Objective: Answering Reviewers

Title: The relationship between adipose tissue dysfunction, vitamin D deficiency and the pathogenesis of NAFLD

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ANSWERS TO REVIEWERS

We thank the reviewers for their comments and have now answered to all the points raised in the review.

Please find below a point-by-point list with all the changes made throughout the manuscript:

Referee #1

1. Authors mentioned that vitamin D receptor (VDR) is expressed in adipocytes and is dynamically up-regulated during adipogenesis. However, there are no evidences regarding VDR expression in adipocyte of human or animal model. Do you have any information regarding the changes of vitamin D receptor in human study or some animal models?

We thank the reviewer for this comment which allow us to add further evidence on the VDR expression in adipocytes both in animals models and in humans. In particular, the expression of VDR gene has been reported in cultured adipocytes (Kamei Y et al 1993), in human pre-adipocytes (Trayhurn P et al 2011) and human subcutaneous and visceral adipose tissue (Ding C et al 2012; Nimitphong H et al 2012). These studies have been now mentioned in the manuscript (page 9, lines 2-5) and the corresponding references have been added in the references' section (page 24, references number 110-113).

2. I cannot understand why authors focus on the relationship between adipocyte dysfunction and vitamin D. Because VDR is expressed in the immune system (T and B cells, macrophages,

and monocytes), the reproductive system (uterus, testis, ovary, prostate, placenta, and mammary glands), the endocrine system (pancreas, pituitary, thyroid and adrenal cortex), in muscles (skeletal, smooth and heart muscles), and in brain, skin, and liver there are many action site of vitamin D as shown in Eliades M et al. (2014). Therefore, authors change title or include reports regarding relationship between adipocyte dysfunction and vitamin D. Please change this title.

Besides the effects which vitamin D exerts on bone, immune-reproductive-endocrine system and on several organs, such as liver, brain and skin, recent studies show that vitamin D has a role in modulating the homeostasis of adipose tissue improving local insulin sensitivity and regulating immune processes such as the secretion of many chemokines and adipokines. For this reason, we do consider central to explore the interaction between vitamin D action, adipose tissue dysfunction in the course of dysmetabolic conditions and the development of NAFLD in this review. As an example, very recently, Karkeni E et al demonstrated that vitamin D modulates the expression of miRs in adipocytes in vitro and in adipose tissue in vivo through its impact on NF-κB signaling pathway, which could represent a new mechanism of regulation of adipose tissue inflammation by vitamin D (Karkeni E et al 2017). This study along with other insightful and recent studies on this topic have been illustrated and commented in the review (page 9, lines 10-24).

3. "Symbol "font such as????????? is disappeared in all texts.

We thank the reviewer for noticing this editing mistake and we have now revised the manuscript.

4. Page 8, line 22 – 24. Interestingly, many studies have suggested that adipose tissue could be a direct target of vitamin D, and that this hormone might have a role in modulating adipose tissue pathophysiology (106-113). Vitamin D is not hormone.

Since vitamin D is released and transported in the bloodstream and exerts its action in sites far from where is produced, it could be defined as hormone. Nevertheless, we agree with the Reviewer that the term "hormone" referring to vitamin D could lead to an ambiguous interpretation and we have substituted this term with "molecule" throughout the text (page 8, line 28).

5. Authors should compare review as follow: "Eliades M et al 2015 Vitamin D: A new player in non-alcoholic fatty liver disease?"

We thank the reviewer for this suggestion. Since the recurrent coexistence of NAFLD and vitamin D deficiency, in the interesting article entitled "Vitamin D: a new player in non-alcoholic fatty liver disease?" which we quoted in the reference n. 131 (page 27, reference number 131), Eliades et al. explored possible links between these two pathological conditions, particularly focusing on common epidemiology and pathophysiology. In addition, they provided an overview on clinical data from vitamin D supplementation trials in subjects affected by NAFLD.

Conversely, our review explores the relationship between adipose tissue dysfunction, vitamin D deficiency and NAFLD in subjects with metabolic diseases. Indeed, among the several hits determining NAFLD, we focused on metabolic modifications occurring in presence of adipose tissue dysfunction. In particular, we summarized evidence on the association between adipose tissue inflammation and low vitamin D levels/tissue VDR expression exploring the possible role of these conditions in determining insulin-resistance, aberrant FFAs, chemokines and adipokines release and, subsequently, the development of NAFLD. Finally, we provided an update of results from novel supplementation trials which have been published during the last year (Barchetta I et al. 2016; Lorvand Amiri et al 2016; Kitson MT et al. 2016).

Referee #2

1. The manuscript is good, but I suggest to add more clinical data about the NAFLD and vitamin D.

We thank the reviewer for this comment; we have now added further important clinical data from both cross-sectional and interventional studies on NAFLD and vitamin D (page 9, lines 26-27; page 11, lines 5-9) and the corresponding references have been added in the references' section. Although clinical trials are very limited, and have given controversial and conflicting results, more clinical data are now reported in the text (pages 10-11) and in the references (number 131-143; 158-161).