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2. 게재예정학술지

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

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Kim Y *et al.* Anti-adiposity effects and hepatotoxicity

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

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the manuscript; Jung UJ designed the study, performed experiments, analyzed the data and wrote the manuscript.

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ABSTRACT

AIM: to investigate long-term supplementation effects of Garcinia Cambogia (GC) on adiposity and non-alcoholic fatty liver disease in diet-induced 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 weeks. 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 and MCP-1 and TNF-α mRNA expression as well as plasma ALT and AST levels, although HFD-induced hepatic steatosis did not change.

CONCLUSION: GC protects against HFD-induced obesity partially by modulating the enzymatic activity and gene expression involved in fatty acid synthesis and β-oxidation, but induces hepatic fibrosis and inflammation.

Key words: *Garcinia Cambogia*, Anti-adiposity, Metabolic changes, Hepatic collagen accumulation, Inflammation

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删除的内容: **Long-term supplementation of *Garcinia Cambogia* attenuates high-fat diet-induced adiposity and glucose intolerance but exacerbates hepatic collagen accumulation and inflammation in mice**

Young-Je Kim[a#], Myung-Sook Choi[a,b#], Yong Bok Park[c], Sang Ryong Kim[c,d], Mi-Kyung Lee[e], Un Ju Jung[b*]

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Core tip:

✓*Garcinia Cambogia* (GC) is a popular dietary supplement for weight loss.

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✓ However, little is known about the efficacy and hepatotoxicity of long-term GC supplementation in mice fed a high-fat diet (HFD).

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✓ GC ameliorated HFD-induced adiposity by modulating enzymatic activity and gene expression involved in fatty acid metabolism.

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✓ GC also reduced the plasma resistin level and glucose intolerance.

✓ However, GC caused hepatic collagen accumulation and pro-inflammatory gene expression without affecting HFD-induced steatosis.

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INTRODUCTION

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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 (Gallagher et al., 2010). According to the World Health Organization global estimates from 2008, more than 1.4 billion adults are overweight and at least 500 million of them are obese (http://www.who.int/mediacentre/factsheets/fs311/en/). It is estimated that by 2020, two-thirds of the global burden of disease will be attributed to chronic disorders associated with obesity (Chopra et al., 2002). As the prevalence of obesity has increased, the use of dietary supplements for weight loss has been common (Pillitteri et al., 2008). However, the optimal dose and safety profiles of many dietary supplements are poorly studied, because they are not regulated by the FDA in a manner observed for pharmacological agents (Pittler et al., 2005; Hurt & Wilson, 2012).

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Garcinia Cambogia (GC), a fruit native to southeastern Asia and western Africa, has been widely used as an anti-obesity herbal supplements for decades over the world. Its main component hydroxycitric acid (HCA) not only inhibited ATP-citrate lyase, the enzyme response for *de novo* fatty acid synthesis, but also increased hepatic glycogen synthesis, reduced food intake by suppressing appetite and decreased body weight gain (Heymsfield et al., 1998; Leonhardt et al., 2001; Sullivan et al., 1974; Nageswara & Sakeriak, 1988). Although extensive experiments reported the weight loss and body fat-lowering effects of GC,

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some animal and clinical studies have shown controversial findings (Márquez et al., 2012; Heymsfield et al., 1998; Kim et al., 2008; Saito et al., 2005; Hayamizu et al., 2003) and no studies have shown whether these effects persist beyond 13 weeks of GC treatment. Furthermore, some studies have reported the potential for hepatotoxicity of hydroxycut, a formulation that contains GC among other ingredients (Stevens et al., 2005; Dara et al., 2008).

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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) that are commonly used to study obesity and its related metabolic disease because they have all the hallmarks of obesity, insulin resistance and NAFLD (Collins et al., 2004). We also examined the effect of GC on liver dysfunction, collagen accumulation and inflammation.

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METERIALS AND METHODS

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Male C57BL/6J mice (four-week-old) were purchased from Jackson Laboratories (Bar Harbor, ME, USA). The mice were individually housed in polycarbonate cages, which were kept in a room maintained at a constant temperature (24 ℃) with a 12-hour light/dark cycle. The mice were fed a normal chow diet for acclimation for 1 week after delivery. At 5

weeks of age, they were randomly divided into two groups of 10 mice each and fed a high-fat diet (HFD, D12451, Research Diets, New Brunswick, NJ, USA) with or without GC (1%, w/w, 60% hydroxyl citric acid; Newtree Inc., USA) for 16 weeks. 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, USA) after a 12-hour 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 1,000×g for 15 min at 4℃, and the plasma was separated. After blood collection, epididymal white adipose tissue (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℃ until enzyme activity and RNA analyses. All experimental procedures were performed in accordance with the protocols for animal studies approved by the Kyungpook National University Ethics Committee (Approval No. KNU-2011-28).

Fasting blood glucose and intraperitoneal glucose tolerance test

The blood glucose concentration was measured with whole blood obtained from the tail

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veins after withholding food for 12 hours using a glucose analyzer (Glucocard, Arkray, Japan) based on the glucose oxidase method. The intraperitoneal glucose tolerance test was performed on the 15th week. After a 12-hour 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.

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Plasma biomarkers

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Plasma insulin, adipokines (resistin and leptin) and cytokines (tumor necrosis factor-α, TNF-α and monocyte chemoattractant protein-1, MCP-1) were measured with a multiplex detection kit from Bio-Rad (Hercules, CA, USA). All samples were assayed in duplicate and analyzed with a Luminex 200 Labmap system (Luminex, Austin. TX, USA). Data analyses were done with Bio-Plex Manager software version 4.1.1 (Bio-Rad, Hercules, CA, USA).

Plasma lipid and apolipoprotein concentrations were determined with commercially available kits: Plasma free fatty acid, phospholipid, apolipoprotein A and apolipoprotein B levels were measured using the Wako enzymatic kit (Wako Chemicals, Richmond, VA, USA), and triglyceride, total cholesterol and HDL-cholesterol levels were determined using Asan enzymatic kits (Asan, Seoul, Republic of Korea).

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Plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined using enzymatic kits (Asan, Seoul, Republic of Korea).

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Hepatic lipids contents

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Hepatic lipids were extracted (Folch et al., 1957), 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 μL 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

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To measure the lipid-regulating enzymes activities in the epididymal WAT and liver, samples were prepared and analyzed as previously described (Kim et al., 2008). Briefly, fatty acid synthase (FAS) activity was determined with a spectrophotometric assay according to the method by Carl et al. (1975); one unit of FAS activity represented the oxidation of 1 nmol of NADPH per minute at 30 ℃. Carnitine palmitoyltransferase (CPT) activity was determined according to the method by Markwell et al. (1973) and the results were expressed as nmol/min/mg protein. Fatty acid β-oxidation was measured spectrophotometrically by monitoring the reduction of NAD to NADH in the presence of palmitoyl-CoA as described

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by Lazarow (1981), with slight modification. Protein concentration was measured by the Bradford method using BSA as the standard (Bradford, 1976).

Analysis of gene expression

Epididymal WAT and liver were homogenized in TRIzol reagent (Invitrogen Life Technologies, Grand Island, NY) and total RNA was isolated according to the manufacturer's instructions. The total RNA was converted to cDNA using the QuantiTect Reverse Transcription Kit (QIAGEN Gmbh, Hilden, Germany). The RNA expression was quantified by quantitative real-time PCR using the QuantiTect SYBR green PCR kit (QIAGEN Gmbh, Hilden, Germany) and the SDS7000 sequence-detection system (Applied Biosystems, CA, USA). 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 ℃, 15 sec at 95 ℃ and 60 sec at 60 ℃ 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 (Schmittgen & Livak, 2008). The following gene-specific primers were used: for cell death-inducing DNA fragmentation factor-α-like effector A (CIDEA), 5′-TTT CAA ACC ATG ACC GAA GTA GCC-3′

(forward), 5′-CCT CCA GCA CCA GCG TAA CC-3′ (reverse); for CPT, 5′-ATC TGG ATG GCT ATG GTC AAG GTC-3′ (forward), 5′-GTG CTGTCA TGC GTT GGA AGT C-3′ (reverse); for FAS, 5′-CGC TCC TCG CTT GTC GTC TG -3′ (forward), 5′-AGC CTT CCA TCT CCT GTC ATC ATC-3′ (reverse); for fatty acid translocase/cluster of differentiation 36 (FAT/CD36), 5'- ATT GGT CAA GCC AGC T-3' (forward), 5'- TGT AGG CTC ATC CAC TAC-3' (reverse); for glyceraldehyde-3-phosphate dehydrogenase (GAPDH), 5′-ACA ATG AAT ACG GCT ACA GCA ACA G-3′ (forward), 5′-GGT GGT CCA GGG TTT CTT ACT CC-3′ (reverse); for MCP-1, 5′-TTC CTC CAC CAC CAT GCA G-3' (forward), 5′-CCA GCC GGC AAC TGT GA-3′ (reverse); for peroxisome proliferator-activated receptors (PPAR)α, 5'-CCT GAA CAT CGA GTG TCG AAT AT (forward), 5'- GGT CTT CTT CTG AAT CTT GCA GCT-3' (reverse); for TNF-α, 5'-GCA GGT CTA CTT TAG AGT CAT TGC-3' (forward), 5'-TCC CTT TGC AGA ACT CAG GAA TGG-3' (reverse); for stearoyl-CoA desaturase (SCD1), 5′-CCC CTG CGG ATC TTC CTT AT-3′ (forward), 5′-AGG GTC GGC GTG TGT TTC T-3′ (reverse); and for sterol-regulatory-element-binding protein 1c (SREBP1c), 5′- GGA GCC ATG GAT TGC ACA TT-3′ (forward), 5′-CCT GTC TCA CCC CCA GCA TA-3′ (reverse).

Histological analysis

Epididymal WAT and liver were fixed in a buffer solution of 10 % formalin and

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embedded in paraffin for staining with hematoxylin and eosin (H&E) and Masson's trichrome. Stained areas were viewed using an optical microscope (Nikon, Tokyo, Japan) with a magnifying power of ×200.

Statistical analysis

Data were expressed as the mean ± SEM. 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

The 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-week-old male C57BL/6J mice with HFD or 1% (w/w) GC supplemented HFD for 16 weeks. During the experimental period, there was no significant difference in daily food intake between the groups (HFD, 3.96±0.14g; GC, 3.87±0.07g). 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 (p=0.09) (Fig. 1 A&B). Thus, the food efficiency ratio was not significantly different between the groups (Fig. 1C). However, the weight of the visceral WAT, including the epididymal, perirenal, retroperitoneal and mesentery WAT, was significantly lower in the GC-supplemented mice than in the HFD control mice (Fig. 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 (Fig. 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 (Fig. 2A). Furthermore, GC-supplemented mice showed a significant increase in the activity of CPT and β-oxidation in the epididymal

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WAT (Fig. 2B&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 (Fig. 2D). Moreover, GC-supplemented mice showed a significant increase in the mRNA expression of transcription factor PPARα 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 (Fig. 2D&E).

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

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We next determined whether GC influenced HFD-induced glucose intolerance. The fasting blood glucose and plasma insulin 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 (Fig. 3A). The level of AUC was also markedly decreased in the GC-supplemented mice compared to the control obese mice.

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No significant differences were observed in the levels of plasma lipids (triglycerides,

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total cholesterol, HDL-cholesterol, phospholipids and free fatty acids) and apolipoproteins (apolipoprotein A and apolipoprotein 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 (Fig. 3B&C).

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

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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 (Fig. 4A&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, including FAS, SCD1, CPT, CIDEA, SREBP1c and PPARα, between the two groups (Fig. 4C&D). However, trichrome staining of the liver revealed GC supplementation increased collagen deposition (blue staining) compared to the control mice (Fig. 4E). Furthermore, GC supplementation caused significant increases of hepatic TNF-α and MCP-1 mRNA levels compared to the control mice (Fig. 4F). Plasma ALT and AST levels were also significantly increased in the GC group compared to the control mice (Fig. 4G).

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DISCUSSION

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Since poor eating habits combined with limited activity are a major contributor to obesity, long-term dietary lifestyle changes may present a cost-effective first-line of intervention for obesity (Ross et al., 2000; Blackburn, 2001). Thus, a number of natural dietary supplements are popular and widely used as a part of a nutritional lifestyle intervention for weight management (Pittler & Ernst, 2004). Among them, HCA-containing GC has been shown to be efficacious in lowering body weight and body fat (Márquez et al. 2012; Heymsfield et al., 1998; Kim et al., 2008; Saito et al., 2005; Hayamizu et al., 2003). However, some clinical trials and animal studies have shown conflicting results (Heymsfield et al. 1998; Saito et al. 2005), and a case series on hepatotoxicity has been reported in patients taking GC-containing hydroxycut, although the individual chemical component underling liver injury remains poorly understood (Stevens et al., 2005; Dara et al., 2008). 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.

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In the present study, GC supplementation (1%, w/w) in a HFD for 16 weeks 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

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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 PPARα in the epididymal WAT. The CPT is a major rate-limiting enzyme for fatty acid oxidation, and its gene expression is regulated by PPARα in adipocytes (Lee et al., 2011). The PPARα mRNA expression was decreased in the WAT of both genetic and HFD-induced obese mice, and the down-regulation of PPARα in obese WAT was involved in obesity-induced mitochondrial dysfunction and metabolic disorders (Goto et al., 2011). 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 (Saito et al. 2005). The ACL is another lipogenic enzyme catalyzing the cleavage of citrate to oxaloacetate and acetyl-CoA for *de novo* fatty acid synthesis (Sullivan et al., 1977). The inhibitory action of HCA on ACL reduces the acetyl-CoA pool, which can lead to a decreased

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concentration of malonyl-CoA, a physiological inhibitor of CPT (McGarry et al., 1977), and thus results in the suppression of body fat accumulation through stimulation of fatty acid oxidation (Ishihara et al., 2000). Kim et al. (2008) also reported that HCA-containing GC (1%, w/w) supplementation in HFD-fed mice for 12 weeks 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 (Asghar et al., 2007; Cheng et al., 2012). HCA increases the cellular pool of citrate by inhibiting ACL, which in turn can increase glycogen production (Soni et al., 2004). Recently, HCA supplementation enhanced the glycogen synthesis rate in skeletal muscles and improved post-meal insulin sensitivity (Cheng et al., 2012). 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 (Steppan et al., 2001), and HFD significantly increased plasma resistin levels in mice compared to a standard low-fat/high-carbohydrate diet (Muse et al., 2004). Resistin deficiency ameliorated glucose homeostasis in mice (Banerjee et al., 2004), whereas administration of resistin impaired glucose tolerance and insulin action (Rajala et al., 2003; Steppan et al., 2001).

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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 (Jo et al., 2009).

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 (Pickens et al., 2009; Rinella et al., 2008). Steatohepatitis is the combination of steatosis with hepatic inflammation and fibrosis. Liver fibrosis is excess synthesis and deposition of extracellular matrix proteins including collagen (Friedman 2008), 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 (Deshmane et al., 2009). Several studies indicate that MCP-1 is an important mediator of liver fibrosis (Seki et al., 2009; Ramm et al., 2009; Wynn, 2008). MCP-1 mRNA expression was markedly increased in the

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livers of patients with steatohepatitis and in murine models of steatohepatitis such as mice fed a HFD or methion-cholin deficient diet (Haukeland et al., 2006; Westerbacka et al., 2007; Marsillach et al., 2009; Greco et al., 2008; Kanda et al., 2006; Rull et al., 2009; Tous et al., 2006; Rinella & Green 2004). CCR2 inhibitor suppressed the early and late features of steatohepatitis including fibrosis (Miura et al. 2012), 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 (Ito et al., 2007). 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 (Kassel et al., 2010).

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 [Dara et al., 2008; Stevens et al., 2005; Shim & Saab, 2009; Jones & Andrews, 2007]. Our experiments provide new information regarding

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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.

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 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 Fig. 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.

Acknowledgements

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Table 1. Effects of GC supplementation on plasma lipids and apolipoproteins levels in mice fed a HFD for 16 weeks

	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 ±SEM (n=10).

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FIGURE LEGENDS

Figure 1. Effects of GC supplementation on body weight gain (A&B), food intake (C), fat-pad weight (D) and adipocyte size (E) in mice fed a HFD for 16 weeks. A-D: Data are expressed as the mean±SEM (n=10). *p<0.05 versus control group. E: H&E staining is shown. Representative photographs of epididymal WAT (original magnification ×200). HFD, mice fed a high-fat diet alone; HFD + GC, mice fed a high-fat diet containing *Garcinia Cambogia* (1%, w/w); WAT, white adipose tissue.

Figure 2. Effects of GC supplementation on fatty acid-regulating enzyme activity (A-C) and gene expression (D-F) in epididymal WAT of mice fed a HFD for 16 weeks. Data are expressed as the mean±SEM (n=10). *p<0.05, **p<0.05, ***p<0.05, versus control group. HFD, mice fed a high-fat diet alone; HFD + GC, mice fed a high-fat diet containing *Garcinia Cambogia* (1%, w/w); WAT, white adipose tissue; FAS, fatty acid synthase; CPT, carnitine palmitoyltransferase; PPARα, peroxisome proliferator-activated receptor α.

Figure 3. Effects of GC supplementation on glucose tolerance and plasma adipocytokine levels in mice fed a HFD for 16 weeks. Data are expressed as the mean±SEM (n=10). *p<0.05 versus control group. A: The intraperitoneal glucose tolerance test was performed on the 15th week of GC supplementation in HFD-fed mice. Following a 12-hour fast, the mice were injected intraperitoneally with glucose (0.5 g/kg body weight). Blood glucose

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was then measured via the tail vein at the indicated time. Left, blood glucose values. Right, areas under the curves (AUC). B&C: Plasma levels of leptin, resistin, MCP-1 and TNF-α were assayed after 16 weeks of GC supplementation in HFD-fed mice. HFD, mice fed a high-fat diet alone; HFD + GC, mice fed a high-fat diet containing *Garcinia Cambogia* (1%, w/w); MCP-1, monocyte chemoattractant protein-1; TNF-α, tumor necrosis factor-α.

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Figure 4. Effects of GC supplementation on liver histology (A&E), hepatic lipids contents (B), activity of hepatic fatty acid synthase and β-oxidation (C), mRNA expression of hepatic genes involved in fatty acid synthesis, β-oxidation, lipid storage and inflammation (D&F) and levels of plasma AST and ALT (G) in mice fed a HFD for 16 weeks. A&E: The liver tissue sections were stained with H&E (A) and Masson's trichrome (E). Representative images are shown (original magnification ×200). B-D, F: Data are expressed as the mean±SEM (n=10). $^{*}p<0.05$, $^{**}p<0.01$ versus control group. HFD, mice fed a high-fat diet alone; HFD + GC, mice fed a high-fat diet containing *Garcinia Cambogia* (1%, w/w); SREBP1c, sterol-regulatory-element-binding protein 1c; PPARα, peroxisome proliferator-activated receptor α; FAS, fatty acid synthase; SCD1, stearoyl-CoA desaturase; CPT, carnitine palmitoyltransferase; CIDEA, cell death-inducing DNA fragmentation factor-α-like effector A; MCP-1, monocyte chemoattractant protein-1; TNF-α, tumor necrosis factor-α.

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Figure 5. Summary of the long-term GC supplementation effects on adiposity, glucose tolerance and steatohepatitis in HFD-induced obese mice. The GC supplementation decreased FAS mRNA expression and its activity, while PPARα and 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 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 MCP-1 and TNF-α mRNA expression and collagen accumulation in the liver. Plasma AST and ALT 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 and inflammation without affecting hepatic fat accumulation. ALT, alanine aminotransferase; AST, aspartate aminotransferase; CPT, carnitine palmitoyltransferase; FA, fatty acid; FAS, fatty acid synthase; GC, *garcinia cambogia*; MCP-1, monocyte chemoattractant protein-1; PPARα, peroxisome proliferator-activated receptor α; TNF-α, tumor necrosis factor-α.