Author's response to reviews

Title: Plasma branched-chain and aromatic amino acid concentration after ingestion of an urban or rural diet in rural Mexican women.

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Author's response to reviews: see over
Dear Editor,

Thank you very much for giving us an opportunity to resubmit a revised version of our manuscript "Plasma branched-chain and aromatic amino acid concentration after ingestion of an urban or rural diet in rural Mexican women". Please find enclosed a new version of our manuscript as well as a detailed point-by-point answer letter addressing all issues raised by the reviewers.

We have now included a table that incorporates other biochemical parameters such as glucose, cholesterol, triglycerides and HDL cholesterol. These new data, together with a number of text changes, were integrated in the revised manuscript to satisfy all the reviewers' concerns.

We hope that this new version will satisfy the referees and qualify our work for publication in *BMC Obesity*.

We look forward to hearing from you.

With best regards,

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Answers to Referees’ comments:

Referee #1 (Revision Comments):

Lopez et al. describe post-prandial differences in selected plasma essential and non-essential amino acids in rural Mexican women fed either a typical Mexican “rural” diet versus an “urban” diet. In a crossover design, Lopez et al. reported significantly higher post-prandial AUC for isoleucine, valine, total BCAA, tyrosine, and proline after consumption of the “urban” diet. The results are not unexpected, but they do provide a clear example of how mixed meals of different composition can impact blood amino acid patterns in humans. Such examples may prove useful in the literature in terms of design of diets that alter amino acids for research purposes, and thus the paper may be better suited for a modest nutrition-specialty journal. A major flaw that requires attention for any revision and for any journal format is the assertion that BCAA etc. patterns acutely following each meal somehow promotes dysmetabolic phenotypes, which is far too speculative and does not have a solid foundation in the literature. The urban vs. rural diets have a huge variety of differences beyond protein, and physical activity is also a factor in driving insulin resistance and obesity outcomes. Changes in BCAA etc. seem to reflect, not cause insulin resistance.

We thank this referee for his/her comments and suggestions that have enabled to improve the manuscript. A detailed point-by-point answer to his/her comments is provided below.

1. Lines 85-86: Please report BMI; in the statistics, presumably a paired t-test was employed (this is not clear from the write up)

To satisfy this reviewer we have now included the BMI in the subjects’ section of material and methods. In addition, we apologize for our lack of clarity in the statistical analysis. We have now rewritten this section.

2. Was there any attempt to determine historical dietary data in these women? Also, in the Introduction there is an assertion that a diet more rich in protein or certain proteins would lead to the associations of BCAA, Phe, and Tyr and insulin resistance; yet, the latter literature was conducted during the overnight-fasted period and so it is not clear how that would impact overnight-fasted amino acid patterns.

Yes, we performed a 24-hours food recall in these women. We have now included the main results of this analysis in the subjects’ section. We would like to point out that we established the composition of the typical diets according to the analysis of a 24-hours food recall from women of a rural and urban area.

Also, we would like to apologize if we have given the impression in the background of the assertion that a diet more rich in protein or certain proteins would lead to the associations of BCAA and insulin resistance. We have modified the text to make clearer our rational: 1) BCAA’s are increased during obesity; 2) There are several mechanisms that would lead to this increase, including a decrease in BCAA oxidation; and 3) One of the mechanisms could be the ingestion of different type of proteins since the literature shows that this is possible for other amino acids.
3. It is not clear how the test meal (i.e., after time 0) was administered. Table 1 appears to provide the total daily composition of urban or rural diets, but the plasma measurements occurred presumably after single meal ~ 7am. Was the test meal administered and consistent among participants? What was the composition of the meal before the post-prandial measurements?

We thank this reviewer for his/her remark. The diet was administered as a single meal at time 0, and we indicate in material and methods (experimental diets section) the exact composition of each diet. As we mention before the composition of the diet was established from the analysis of a 24-hour food recalls performed in women from rural and urban areas. The meal was consistent among participants and they had 12 hours fast before they ingested this meal.

4. Was glucose or insulin measured? These measurements would provide context for many of the author’s conclusions.

We completely agree with the reviewer’s observation. We have now included a table that incorporates other biochemical parameters such as glucose, cholesterol, triglycerides and HDL cholesterol. Unfortunately, we weren’t able to measure any hormone, and we recognize in the discussion that measuring insulin and other hormones in further studies will provide a better understanding of the effect of these diets in insulin signaling and secretion.

5. There is very little discussion about the other macronutrient differences between the “rural” and “urban” diet. In addition to differences in protein, there are stark differences in carbohydrates, lipids and dietary fiber; all of which will impact insulin and energy dynamics. This is a major confounder in terms of interpretations and must be fully acknowledged.

We thank this reviewer for his/her concern. We have now included a paragraph in the discussion describing how other components of the diet could also impact insulin sensitivity.

6. The authors conclude that the elevations in post-prandial plasma BCAA concentrations seen after consumption of an “urban” diet may increase the risk of obesity and diabetes. Contrary to this conclusion, there is considerable evidence that diets high in leucine improve insulin sensitivity. There is, actually, a far stronger case that elevations in BCAA, Phe, Tyr in the insulin resistant state are a result, not a cause of the resistance (see, e.g., S.H. Adams, Advances in Nutrition). Additional discussion about the counter evidence is needed.

We completely agree with the reviewer and we have modified the discussion accordingly.

7. The data presented herein is really insufficient (i.e., a single post-prandial meal response) to draw such conclusive statements regarding obesity and diabetes risk. Although the reviewer agrees that the risk of obesity and diabetes is greater in a Mexican “urban” environment compared to a “rural” environment, a single post-prandial BCAA response is not enough evidence to justify the final sentence of the manuscript (lines 183 through 185, and last lines in
the Abstract), or the bulk of L. 164-179. This reviewer suggests an alternative conclusion that addresses the limitation in their study and a statement regarding the need for future controlled longitudinal studies.

We thank this reviewer for his/her suggestion. We have now stated in the discussion the need for future studies to assess the short- and long-term effects of the consumption of these diets on other parameters including, hormones, genetic variability between rural and urban population, environmental conditions, physical activity, among others.

Referee #2 (Revision Comments):

The manuscript of Lopez et al. (BMC- Obesity- Plasma branched-chain and aromatic amino acid concentration after ingestion of an urban or rural diet in rural Mexican women) provides data on amino acid concentrations after ingestion of two different meal types in rural Mexican women. The background for the information is the rise in obesity after rural people move to urban settings and the insulin resistance associated with obesity. The study is reasonably well done although, there are some issues that would need to be addressed before publication could be considered.

We would like to thank this reviewer for his/her positive comments. A detailed point-by-point answer is provided below.

Major concerns
Was there an internal or external standard for the amino acid analysis?
Yes, we use L-Norleucine as internal standard and we have modified materials and methods to clearly state its use.

Did each woman serve as her own control or were the results averaged over all women? It appears that the data are pooled across diet and this should be stated.
We have pooled the results of the 15 women in each diet. In the revised version we now include this statement in the statistical analysis section.

The fact that the women were from a rural population is interesting although one would have expected the same result if the women were from an urban background, correct? The differences are likely just a function of the diet consumed, not where the subjects were from.
We thank this reviewer for his/her concern. We agree that the differences are likely just a function of the diet consumed, not where the subjects were from. Evaluating women from an urban area will help to clarify this but also could provide more information regarding the basal amino acids levels, since it is possible that a constant stimulus with an urban diet could lead to an increase in the basal levels. This would be also important to evaluate in further studies. We have now included a paragraph in the discussion addressing this issue.
Discretionary Revisions
L129 – consider putting P-values in for each amino acid since some are significant and some are not.
L162 – ...to proteins at a higher rate
L169 – ...such as norepinephrine which is known to modify food intake. Intriguingly, we also...
L172 – ...the greater increase
L175 – ...in feeding behavior affect rural populations.
L177 – ...companies that increase access to energy dense foods.
L178 – ...is reflected in the greater increase in the prevalence of overweight and obesity in rural (3.9%) compared to urban (2.5%) women in the last 6 years.

We thank this referee for his/her suggestions. We have made the pertinent changes that have improved the manuscript.

Referee #3 (Revision Comments):

In the present study the effect on plasma amino acid pattern following ingestion of either an urban or rural diet is presented. The study involves 15 subjects in a cross-over design. The authors show differences with a more pronounced increase of postprandial BCAA, tyrosine and proline concentrations after ingestion of an urban more protein- and fat-containing diet. This finding is of interest and may have implications for health and possibly for development of illnesses related to metabolic conditions such as obesity, metabolic syndrome and diabetes.

We would like to thank this reviewer for his/her positive comments. A detailed point-by-point answer is provided below.

Minor essential revisions
It could be of value to include a classical reference by Elia et al. Effects of ingested steak and infused leucine on forelimb metabolism in man and the fate of the carbon skeletons and amino groups of branched-chain amino acids. Clin Sci, 64; 517-526, 1983.

We have now included this reference in the discussion section.

A suggestion is to increase the bars and the p-value incorporated in the figures to make it easier to read.

We thank this reviewer for his/her suggestion. We have modified the figures accordingly.
It would be of interest if glucose and insulin values have been collected during the same time-frame as the amino acids in order to cover the different nutrients effect on insulin release and glucose level. If so, it is a good idea to present them.

We completely agree with the reviewer. We have now included a table with another biochemical parameters including glucose. Unfortunately we weren’t able to measure any hormones. In the revised version, we address this issue in the discussion.