Author's response to reviews

Title: Effects of leucine supplemented diet on intestinal absorption in tumor bearing pregnant rats.

Authors:
Gislaine Ventrucci (ventrucc@unicamp.br)
Maria Alice R de Mello (mellomar@rc.unesp.br)
Maria Cristina C Gomes-Marcondes (cintgoma@unicamp.br)

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PDF covering letter
Reviewer's report
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Authors: Gislaine Ventrucci (ventrucc@unicam.br)
Maria Alice R de Mello (mellomar@rc.unes.br)
Maria Cristina C Gomes-Marcondes (cintgoma@unicam.br)
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Answers to Reviewer: Dr Peter Taylor

COMPULSORY REVISIONS
1. There is some confusion throughout caused by nomenclature of experimental groups. For example, it is not immediately clear (especially in Abstract) that tumor-bearing and pair-fed rats are also pregnant. Equally, groups C, P and L are all referred to as "control" at different stages. Group names and text should be altered to fully reflect these points.

A1) Attending the reviewer suggestion we have now described, and added to the main text, 7 groups: one group is C, non pregnant rat, and 6 pregnant groups which we renamed them as N, pregnant; WN, pregnant tumor-bearing rats; Np pregnant pair-fed; these three groups were fed with normal protein diet (18% protein); and other three groups fed with leucine supplemented diet named as L, pregnant; WL pregnant tumor-bearing and Lp pregnant pair-fed. All groups' names were altered in whole text.

2. Was ethical approval obtained / required for these studies?

A2) This study was approved by Ethical Committee for Animal Research CEEA and COBEA, Brazil, no. 034-2, and General UKCCR (1988, United Kingdom Coordinating Committee on Cancer Research) for animal welfare were followed.

3. Some detail of perfusion system (e.g. whether perfused loop, in situ, recirculated, perfusate volume) is required.

A3) We have explained better the intestinal perfusion methodology; it is a continuing intestinal flux and the intestinal perfusate was collected to measure glucose, methionine and leucine content.

4. Table 1. Does body weight gain represent total weight or carcass minus fetus / tumor (as appropriate)? Fetal weight in tumor-bearing rats seems to be markedly lower than in other groups, although no indication of statistical significance of these results is shown.

A4) The body weight gain represents the total body weight minus fetus or tumor at the final day of the experiment (20th day). The fetal weight in tumor-bearing groups is different than the other groups and it was corrected and now it is showing in the table.
5. Which muscle is being studied (e.g. gastrocnemius)? It is difficult to know exactly whether tumor growth causes a real "decrease" in muscle weight rather than a reduced increase from onset of experiment.

A5) The muscle studied was gastrocnemius and it is added to the main text. All animals started the experiment with the same initial body weight, so in this case muscle weight reduction could reflect the tumor effects on reduced increase in muscle weight.

6. I assume the absorption studies reflect disappearance of solute from recirculating perfusate, in which case "absorption" includes solute metabolised / assimilated by gut tissue as well as solute entering the blood. On this basis, the following points need to be addressed:-

(a) Given that absorption is measured per cm of gut, it is extremely important to establish whether changes in gut mucosal mass / protein content have occurred and whether these may account for some underlying changes in absorption.

A6a). Although we have not analysed the gut mass/ protein content in the present study, in previous studies, we have showed that the reduction of the ratio gut mass/ protein content occurred in groups which were submitted to food restriction or tumor implant and there was a decrease in nutrient intestinal absorption (Gomes-Marcondes et al, 1998). The present study, this ratio can not be related with leucine absorption in pregnant tumor bearing rats, so we believe that the decrease in glucose intestinal absorption was due to reduction in the food intake; as glucose absorption is up-regulated and depends on its carrier, pregnant groups (P and L) were adapted to a non-specific regulation of carriers which can be accompanied by mucosal hyperplasia. The W and WL group showed decrease in glucose and methionine absorption compared to respective groups (P and L) but analysing leucine absorption the tumor bearing groups showed changes in this amino acid absorption which can not be related to reduction in rat mass/ protein gut. We are now developing another experiment, that we intent to publish earlier, demonstrating the change in presence and number increase of glucose (GLUT1) transporter and amino acid (L) transporter in presence or not of growing tumor during pregnancy.

(b) Equally, could differences in water absorption produce anomalous "apparent" rates of solute absorption?

Nevertheless, the observation that Leu, Met and glucose absorptions respond differently to treatments excludes the above as sole reason(s) for observed changes.

A6 b) There is no difference in water absorption as it was verified by perfunding polyethylene glycol (PEG, 4000 mol. Wt) as a tracer in nutrients uptake studies, so we believe that there is no differences or apparent changes in rates of solute absorption.

7. I do not think the statements in the first paragraph of Discussion concerning "somatic parameters" are fully supported by the data and they should be modified in light of the following observations:-

(a) Much of the reduction in weight gain, muscle protein balance etc in tumor-bearing rats can readily be ascribed to reduced food intake (i.e. effects of tumor-burdon or pair-feeding are essentially equivalent), although there do appear to be substantial and potentially-important differences in effect on fetal weight.

A7a ) It was a typing mistake we have now corrected the text as some parameters, instead ‘somatic’. 
We have already described in previous results that the effects of tumor growth or pair feeding are not the only effect on fetal weight reduction but the indirect tumor influence, such as probably cytokines and/or substances produced by tumor cells, which produced the same effects induced in tumor-bearing pregnant rats and pregnant which received ascite tumoral fluid without the cellular elements (Gomes-Marcondes et al., 1998).

(b) The major effects of Leu supplementation are to prevent experimental hypoglycaemia (Table 1) and to stimulate Amino Acid absorption / assimilation by the perfused intestine (only for Met u take are there appreciable tumor-related differences).

A7 b)

DISCRETIONARY REVISIONS

1. The specific relevance to pregnancy should be made more clear in the Background section

A1) The relevance of pregnancy studies was added in the background section.

2. BCAA are a source of metabolic fuel for skeletal muscle (particularly during prolonged exercise) but I question whether they are "the major source" (as stated in Background).

A2) BCAA are the source of metabolic fuel and we agreed that BCAA are a great source to metabolic fuel for muscle.

3. I think the Discussion section is too long, repetitious and contains some material of only peripheral relevance. It could easily be reduced in length by 30% without loss of coherence, particularly the long section (3 ages) on effects of Leu and cancer on muscle protein turnover.

A3) We have reduced the discussion section text trying not to lose the coherence.

4. The work of Diamond and colleagues (e.g. Am J Physiol 26, R793; 27, G969) on nutritional adaptations of intestinal transport processes might be further considered in the Discussion.

A4) As we have noticed in Diamond and colleagues work with hypercabohidrate diet or hyperprotein diet that there are some intestinal adaptations, which were considered in the discussion sections in the present study.

Competing interests:
None declared.