Reviewer 1

The manuscript by Ippea Kanazawa “Osteocalcin as a hormone regulating glucose metabolism” summarizes relevant studies in regards to glucose metabolism and endocrine regulation between bone, fat, pancreas, and muscle. On top of data initiated in the Karsenty laboratory showing that bone is an endocrine organ studies from humans are included. The review is up to date and well written.

Response to reviewer 1,

I would like to thank you for your kind comments.

Reviewer 2

Overall this is a nice review of the actual evidence from basic science about the role of osteocalcin as a putative regulator of glucose metabolism. The topic is actual and of interest. The author reviewed many pathways involved in the role of osteocalcin in the regulation of glucose metabolism. However, it seems that the revision of the literature was not very accurate for all pathways. Indeed, as outlined below, for some pathways the author report just one side of current evidence and contrasting evidence have not been discussed. I would appreciate a more balanced review of the literature and a fairly discussion about unsolved controversies. Moreover, I would appreciate if the author can extend the section about human studies.

Major revisions

1. Cover Tip. The last cover tip suggested by the author is: “Osteocalcin may be a novel candidate for treatment of type 2 diabetes”. In my opinion, to date there is no enough evidence to support this cutting-edge position. Even though the author nicely report what is currently known about role of osteocalcin in the regulation of glucose metabolism, most of the data reported in this review are from preclinical models and few human data have been reported. Thus, I would suggest the author to balance this statement.

Response to comment 1,

Thank you very much for your appropriate comment. As you pointed it out, there are no clinical studies showing the intervention with osteocalcin in human although several clinical studies showed the relationship between serum osteocalcin and glucose metabolism in human. According to your comment, we have changed the last sentence to “Therefore, osteocalcin may be an important factor linking between bone and glucose homeostasis”.

2. The author should add the criteria he used for the revision of the literature (database searched, words used, timespan) and for the decision to include or exclude studies.

Response to comment 2,
I agree with your comment that the information is important. However, this is a mini-review article, I did not comprehensively search database at this time with specific criteria.

3. Page 5, lines 27-28. Mizokami et al showed that exendin, a GLP1 receptor antagonist, blocked the effects of osteocalcin on glucose metabolism and insulin secretion, but they did not show an effect of exendin on the osteocalcin dependent increase of serum GLP1. This sentence could be confusing for readers and thus I would be more precise.

Response to comment 3,
Mizokami et al. examined whether ucOC increase insulin secretion through stimulating GLP-1 secretion. Thus, they used a DPP-4 inhibitor, which enhance the action of GLP-1, and exendin(9-39) as an antagonist of GLP-1 receptor. They did not need to examine the effect of exendin on the ucOC-dependent increase of GLP-1 secretion in their study. I have added a sentence explaining the conclusion of Mizokami et al.'s studies (page 6, line 8-9).

4. Page 6 lines 7-9. Here the author reports data suggesting that uOC do not affect insulin sensitivity. However, there is also evidence showing that osteocalcin is able to increase insulin sensitivity. I would suggest writing about these data too, in order to give reader a more balanced evaluation of the actual knowledge in the field. How does the author explain these contrasting findings?

Response to comment 4,
As you pointed out, previous studies have shown that osteocalcin increases insulin sensitivity. However, Mizokami et al. demonstrated that oral administration of osteocalcin did not improved insulin sensitivity by using insulin tolerance tests. The contrasting findings may depend on the different ways between oral administration and injection of osteocalcin. I have added a sentence about the necessity of further examination (page 7, line11-13).

5. Page 6 line 24 and page 7 line 4. Reference 18 is not correct. Please add the right reference.

Response to comment 5,
Thank you for notifying my mistake. I have corrected the reference 18 (revised manuscript ref

24).

6. Page 6 22-24. Here the author writes that “Ferron et al. previously showed that decreased expression of FoxO1 […] was associated with the glucose intolerance of Ob_IR-/- mice.” However Ferron et al showed that FoxO1 haploinsufficiency (decreased expression of FoxO1) rescued the Ob_IR-/- phenotype ameliorating the glucose intolerance associated with Ob_IR-/-. This is consistent with the inhibitory effects of insulin on FoxO1. Please check and correct the statement. Thank you.

Response to comment 6,
I greatly appreciate your useful comment. I have referred your comment and revised the manuscript (page 8, line 22-24).

7. Page 6 line 28-29. There is evidence showing that the interaction between FoxO1 and ATF4 increases osteocalcin inactivation (Kode A et al. J Biol Chem 2012). This seems to be in contrast with the data reported by Rached MT in Cell Metab 2010 that have been discussed by the author. Could please the author critically evaluate, report and discuss these contrasting data? If insulin has an anabolic effect on bones, it is expected that FoxO1 knockout causes an increase in bone mass, but this is not the case of the data reported by the author. How does the author explain this? Moreover, since many WJD readers are clinicians, I would suggest to better explain the insulin/FoxO1/ATF4 pathway not assuming that the readers have a deep knowledge of this pathway. In particular, it is not clear if the author suggests that insulin has anabolic effects by inhibiting Fox01 or not.

Response to comment 7,
Thank you very much for notifying the article by Kode A et al. As Kode et al showed that the interaction of FoxO1 and ATF4 increased the expression level of osteocalcin, but inactivated the osteocalcin function by reducing the undercarboxylated form of osteocalcin, resulting in glucose intolerance. I have cited the study and rewrote the manuscript according to your comment (page 8 line 24-27).
As you pointed out, the underlying mechanism of insulin in osteoblasts is not fully understood. Based on the clinical feature of type 1 diabetes and the bone phenotype of insulin receptor knockout mice, insulin signal may have an anabolic function. FoxO1 is known to be a target molecule of insulin signal, and insulin suppresses FoxO1 action by removing it from nuclear. However, FoxO1 is also known to protect against oxidative stress, which suppresses osteoblast function. According to your comment, I rewrote the paragraph of insulin effect on bone

formation (page 7, line 16-19, line 25-page 8, line 1).

8. Page 9 Lines 21-27. The author writes that his own data (ref 41) are in contrast with those published by Luo (Ref 40). However, to the reviewer's understanding, this is not the case. Lou et al. showed that adiponectin increases ALP activity and osteocalcin expression in human osteoblasts. As well, Kanazawa's pap

Response to comment 8,
Thank you very much for pointing out our mistake. The conclusions of Luo et al (Ref 40) and ours (Ref 41) are basically same; adiponectin signal increases osteoblast differentiation in vitro. I have revised it (page 11, line 17).

Reviewer 3
This manuscript provides an overview on Osteocalcin and its functions in glucose metabolism as well as their implications in diabetes and obesity. In general, the manuscript was well and concisely written, different aspects of osteocalcin were covered, described and explained with references and logical flow. It is a topic of current biological and biomedical significance with potential impacts in the field of glucose metabolism and diabetes research and future diabetes treatment. However, the relative scarcity of the human data dampens this reviewer's enthusiasm for the protein and for the manuscript. In addition, there are some major and minor concerns associated with the current version of the manuscript. Modifications and updates should be made before the manuscript can be accepted for publications.

1. Major concerns.
1-1. This is a review article. However, only 60 references were listed. This indicates either the narrowness of the field or the scarcity of the research on this topic, which may impact the readership. Author may consider additional references if they are available, particularly those in the recent 10 years.

Response to major concern 1-1,
Thank you very much for your appropriate comment. This mini-review article reviewed the endocrine function of osteocalcin in glucose metabolism. As you know, the endocrine function of osteocalcin was firstly found in 2007, and second article about it was reported in 2008. Although the number of articles on this topic is now increasing, there is not enormous thus far. According to your comment, I have added several articles.

1-2. This is a review on osteocalcin and its functions or potential functions in glucose metabolism. However, in the Conclusion of the manuscript, the word osteocalcin was not even mentioned. This is both surprising and puzzling. The word of osteocalcin should be added to the Conclusion, which should be rewritten.

Response to major concern 1-2,
I would like to thank you for notifying that. According to your comment, I have revised the conclusion section (page 15, line 32-page 16, line 5).

1-3. Positive feedforward loop was mentioned in the Abstract. However, this point was not sufficiently emphasized in other parts of the manuscript including figures. A little more on the loop should be added in the text (particularly the section of "An endocrine loop between bond and pancreas) and/or figures. In the section of "The cross relationships …..", on page 9, lines 10 and 11, "negative feedback loops" were suggested. The relationship between the potential positive loop and this negative loop, if there is any, should be described and discussed.

Response to major concern 1-3,
I appreciate your good suggestion. I have added the discussion about a negative loop between leptin and insulin as well as a positive loop between adiponectin and insulin (page 12, line 12-16), and three figures.

1-4. According to the author, osteocalcin is apparently anti-diabetic. This reviewer is wondering the reason that not much has been done with this important protein in human and its potential use in treating diabetes. The side effects or potential side effects of the protein should also be covered. In addition, potential technical obstacles, if there are any, in human studies should also be discussed.

Response to major concern 1-4,
I agree with your comment that the effect of osteocalcin on glucose metabolism in human is the most important. Although there are clinical studies showing that the relationships between serum osteocalcin and glucose metabolism in clinical settings, no intervention studies were reported thus far.

1-5. Figure legends may include potential positive or negative loop descriptions. If these loops are inconclusive, dotted lines can be used.

Response to major concern 1-5,
As you pointed out, some of the loops are not completely verified so far. However, each arrow in figures is based on the published articles.

2. Minor concerns
2-1. On page 4, line 34, “and can’t” should be “and can not”.
2-3. Page 7, line 13, the word “resistant” should be changed to “resistance”.
2-4. Page 13, line 9, “serum total osteocalcin” should be “total serum osteocalcin”.

Response to minor concerns 2-1, 2-3, 2-4,
Thank you very much for the efforts to find my grammatical errors. I have changed according to your comments.

2-2. Page 6, line 32, the abbreviation “BMD” should be spelled out if it is mentioned first time.

Response to minor concern 2-2,
BMD was firstly spelled out in page 4, line 6.

2-5. Some of the papers used for resistin may be too old. Newer papers should be used.

Response to minor concern 2-3,
I looked for the recent papers on effects of resistin on bone as much as possible. However, new experimental studies were not reported since 2006.

2-6. Figures should be cited in the text. The inserted figures might not have placed in the most appropriate sites in the manuscript. In addition, without referring to the figures in the text, it is very difficult for readers to go back and forth for the contents covered in the figures.

Response to minor concern 2-6,
According to your comment, I have cite the figure in the text and added two figures.