Author’s response to reviews

Title: Effect of long-term feeding of the Obudu natural honey and table sugar-sweetened diets on obesity and pro-inflammatory biomarkers in rats.

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SN Reviewer’s comment Response/rebuttal
Th Reviewer 1
1 The authors explore the effects of honey and table sugar on several zoometric and plasma parameters in male and female Wistar rats. The study is descriptive, and the conclusions would be reinforced if the authors could perform some new experiments. Specifically, the authors could assess whether insulin signaling is impaired, as they suggest in the discussion. Moreover, it would be interesting to assess whether sugar and honey supplementation cause an increase in liver lipids. These suggestions are well-noted and shall be fully addressed in further and follow-up studies on the subject
2 Ln 160: please explain how were the sugars incorporated to the diet
   The table sugar and honey were incorporated by wet mixing with the rat chow (w/w) and repelleting. See section 2.3
3 Ln 162: please, change this cite of a webpage for a reference
   An appropriate reference has been cited. See section 2.3
4 Ln 201-215, please remove, it is not necessary to give the details
   The portion has been removed. See section 2.5.
5 Figure 1 and 2: Graphs are not clear enough, could you try to express the results as the area under the curve?
   The graphs have been presented in TIFF format and 600dpi resolution.
6 Regarding the diet intake, it would be nice to calculate the caloric intake in the different treatment groups, based on the amount of food consumed and the energy supplied
   Calculation of caloric intake would require that proximate composition of the formulated diets be known. These were not determined in the present study. However, there is a strong positive correlation between the amount of diet consumed and caloric intake which allows for interpretation of the result in the light of energy intake. Subsequent studies will make for caloric intake evaluation/calculations.
7 High consumption of sugars, in particular fructose, can cause fatty liver, but this effect may depend also on the length of treatment. It would be interesting to assess whether under the conditions of the present study sugar and/or honey cause an increase in hepatic lipid levels
   Further research in
our laboratory will definitely evaluate hepatic lipid levels and activities of the associated regulatory enzymes.

It is intriguing why H20% causes an increase in female weight gain but a decrease in the case of males. Do the authors have any explanation for this discrepancy? Similarly, there are divergent results of S8%, which increases male's weight gain, but not in females. All these differences between sexes are interesting and should be discussed. They are difficult to explain with certainty, yet those were our observations. Be that as it may, it is a known fact that females differ remarkably from males in terms of the mechanisms that regulate substrate utilization and energy homeostasis. Physiologically, females have stronger propensity toward retention of fat mass during times of energy surfeit (Cortright & Koves, 2000). According to Cryan & Wolf (2003), the greatest energy demand in males coincides with spermatogenesis. In addition, the timing of reproductive demands differs between the sexes. Moreover, the rate at which males lost water increased more rapidly than females. There are several potential explanations for higher rates of water loss in males, including sex differences in breathing rate, body size and metabolism.


There are also important differences in the bioavailability, metabolism, distribution, and elimination of foods and beverages in males and females (Marimo, M.; Masella, R.; Bulzomi, P.; Campesi, I.; Malomi, W. & Flavia, F. (2011). Nutrition and human health from a sex – gender perspective. Molecular Aspects of Medicine, 32 (1), 1 – 70).

Liver tissues were not fixed in 10% formalin, to allow for histological studies, because it was not in the scope for the present study. These shall be performed in further studies.

If the authors have not measured the weight of subcutaneous adipose tissue, they cannot make this assumption This portion has been revised. See lines 357 – 358.

The increase in TNF-alpha is only an indirect evidence The use of the word “affirm” has been revise. See line 376.

Table sugar was used. The sentence has been re-written. See lines 29 – 30.

The conclusion has been re-written. See lines 50 – 53.

This has been corrected. See line 83.
The work presented by Atangwho and co-workers entitled "Effect of long-term feeding of the Obudu natural honey and table sugar-sweetened diets on obesity and pro-inflammatory biomarkers in Wistar rats (Rattus norvegicus)" compares the effects of diets enriched with table sugar and Obudu honey in insulin and glucose homeostasis and inflammation in rats. As I understood, the motivation of the study relies on the fact that obesity has reached epidemic proportions in developing countries and table sugar consumption is one part of the problem. And honey, as a sweetener, could be used as a replacement for table sugar but little is known about the metabolic effects of honey consumption. Despite the fact this is a scientifically sound study, in the way the background/introduction is presented and also how the study was designed, there must be major changes for the rationale of the study to be clear and for the conclusions to reflect the results, as explained below: The motivation for the study as understood by this reviewer is not entirely correct: There is a claim that honey is a better alternative for table sugar, which allows for abuse and indiscriminate use of honey, thereby worsening the already strained obesity epidemic. A long-term exposure to both honey and sugar in equivalent amount (on dry weight basis) would clarify whether or not honey does not pose the risk of obesity, its metabolic effects.

English is poor and the manuscript must be carefully revised. It is very hard to follow the authors' message in several parts of the manuscript: Some examples: The entire manuscript has been subjected to English Language scrutiny.

Line 35: "increasingly eat less compared to the normal control (NC) and the corresponding S16%...". This has been revised. See line 37.

Line 49: "but sublime effects that only require..." sublime is not a technical term and, for the sake of clarity, must be replaced.

Line 80: "sweet test to food thereby improving its palatability". The word “test” was used instead of “taste” in error. The sentence has been revised. See lines 82 – 83.

Results (Lines 232-33): "Both honey and sugar at their highest percent incorporation increased body weight gain successively in the female rat groups compared to control (P < 0.05) The sentence has been revised. See lines 237 – 239.

The motivation of the study is not clear. Why to study honey? How is the trend of honey consumption in the world and in Nigeria? What is the motivation to study Obudu honey?

The last sentence of the Background states (Lines 130-32): "Since the chemical composition of natural honey, as noted earlier depends largely on its geographical origin, it is expected that the nutritional and hence the physiological effect may also vary, justifying the need for the current study." The chemical characterization of the Ubuntu Honey would significantly improve the data and would shed light into the biological effects of the honey diet. I understand this was not the scope of the manuscript, but the way it is written, it seemed that the motivation of this study was the potential beneficial effects of this particular honey. Therefore, the authors should address, at least in the discussion, the potential composition of the Obudu honey, accordingly to what was stated in the introduction. Honey is a highly regarded product in Nigeria. Apiculture i.e. honey production or bee farming is widely practiced in many areas in Nigeria (Aiyeloja, A.; Popoola, L. & Ogunjinmi, A. (2010). Economic analysis of honey production in Southwest Nigeria. Asian-Pacific Journal of Rural Development, 20, 1.).

Lawal et al. (2017) have shown the basic chemical composition of the Obudu honey, where they indicated that the Obudu honey and Ogoja honey were the only two honey samples out of the 7 samples studied that met the Codex Alimentarius specifications for fructose and glucose (Olajumoke Omobola Lawal, Ekpe Onot Obolo, Stella Celestine Bassey, Okey Ogbaka Obeten (2017). Composition of Sugars in Honey Produced in the South-South and South-West Regions of Nigeria International Journal of Sciences 6: 178 -185).
Aspects of the discussion relating to this has been revised.

25 The background should be re-written not only to provide scientifically sound evidences that it is important to investigate the metabolic effects of honey in general and Obudu honey in particular, but also to revise some paragraphs that does not provide critical information to the study, as the 3rd paragraph (Lines 91-100) - which describes what honey and table sugar is - which, in my opinion, is a textbook or Wikipedia information. The background has been revised in line with the recommendations. See portions in colour. Moreover, the 3rd paragraph has been revised.

26 The experimental designed should be critically revised: There were 4 types of diets, in addition to control diet: with 8 and 16 % of sugar and 10 and 20 % of honey. To sum up 100 %, what was done was to decrease the % of the regular diet. And not only the % of glucose, dextrose or starch. There are important implications of this type of design: Percent supplementation of a substance in a diet is a common model that is used in several studies. Even with honey, several levels of supplementations have been used and reported by other authors. For instance, Chepulis and Starkey had used 8 and 10% supplementation with sucrose and honey, respectively.


27 Possible alterations in the consistency of the diet - did it change? The decrease in the intake by female rats in the 20 % honey might be due to altered consistency and, therefore, acceptance of the diet by the animals. It is obvious that the observation could not derive from altered consistency. Altered consistency effect would not have been restricted to the female rats only - sex dependent. Moreover, the animals were acclimatized to the diets before commencement of experimental feeding.

28 More importantly, decreasing 20 % of regular diet implicates in the reduction of essential nutrients, as protein, essential fatty acid and vitamin/mineral. Therefore, the authors cannot conclude if the differences observed between groups were due to the increase in sugar or honey consumption and/or reduction in the other dietary components. Again, the effect of nutrient deficiency would not be restricted to female animals and in the 20% treatment group only. Honey, being a natural substance contains more nutrients than table sugar which is largely sucrose. The case of nutrient deficiency would have been more pronounced with table sugar incorporation.

29 Did the study include pair-feeding protocol? If not, the authors cannot conclude if the differences observed between groups were due to the increase in sugar or honey consumption and/or reduction in total food intake in the case of female rats.

30. Yes, the diet consumed by test groups were matched with that of control.