Dear Editor,

Thank you and the reviewers for their comments and criticisms. We carefully read the reviewers' comments and below you will find a point by point answer to their concerns. The changes in the ms are underlined.

Reviewer's code: 02446101

SPECIFIC COMMENTS TO AUTHORS

- *What the said in the Para 1 and 2 in the Discussion section should be simplified. 2. Place add some results about the identification of BMSCs*

We added this in material and methods: We evaluated on MSCs the expression of markers recognized as one of the criteria to identify MSCs (Dominici, 2006). We verified by immunocytochemistry that MSCs expressed the surface antigens CD73, CD90 and CD105.

You are right, the discussion could appear complex. On the other hand, we aimed to explain the role of cytokines we identified in the sera of skinny people, this to make an hypothesis on the circulating factors that may promote brown adipogenesis. On this premise, we think that any discussion curtail may alter the conclusion of our finding.

Reviewer's code: 02566952

SPECIFIC COMMENTS TO AUTHORS

- *The manuscript presents an interesting approach in ascertaining the role of circulating molecules extracted from blood stream of lean human healthy subjects, in maintaining metabolic balance and balanced white adipose tissue quantity and function possible having a role in converting adipose tissue metabolism to ATP generator brown adipose. Introduction is relevant to the topic and accurately presents state of the art in the field. A minor suggestion, even though it is very interesting, the description of obesity and its classification might be reduced to several phrases in order to allow an increased attention of the reader to the introduction of skinny people (SP) concept and proposed explanatory theories.*

As suggested, we deleted some sentences to allow an increased attention on the Skinny people.

- *In a previous work of the team involving the effect of obese and overweight subjects on in vitro differentiation is introduced. Material and methods Are correctly described. Why were only male SP donnors used for this study? What was the age group of the donners? Age as well as sex might influence the amount of " SP factors" and maybe it would be good to mention the choice of a special group (if it was the case)*

In the results we added: Serum samples were collected from 12 adult men with normal BMIs. We selected only male since estrogen fluctuation may introduce further complexity to data analysis. Indeed, estrogen may influence MSC osteo-adipo commitment.

Please, see underlined text in the ms. In the method section we added age range (20 – 30 yold).

- *Why did the authors choose to use bFGF for culturing (and priming) of MSCs?*

We study senescence since a long time ago, and we use bFGF since it reduces senescence. Please see: Ito T, Sawada R, Fujiwara Y, Seyama Y, Tsuchiya T. FGF-2 suppresses cellular senescence of human mesenchymal stem cells by down-regulation of TGF-beta2. Biochem Biophys Res Commun. 2007 Jul 20;359(1):108-14. Epub 2007 May 21. PubMed PMID: 17532297.

- *Results are very well presented Discussion is well argumented to support the hypothesis What authors think about a further study on detecting the exact factors that might have a role in shifting WAT to BAT (or beige) metabolism ? Breaking down results from cytokine array and separately testing their role (for example for*

the proposed adiponectin or IGFBP-1? This is an important study orienting research towards elucidating the specificity of metabolic balance in a group of individuals, possible generating a modality to treat excess metabolic disorders such as obesity. Study could be extended to provide age and sex related specificity of this feature as well as to identify the exact cytokines involved in keeping metabolic and thermal balance in lean subjects.

Indeed, our aim is to identify which factor(s) may play a major role in promoting brown adipogenesis. We aim to prime cells with skinny people sera in presence of neutralizing antibodies against one of the candidate factors that could affect adipogenesis. This study could allow us to evaluate which specific factors have a role in adipogenesis of MSCs. This is the next step in our studies.

Reviewer's code: 02728252

SPECIFIC COMMENTS TO AUTHORS

- *It is a well-designed comprehensive in vitro study aimed to evaluate the effect of priming the bone marrow mesenchymal stromal cells with sera obtained from the skinny people compared to those obtained from normal people, regarding proliferation, apoptosis, senescence, cell commitment, and differentiation. The authors concluded that sera from skinny people may promote brown adipogenesis rather that white adipocyte differentiation and they hypothesized that some circulating components present in the blood of these individuals may favor brown adipogenesis at expense of white adipocyte production. The study is interesting, well written and has a rational with no further comments.*

Thank the reviewer for his/her comment.

Reviewer's code: 00504800

SPECIFIC COMMENTS TO AUTHORS

- *The authors desire to demonstrate that circulating factors in the serum of skinny people influence adipocyte differentiation. This is an interesting question and the study here, though preliminary, is well conceived. The main question raised by this manuscript is very basic: how do the authors define "skinny people"? Both normal people and skinny people are define as having a BMI of <25. The only criteria used to define skinny people is excess calorie intake, which seems to be 30-40% more caloric intake than normal people while maintaining a BMI <25. Is there precedent for this definition?*

In the literature there are data regarding lean people. For example, scientists at Medical Research Council's Epidemiology Unit of Cambridge (UK) identified dozens of genes that may be related to body mass index (BMI). In detail, they found that a variation of one of them, a gene called IRS1, is linked to a low percentage of body fat (Nat Genet. 2011 Jun 26;43(8):753-60. doi: 10.1038/ng.866). Others identified extreme BMI phenotypes associated with gene dosage (Nature 2011 Aug 31;478(7367):97-102. doi: 10.1038/nature10406). Nevertheless, these data refer to slim people (BMI <20), whose leanness may be pathological. In this study we aimed to analyze sera from healthy people with normal BMI, whose caloric intake by far exceed the physiological need. These are people that on popular magazines are defined as annoying people who claim that they can eat what they like but never put on weight. Indeed, at Weight Control Center of our University we evaluated many healthy persons that daily ingest an excess of caloric intake (more than 30% of their needs) but preserve their physiological weight. We defined these persons: Skinny People.

- *Did the authors examine serum from individuals with a BMI of <20 compared to 20-25? I understand and appreciate the results presented, but clarification of the definition of skinny is needed.*

As written above, we are interested in healthy people and not in people showing an extreme leanness.

- *Likewise, bone marrow MSC were used in these experiments. Did the authors perform any experiments or consider using MSC from adipose tissue? Do the authors think there would be significant differences between bone marrow MSC and MSC from other tissues? I think this would be of great interest to the readers.*

Thanks for this comment. In the introduction we added:

We focused our attention on bone marrow MSCs since they are precursor of fat cells present in this semi-solid tissue. Bone marrow adipocytes regulates functions of resident stem cells and may also play a role in energy storage and thermogenesis. [19]

- *Minor comments: The authors state that a panel of 17 cytokines was examined, but only the ones with significant differences between skinny and normal sera are noted. What are the others?*

We did a mistake, indeed the kit allows identification of 62 cytokines related to obesity. We modified the results as it follows:

We used the Human Obesity Antibody Array C1 to accurately profile the expression of 62 cytokines involved in obesity. The expression levels of several investigated cytokines did not differ significantly between the SP and NP samples. We identified 17 cytokines whose expression differed between SP and NP samples (ACE-2, Adiponectin (ACRP30), Adipsin (Complement Factor D), Angiopoietin-1, Angiopoietin-2, Fas (TNFRSF6/Apo-1), HCC-4 (CCL16), IGFBP-1, IL-1 alpha (IL-1 F1), LIF, MSP alpha/beta, PAI-1, RANTES (CCL5), Resistin, SAA (Serum Amyloid A), TNF RII (TNFRSF1B), TIMP-1, TIMP-2). We found statistically significant differences only for some of them. In particular, we observed an increased level of ACRP30, ANGPT1, FAS, HCC-4, IGFBP1, RANTES, RESISTIN, and SAA (Figure 6).

- *Figure 2C, X axis label should be annexin, not nexin Figure 5F, X axis label of SP is missing*

In figure 2C we modified Nexin with Annexin. In figure 5F we added SP.

- *In some figures, two asterisks are used to denote statistical significance, but P values for only a single asterisk are provided. I assume * = P <0.05 and ** = P <0.01, but this should be clarified.*

We corrected the mistakes.

Reviewer's code: 02495033

SPECIFIC COMMENTS TO AUTHORS

WJSC-43422 In the present review, the authors demonstrated that factors from SP sera affect commitment and adipocyte differentiation of MSCs. The results are interesting, and may provide readers and investigators with good information. 1) However, there is no clear criteria between NP and SP: i.e., both groups have similar parameters, especially BMI (22.10 vs 20.50) and relatively-high total cholesterol (185.6~190.6 mmol/l). I wonder if the authors did not compare among lean (normal), overweight, and obese people.

Our study aimed to evaluate the difference in sera composition between two groups of normal weight people (20<BMI<25). One group had physiological caloric intake (Normal People NP), the other group had an excess of caloric intake, which exceeded by at most 30%–40% the daily caloric intake (SP, skinny people).

In the results we further clarified this issue. Please see the first two paragraphs (underlined).

2) Please clearly explain the different results of adipogenic genes in Figure 4 and Figure 5c that make readers confused.

- We guess the reviewer refers to figure 3 and 5C. In the legends of these figures we added an explanation of early and late differentiation genes.

On behalf of all the authors, thank you in advance for your kind consideration of our work.

Yours sincerely,

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