ANSWERING REVIEWERS

May 15, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: revised manuscript (kim et al.)).

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

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Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 2901

The manuscript has been improved according to the suggestions of reviewers:
1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer

(1) Reviewed by 00043561

I carefully evaluated the manuscript titled ''Garcinia Cambogia attenuates diet-induced adiposity but exacerbates hepatic collagen accumulation and inflammation'' In this experimental study, the authors investigated long-term supplementation effects of Garcinia Cambogia (GC) on adiposity and non-alcoholic fatty liver disease 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 WAT. 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. I can tell the manuscript has no structural flaws. The hypothesis is relevant and methods to test the hypothesis were uptodate. I have several minor comments;

1. Running head should be corrected.
➔Thank you for your kind suggestion. We corrected running head: "Anti-adiposity effects and hepatotoxicity" → "Hepatotoxic effect of *Garcina Cambogia*" (in page1, line5)

2. Introduction; the 3rd and 4th sentences are not required for our readers.
➔ We deleted 3rd sentence and revised 4th sentences.

Deleted 3rd sentence:
"It is estimated that by 2020, two-thirds of the global burden of disease will be attributed to chronic disorders associated with obesity[3]."

Revised 4th sentence (in page5, line6-13):

"As the prevalence of obesity has increased, the use of dietary supplements for weight loss has been common[4]. 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[5,6]."

→ "Although the use of dietary supplements for weight loss become common[3], the optimal dose and safety profiles of many dietary supplements are poorly studied. The US 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."

3. Materials and methods; I understand some type of EIA array was used. However, ''adipokines'' and ''cytokines'' should be replaced by specific proteins tested.

➔ **We revised several sentences to explain it in more detail based on your comment.**

In Materials and Methods (in page8, line13-page9, line1):

"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 adipokines were measured with a multiplex detection kit (#171-F7001M, Bio-Rad, Hercules, CA, USA). 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, USA). Data analyses were done with Bio-Plex Manager software version 4.1.1 (Bio-Rad, Hercules, CA, USA). Plasma cytokines were measured with a multiplex detection kit (#M60-009RDPD, Bio-Rad, Hercules, CA, USA). 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."

4. Materials and methods; the information regarding the assays should be given in full including lot Nr

➔ **We inserted the lot Nr in Materials and Methods.**

(2) Reviewed by 02462111

1. suggest to check the oxidative stress, leptine and HOMA-IR after and befor GC.

➔ **Thank you for your reasonable comment. Unfortunately, we could not check the oxidative stress, leptin and HOMA-IR after and before GC supplementation. The reason why we did not include before GC group is that we mainly focused on the long-term supplementation of GC on HFD-induced adiposity, glucose intolerance and nonalcoholic liver disease by comparing with a negative control group. Although we do not have "before GC samples", the effect of GC on oxidative stress and HOMA-IR was compared with the HFD control group. These are now shown in the Fig. 4. We also have revised or inserted several sentences in abstract, key words, core tip, introduction, materials and methods, results, discussion and figure legends.**

In Abstract (in page3, line12-line17):

"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 by modulating the enzymatic activity and gene expression involved in fatty acid synthesis and β-oxidation but induces hepatic fibrosis and inflammation."

→ "However, we first demonstrated that it increased hepatic collagen accumulation, lipid peroxidation and mRNA levels of genes related to oxidative stress (SOD, GSH-Px) and inflammatory responses (TNF-α, MCP-1) as well as plasma ALT and AST 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."

In Key words (in page3, line18-line19):

"*Garcinia Cambogia*, Anti-adiposity, Metabolic changes, Hepatic collagen accumulation, Hepatic inflammation"

→ "*Garcinia Cambogia*, Anti-adiposity, Metabolic changes, Hepatic collagen accumulation, Hepatic inflammation, Hepatic oxidative stress"

In Core tip (in page4, line3-line4):

"However, GC caused hepatic collagen accumulation and pro-inflammatory gene expression without affecting hepatic steatosis"

→ "However, GC caused hepatic collagen accumulation, inflammation and oxidative stress without affecting hepatic steatosis."

In Introduction (in page6, line1-line2):

"We also examined the effect of GC on liver dysfunction, collagen accumulation and inflammation."

→ "We also examined the effect of GC on liver dysfunction, collagen accumulation, inflammation and oxidative stress."

In Materials and Methods (in page7, line24-page8, line10):

"Fasting blood glucose and intraperitoneal glucose tolerance test

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

→ ***"Fasting blood glucose, intraperitoneal glucose tolerance test and homeostatic index of insulin resistance (HOMA-IR)***

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

withholding food for 12 hours using a glucose analyzer (OneTouch Ultra , Lifescan Inc, USA) 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. Homeostatic index of insulin resistance (HOMA-IR) was calculated according to the homeostasis of the assessment as follows:

HOMA-IR = [fasting glucose (mmol/L) × fasting insulin (μL U/mL)]/22.51"

In Materials and Methods (in page10, line5- line13):

"2.5 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 800 ×g for 15 min. The resulting colored layer was measured at 535 nm."

In Materials and Methods (in page11, line2- line24):

"The following gene-specific primers were used: for catalase (CAT), 5′- GCG TCC GTC CCT GCT GTC-3′ (forward), 5′- TGC TCC TTC CAC TGC TTC ATC TG -3′ (reverse); 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 glutathione peroxidase (GHS-Px), 5′- TCG CAA TGA GCC AAA ACT GAC G-3′ (forward), 5′- GCC AGG ATT CGT AAA CCA CAC TC-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); for superoxide dismutase (SOD), 5′- TGG TTG AGA AGA TAG GCG ACA-3′ (forward), 5′- CAT CTC GGC AGC ATC CAC CTC-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)."

In Results (in page14, line15- line17):

"The fasting blood glucose and plasma insulin levels were not significantly altered by GC supplementation (data not shown)."

→ "The fasting blood glucose, plasma insulin and HOMA-IR levels were not significantly altered by GC supplementation (data not shown)."

In Results (in page15, line2- line3):

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

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

In Results (in page15, line11- line17):

"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).”

→ “Furthermore, Plasma ALT and AST levels were significantly increased in the GC group compared to the control mice (Fig. 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 (Fig. 4G). GC supplementation also caused significant increases of hepatic SOD and 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 (Fig. 4H&I).”

In Discussion (in page18, line9- line14):

“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].”

In Discussion (in page19, line24- page20, line11):

“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 amplifie 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.”

In Discussion (in page20, line15-line19):

“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.”→

“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.”

In Figure legends (in page33, line1-line14):

“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.”

→ “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, inflammation and oxidative stress (D, G, H), lipid peroxidation (I) and levels of plasma AST and ALT (F) 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-I: 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); CAT, catalase; CIDEA, cell death-inducing DNA fragmentation factor-α-like effector A; CPT, carnitine palmitoyltransferase; FAS, fatty acid synthase; GC, *garcinia cambogia*; GSH-Px, glutathione peroxidase; MCP-1, monocyte chemoattractant protein-1; PPARα, 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."

In Figure legends (in page33, line25-page34, line4):

"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."

→ "However, GC increased pro-inflammatory MCP-1 and TNF-α mRNA expression, lipid peroxidation 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, inflammation and oxidative stress without affecting hepatic fat accumulation."

2. suggest to check HSC function and CD4/CD8 activity before and after GC treatment

➔ **Sorry that we could not check the HSC function and CD4/CD8 activity after and before GC. As stated above, we mainly investigated the effects of long-term GC supplementation on HFD-induced adiposity, glucose intolerance and nonalcoholic liver disease by comparing with HFD-fed negative control group. To elucidate how long-term GC supplementation exacerbates steatohepatitis, we plan to check HSC function and CD4/CD8 activity in the future experiment, and this will be reported in another paper. Thank you for your helpful suggestions.**

(3) Reviewed by 00000456

1. Abstract - should briefly mention what is Garcinia Cambogia

➔ **We changed from "AIM: to investigate long-term supplementation effects of *Garcinia Cambogia* (GC) on adiposity and non-alcoholic fatty liver disease in diet-induced obese mice." to "AIM: to investigate long-term effects of *Garcinia Cambogia* (GC), weight-loss supplement, on adiposity and non-alcoholic fatty liver disease in obese mice." (in page3, line2-line3). Please consider that World Journal of Gastroenterology limits word number of AIM (no more than 20 words) in Abstract.**

2. Introduction – "more than 1.4 billion adults are overweight and at least 500 million of them are obese"? omit "of them"

➔ **We changed from "of them" to "adults".**

In Introduction (in page5, line4-line6):

"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[2]." →

"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]."

3. "for weight loss has been common" -? become common.

➔ **We changed from "has been" to "become".**

In Introduction (in page5, line6-line8):

"As the prevalence of obesity has increased, the use of dietary supplements for weight loss has been common[4]." →

"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."

4. "they are not regulated by the FDA in a manner observed for pharmacological agents" -? sentence unclear: are they regulated at variance with drugs ? are they unregulated at all ?

➔ **We revised the sentence and inserted one sentence to make clear statements.**

In Introduction (in page5, line8-line13):

"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[5,6]."

→ "The US Food and Drug Administration (FDA) do not regulate dietary supplements in the same manner as pharmacological agents[5,6]. Accordingly, 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. "

5. "been widely used as an anti-obesity herbal supplements for decades over the world" This contention is poorly supported by medical literature: this reviewer was able to find no more than 39 reports referring to Garcinia Cambogia (PubMed accessed on March 24th 2013; limits: humans)

indicating that the use of Garcinia Cambogia definitely belings to local medicine. Clearly this limitations heavily restricts the interest for this submission to a vast audience of readers worldwide.

➔ **We changed the sentence by considering reports from medical literature. However, there are many web-site in which Garcinia Cambogia is advertised to be a popular anti-obesity herbal supplements. As referred in introduction, although several studies have found that the supplementation of Garcinia Cambogia extracts is associated with body weight and fat loss, some animal and clinical studies have shown controversial findings. In addition, none of them have shown whether these effects persist beyond 13 weeks of intervention. Therefore, there is still little evidence to support the potential long-term efficacy and safety of Garcinia Cambogia extracts. In this respect, we consider that the present study is meaningful as it is.**

In Introduction (in page5, line13-line15):

"*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.**"**

→ **"**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].**"**

6. "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"-? The verbs should all be used in the present tense inhibt increase and reduce.

➔ **We changed verbs.**

In Introduction (in page5, line15-line19):

"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]."

7. "that are commonly used to study obesity and its related metabolic disease because they have all the hallmarks of obesity, insulin resistance and NAFLD"? is this sentence necessary ? I think the main rationale for this study is to ascertain the mechanism of hepatotoxicity due to Garcinia Cambogia.

➔ **We agree with your comment and deleted "that are commonly used to study obesity and its related metabolic disease because they have all the hallmarks of obesity, insulin resistance and NAFLD".**

8. Discussion – "poor eating habits" -? Unhealthy

➔ **We changed from "poor eating habits" to "**unhealthy eating habits". Thank you.

9. "lifestyle changes may present a cost-effective first-line of intervention for obesity[25,26]. Thus, a number of natural dietary supplements are popular and widely used as a part of a nutritional lifestyle intervention for weight management"-? please reword this ambiguous sentence stating clearly as follows (a-d): a) dietary supplements have nothing to do with lifestyle changes (Scaglioni F, et al. 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 Dec 26. doi:pii: S2210-7401(12)00328-2. 10.1016/j.clinre.2012.10.009.) b) such supplements are not even mentioned in any of the position papers/guidelines for management of NAFLD (Ratziu V, et al. A position statement on NAFLD/NASH based on the EASL 2009 special

conference. J Hepatol. 2010;53:372-84.; Farrell GC, Asia-Pacific Working Party on NAFLD. Guidelines for the assessment and management of non-alcoholic fatty liver disease in the Asia-Pacific region: executive summary. J Gastroenterol Hepatol. 2007;22:775-7.; Fan JG, et al. Guidelines for the diagnosis and management of nonalcoholic fatty liver disease: update 2010 (published in Chinese on Chinese Journal of Hepatology 2010; 18:163-166). J Dig Dis. 2011;12:38-44.;. Loria P, et al. 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;42:272-82.; Chalasani N, et al. The diagnosis and management of non-alcoholic fatty liver disease: Practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. Hepatology 2012; 55:2005-23.; Vajro P, et al. Diagnosis of nonalcoholic fatty liver disease in children and adolescents: position paper of the ESPGHAN Hepatology Committee. J Pediatr Gastroenterol Nutr. 2012;54:700-13.) c) have been associated with increased mortality in some studies (Bjelakovic G, et al Antioxidant supplements for prevention of mortality in healthy participants and patients with various diseases. Cochrane Database Syst Rev. 2012 Mar 14;3:CD007176. doi: 10.1002/14651858.CD007176.pub2.) and that d) herbal products represent an a

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➔ **Thank you for your kind suggestion. We revised it according to your suggestion in a manner of inadequate use of some dietary supplements.**

In discussion (in page16, line4-line8):

"Thus, a number of natural dietary supplements are popular and widely used as a part of a nutritional lifestyle intervention for weight management[27]."

→ **"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 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]."**

10. References and typesetting were corrected

➔**We checked and revised them.**