

**Reviewer #1:**

**Scientific Quality:** Grade A (Excellent)

**Language Quality:** Grade B (Minor language polishing)

**Conclusion:** Accept (High priority)

**Specific Comments to Authors:** 6/16/20 Review for World J Gastroenterology.

“Acupuncture improved lipid metabolism by regulating intestinal absorption in mice”

General Comments The authors conducted an animal study to investigate a potential role of acupuncture treatment in lipid metabolism and the therapeutic modality for NAFLD. They found positive effect of acupuncture in regulation of lipid metabolism possibly through regulation of intestinal absorption of lipid. The findings of reduced intra-abdominal fat, low lipid absorption in the small intestine and lower blood lipid levels in the acupuncture treated mice are highly significant. Furthermore, more fat deposition in the intestinal epithelium suggests that acupuncture may suppress lipid absorption by downregulating the expression of apolipoproteins in the small intestine. These findings in the mice experiment are highly commendable. It is hoped that acupuncture may play a role in preventing/management of NAFLD in humans. The major concerning points for this work Food intake at the beginning and throughout the treatment with acupuncture was not assessed. Therefore, the conclusion that weight loss and low fat absorption are attributed to acupuncture alone becomes questionable.

Questions and suggestions

1. For the readers unfamiliar with Traditional Chinese Medicine, it would be courteous that authors provide the significance and function of the three acupoints, ST36, CV4, KI1 and the respective location in the body.

Response:

First of all, we would like to thank you for the helpful suggestions and comments. According to these suggestions and comments, we have revised our manuscript with more detail of Traditional Chinese Medicine and the function of ST36, CV4, KI1 in the introduction, while the location of ST36, CV4, KI1 in the method.

The revised introduction is as follows: (The revised part is marked with yellow color.)

Acupuncture is one of the most important external interventions in traditional Chinese medicine (TCM). It has a long history of more than 2000 years as a treatment for many diseases on a basis of *the Yellow Emperor's Classic of Internal Medicine*. According to the TCM theory, all diseases were caused by a stagnation of “qi” and “blood” which is a result of the imbalance between “Yin” and “Yang”.

And

Most of the acupoints are located on different meridians which correspond to different systems and have different functions. According to the TCM theory, ST36 which belongs to the stomach meridian of foot-Yangming has a function in generating stomach qi to regulate digestion and absorption. CV4 which belongs to conception vessel is front Mu point of small intestine which is a key acupoint in regulating the motion of qi. While KI1 which belongs to kidney meridian of foot-Shaoyin is an important acupoint in regulating the motion of both qi and blood.

2. At the end of inducing the NAFLD with MCT+high fat (HF), the mice were divided into two groups, AG and NG. Q. Were their weight comparable between two groups?

Response:

Thank you for the question. All experiment mice were randomly separated into AG and NG group. Before the needling treatment, their body weight showed no statistical difference (NG  $16.28 \pm 0.41$ g vs AG  $16.1 \pm 0.71$ g).

Although we did not find a significant difference in food consumption between the two groups of mice, since we did not use metabolic cages, it was not possible to confirm that the two groups had the same amount of food in this study. Thus, the reduction of food intake may be an important reason for the AG mice having lower body weight. However, as mentioned in the discussion section, "Food retention was clearly observed in the AG intestine (Figure 2A), suggesting that gastrointestinal motility may be relatively repressed in these mice. (Page 13, paragraph 2, line5~6)" acupuncture can inhibit gastrointestinal peristalsis by stimulating certain neural circuits, further leading to decreased appetite and diet. Therefore, if the weight difference is caused by the change of food intake, we have reasons to consider that acupuncture treatment indirectly caused the weight loss of mice. Of course, these conjectures need further experiments to prove.

Q. During the acupuncture treatment for 2 weeks, how many times per day were the mice treated?

Response:

During the acupuncture treatment for 2 weeks, the mice were needled once a day both AG and NG. Each acupoint was needled for 2 minutes with rotated slowly at 60 rotations without retaining the needle.

Q. Were they under anesthesia during acupuncture treatment? If not how were the mice restrained during the treatment?

Response:

During the acupuncture treatment, the mice were not under anesthesia. We used a mouse retainer to restrain the mice when needling. The treatment was just like the

following photo and we upload a video about how we needled the mouse.



Q. If anesthetized for the treatment, would that have influenced the appetite following the treatment under anesthesia.

Response:

We did not anaesthetize the mice. All mice were needled at awake state. So, there is no influence of anaesthetization.

Q. Were the food intake comparable between AG and NG mice during the 2 weeks' treatment period.

Response:

Thank you for point out this question. In fact, according to the TCM theory, Zu san li (ST36), Guan yuan (CV4) and Yong quan (KI1) have the effect on regulating appetite. Although we found no obvious difference in the food intake between AG and NG mice, we also considered the food intake was adjusted by needling treatment.

Suggestion in Methods In methodology, it would be helpful for the readers to describe the experiment chronologically; briefly, after 2 weeks MCD diet, HF continued. At the beginning of HF diet, they were randomized and acupuncture treatment for one group. After the end of 2 weeks on HF diet, acupuncture treatment, mice put under anesthesia, exsanguination, autopsy and etc.

Response:

Thank you so much for these helpful suggestions and we have improved the method in the revision manuscript file.

The *experimental animals* as follows:

All mice were fed with an MCD+ high fat (HF) diet (60% fat; KBT Oriental Corporation, Saga, Japan) for 3 weeks to induce NAFLD. After that, MCD diet stopped. They were then fed an HF (60% fat; KBT Oriental Corporation, Saga, Japan) diet for 2 weeks to maintain their hyperlipidemia. When HF diet started, they were separated randomly into two groups: the acupoints group (AG, n=10) and the non-acupoint group (NG, n=10) for the needling treatment. After two weeks' treatment, mice were anesthetized with an injection of ketamine-medetomidine and euthanized by exsanguination.

And the *acupuncture treatment* as follows:

The mice were randomly separated into AG and NG. Needling treatment started as well as HF diet started. As described previously, three acupoints, ST36, KI1 and CV4, or corresponding non-acupoints were needled with 13-mm needles (Suzhou Medical Appliance Factory 0.25 mm × 13 mm), which were rotated slowly at 60 rotations per minute for 2 minutes, without retaining the needle. The location of ST36 in mouse is 1.5 mm below the anterior tibial tubercle of both sides' hind limbs. KI1 is located in the centre of both sides' hind soles. CV4 is 10 mm below the mouse's navel.

**Reviewer #2:**

**Scientific Quality:** Grade B (Very good)

**Language Quality:** Grade A (Priority publishing)

**Conclusion:** Minor revision

**Specific Comments to Authors:** well written manuscript. i have few suggestions.

Thank you very much for taking time to review this manuscript. It is my great honor for your appreciation. My responses are as following and my revisions are in the revision manuscript files.

1- what is the statistical method?

Response:

Thanks very much for the question. All data in our manuscript are expressed as the mean ± standard deviation (SD). In this study, the sample size was not big, and the samples' mean and standard deviation can be obtained. As well as the samples fit a nearly normal distribution. So, we used 2-sided Student's t-test or Welch's t-test as appropriate to analyze statistical significance. And we used Microsoft Excel for calculation. P values of <0.05 were considered to indicate statistical significance.

2- (DOI: 10.1111/iwj.13265) and (<https://doi.org/10.1111/tbj.13174>) I suggest these uptodate studies for the references.

Response:

Thank you for the references. We studied more information about acupuncture in treating these diseases and added the references.