ANSWERING REVIEWERS

April 16, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 2839-review.doc).

Title: Dietary-suppression of hepatic lipogenic enzyme expression in intact male transgenic mice

Author: Maria Notarnicola, Maria Gabriella Caruso, Angela Tafaro, Valeria Tutino, Giusy Bianco, Mario Minoia , Antonio Francavilla

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 2839

The manuscript has been improved according to the suggestions of reviewers and all the revisions are highlighted in the revised manuscript.

Below you will find the point-by-point reply to three reviewers

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,

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Reviewer 00503516

Point by point reply to reviewer:

Major point:

1. According to reviewer's suggestion, in the revised manuscript, to substantiate the inhibition of cell proliferation in liver mice after nutritional treatment, we have evaluated the expression level of cyclin E gene in the same liver samples used to measure the expression of the lipogenic enzymes. Obtained data have been added in the Result section (page 9, lines 18-21) and in Figure 3 (page 21). Moreover, cyclin E gene expression levels have been discussed in the Discussion section (page 10, lines 26-31 and page 11, lines 1-3).

Minor point:

1. In the Abstract, in order to clarify the connection between the "aim" and the "conclusion", the "aim" has been rephrased, as suggested by reviewer (page 2, lines 2-4).

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Reviewer 00467187

Point by point reply to reviewer:

1. According to reviewer's suggestion, to prove that cell proliferation was inhibited by nutritional treatments, in the revised manuscript we have evaluated the expression level of cyclin E gene in the same liver samples used to measure the expression of the lipogenic enzymes. Obtained data have been added in the Result section (page 9, lines 18-21) and in Figure 3 (page 21). Moreover, cyclin E gene expression levels have been discussed in the Discussion section (page 10, lines 26-31 and page 11, lines 1-3).

2. The ability of natural compounds, such as eicosapentaenoic acid (EPA), Arachidonic acid and hydroxytyrosol, to inhibt cell proliferation has been demonstrated in our previous in vitro studies, using human hepatoma and colon cancer cells. In future, as suggested by referee, the use of a model in vivo of hepatocellular carcinoma can be useful to better understand the molecular mechanisms of cell inhibition of these substances.

3. In our preliminary experiments, lipogenic enzyme activities and gene expression have been evaluated in liver samples from mice treated with lovastatin and orlistat alone. The findings demonstrated that the combinations of olive oil with the drugs elicited a synergic effect, suggesting that the inhibition of these enzymes with natural components in association with canonical drugs counteracts more effectively hepatic cell proliferation in mice compared to the use of drug alone.

4. Experiments evaluating the effects of olive oil and other natural diet component in normal intestinal tissue and adenoma from APC Min/+ mice are in progress in our laboratory.

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Reviewer 00037961

Point by point reply to reviewer:

Major comments:

1. According to reviewer's suggestion, in the Results section, the statement indicating histological analysis has been removed in the revised manuscript (page 9).
2. In order to justify the role of lipogenic enzymes as markers for hepatic cell proliferation, we have evaluated the expression level of cyclin E gene in the same liver samples used to measure the expression of the lipogenic enzymes. Obtained data have been added in the Result section (page 9, lines 18-21) and in Figure 3 (page 21). Moreover, cyclin E gene expression levels have been discussed in the Discussion section (page 10, lines 26-31 and page 11, lines 1-3).