Reviewer's report

Title: Effect of Lactobacillus acidophilus supernatants on body weight in rodents

Version: 2 Date: 22 October 2007

Reviewer: Kendall Frazier

Reviewer's report:

General

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Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

1) Given the title, the major objective and the experimental design, it is very difficult to understand why the cytokine array data was included in this manuscript. I can appreciate that the array demonstrates there is upregulation of leptin. However, this experiment was performed in bovine tissue, was from endothelial cells, and an angiogenic array was utilized, rather than one which includes major neurogenic peptides and receptors that would be involved in a centrally mediated or peripherally mediated anorexic or fat deposition response. The inclusion of bovine angiogenic or cytokine endothelial data has very little to do with the purposes of brain expression experiments in rodents and detracts greatly from the quality and intent of this paper. I strongly encourage the authors to delete this data, and simply refer to it in the introduction as a serendipitous anecdotal finding that spurred further work (data not shown). Instead I would do a simple taqman experiment showing that in rodent (rat) cultures of neurogenic origin there is upregulation of leptin with exposure to the supernatant. There are multiple cell lines that could be used, and although I understand that it is never easy to have a reviewer request an additional experiment, this is such an easy, straightforward experiment that I can see no reason why it should not be included in your experimental design. Using taqman and western blot (or other measure of leptin protein synthesis) you will be able to bridge your immunohistochemical results to earlier experiments. Without it, the bovine endothelial data is just hanging there without any real apparent relevance. Where is the expression or synthesis data that shows that endothelium (or even choroid plexus) was a major contributor to the leptin immunohistochemical staining? Rather, it sounds like neurons gave the most positive signal, and hence these should be the focus of supporting expression data. This is the major flaw in the paper as written, but one I think that could be easily corrected and improved upon.

2) The figures and figure legends need improvement. In figure 1 legend, please add “weight” after “body”. Where is the value for 120 hours for LA in the graph? I understand there was only 1 animal taken at this timepoint, but how can you draw the conclusion that there is a significant effect at this timepoint and not show the data? Also and importantly, in all figures, there is a substantial
difference in the white balance between controls and treated. The intensity of the blue background suggests that the temperature/light level was at least 20% less in these photographs as compared to the supernatant treated and this not only makes it difficult to compare but also the blueish photos are of insufficient quality to be reproduced in any journal. They should be redone, and of the same white balance background, and of the same magnification. There is approximately a 30% difference in the diameter of the choroids plexus between photomicrographs in one figure, which makes it very difficult to imagine that these were taken at the same level of magnification. Similarly in the composite photograph of the immunos in the brain, either these were not taken in the exact same location or there is a difference in magnification (and this is unacceptable for publication). Please use the same location, same orientation and same magnification if trying to show comparisons of immunostaining. Finally the photomicrograph of control choroid plexus "A" is not in focus, at least in the version I have on-line, so it is very difficult to compare to the treated in "B".

3) Please justify the choice of the numbers of group size using statistical rationale. I appreciate these are very difficult and expensive in vivo experiments to run and that it is hard in an academic institution to adequately power such experiments. However, the major conclusion in this study is that body weight as an endpoint is statistically different at 96 or 120 hours from control. How can one way ANOVA be used to say there is statistically significant differences in 2 group control populations in which one only has an N of 2 and the other has an N of only 1. In this regard, I think consultation with a statistician must be used so that readers are convinced that these endpoints are adequately powered. Simply using the stats program and deriving a p value does not sufficiently rationalize the significance of a finding. There needs to be a paragraph in the discussion explaining how an N of 2 in controls still warrants deriving the conclusion of decreased body weight. From this standpoint, the low pH control would be the more justifiable comparator than the saline infusion group. There are plenty of rats in the early timepoints for weight, but losing 2 each timepoint, 8,6,4, etc. makes it harder to power, and unfortunately the difference is only apparent in the later timepoints.

4) What age were the rats? Were they randomized to group? 6-8 weeks would be the age I would guess for 250-350 g rats, but if weight loss is the major endpoint, it is imperative that more data about starting weights in each group is included.

5) The conclusion that leptin is the mechanism for the weight loss is not supported by the data in this study. All we know is that there is increased expression of leptin in neurons, and that there is some weight loss noted without a concomitant decrease in food consumption. This is too little evidence to support a conclusion that “leptin may be involved”. Rather the conclusion should be reworded to support what actually was observed in this study, eg. that Lactobacillus supernatant ICV infusion resulted in weight loss (as long as this is supported by reevaluation by statistician) at 96 and 120 hours, without a decrease in food consumption and was associated with increased expression of leptin in neurons.
Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

1) Why was the body weight loss called “transient” in the text? I see no reference or data saying that it reversed or came back to normal.

2) There needs to be a little more in the discussion explaining where leptin has been shown to be upregulated in the brain during weight loss and how does this compare to the findings in this study if we are to better understand the authors’ assertion that leptin is the mechanism here. A little more discussion of potential mechanisms of leptin effects on weight loss would also have been helpful.

3) What was the osmolality of the supernatant and the lactic acid solution control? The pH of the solution is one variable, but administration of hyperosmolar infusions may also have a tremendous effect on gene regulation and protein synthesis and local necrosis.

4) Was any antigen retrieval necessary in the immunohistochemical procedure? If not, this would be unusual given these were formalin fixed paraffin embedded samples, but in either case it should be noted in methods.

Discretionary Revisions (which the author can choose to ignore)

1) It would have been helpful to have plasma leptin levels in these rats to better strengthen the conclusion.

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:

I declare that I have no competing interests.