Reviewer 3

Title: **The longitudinal changes in subcutaneous and visceral abdominal adipose tissue volumetries are modulated by iron status.**

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General comment:
Epidemiological studies suggest that iron deficiency affects 20-40% of obese individuals. The association between obesity and iron deficiency is not fully understood and may be bidirectional. On the one hand, obesity may disrupt iron homeostasis, resulting in iron deficiency. On the other – iron status might determine metabolic health e.g. its cumulation in the liver impacts insulin sensitivity. In their work, Alejandro Hinojosa-Moscoso et al., in a prospective study, investigated whether iron status is linked to changes in adipose tissue distribution in both: obese and normal- to overweight individuals. They found that baseline serum hepcidin and serum ferritin concentrations, low transferrin, and TIBC, were the main iron parameters associated with an increase in subcutaneous and visceral adipose tissue after one year of follow-up. The concept of the study is appealing and the methodology is properly chosen; however, there are some issues regarding the manuscript structure that should be considered before the manuscript is accepted for publication.

The authors are grateful for the reviewer comments which have contributed to clarify the message of our paper and to improve the quality of our submission. The specific comments are addressed below:

Major revisions:

The structure of the manuscript suggests that it has been revised or the Authors changed their concept. The abstract and the introduction do not suggest that the Authors initially aimed to include the results regarding adrenal and gonadal steroids in the analysis (parts of the text marked in red font). Instead of that the Authors write: "Therefore, we aimed to evaluate whether different parameters affected by iron exposure were associated with changes in adiposity and insulin action in a longitudinal study, controlling for inflammatory and oxidative stress markers". It seems that the initially not gonadal/adrenal steroids but markers of oxidative stress were to be investigated.

Even though the analyses regarding gonadal/adrenal steroids, iron status, and adipose tissue distribution were mainly negative, the mere fact that they were carried out requires a few words of commentary in the introduction and a broader commentary in the discussion.
However, if the parts of the manuscript written in red correspond to the Authors' revisions, I would appreciate sharing the view of the previous reviewers on the manuscript quality.

The parts of the manuscript written in red correspond to the Authors' revisions. In the previous revisions, Academic Editor recommended assessing other factors that also impact obesity and insulin regulation such as adrenal and gonadal steroid hormones, for this reason these hormones were included in the manuscript.

To clarify this point, the following text has now been included in introduction:

"Considering that adrenal or gonadal steroids hormones are important metabolism modulators that contribute to body fat distribution [18-20] and iron homeostasis [21,22], levels of cortisol, estradiol, dehydroepiandrosterone sulphate, testosterone, progesterone, corticosterone and

aldosterone were also analysed in relation to adipose tissue volumetry and distribution and baseline levels of iron metabolism related parameters."

21. Hennigar SR, Berryman CE, Harris MN, et al. Testosterone Administration During Energy Deficit Suppresses Hepcidin and Increases Iron Availability for Erythropoiesis. J Clin Endocrinol Metab. 2020;105:dgz316.
22. Beggs LA, Yarrow JF, Conover CF, et al. Testosterone alters iron metabolism and stimulates red blood cell production independently of dihydrotestosterone. Am J Physiol Endocrinol Metab. 2014;307:E456-E461.

Minor revisions:

1) Abstract: Please include information regarding the statistical significance (P values) in the part of the abstract referring to the description of the results.

This information is now included accordingly. Please see below:

Baseline serum hepcidin (p=0.005 and p=0.002) and ferritin (p=0.02 and p=0.01)) were associated with an increase in VAT and SAT over one year in all subjects, while serum transferrin (p=0.01 and p=0.03) and total iron binding capacity (p=0.02 and p=0.04) were negatively associated. These associations were mainly observed in women and in subjects without obesity and were independent of insulin sensitivity. After controlling for age and sex, serum hepcidin was significantly associated with changes in iSAT (β = 0.406, p = 0.007) and iVAT (β = 0.306, p = 0.04), while changes in insulin sensitivity (β = 0.287, p = 0.03) and fasting triglycerides (β = -0.285, p = 0.03) were associated with changes in pSAT.

2) Material and Methods: Please provide data if any of the study participants used iron supplements during the follow-up period.

No participants used iron supplements during the follow-up period. This information is now added in methods. Please see below:

"Exclusion criteria were type 2 diabetes mellitus, NAFLD or liver cirrhosis, major eating or psychiatric disorder antecedents, anemia or hemoglobinopathy, language disorders, neurological diseases, history of trauma or brain injury, alcohol intake > 20g per day, infection in the last month, serious chronic illness, pregnancy, lactation and MRI contraindications, such as claustrophobia or ferro-magnetic implants. No participants used iron supplements during the follow-up period."

3) Discussion: Compared to the plethora of results, the discussion is relatively brief. Please consider broader commentaries on the study results including the directions for further research.

Thank you. Discussion text has now been expanded to discuss better some results and suggest the directions for further research. Please see below:

"We also analyzed adrenal or gonadal steroids hormones, which are important metabolism modulators that contribute to body fat distribution [18-20] and iron homeostasis [21,22], and no significant association between these hormones and

circulating iron metabolism-related parameters or changes in iSAT, iVAT and pSAT were found, suggesting a minor role of these hormones in the relationship between iron and adiposity. However, these data should be cautiously considered, because important aspects such as the reproductive cycle were not taken into account during sampling."

"Future studies should be designed to investigate in humans whether the early correction of iron excess might prevent unhealthy expansion of abdominal adipose tissue."