POINT-BY-POINT ANSWER TO REVIEWERS

We thank the reviewer for the comments to improve the manuscript. as shown below.

1) It is unclear what age patients the MSC used for the in vitro experiments were obtained from.

Answer: The MSCs used in the study were from adults, but younger than those used in the intervention trial. Specifically, they came from the collection of donors recruited through the Hematology Service, under the Bone Marrow Transplantation Program, at the Reina Sofía University Hospital (Córdoba, Spain) (Casado Díaz et al., 2008; Cytotherapy Vol. 10, No. 5, 460-468, DOI: 10.1080/14653240802192644). To avoid confusion, we have added this information in the revised article.

2) Would it be worth exploring differences in MSCs from patients of different ages? I also find the link between the in vitro and human experiments somewhat tenuous.

Answer: The reviewer's suggestion is very interesting, although the aim of the study was not to evaluate the possible differences between MSC of donors of different ages versus CH. Probably, the most interesting approach would have been to obtain MSC from people involved in the study and subject them to hypoxia treatments. However, bone marrow collection is an invasive technique. Therefore, it is difficult to obtain volunteers, especially if they are elderly and there is no clinical need for it. However, although the behavior of MSC may vary with age in certain aspects, such as proliferation capacity, there are studies that show that the capacity for in vitro differentiation is similar between young and older people (Fickert et al., J Bone Miner Metab (2011) 29:224-235; DOI: 10.1007/s00774-010-0215-y). So, probably MSC from people of different ages will behave in vitro similarly to cyclic hypoxia treatments.

Our results on the differentiation of MSC in CH show that with long periods of hypoxia, differentiation decreases into both osteoblasts and adipocytes. This, as indicated in the discussion of the paper, may partly explain the results obtained by different authors, who have shown that long periods of hypoxia have negative effects on bone and fat formation. Therefore, with respect to our in vivo observations, we believe that the effect of CH on MSC is part of the mechanism of the observed effects on bone health. However, we are aware that CH in vivo will affect the metabolism of the whole organism. Therefore, the interactions between different physiological systems, and signaling pathways, may result in more complex responses from MSC in bone marrow than those observed in vitro.

Why was the drop out rate so high?

Answer: We are aware that a limitation of this study is the reduced sample size. That is a global problem in this kind of randomized control trials. Indeed, it is difficulty to recruit volunteers of this age to take part in this kind of experiments, with limits the potential of these studies (Duckham, 2018, BMC Med Res Methodol 18:173. DOI: 10.1186/s1287 4-018-0633-4). Previous works have also reported a high rate of dropout in such populations (Beaudart, 2013; BMC Geriatr 13:42. DOI: 10.1186/1471-2318-13-42). In fact, currently, there is limited data on successful recruitment strategies of older adults (Duckham, 2018, BMC Med Res Methodol 18:173. DOI: 10.1186/s1287 4-018-0633-4).

Are there other factors that could affect bone mineral density other than OPG/RANKL ratio?

Answer: We have highlighted that CH can affect the *OPG/RANKL* ratio, increasing it. Therefore, that could decrease the osteoclastic activity and bone resorption.

Bone metabolism involves a whole series of complex biological phenomena, integrated into bone remodeling, which depends on multiple instances; not only cellular, but also endocrine of various kinds (estrogen, endocrine system of vitamin D, PTH, calcitonin, even corticoids, etc). Likewise, auto and paracrine ones, with various growth factors, interleukins and leukotrienes involved, among others. Together with a real bone mechanostat, they all converge into a common final effector system, regulating the balance between formation and resorption, which is the RANK-RANKL-OPG system (Vega D et al., J Clin Endocrinol Metab. 2007 Dec;92(12):4514-21. DOI: 10.1210/jc.2007-0646; Boyce et al., Osteoporosis Reports. 2007;5(3):98–104. doi: 10.1007/s11914-007-0024-y).

The *OPG/RANKL* ratio is a determining factor in bone remodeling. Thus, one of the main drugs currently used to treat severe osteoporosis is denosumab. It is a monoclonal anti-RANKL antibody, preventing its binding to RANK in osteoclasts, and therefore inhibiting bone resorption. In our case, such fact may also help to explain what has been described by other authors. They suggested that the positive effect of CH on bone may be due more to the inhibition of osteoclastogenesis than to the induction of osteoblastogenesis (Guner I et al., Biol Trace Elem Res 2013;154:262-7 DOI: 10.1007/s12011-013-9722-8), as we discuss in the paper. However, our results also suggest that CH may additionally affect bone formation through other ways, such as the Wnt/β-catenin signaling pathway. The latter is fundamental in the differentiation of MSC into osteoblasts or adipocytes. Therefore, the hypoxia time, as well as the recovery time in normoxia, CH exposure, may be determining factors modulating MSC differentiation. Thus, while activation of such pathway may promote osteoblastic differentiation, its maintenance over time may keep cells in an undifferentiated state, inhibiting bone mineralization, as we have discussed in the paper. Therefore, together with the *OPG/RANKL* ratio, the effect on the Wnt/β-catenin pathway is a factor to be taken into account in the effect of CH on bone.