Author's response to reviews

Title: Inhibitory effect of green coffee bean extract on fat accumulation and body weight in mice

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Version: 2 Date: 14 September 2005

Author's response to reviews: see over
I send revised manuscript aEoeInhibitory effect of green coffee bean extract on fat accumulation and body weight in micesaE with answer for refereeaETMs comments.

For the comments of Dr. Pin-Der D. Duh
1) Page 2, line 4-5,
The sentence has no significant corrections. It needs to be corrected.
(A) We have revised the expression.
2) Page 3, line 5,
Authors explained that caffeine inhibited the fat absorption and upregulated CPT activity. However, caffeine did not passively modulate CPT activity.
(A) We examined the effects of other polyphenolic compounds such as neochlorogenic acid and mixture of feruloylquinic acids. As a result, both compounds obviously enhanced CPT activity. We insert the new column of these data in Fig. 4 and revised the conclusion as bellow.
[These results suggest that GCBE is beneficial against weight gain and fat accumulation. The inhibition of fat absorption by caffeine and enhancement of CPT activity by polyphenolic compounds except chlorogenic acid seemed to be involved in the anti-obesity property of GCBE. ]
3) Page 8, Fig. 2
The effect of GCBE on reducing TG level was less than Chlorogenic acid, however, the latter is present in GCBE, the authors need to explain and discuss discrepancy is.
(A) Coffee beans contain compounds*) with fat accumulating activity. The possibility of interference of these compound with negative activity on hepatic TG reducing effect of chlorogenic acid. We commented in the discussion (page 10, line 17) as bellow.
[Other constituents of GCBE such as cafestol and kawehol which promote the synthesis of lipids may cause discrepancy in activities between GCBE (crude chlorogenic acid) and pure chlorogenic acid.]
4) Page 9, GCBE was able to enhance CPT activity, but chlorogenic acid and caffeine did not have this property. This finding revealed that some other active components in GCBE might revealed that some other active components in GCBE might contribute to this property. The authors should try to explain.
(A) As described in (3), we found CPT enhancing activity in neochlorogenic acid and feruloylquinic acids mixture. Detail on new observation was included in Materials and method, Result, Discussion, Figure 5 and Abstract.

For the comments of Dr. Takatoshi Murase
1) P3, Background: The contents and structure should be reviewed.
A) Chemical structures of constituents in GCBE was inserted in Background section as Figure 1. The contents of these compounds were listed in aEoePreparation of GCBEaE in Methods (P5, line 3).

2) P9-11, The contents of the Discussion repeated the contents of the results. The point is not clear.
   A) We clarified the active constituents in GCBE possess CPT enhancing activity. The abstract, result and discussion were wholly revised.

3) The explanation of the mechanism involved in the anti-obesity effect of GCBE is not clear. The anti-obesity effect of GCBE and the result of analyses are not consistent. How do the authors explain the difference?
   We think that caffeine express lipolytic citivity and inhibition of fat absorption. On the other hand, polyphenolic compounds including chlorogenic acid are suggested to improve hepatic lipid metabolism. These positive effects may be interfered by negative activity of the other compounds such as cafestol and kawehole.

4) P5, What are the components of GCBE other than chlorogenic acid and caffeine, and what percentages do they comprise?
   A) The contents of 3-caffeoylquinic acid (neochlorogenic acid), mixture of feruloyl quinic acid and 4,5-dicafeoyl quinic acid were described in aEoePreparation of GCBEaE under Methods.

5) There are some typographical errors and improper wording.
   A) We checked and corrected accordingly.

6) P4, Methods: Did the authors obtain the approval of the Ethics Committee for Use of Experimental Animals?
   A) All animal experiments were approved by the Ethical Committee for Use of Experimental Animals in our company.

7) P6, Paragraph 4: Why is the study period 6 days, not 14 days. The experimental condition is not standardized.
   A) Fourteen days are not required to enhance CPT activity. If test sample possess CPT enhancing activity, consecutive 6-day treatment satisfy the term to confirm the activity. This observation has been confirmed by previous experiment.

8) P8, L2: Food intake should be described.
   A) The amount of food intake was inserted in Table 1.

9) Table 1: Dose-independent effects of GCBE on body weight and visceral fat amount are not demonstrated. How do the authors explain this findings?
   A) Coffee beans contain compounds*) with fat accumulating activity. These compounds may interfere the
fat reducing effect of caffeine or polyphenolic compound in GCBE. This phenomena is often seen seldom in natural crude extract with various pharmacological active constituents. In this case, the interaction of the compounds with negative activity was manifested and fat reducing effects was inhibited in group fed GCBE (1%).


10) In this study, the authors may be demonstrating growth inhibition. A) We are not able to replay precise comments for this serious question. Caffeine is regarded as toxic or a habitual compound. Indeed upon excessive consumption is regarded as toxic for CNS, circulatory system and growth. The caffeine content in regular coffee or beverages are low. These doses are not toxic. It was reported that proper dose of caffeine can reduce ratio of body fat and enhance fat metabolism*. **) The mechanism is dependent on lipolytic activity caused by hormone sensitive lipase and enhancement of UCPs expression. These effects are pharmacological effect instead of toxicity. So, proper dose of caffeine can reduce the ratio of fat mass to body weight which is not toxicity.

GCBE can reduce body weight of mice fed with both normal and high fat diets. We think fat reducing effect of GCBE is partially based on pharmacological effect of caffeine, not toxicity. 


11) P8, Paragraph 2: An inhibitory effect of chlorogenic acid on hepatic TG level is observed. Dose GCBE that contains the same amount of chlorogenic acid not have the same effect? If not, it is not suggested that chlorogenic acid is an active substance.

A) As your suggestion, the hepatic TG reducing effect of GCBE was attenuated than that of pure chlorogenic acid. On this attenuation interaction of the other compound with TG accumulating activity such as cafestol and kahwole is considered. If we have to demonstrate contents in GCBE and activity of these compounds, we will eliminate this description.

12) P10, L2: Are there any findings suggesting the synergistic effects of chlorogenic acid and caffeine?

A) We only have a reference* that reveals the antagonistic effect of caffeine and chlorogenic acid on
glucose tolerance. Synergistic effects of both compounds have not been reported yet. Should we eliminate this description? If so, we will eliminate this description.


13) P6-7: The description of centrifugation should be standardized.
A) We change expression aEoerpmaE to aEoexgaE.

14) P6, L11: Is it possible to determine extracted TG concentration in suspension with accuracy?
A) We can determine TG concentration in the liver homogenate or its mitochondrial fraction. Although, we did not determine the concentration in the experiments, correlation between CPT activity and TG concentration should be evaluated. In the course of our study about the effect of GCBE on hepatic TG, we will examine these parameters concomitantly.

15) P6: Paragraph 3: What are the values for concentration and absolute amount of suspension?
A) The concentration of mitochondrial fraction and its adding procedure were lacked in the paragraph. The description about the fraction aEoeaEaE 37.5 mM palmitoyl CoA (20 mL) and 20 mL of mitochondrial fraction (6 mg/mL) were added into a reaction cuvette aE was added to the paragraph.

16) P10, L2: References should be used to quote published papers. Proceedings should not be listed.
References 16 and 17 were excluded.

17) How many times did the authors assess the reproducibility of each experiment described in the manuscript?
A) All experiments except examination of CPT activity were performed once. Because these experiments had been performed routinely in our laboratory. CPT examination was done twice in the case that was observed the significant activity.