α-Mangostin Regulates Hepatic Steatosis and Obesity through SirT1-AMPK and PPARγ Pathways in High-Fat Diet-Induced Obese Mice

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ABSTRACT: Previous studies have shown that α-mangostin (α-MG) suppresses intracellular fat accumulation and stimulation of lipolysis in in vitro systems. Together with the relatively high distribution of α-MG in liver and fat, these observations made it possible to propose a plausible hypothesis that an α-MG supplement may regulate hepatic steatosis and obesity. An α-MG supplement (50 mg/kg) reduced the body weight gain (13.8%) and epidymal and retroperitoneal fat mass accumulation (15.0 and 11.3%, respectively), as well as the biochemical serum profiles such as cholesterol [TC (26.9%), LDL-C (39.1%), and HDL-C (15.3%)], glucose (30.2%), triglyceride (29.7%), and fatty acid (30.3%) levels in high-fat fed mice compared with the high-fat diet-treated group, indicating that α-MG may regulate lipid metabolism. In addition, an α-MG supplement up-regulated hepatic AMPK, SirT1, and PPARγ levels compared with the high-fat diet states, suggesting that α-MG regulates hepatic steatosis and obesity through the SirT1-AMPK and PPARγ pathways in high-fat diet-induced obese mice.

KEYWORDS: α-mangostin, hepatic steatosis, obesity, SirT1, AMPK

INTRODUCTION

The obesity-induced metabolic disorders have been known mainly to be caused by an imbalance of dietary intake such as excess fat and lack of exercise, which are related to hyperglycemia, hyperlipidemia, hepatic steatosis, and cardiovascular diseases and, hence, have become a global health threat. The general characteristics of obesity include an increase in adipose tissue masses from increased fat cell numbers (hyperplasia) and size (hypertrophy). In addition, lipid accumulation and changes in lipid forms (e.g., triglycerides, fatty acids, and various cholesterols) in adipose tissue, liver, and other tissues are associated with the development of obesity. Thus, along with adipose tissue, the modulation of various targets in referential tissues may be able to regulate body weight and metabolic disorders (e.g., hepatic steatosis and diabetic mellitus). It seemed to be valuable to investigate the changes in fat mass in adipose tissue, the circulating lipid fuels in the blood, and signaling pathway in the liver, a representative organ related to fatty acid oxidation.

A high-fat diet is associated with impairment of the hepatic sirtuin 1 (SirT1)-AMP-activated protein kinase (AMPK) axis, a central signaling system controlling the lipid metabolism pathways. In particular, the activation of the SirT1-AMPK pathway in metabolic tissues, including the liver, has been found to increase rates of fatty acid oxidation and to repress lipogenesis, largely by modulating peroxisome proliferator-activated receptor γ (PPARγ) and retinoid X receptor (RXR) activities.

Mangosteen, Garcinia mangostana L., is a tropical fruit that has been used for hundreds of years around the world as a traditional herbal medicine, containing biologically active compounds including xanthones, terpenes, anthocyanins, tannins, phenols, and multiple vitamins. Recently, mangosteen has gained popularity as a functional food and botanical dietary supplement due to its purported health benefits. α-Mangostin (α-MG), a major xanthone derivative, possesses a variety of pharmacological efficacies such as antioxidant, anti-inflammatory, and anti-diabetic activities. Moreover, AMPK activation by α-MG in glioblastoma cells has been reported to be beneficial to autophagy. Interestingly, the suppression of intracellular fat accumulation and the stimulation of lipolysis by α-MG in 3T3-L1 cells, as well as the high distribution of α-MG in liver and fat in vivo, provide a substantial hypothesis that α-MG may be valuable in the prevention or treatment of obesity-related diseases such as hepatic steatosis. To date, the role of α-MG in a high-fat diet-induced obese model has not yet been established; thus, here, we focus on the regulatory effects of α-MG on hepatic steatosis and obesity in high-fat diet fed mice, associated with the SirT1-AMPK and PPARγ pathways in an in vivo system, along with the regulation of body weight, fat mass, and lipid metabolism.

MATERIALS AND METHODS

Chemicals. α-MG was purified (>98.0% of purity) at the College of Pharmacy, Dongguk University (Seoul, South Korea), according to...
the previously reported protocol.\textsuperscript{14} Polyethylene glycol 400 (PEG 400) was from Showa Chemical Co. (Tokyo, Japan). Antibodies against β-actin, SirT1, p-AMPK, AMPK, PPARγ, and RXRα were purchased from Cell Signaling Technology, Inc. (Danvers, MA, USA).

**Animals**. The protocols for the animal studies were approved by the Institute of Laboratory Animal Resources of Dongguk University, Seoul, South Korea (2012-0673; July 25, 2012). Male C57BL/6 mice were obtained from Charles River Orient (Seoul, South Korea) and acclimated for 1 week before the study was begun. Upon arrival, animals were randomized and housed at three per cage under strictly controlled environmental conditions (20°C ± 2°C and 48–52% relative humidity). A 12 h light/dark cycle was used at an intensity of 150 lux. C57BL/6 mice were started on either a normal diet (ND, 10% of kilocalories as fat; product D12592, Research Diets, New Brunswick, NJ, USA) for 80 days. After 5 weeks (at day 35) of ND or HD, the mice were distributed into six treatment groups (n = 8 for each) every day during the last 6 weeks of diet feeding. ND and HD groups (n = 8 for each) were orally administered a vehicle instead of α-MG.

**Measurement of Liver, Fats, and Kidney Weights and Triglyceride (TG) Levels in the Liver**. At the end of the experimental period, the liver, fats, and kidney were extracted and the weights were measured. In the case of fats, the weights of total, epididymal, and retroperitoneal fats were measured separately. For the determination of protein concentration, 20 μg of protein was mixed with 2× sample buffer (20% glycerol, 4% SDS, 10% 2-ME, 0.05% bromophenol blue, and 1.25 M Tris-buffer, pH 6.8), loaded onto an SDS-PAGE gel (8 or 15%), and run at 150 V for 90 min. The tissue proteins were transferred to an ImmunoBlot polyvinylidene difluoride membrane using a Bio-Rad semidry transfer system. Following blocking, membranes were incubated with primary antibody at dilutions of 1:500–1:1000 at 4 °C overnight, washed three times in PBS, and further incubated with a HRP-conjugated secondary anti-IgG antibody at dilutions of 1:2000–1:8400 for 1 h. After three washings, the immunoreactive proteins were visualized by ECL chemiluminescence detection (Amersham Biosciences, Piscataway, NJ, USA). Equal loading of proteins was verified by β-actin immunoblotting.

**Statistical Analysis**. A P value <0.05 was deemed to be statistically significant using Duncan’s multiple-range test of the Social Package of Statistical Sciences (SPSS) posteriori analysis of variance (ANOVA). All data are expressed as the mean ± standard error.

## RESULTS

**Effects of α-MG on Body Weight Gain and Food Intake**. Effects of α-MG on body weight changes in C57BL/6
mice fed a normal diet (ND) or a high-fat diet (HD) are shown in Figure 1. The body weight gains in the high-fat diet groups were significantly greater than those in the normal diet groups from day 14 to the end of this experiment (e.g., body weight gain compared with initial body weight: 12.3 vs 28.8% at day 14 and 39.6 vs 130% at day 80, respectively, for the ND and HD groups). The body weight loss appeared 26 days after starting the treatment with 50 mg/kg α-MG (at day 61 after the start of the HD) in the HMG50 group compared with the HD group. However, a body weight loss did not appear in the HMG10 group compared with the HD group. The mean value of body weight in the NMG50 group seemed to be greater than in the ND or NMG10 group; however, there was no significant difference. The food intakes were comparable among all groups of mice (data not shown).

Effects of α-MG on Hepatic Steatosis. In an effort to assess the effect of α-MG on hepatic steatosis, measurements of liver weight and hepatic TG level and histopathological analysis of the liver were conducted. In the HMG50 group, the high-fat diet induced an increase in liver weight and hepatic TG level in the HD group (by 44.1 and 262% increase for liver weight and hepatic TG, respectively, vs the ND group), and the increase in liver weight and hepatic TG level was restored (by 12.2 and 44.1% decrease, respectively, vs the HD group) (Figure 2A,B). As shown in Figure 2C, the histopathological analysis of the liver revealed that HD caused the accumulation of fat droplets in the liver, accompanying hepatic steatosis. Arrestingly, the hepatic fat accumulation decreased in the HMG50 group according to liver histology. The conditions of liver weight, hepatic TG level, and hepatic steatosis in the HMG10 group

Figure 2. Effects of α-MG on liver from ND, NMG10, NMG50, HD, HMG10, and HMG50 groups. The weights of liver and hepatic TG contents were measured (A, B). Also, the histology of liver was compared after H&E staining: vein (blue arrow); bile duct (red arrow) (C). The liver functions were determined by ALT and AST in serum (D, E). (*) Means not sharing a common symbol differ, P < 0.05.
were still abnormal and similar to the HD group. According to Figure 2D,E for the measurement of liver functions, the serum ALT and AST levels were significantly higher in the HD group (by 144 and 128% increases respectively, vs the ND group), but were recovered to control levels in the HMG10 (by 45.7 and 42.3% decreases respectively, vs the HD group) and HMG50 (by 59.8 and 53.6% decreases respectively, vs HD group) groups. The albumin levels were comparable among the three

Figure 3. Effects of α-MG on fat from ND, NMG10, NMG50, HD, HMG10, and HMG50 groups. The weights of total fat, epididymal fat, and retroperitoneal fat were measured (A–C). The histologies of epididymal and retroperitoneal fats were compared after H&E staining (D, E). Adipocyte size of epididymal fat (F) and retroperitoneal fat (G) was measured. (*) Means not sharing a common symbol differ, P < 0.05.
groups of mice fed HD (data not shown). With the ND, in both the NMG10 and NMG50 groups, all conditions of the liver maintained a normal state. Collectively, these results demonstrate that 50 mg/kg α-MG treatment attenuated hepatic steatosis in high-fat diet-fed mice.

**Effects of α-MG on Fat Accumulation.** To investigate whether α-MG regulated the fat accumulation in adipose tissues, the weight and size of epididymal and retroperitoneal fats were examined. The weights of total, epididymal, and retroperitoneal fats were increased in the HD group (by 614, 549, and 804% increases respectively, vs the ND group), which were significantly reduced (by 13.8, 15.0, and 11.3% decreases respectively, vs HD group) in the HMG50 group (Figure 3A–). As shown in Figure 3D,E, the sizes of epididymal and retroperitoneal fats in the HD group were significantly increased, by 64.9 and 104.6%, respectively, compared with
those of the ND group. In the HMG50 group, the sizes of adipose tissue were slightly reduced, by 24.2 and 44.5% in both epididymal and retroperitoneal fats, respectively, as compared with the HD group (Figure 3F). However, 10 mg/kg α-MG treatment did not significantly affect the fat weights and sizes from the HD group. In normal diet groups (ND, NMG10, and NMG50), there were no changes in fat weights or sizes regardless of treatment with α-MG.

**Effects of α-MG on Lipid Metabolism.** To determine whether α-MG improved lipid metabolism, the serum glucose, FFA, TG, TC, HDL-C, and LDL-C levels were measured (Figure 4). All parameters in the HD group were higher (by 76.6, 45.7, 37.1, 111, 24.8, and 172% increases respectively) than in the ND group, and all of them were decreased in the HMG50 group (by 30.2, 30.3, 29.7, 26.9, 15.3, and 39.1% decreases respectively, vs the HD group). In the HMG10 group, FFA, TC, and LDL-C levels were reduced (by 28.5, 23.3, and 18.4% decrease, respectively, vs the HD group). In particular, in the HMG50 group, the reduction in the LDL-C level was greater than that of the HDL-C level (by 39.1 and 15.3% decreases for LDL-C and HDL-C levels, respectively, vs the HD group). The ratio of HDL-C/LDL-C in the HMG50 group was significantly increased (by 38.5%) compared with the HD group. These results suggest that α-MG improved the serum lipid profile, which may result from the decrease in lipogenesis.

**Effects of α-MG on Hepatic Fatty Acid Metabolism through the SirT1-AMPK Pathway.** To investigate the involvement of α-MG in hepatic fatty acid metabolism, Western blotting analysis was performed as shown in Figure 5. First, the protein levels of hepatic PPARγ and AMPK were increased in the HD group (vs the ND group); however, those of hepatic SirT1 and phosphorylated AMPK were much the same in the ND and HD groups. When ND-fed mice were supplied with 10 or 50 mg/kg α-MG, the protein levels of hepatic PPARγ and AMPK were remarkably amplified; however, α-MG seemed to negligibly affect the level of SirT1 and phosphorylated AMPK proteins. In HD-fed mice, an α-MG supplement up-regulated the hepatic PPARγ protein level in HD-fed mice, suggesting that α-MG acted as a hepatic PPARγ agonist. Interestingly, the protein levels of hepatic SirT1 and phosphorylated AMPK showed a different phenomenon with the α-MG supplement between ND- and HD-fed mice. In the HMG10 and HMG50 groups, protein levels of both hepatic SirT1 and phosphorylated AMPK were outstandingly increased compared with the HD group, suggesting that the hepatic SirT1 and AMPK axis was implicated in changes in fat accumulation in the liver, in parallel with the induction of hepatic PPARγ by α-MG. On the basis of these results, α-MG regulated the hepatic fatty acid metabolism through the SirT1 and AMPK pathways. Subsequently, the effects of α-MG treatment on the levels of lipogenic genes involved in fatty acid oxidation were determined in the liver. The protein expression of RXRα was stimulated in the HMG10 and HMG50 groups compared with the HD group, suggesting that the activated PPARγ and RXRα may form a heterodimer and bind to responsive DNA elements more effectively, thereby stimulating the transcription of genes related to lipid metabolism in the liver.

### DISCUSSION

The present study provides the first in vivo evidence that α-MG attenuated hepatic steatosis through hepatic SirT1-AMPK and PPARγ pathways. As found in the experimental data, the hepatic fat accumulation was significantly attenuated, and the increase in hepatic TG content was decreased by α-MG compared with the HD group. All of these results were consistent with the changes in metabolic parameters.

A metabolic syndrome is defined as the combination of various symptoms of abdominal obesity, dyslipidemia, impaired glucose metabolism, hypertension, inflammatory responses, and hepatic steatosis, the latter of which is referred to as a representative hepatic component of metabolic syndrome. Typically, the high-fat diet is emerging as the most common cause for the overproduction and accumulation of lipids (mostly TG) within hepatocytes, which evolves into a line of diagnostic symptoms in hepatic steatosis with obesity patients. Hepatic steatosis leads to the elevated influx of fatty acids, hepatic fat accumulation, and impaired export of TG and other cytotoxic factors, including oxidative stress, lipid peroxidation, and inflammatory cytokines after excess intake of nutrients. Thus, the hepatic steatosis model in obese mice induced by a high-fat diet was utilized in this study.

The gross inspection of the HMG50 group indicates a clear recovery in body weight, fat mass, hepatic steatosis, and serum biochemical profiles relevant to lipid metabolism. The average values of food intake for 80 days were comparable among all groups, indicating that α-MG did not control appetite, but had a beneficial influence on fatty acid oxidation or cholesterol homeostasis. In particular, the up-regulated serum glucose, FFA, TC, LDL-C, and TG were gradually reduced by α-MG in the HMG10 and HMG50 groups, and notably a significantly increased HDL-C and HDL/LDL-C ratio were observed. Moreover, the decreased TG content may result from decreased lipid synthesis. Because the high HDL-C/LDL-C ratio showed a positive correlation with a lower risk of cardiovascular disease, the 50 mg/kg α-MG treatment showed potential efficacy to ameliorate metabolic diseases. In the HMG50 group, hepatic steatosis and abnormal liver function seemed to be recovered by 50 mg/kg α-MG supplementation, but 10 mg/kg α-MG seemed to reduce hepatic steatosis negligibly. The elevated ALT and AST levels in the HD groups were restored in the HMG10 and HMG50 groups, indicating that a high-fat diet resulted in abnormal liver function, and α-MG attenuated hepatic injury. With supplementation of 10 and 50 mg/kg α-MG with the ND, the normal liver state was
maintained, suggesting that α-MG itself did not cause liver injury. In addition, hepatic steatosis was recovered in the HMG50 group with respect to morphological aspects. Furthermore, no renal injuries were observed in any group of mice (Supporting Information 1). Taken together with body weight changes, hepatic steatosis, fat accumulation, and/or lipid metabolism, α-MG seemed to regulate hepatic steatosis and obesity. In particular, the body weight and liver weight changes seemed to be dose-dependent, because 50 mg/kg α-MG treatment significantly decreased body and liver weights, but there was no significant decrease at 10 mg/kg α-MG treatment compared to those in the HD group. A dose of 50 mg/kg of α-MG administered to mice is equivalent to approximately 320 mg/kg in the human adult, according to the interspecies dose conversions of NOAELs and previous reports.

The Western blotting results clearly indicate that the hepatic SirT1 and phosphorylated AMPK levels were activated by α-MG in the HMG10 and HMG50 groups. Moreover, the protein expression of hepatic PPARγ and RXRα was stimulated by α-MG in the HMG10 and HMG50 groups. AMPK, a downstream component of a protein kinase cascade, acts as an intracellular energy sensor to maintain energy balance. This pivotal role of AMPK makes it an ideal candidate for regulating whole-body energy metabolism such as glucose homeostasis and lipid metabolism, and additionally AMPK may play a role in protecting the body from metabolic diseases with obesity. Along with AMPK phosphorylation, SirT1, a nuclear protein, plays a key role in controlling the transcription factors related to energy metabolism. The specific SirT1 activator enhances oxidative metabolism in liver and skeletal muscle, which may regulate whole-body glucose metabolism both systemically and locally. Activation of SirT1 mimics several metabolic aspects of calorie restriction that enhance selective nutrient utilization and mitochondrial oxidative function. The AMPK/SirT1 signaling pathway is, furthermore, the mechanism by which hormones enhance mitochondrial metabolism. Regulation of AMPK and SirT1 is a potential therapeutic target for attenuation of fatty acid oxidation in liver. The functions of these enzymes are like a fuel gauge: they are activated under conditions of high-energy depletion. Phosphorylation of AMPK leads to the down-regulation of lipid biosynthesis by phosphorylating of acetyl-CoA carboxylase, which decreases the amount of malonyl-CoA and, in turn, suppresses carnitine palmitoyl-CoA transferase, resulting in an increase of fatty acid oxidation. In addition, PPARγ forms heterodimers with the RXR that regulate the lipid metabolism. SirT1 and PPARγ might coordinate the regulation of adipogenesis. We have found that α-MG suppressed lipid accumulation in the mouse model. Although α-MG has also been found to be effective in fatty liver by PPARγ activation, the interplay between the AMPK signaling pathway and regulation of lipid disorders remains unclear.

The administration of α-MG to HF-induced obese mice reduced body-weight gain, adipose mass, serum triglyceride, total cholesterol, and low-density lipoprotein concentration. Our results suggest that α-MG exerts antiobesity effects in HF-induced obese mice by activating hepatic AMPK, SirT1, and PPARγ expression. The antiobesity effect of α-MG in HF-induced mice may support the potential of α-MG to reduce the risks of metabolic diseases.


