiffman S, Rohay JM, Harkins AM, Burton SL, Wadden TA. Use of dietary supplements for weight loss in the United States: results of a national survey. *Obesity* (Silver Spring) 2008; **16**: 790-796 [PMID: 18239570 DOI: 10.1038/oby.2007.136]
 - 4 **Pittler MH**, Schmidt K, Ernst E. Adverse events of herbal food supplements for body weight reduction: systematic review. *Obes Rev* 2005; **6**: 93-111 [PMID: 15836459 DOI: 10.1111/j.1467-789X.2005.00169.x]
 - 5 **Hurt RT**, Wilson T. Geriatric obesity: evaluating the evidence for the use of flavonoids to promote weight loss. *J Nutr Gerontol Geriatr* 2012; **31**: 269-289 [PMID: 22888842 DOI: 10.1080/21551197.2012.698222]
 - 6 **Heymsfield SB**, Allison DB, Vasselli JR, Pietrobelli A, Greenfield D, Nunez C. *Garcinia cambogia* (hydroxycitric acid) as a potential antiobesity agent: a randomized controlled trial. *JAMA* 1998; **280**: 1596-1600 [PMID: 9820262 DOI: 10.1001/jama.280.18.1596]
 - 7 **Leonhardt M**, Hrupka B, Langhans W. Effect of hydroxycitrate on food intake and body weight regain after a period of restrictive feeding in male rats. *Physiol Behav* 2001; **74**: 191-196 [PMID: 11564468 DOI: 10.1016/S0031-9384(01)00547-9]
 - 8 **Sullivan AC**, Triscari J, Neal MO. The influence of (-)-hydroxycitrate on in vivo rates of hepatic glycogenesis: lipogenesis and cholesterol-genesis. *Fed Proc* 1974; **33**: 656
 - 9 **Nageswara RR**, Sakeriak KK. Lipid-lowering and antiobesity effect of (-) hydroxycitric acid. *Nutr Res* 1988; **8**: 209-212 [DOI: 10.1016/S0271-5317(88)80024-1]
 - 10 **Márquez F**, Babio N, Bulló M, Salas-Salvadó J. Evaluation of the safety and efficacy of hydroxycitric acid or *Garcinia cambogia* extracts in humans. *Crit Rev Food Sci Nutr* 2012; **52**: 585-594 [PMID: 22530711 DOI: 10.1080/10408398.2010.500551]
 - 11 **Kim KY**, Lee HN, Kim YJ, Park T. *Garcinia cambogia* extract ameliorates visceral adiposity in C57BL/6J mice fed on a high-fat diet. *Biosci Biotechnol Biochem* 2008; **72**: 1772-1780 [PMID: 18603810 DOI: 10.1271/bbb.80072]
 - 12 **Saito M**, Ueno M, Ogino S, Kubo K, Nagata J, Takeuchi M. High dose of *Garcinia cambogia* is effective in suppressing fat accumulation in developing male Zucker obese rats, but highly toxic to the testis. *Food Chem Toxicol* 2005; **43**: 411-419 [PMID: 15680676 DOI: 10.1016/j.fct.2004.11.008]
 - 13 **Hayamizu K**, Hirakawa H, Oikawa D, Nakanishi T, Takagi T, Tachibana T, Furuse M. Effect of *Garcinia cambogia* extract on serum leptin and insulin in mice. *Fitoterapia* 2003; **74**: 267-273 [PMID: 12727492 DOI: 10.1016/S0367-326X(03)00036-4]
 - 14 **Stevens T**, Qadri A, Zein NN. Two patients with acute liver injury associated with use of the herbal weight-loss supplement hydroxycut. *Ann Intern Med* 2005; **142**: 477-478 [PMID: 15767636 DOI: 10.7326/0003-4819-142-6-200503150-00026]
 - 15 **Dara L**, Hewett J, Lim JK. Hydroxycut hepatotoxicity: a case series and review of liver toxicity from herbal weight loss supplements. *World J Gastroenterol* 2008; **14**: 6999-7004 [PMID: 19058338 DOI: 10.3748/wjg.14.6999]
 - 16 **Folch J**, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem* 1957; **226**: 497-509 [PMID: 13428781]
 - 17 **Kim HJ**, Lee KT, Park YB, Jeon SM, Choi MS. Dietary docosahexaenoic acid-rich diacylglycerols ameliorate hepatic steatosis and alter hepatic gene expressions in C57BL/6J-Lep(ob/ob) mice. *Mol Nutr Food Res* 2008; **52**: 965-973 [PMID: 18481331 DOI: 10.1002/mnfr.200700315]
 - 18 **Carl MN**, Lakshmanan MR, Porter JW. Fatty acid synthase from rat liver. *Methods In Enzymology* 1975; **35**: 37-44 [DOI: 10.1016/0076-6879(75)35136-7]
 - 19 **Markwell MA**, McGroarty EJ, Bieber LL, Tolbert NE. The subcellular distribution of carnitine acyltransferases in mammalian liver and kidney. A new peroxisomal enzyme. *J Biol Chem* 1973; **248**: 3426-3432 [PMID: 4702872]
 - 20 **Lazarow PB**. Assay of peroxisomal beta-oxidation of fatty acids. *Methods Enzymol* 1981; **72**: 315-319 [PMID: 7031421 DOI: 10.1016/S0076-6879(81)72021-4]
 - 21 **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 [PMID: 942051 DOI: 10.1016/0003-2697(76)90527-3]
 - 22 **Ohkawa H**, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 1979; **95**: 351-358 [PMID: 36810 DOI: 10.1016/0003-2697(79)90738-3]
 - 23 **Schmittgen TD**, Livak KJ. Analyzing real-time PCR data by the comparative C(T) method. *Nat Protoc* 2008; **3**: 1101-1108 [PMID: 18546601]
 - 24 **Ross R**, Dagnone D, Jones PJ, Smith H, Paddags A, Hudson R, Janssen I. Reduction in obesity and related comorbid conditions after diet-induced weight loss or exercise-induced weight loss in men. A randomized, controlled trial. *Ann Intern Med* 2000; **133**: 92-103 [PMID: 10896648 DOI: 10.7326/0003-4819-133-2-200007180-00008]
 - 25 **Blackburn GL**. Treatment approaches: food first for weight management and health. *Obes Res* 2001; **9** Suppl 4: 223S-227S [PMID: 11707545 DOI: 10.1038/oby.2001.122]
 - 26 **Scaglioni F**, Marino M, Ciccia S, Procaccini A, Busacchi M, Loria P, Lonardo A, Malavolti M, Battistini NC, Pellegrini M, Carubbi F, Bellentani S. Short-term multidisciplinary non-pharmacological intervention is effective in reducing liver fat content assessed non-invasively in patients with nonalcoholic fatty liver disease (NAFLD). *Clin Res Hepatol Gastroenterol* 2012; Epub ahead of print [PMID: 23273500]
 - 27 **Ratziu V**, Bellentani S, Cortez-Pinto H, Day C, Marchesini G. A position statement on NAFLD/NASH based on the EASL 2009 special conference. *J Hepatol* 2010; **53**: 372-384 [PMID: 20494470 DOI: 10.1016/j.jhep.2010.04.008]
 - 28 **Farrell GC**, Chitturi S, Lau GK, Sollano JD. Guidelines for the assessment and management of non-alcoholic fatty liver disease in the Asia-Pacific region: executive summary. *J Gastroenterol Hepatol* 2007; **22**: 775-777 [PMID: 17565629 DOI: 10.1111/j.1440-1746.2007.05002.x]
 - 29 **Fan JG**, Jia JD, Li YM, Wang BY, Lu LG, Shi JP, Chan LY. Guidelines for the diagnosis and management of nonalcoholic fatty liver disease. *J Dig Dis* 2011; **12**: 38-44 [PMID: 21276207 DOI: 10.1111/j.1751-2980.2010.00476.x]
 - 30 **Loria P**, Adinolfi LE, Bellentani S, Bugianesi E, Grieco A, Fargion S, Gasbarrini A, Loguercio C, Lonardo A, Marchesini G, Marra F, Persico M, Prati D, Baroni GS. Practice guidelines for the diagnosis and management of nonalcoholic fatty liver disease. A decalogue from the Italian Association for the Study of the Liver (AISF) Expert Committee. *Dig Liver Dis* 2010; **42**: 272-282 [PMID: 20171943 DOI: 10.1016/j.dld.2010.01.021]
 - 31 **Chalasani N**, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, Charlton M, Sanyal AJ. The diagnosis and management of non-alcoholic fatty liver disease: practice guideline by the American Gastroenterological Association, American Association for the Study of Liver Diseases, and American College of Gastroenterology. *Gastroenterology* 2012; **142**: 1592-1609 [PMID: 22656328 DOI: 10.1053/j.gastro.2012.04.001]
 - 32 **Vajro P**, Lenta S, Socha P, Dhawan A, McKiernan P, Baumann U, Durmaz O, Lacaille F, McLin V, Nobili V. Diagnosis of nonalcoholic fatty liver disease in children and adolescents: position paper of the ESPGHAN Hepatology Committee. *J Pediatr Gastroenterol Nutr* 2012; **54**: 700-713 [PMID:

- 22395188 DOI: 10.1097/MPG.0b013e318252a13f]
- 33 **Bjelakovic G**, Nikolova D, Gluud LL, Simonetti RG, Gluud C. Antioxidant supplements for prevention of mortality in healthy participants and patients with various diseases. *Cochrane Database Syst Rev* 2012; **3**: CD007176 [PMID: 22419320 DOI: 10.1002/14651858.CD007176.pub2]
- 34 **Pittler MH**, Ernst E. Dietary supplements for body-weight reduction: a systematic review. *Am J Clin Nutr* 2004; **79**: 529-536 [PMID: 15051593]
- 35 **Lee JY**, Hashizaki H, Goto T, Sakamoto T, Takahashi N, Kawada T. Activation of peroxisome proliferator-activated receptor- α enhances fatty acid oxidation in human adipocytes. *Biochem Biophys Res Commun* 2011; **407**: 818-822 [PMID: 21443859 DOI: 10.1016/j.bbrc.2011.03.106]
- 36 **Goto T**, Lee JY, Teraminami A, Kim YI, Hirai S, Uemura T, Inoue H, Takahashi N, Kawada T. Activation of peroxisome proliferator-activated receptor- α stimulates both differentiation and fatty acid oxidation in adipocytes. *J Lipid Res* 2011; **52**: 873-884 [PMID: 21324916 DOI: 10.1194/jlr.M011320]
- 37 **Sullivan AC**, Singh M, Srere PA, Glusker JP. Reactivity and inhibitor potential of hydroxycitrate isomers with citrate synthase, citrate lyase, and ATP citrate lyase. *J Biol Chem* 1977; **252**: 7583-7590 [PMID: 334761]
- 38 **McGarry JD**, Mannaerts GP, Foster DW. A possible role for malonyl-CoA in the regulation of hepatic fatty acid oxidation and ketogenesis. *J Clin Invest* 1977; **60**: 265-270 [PMID: 874089 DOI: 10.1172/JCI108764]
- 39 **Ishihara K**, Oyaizu S, Onuki K, Lim K, Fushiki T. Chronic (-)-hydroxycitrate administration spares carbohydrate utilization and promotes lipid oxidation during exercise in mice. *J Nutr* 2000; **130**: 2990-2995 [PMID: 11110858]
- 40 **Asghar M**, Monjok E, Kouamou G, Ohia SE, Bagchi D, Lokhandwala MF. Super CitriMax (HCA-SX) attenuates increases in oxidative stress, inflammation, insulin resistance, and body weight in developing obese Zucker rats. *Mol Cell Biochem* 2007; **304**: 93-99 [PMID: 17503004 DOI: 10.1007/s11010-007-9489-3]
- 41 **Cheng IS**, Huang SW, Lu HC, Wu CL, Chu YC, Lee SD, Huang CY, Kuo CH. Oral hydroxycitrate supplementation enhances glycogen synthesis in exercised human skeletal muscle. *Br J Nutr* 2012; **107**: 1048-1055 [PMID: 21824444 DOI: 10.1017/S0007114511003862]
- 42 **Soni MG**, Burdock GA, Preuss HG, Stohs SJ, Ohia SE, Bagchi D. Safety assessment of (-)-hydroxycitric acid and Super CitriMax, a novel calcium/potassium salt. *Food Chem Toxicol* 2004; **42**: 1513-1529 [PMID: 15234082 DOI: 10.1016/j.fct.2004.04.014]
- 43 **Steppan CM**, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM, Patel HR, Ahima RS, Lazar MA. The hormone resistin links obesity to diabetes. *Nature* 2001; **409**: 307-312 [PMID: 11201732 DOI: 10.1038/35053000]
- 44 **Muse ED**, Obici S, Bhanot S, Monia BP, McKay RA, Rajala MW, Scherer PE, Rossetti L. Role of resistin in diet-induced hepatic insulin resistance. *J Clin Invest* 2004; **114**: 232-239 [PMID: 15254590]
- 45 **Banerjee RR**, Rangwala SM, Shapiro JS, Rich AS, Rhoades B, Qi Y, Wang J, Rajala MW, Poci A, Scherer PE, Steppan CM, Ahima RS, Obici S, Rossetti L, Lazar MA. Regulation of fasted blood glucose by resistin. *Science* 2004; **303**: 1195-1198 [PMID: 14976316 DOI: 10.1126/science.1092341]
- 46 **Rajala MW**, Obici S, Scherer PE, Rossetti L. Adipose-derived resistin and gut-derived resistin-like molecule- β selectively impair insulin action on glucose production. *J Clin Invest* 2003; **111**: 225-230 [PMID: 12531878]
- 47 **Jo J**, Gavrillova O, Pack S, Jou W, Mullen S, Sumner AE, Cushman SW, Periwai V. Hypertrophy and/or Hyperplasia: Dynamics of Adipose Tissue Growth. *PLoS Comput Biol* 2009; **5**: e1000324 [PMID: 19325873 DOI: 10.1371/journal.pcbi.1000324]
- 48 **Tripathy D**, Almgren P, Tuomi T, Groop L. Contribution of insulin-stimulated glucose uptake and basal hepatic insulin sensitivity to surrogate measures of insulin sensitivity. *Diabetes Care* 2004; **27**: 2204-2210 [PMID: 15333485 DOI: 10.2337/diacare.27.9.2204]
- 49 **Ferrara CM**, Goldberg AP. Limited value of the homeostasis model assessment to predict insulin resistance in older men with impaired glucose tolerance. *Diabetes Care* 2001; **24**: 245-249 [PMID: 11213873 DOI: 10.2337/diacare.24.2.245]
- 50 **Pickens MK**, Yan JS, Ng RK, Ogata H, Grenert JP, Beysen C, Turner SM, Maher JJ. Dietary sucrose is essential to the development of liver injury in the methionine-choline-deficient model of steatohepatitis. *J Lipid Res* 2009; **50**: 2072-2082 [PMID: 19295183 DOI: 10.1194/jlr.M900022-JLR200]
- 51 **Rinella ME**, Elias MS, Smolak RR, Fu T, Borensztajn J, Green RM. Mechanisms of hepatic steatosis in mice fed a lipogenic methionine choline-deficient diet. *J Lipid Res* 2008; **49**: 1068-1076 [PMID: 18227531 DOI: 10.1194/jlr.M800042-JLR200]
- 52 **Friedman SL**. Mechanisms of hepatic fibrogenesis. *Gastroenterology* 2008; **134**: 1655-1669 [PMID: 18471545 DOI: 10.1016/j.jbpg.2011.02.005]
- 53 **Deshmane SL**, Kremlev S, Amini S, Sawaya BE. Monocyte chemoattractant protein-1 (MCP-1): an overview. *J Interferon Cytokine Res* 2009; **29**: 313-326 [PMID: 19441883 DOI: 10.1089/jir.2008.0027]
- 54 **Seki E**, de Minicis S, Inokuchi S, Taura K, Miyai K, van Rooijen N, Schwabe RF, Brenner DA. CCR2 promotes hepatic fibrosis in mice. *Hepatology* 2009; **50**: 185-197 [PMID: 19441102 DOI: 10.1002/hep.22952]
- 55 **Ramm GA**, Shepherd RW, Hoskins AC, Greco SA, Ney AD, Pereira TN, Bridle KR, Doecke JD, Meikle PJ, Turlin B, Lewindon PJ. Fibrogenesis in pediatric cholestatic liver disease: role of taurocholate and hepatocyte-derived monocyte chemoattractant protein-1 in hepatic stellate cell recruitment. *Hepatology* 2009; **49**: 533-544 [PMID: 19115220 DOI: 10.1002/hep.22637]
- 56 **Wynn TA**. Cellular and molecular mechanisms of fibrosis. *J Pathol* 2008; **214**: 199-210 [PMID: 18161745]
- 57 **Haukeland JW**, Damås JK, Konopski Z, Løberg EM, Haaland T, Goverud I, Torjesen PA, Birkeland K, Bjørø K, Aukrust P. Systemic inflammation in nonalcoholic fatty liver disease is characterized by elevated levels of CCL2. *J Hepatol* 2006; **44**: 1167-1174 [PMID: 16618517 DOI: 10.1016/j.jhep.2006.02.011]
- 58 **Westerbacka J**, Kolak M, Kiviluoto T, Arkkila P, Sirén J, Hamsten A, Fisher RM, Yki-Järvinen H. Genes involved in fatty acid partitioning and binding, lipolysis, monocyte/macrophage recruitment, and inflammation are overexpressed in the human fatty liver of insulin-resistant subjects. *Diabetes* 2007; **56**: 2759-2765 [PMID: 17704301 DOI: 10.2337/db07-0156]
- 59 **Marsillach J**, Oliveras-Ferraro C, Beltrán R, Rull A, Aragonès G, Alonso-Villaverde C, Vázquez-Martín A, Joven J, Menéndez JA, Camps J. Serum concentrations of extracellular fatty acid synthase in patients with steatohepatitis. *Clin Chem Lab Med* 2009; **47**: 1097-1099 [PMID: 19728851 DOI: 10.1515/CCLM.2009.259]
- 60 **Greco D**, Kotronen A, Westerbacka J, Puig O, Arkkila P, Kiviluoto T, Laitinen S, Kolak M, Fisher RM, Hamsten A, Auvinen P, Yki-Järvinen H. Gene expression in human NAFLD. *Am J Physiol Gastrointest Liver Physiol* 2008; **294**: G1281-G1287 [PMID: 18388185 DOI: 10.1152/ajpgi.00074.2008]
- 61 **Kanda H**, Tateya S, Tamori Y, Kotani K, Hiasa K, Kitazawa R, Kitazawa S, Miyachi H, Maeda S, Egashira K, Kasuga M. MCP-1 contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis in obesity. *J Clin Invest* 2006; **116**: 1494-1505 [PMID: 16691291 DOI: 10.1172/JCI26498]
- 62 **Rull A**, Rodríguez F, Aragonès G, Marsillach J, Beltrán R, Alonso-Villaverde C, Camps J, Joven J. Hepatic monocyte

- chemoattractant protein-1 is upregulated by dietary cholesterol and contributes to liver steatosis. *Cytokine* 2009; **48**: 273-279 [PMID: 19748796 DOI: 10.1016/j.cyto.2009.08.006]
- 63 **Rinella ME**, Green RM. The methionine-choline deficient dietary model of steatohepatitis does not exhibit insulin resistance. *J Hepatol* 2004; **40**: 47-51 [PMID: 14672613]
- 64 **Miura K**, Yang L, van Rooijen N, Ohnishi H, Seki E. Hepatic recruitment of macrophages promotes nonalcoholic steatohepatitis through CCR2. *Am J Physiol Gastrointest Liver Physiol* 2012; **302**: G1310-G1321 [PMID: 22442158 DOI: 10.1152/ajpgi.00365.2011]
- 65 **Ito M**, Suzuki J, Tsujioka S, Sasaki M, Gomori A, Shirakura T, Hirose H, Ito M, Ishihara A, Iwaasa H, Kanatani A. Longitudinal analysis of murine steatohepatitis model induced by chronic exposure to high-fat diet. *Hepatol Res* 2007; **37**: 50-57 [PMID: 17300698 DOI: 10.1111/j.1872-034X.2007.00008.x]
- 66 **Kassel KM**, Guo GL, Tawfik O, Luyendyk JP. Monocyte chemoattractant protein-1 deficiency does not affect steatosis or inflammation in livers of mice fed a methionine-choline-deficient diet. *Lab Invest* 2010; **90**: 1794-1804 [PMID: 20697377 DOI: 10.1038/labinvest.2010.143]
- 67 **Shim M**, Saab S. Severe hepatotoxicity due to Hydroxycut: a case report. *Dig Dis Sci* 2009; **54**: 406-408 [PMID: 18661239 DOI: 10.1007/s10620-008-0353-4]
- 68 **Jones FJ**, Andrews AH. Acute liver injury associated with the herbal supplement hydroxycut in a soldier deployed to Iraq. *Am J Gastroenterol* 2007; **102**: 2357-2358 [PMID: 17897352 DOI: 10.1111/j.1572-0241.2007.01353_10.x]
- 69 **Sánchez-Valle V**, Chávez-Tapia NC, Uribe M, Méndez-Sánchez N. Role of oxidative stress and molecular changes in liver fibrosis: a review. *Curr Med Chem* 2012; **19**: 4850-4860 [PMID: 22709007 DOI: 10.2174/092986712803341520]
- 70 **Galli A**, Svegliati-Baroni G, Ceni E, Milani S, Ridolfi F, Salzano R, Tarocchi M, Grappone C, Pellegrini G, Benedetti A, Surrenti C, Casini A. Oxidative stress stimulates proliferation and invasiveness of hepatic stellate cells via a MMP2-mediated mechanism. *Hepatology* 2005; **41**: 1074-1084 [PMID: 15841469 DOI: 10.1002/hep.20683]

P- Reviewers Mahmud M, Tasci I **S- Editor** Gou SX
L- Editor A **E- Editor** Ma S





百世登

Baishideng®

Published by **Baishideng Publishing Group Co., Limited**

Flat C, 23/F., Lucky Plaza,

315-321 Lockhart Road, Wan Chai, Hong Kong, China

Fax: +852-65557188

Telephone: +852-31779906

E-mail: bpgoffice@wjgnet.com

<http://www.wjgnet.com>



ISSN 1007-9327



9 771007 932045