PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form (**http://bmjopen.bmj.com/site/about/resources/checklist.pdf**) and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

ARTICLE DETAILS

TITLE (PROVISIONAL)	Cross-sectional and longitudinal study protocols of the “ADIposity and BOne metabolism: effects of eXercise-induced weight loss in obese adolescents” (ADIBOX) project.
AUTHORS	CHAPLAIS, Elodie; Dutheil, Frederic; Naughton, Geraldine; Greene, David; Pereira, Bruno; THIVEL, David; Courteix, Daniel

VERSION 1 - REVIEW

REVIEWER	Dr Rachel Duckham Deakin University, Australia
REVIEW RETURNED	24-Feb-2016

GENERAL COMMENTS	Manuscript ID bmjopen-2016-011407 entitled: "ADIposity and BOne metabolism: effects of eXercise-induced weight loss in obese adolescents” (ADIBOX): a protocol." The manuscript entitled "ADIposity and BOne metabolism: effects of eXercise-induced weight loss in obese adolescents” (ADIBOX): a protocol" is a methods paper discussing the protocol of the ADIBOX study which combines two studies a cross-sectional study run in Australia and a longitudinal study run in France. Both studies have been designed to clarify the possible interactions between adiposity and bone in childhood obesity. Although, this is an important area of research and while the study protocols seem robust independently, it remains unclear from this manuscript how these two studies fit together to form the ADIBOX protocol when there is no clear synergy between the studies and study locations. As a reviewer I wonder why the two locations are not both running the same cross-sectional and longitudinal design in both location. It would make more sense to write a protocol paper if this was the case. It would also strengthen the extremely important area of research with a multi-centred approach. Although there are some concerns with this protocol paper these are mainly focused on how the two studies have be packaged together to form the ADIBOX protocol. It may be clearer to discuss the studies separately i.e. all the cross-sectional components and then all the longitudinal components rather than bits and pieces throughout the manuscript especially for the methods section. Specific Comments. Abstract page 2 Line 11: it is advised the authors remove “on this cross-talk” it is not required. Line 18: could the authors clarify the inclusion of obesity and overweight for study one, this seems inconsistent to what is stated in

	the methods should this be >97th percentile. Strengths and Weaknesses Page 2 Line 58: If the Tanner stage is being used to determine maturation I am unsure why there was no mention of menstrual function especially in the longitudinal study. Authors should be able to at least determine age at menarche. If not be able to determine menstrual status in the blood. Introduction Page 3 The introduction generally needs a stronger focus it is advised the authors consider adding a paragraph on cross-talk between adiposity and bone and its implications to children and potentially across the life span. Furthermore, there needs to be a greater link with weight loss, bone loss and exercise. The importance of nutrition and exercise. It is also strongly suggested that the authors remove the second half of the third paragraph. A collaborative team between France and Australia does not provide a strong rationale or justification for the protocol. Methods page 4 As mentioned above in the methods section it would be clearer to the reader if the authors discuss all the methods for the cross sectional study (including protocol recruitment, measures) followed by all the methods for the longitudinal study (including protocol recruitment, measures, compliance). Followed by a paragraph which brings the two studies together, i.e. how will the cross sectional study inform the longitudinal work etc. Then discuss areas such as radiation, data confidentiality etc for both studies together as these will be very similar. This may help to provide a protocol that works together as at the moment I am wondering why both studies are not being run in each country and cannot determine how they fit together. Page 5: inclusion criteria there needs to be stronger justifications throughout the paper to why individuals will be recruited. For example why exclude those participating in 250 mins of PA and how will this be determined. Line 14: Sentence beginning with In order to include …… seems unclear, it is advised this be reworded. 10 month longitudinal study: There needs a justification for the length of time of the study and time point measurements. Furthermore it is advised that the authors include the protocol of the weight loss. I.e. calorie intake and exercise protocol if there is one. How are individuals instructed to lose weight during the time in the residential home? Also with the controls are they also being educated to lose weight or are they maintaining their weight. Power Analysis page 5 Line 49: a reference is required after works. Page 6 Line 5: Should there be a unit value for 1.2 to 1.5 what is the index difference? This is not clearly explained. Page 6:

	Participants: there needs to be a stronger justification why in the longitudinal study there were only girls recruited and why this study was not a randomized study. Line 53: sentence beginning “Both the adolescents …….” This sentence does not make sentence it is advised that the sentence be rewritten. It is also suggested that parents give written consent and minors assent. Page 7: Measurements, for both studies there needs to be further justification for the measurements and all measures need references. For example maturation will be assessed with Tanner please reference Tanner. In this section there also needs to be a mention of the limitation of the self-reported Tanner. Page 8 PA and Nutrition questionnaire: will the same questionnaire be used in both countries. Is the questionnaire validated? Further information is required for the administration of the nutrition questionnaire. What additional information. As the DXA and pQCT are being administered in different labs there needs some mention of the CV between the two labs and whether this is in an acceptable range. PQCT will a scout scan be performed and at what speed will the images be assessed, what will the slice size be. Will muscle and fat cross sectional area be assessed and if so what threshold will be used. Page 10: Energy mentalism: there needs a justification for this measure how will it be used within the longitudinal study or is it just to determine change over time. QUS: there needs a stronger justification for this measure in this protocol has there been past studies that have shown this measure predicts fracture in children. How is this measure better than DXA. Authors contribution: there is mention of study III however this is not mentioned anywhere in the protocol.

REVIEWER	Joseph M. Kindler The University of Georgia, United States of America
REVIEW RETURNED	03-Mar-2016

GENERAL COMMENTS	Abstract 1. The objective of this study is not directly stated. The authors mention the “bone-adipocyte cross-talk,” but don’t directly state the specific intervention or primary outcome measures. Please include a description of the intended intervention. 2. The BMI-for-age percentile cutoffs used in this study differ from those set forth by the Centers for Disease Control and Prevention (CDC) as indicated in reference #10. The authors of the current study define “obese” as > 97th percentile, “overweight” as > 85th percentile, and “normal-

	weight" as < 85th percentile. However, the CDC defines obese as ≥ 95th percentile and overweight as ≥ 85th percentile. Please include rationale for using these alternative cutoffs. 3. Please include the sample size for each group. Introduction 1. Page 3, Line 12-16: The authors present statistics on the prevalence of overweight/obesity in Australia and France. However, it is unclear whether these figures are specific to obesity, overweight, or obesity + overweight. Please clarify this. Methods and analysis 1. Page 4, Line 18-22: As opposed to investigating relationships between adipose tissue and bone metabolism, the protocol design for Study I appears to compare bone and body composition outcomes between overweight/obese and normal-weight groups that are stratified by sex. This is supported in the statistical approach presented on Page 11. 2. Page 4, Line 31-33: The phrase, "…one following a residential weight loss program group versus one control group," is confusing to the reader and should be reworded. 3. Page 5, Line 5: The authors state, "…girls will need to have a regular menstrual cycle." However, it is not clear whether pre-menarcheal girls are eligible for either of these studies. 4. Page 5, Line 5-9: The sentence beginning, "Obese boys and girls…" is confusing to the reader and should be reworded. It appears that a word(s) is missing toward the end of the sentence. 5. Page 5, Line 5-9: Does the "medial stage" refer to stage 3? If so, please state this. Also, please include this information on Page 7 (Lines 51-55). 6. Page 5, Line 5-9: The study's group of participants with higher body fat includes both overweight and obese. Please clarify why only the obese participants (i.e., BMI-for-age percentile > 97th) are required to be beyond the "medial" stage of maturation. Is this a requirement for the overweight (and normal-weight) participants as well? 7. Page 5, Line 18: In the sentence, "Normal weight, overweight and obese adolescents will be not be…" please consider deleting the first "be." 8. Page 5, Line 20: How will you measure physical activity performed outside of school? Please specify the unit of time (i.e., weeks, months, etc.) for this 250-minute threshold. If available, please provide justification for the 250-minute cut-point. 9. Page 5, Line 31: Please correct the spelling error (i.e., change physical active to physically active). 10. Page 5, Line 33-35: Please be more specific about the exclusion criteria specific to the intervention in Study II. How will compliance be measured? Will the data from the participants who are excluded after the commencement of the study be retained for intent-to-treat analyses? 11. Page 6, Line 29: It is stated, "For this study we will recruit either male and female…" However, Figure 1 shows that both male and females will be recruited for Study I. Please address this discrepancy. 12. Page 6, Line 55: Please correct the spelling error (i.e., change signed to sign).

	13. Page 7: Will the children in the control group of Study II be provided the residential program after the completion of the study? 14. Page 8, Line 18-20: Please specify whether the dietary questionnaire is a food-frequency questionnaire, 3-day diet diary, 24-hour recall, etc. 15. Page 8: Please specify which DXA scanner is used at each study location. 16. Page 8, Line 53: Please change “p-QCT” to “pQCT.” 17. Page 8: There may be issues with the 66% mid-tibia site for pQCT measures given the smaller gantry size of the XCT 2000. For this reason, problems with scan acquisition may arise with the study participants having a larger body size. Have the authors considered using the 50% site of the mid-tibia as opposed to the 66% site for these measurements? Please comment. 18. Page 10, Line 40-46: Please expand upon your strategy to track compliance (see: reviewer comment #10 above). 19. Page 12, Line 38: Please change the word “study” to “studies.” Discussion 1. Page 13, Line 18-31: The authors mention that pubertal stages are “targeted” in this study. However, the information provided in the methods section of this manuscript does not support this statement. Author’s Contributions 1. Page 14, Line 16: The authors state, “…DC, DT, FD and EC were responsible for obtaining ethics approval and recruiting participants for study II and III.” However, this manuscript presents protocols for only two studies. Additional comments by the reviewer 1. Specific details of the intervention (i.e., Study II) are not presented in this manuscript. 2. Race and ethnicity of intended study participants are not mentioned.

REVIEWER	Dr. Luis Gracia-Marco University of Exeter (UK)
REVIEW RETURNED	15-Mar-2016

GENERAL COMMENTS	It has been a pleasure reviewing this protocol study on an interesting and necessary study to better understand the link between fat and bone. In addition, it is great that the authors are strengthening an existing link between institutions. I just would like the authors to clarify some minor points: - Could the authors mention the starting and finishing dates (approximately) of each study? Or has the study already started? - Could you please check the first paragraph of the introduction and clarify whether those percentages refer to overweight or obese or the combination of both? - I have not been able to understand why the cross sectional study includes both overweight and obese participants while the longitudinal one includes only obese participants. Is this because you anticipate (based on your experience) that this is going to be

	easier for recruitment? - In the inclusion criteria for the cross-sectional study, the authors mention that doing more than 250 minutes of PA outside school will be considered as an exclusion factor. Is this 250 minutes per week? What intensity do the authors refer to? Moderate-to-vigorous PA? Why 250 minutes? Please clarify this to the reader - Are the inclusion criteria of the longitudinal study the same than those of the cross-sectional study? This is on page 5 and it was not clear to me. - Any reason by which the power calculations for the CS are based on a 80% power and those of the LS based on a 90% power? - At some point the authors mention that the LS will last 40 weeks, but it seems it will be 42 weeks, right? Please correct this. - Methods: Maturation; the authors mention that in addition to Tanner stages, blood analyses will be performed. Maybe you could briefly mention here which biomarkers/hormones will be used to measure maturation. - Physical activity: Is there any chance you could measure PA using objective devices (i.e. accelerometers). This could be used as a confounding variable in your analyses (maybe Vitamin D as well). As it is, you are going to collect PA using questionnaires but previous studies have shown that overweight/obese adolescents tend to overestimate their PA levels. - DXA: Please check all you have in brackets when explain DXA in the methods. It seems there are too many DXA's.

VERSION 1 – AUTHOR RESPONSE

Reviewing: 1
Comments to the Author

★ Reviewer number 1:

Abstract page 2
Line 11: it is advised the authors remove “on this cross-talk” it is not required.
In accordance with the reviewer’s comment, we removed “on this cross-talk”.

Line 18: could the authors clarify the inclusion of obesity and overweight for study one, this seems inconsistent to what is stated in the methods should this be >97th percentile.
In accordance with the reviewer’s comment, a clarification has been made in the manuscript regarding the inclusion of obesity and overweight for study one.

Strengths and Weaknesses Page 2
Line 58: If the Tanner stage is being used to determine maturation I am unsure why there was no mention of menstrual function especially in the longitudinal study. Authors should be able to at least determine age at menarche. If not be able to determine menstrual status in the blood.
We would like to thank you the reviewer for this relevant comment. Indeed, we will be able to obtain information about the age at menarche for each girls as it is provided in the medical file.

Introduction Page 3
The introduction generally needs a stronger focus it is advised the authors consider adding a paragraph on cross-talk between adiposity and bone and its implications to children and potentially across the life span. Furthermore, there needs to be a greater link with weight loss, bone loss and exercise. The importance of nutrition and exercise. It is also strongly suggested that the authors remove the second half of the third paragraph. A collaborative team between France and Australia

does not provide a strong rationale or justification for the protocol.
In accordance with the reviewer's comment, the introduction has been rewrite.

Methods page 4
As mentioned above in the methods section it would be clearer to the reader if the authors discuss all the methods for the cross sectional study (including protocol recruitment, measures) followed by all the methods for the longitudinal study (including protocol recruitment, measures, compliance). Followed by a paragraph which brings the two studies together, i.e. how will the cross sectional study inform the longitudinal work etc. Then discuss areas such as radiation, data confidentiality etc for both studies together as these will be very similar. This may help to provide a protocol that works together as at the moment I am wondering why both studies are not being run in each country and cannot determine how they fit together.
In accordance with the reviewer's comment, we reorganized the article separating both studies and completing with another paragraph explaining the link between both studies.

Page 5: inclusion criteria there needs to be stronger justifications throughout the paper to why individuals will be recruited. For example why exclude those participating in 250 mins of PA and how will this be determined.
In accordance with the reviewer's comment, we clarify the inclusion criteria, and justified our choice of cut-off at 250min of P.A..

Line 14: Sentence beginning with In order to include …… seems unclear, it is advised this be reworded.
In accordance with the reviewer's comment, we reworded the sentence.

10 month longitudinal study: There needs a justification for the length of time of the study and time point measurements. Furthermore it is advised that the authors include the protocol of the weight loss. I.e. calorie intake and exercise protocol if there is one. How are individuals instructed to lose weight during the time in the residential home? Also with the controls are they also being educated to lose weight or are they maintaining their weight.
In accordance with the reviewer's comment, we have justified in the article the length of our 10-month study and include information about the protocol of weight loss. The 10-month period correspond to the school year period. Moreover, we decided to collect our data every 14 weeks because (1) it is a good period in the bone remodeling process, (2) it correspond to the beginning of holidays.

Power Analysis page 5
Line 49: a reference is required after works.
In accordance with the reviewer's comment, we added a reference.

Page 6 Line 5: Should there be a unit value for 1.2 to 1.5 what is the index difference? This is not clearly explained.
We would like to thanks the reviewer for addressing this issue and would like to answer it. This index difference (standard deviation) doesn't have a unit value as it is associated with a ratio. This index difference correspond to the variation index of body fat relative to the variation of a marker of bone mass.

Page 6:
Participants: there needs to be a stronger justification why in the longitudinal study there were only girls recruited and why this study was not a randomized study.
We choose to recruit girls for a convenience reason as the obesity center is only hosting female adolescent. Although, as detailed in the description section of the paper, we cannot randomized since

our intervention group will correspond to the adolescent that will join the obesity center as part of their obesity treatment.

Line 53: sentence beginning “Both the adolescents …….” This sentence does not make sentence it is advised that the sentence be rewritten. It is also suggested that parents give written consent and minors assent.
In accordance with the reviewer’s comment, we rewrote the sentence.

Page 7: Measurements, for both studies there needs to be further justification for the measurements and all measures need references. For example maturation will be assessed with Tanner please reference Tanner. In this section there also needs to be a mention of the limitation of the self-reported Tanner.
In accordance with the reviewer’s comment, we added references, provided more justification and limitation of the self-reported TS.

Page 8
PA and Nutrition questionnaire: will the same questionnaire be used in both countries. Is the questionnaire validated? Further information is required for the administration of the nutrition questionnaire. What additional information.
We are using the IPAQ questionnaire for physical activity. This questionnaire is validated for both countries and have been previously used by our teams. Regarding the nutrition questionnaires, we are using a food frequency, food habits as well as food preferences questionnaire, all validated.

As the DXA and pQCT are being administered in different labs there needs some mention of the CV between the two labs and whether this is in an acceptable range.
We would like to thanks the reviewer to address this point. As our two studies are complementary and that we are not comparing them, there is no need of a CV between the two labs.

PQCT will a scout scan be performed and at what speed will the images be assessed, what will the slice size be. Will muscle and fat cross sectional area be assessed and if so what threshold will be used.
In accordance with the reviewer’s comment, we provided the information needed.

Page 10: Energy mentalism: there needs a justification for this measure how will it be used within the longitudinal study or is it just to determine change over time.
In accordance with the reviewer’s comment, we provided more information to justify it. Energy metabolism is a good and reliable tool that reflect the muscle mass which is also an important indicator in our study. As in our laboratory, energy metabolism is a routine clinical investigation we are performing it.

QUS: there needs a stronger justification for this measure in this protocol has there been past studies that have shown this measure predicts fracture in children. How is this measure better than DXA.
In accordance with the reviewer’s comment, we justified the use of the QUS.
In accordance with the reviewer’s comment we provide other justification of why we are using QUS in addition to DXA. Although, as the literature is conflicting, and as the three tools (DXA, pQCT, QUS) are routinely used in our lab and are part of the clinic investigation, we wanted to have them in this study in order to have easier comparison with other studies that are using either DXA, pQCT and QUS.

DXA is the most common non-invasive method used for assessing pediatric bone strength. No one method can adequalty assess bone health and QUS may provide important information on bone quality (Specker et al. 2005). It has the potential to provide information about bone architecture.

Authors contribution: there is mention of study III however this is not mentioned anywhere in the protocol.
In accordance with the reviewer's comment, we deleted the study III as in the ADIBOX protocol there is no study III.

★ Reviewer number 2:

Abstract
1. The objective of this study is not directly stated. The authors mention the "bone adipocyte cross-talk," but don't directly state the specific intervention or primary outcome measures. Please include a description of the intended intervention.
In accordance with the reviewer comment, we rewrote our objective.

2. The BMI-for-age percentile cutoffs used in this study differ from those set forth by the Centers for Disease Control and Prevention (CDC) as indicated in reference #10. The authors of the current study define "obese" as > 97th percentile, "overweight" as > 85th percentile, and "normal-weight" as < 85th percentile. However, the CDC defines obese as ≥ 95th percentile and overweight as ≥ 85th percentile. Please include rationale for using these alternative cutoffs.
In accordance with the reviewer's comment, we corrected the mistake we made.

3. Please include the sample size for each group.
In accordance with the reviewer's comment, we include the sample size for each group.

Introduction
1. Page 3, Line 12-16: The authors present statistics on the prevalence of overweight/obesity in Australia and France. However, it is unclear whether these figures are specific to obesity, overweight, or obesity + overweight. Please clarify this.
In accordance with the reviewer's comment, we clarified the percentages given in the introduction.

Methods and analysis
1. Page 4, Line 18-22: As opposed to investigating relationships between adipose tissue and bone metabolism, the protocol design for Study I appears to compare bone and body composition outcomes between overweight/obese and normal weight groups that are stratified by sex. This is supported in the statistical approach presented on Page 11.
In accordance with the reviewer's comment, we clarified our aim.

2. Page 4, Line 31-33: The phrase, "…one following a residential weight loss program group versus one control group," is confusing to the reader and should be reworded.
In accordance with the reviewer's comment, we reworded the sentence.

3. Page 5, Line 5: The authors state, "…girls will need to have a regular menstrual cycle." However, it is not clear whether pre-menarcheal girls are eligible for either of these studies.
In accordance with the reviewer's comment, we clarified the issue addressed by the reviewer.

4. Page 5, Line 5-9: The sentence beginning, "Obese boys and girls…" is confusing to the reader and should be reworded. It appears that a word(s) is missing toward the end of the sentence.
In accordance with the reviewer's comment, we reworded the sentence.

5. Page 5, Line 5-9: Does the "medial stage" refer to stage 3? If so, please state this. Also, please include this information on Page 7 (Lines 51-55).

In accordance with the reviewer's comment, we addressed the issue.

6. Page 5, Line 5-9: The study's group of participants with higher body fat includes both overweight and obese. Please clarify why only the obese participants (i.e., BMI-for-age percentile > 97th) are required to be beyond the "medial" stage of maturation. Is this a requirement for the overweight (and normal-weight) participants as well?
In accordance with the reviewer's comment, we addressed the issue. Indeed, all participants are asking to be at least at Tanner stage 3.

7. Page 5, Line 18: In the sentence, "Normal weight, overweight and obese adolescents will be not be…" please consider deleting the first "be."
In accordance with the reviewer's comment, we deleted the first be.

8. Page 5, Line 20: How will you measure physical activity performed outside of school? Please specify the unit of time (i.e., weeks, months, etc.) for this 250-minute threshold. If available, please provide justification for the 250-minute cutpoint.
The amount of physical activity outside school will be obtain during the screening visit by a member of the research team and will be confirmed thanks to the physical activity questionnaire: IPAQ. We did choose 250 minutes as it correspond to 4 hours. We wanted to recruit adolescent that are not too much active. The threshold of 250 min per week as be chosen as it correspond to about 2-3 PA session per week, which is usually used in similar studies.

9. Page 5, Line 31: Please correct the spelling error (i.e., change physical active to physically active).
In accordance with the reviewer's comment, we corrected the spelling error.

10. Page 5, Line 33-35: Please be more specific about the exclusion criteria specific to the intervention in Study II. How will compliance be measured? Will the data from the participants who are excluded after the commencement of the study be retained for intent-to-treat analyses?
In accordance with the reviewer's comment we provided more information about the exclusion criteria and how the compliance will be measured.

11. Page 6, Line 29: It is stated, "For this study we will recruit either male and female…" However, Figure 1 shows that both male and females will be recruited for Study I. Please address this discrepancy.
In accordance with the reviewer's comment, we address the discrepancy mentioned.

12. Page 6, Line 55: Please correct the spelling error (i.e., change signed to sign).
In accordance with the reviewer's comment, we corrected the spelling error.

13. Page 7: Will the children in the control group of Study II be provided the residential program after the completion of the study?
As the residential program is a national health care program, it won't be automatically provided to the adolescents. In order to integrate it, they need to candidate for it via the health care system.

14. Page 8, Line 18-20: Please specify whether the dietary questionnaire is a food frequency questionnaire, 3-day diet diary, 24-hour recall, etc.
In accordance with the reviewer's comment, we provided specification regarding the diary food questionnaire.

15. Page 8: Please specify which DXA scanner is used at each study location.
In accordance with the reviewer's comment, we specify the DXA used for each study.

16. Page 8, Line 53: Please change “p-QCT” to “pQCT.”
In accordance with the reviewer’s comment, we corrected the spelling error.

17. Page 8: There may be issues with the 66% mid-tibia site for pQCT measures given the smaller gantry size of the XCT 2000. For this reason, problems with scan acquisition may arise with the study participants having a larger body size. Have the authors considered using the 50% site of the mid-tibia as opposed to the 66% site for these measurements? Please comment.
We would like to thank you the reviewer for addressing this potential issue. At the moment we haven’t encountered this problem with the recruited adolescents. However, if it occurs, as when we are performing scanners we are also doing the 38% tibia site (programmed scan by the manufacturer), we will use it for all instead of the 66%. We didn’t include this measured site in the method as we did choose to analyze the 4% and 66% only.

18. Page 10, Line 40-46: Please expand upon your strategy to track compliance (see: reviewer comment #10 above).
In accordance with the reviewer’s comment, we explained the compliance assessment.

19. Page 12, Line 38: Please change the word “study” to “studies.”
In accordance with the reviewer’s comment, we corrected the spelling error.

Discussion
1. Page 13, Line 18-31: The authors mention that pubertal stages are “targeted” in this study. However, the information provided in the methods section of this manuscript does not support this statement.
In accordance with the reviewer’s comment, we addressed this issue.

Author’s Contributions
1. Page 14, Line 16: The authors state, “…DC, DT, FD and EC were responsible for obtaining ethics approval and recruiting participants for study II and III.” However, this manuscript presents protocols for only two studies.
In accordance with the reviewer’s comment, we corrected the study error.

Additional comments by the reviewer
1. Specific details of the intervention (i.e., Study II) are not presented in this manuscript.
In accordance with the reviewer’s comment, we provided information about the intervention offered by the obesity center.

2. Race and ethnicity of intended study participants are not mentioned.
In accordance with the reviewer’s comment, we provided race and ethnicity of participants. As the obesity center is a national health care program, participants have different race and ethnicity.

★ Reviewer number 3:

- Could the authors mention the starting and finishing dates (approximately) of each study? Or has the study already started?
In accordance with the reviewer’s comment, we included in the manuscript the starting and finishing dates. The longitudinal study is running from September 2015 until June 2016, while the cross-sectional study doesn’t have fix date as it will depend on the recruitment.

- Could you please check the first paragraph of the introduction and clarify whether those percentages refer to overweight or obese or the combination of both?

In accordance with the reviewer's comment, a clarification has been made in the manuscript.

- I have not been able to understand why the cross sectional study includes both overweight and obese participants while the longitudinal one includes only obese participants. Is this because you anticipate (based on your experience) that this is going to be easier for recruitment?
We did choose to include both overweight and obese participant for the cross-sectional study, as based on our experience it is a population quite hard to recruit if not institutionalized.

- In the inclusion criteria for the cross-sectional study, the authors mention that doing more than 250 minutes of PA outside school will be considered as an exclusion factor. Is this 250 minutes per week? What intensity do the authors refer to? Moderate-to-vigorous PA? Why 250 minutes? Please clarify this to the reader
In accordance with the reviewer's comment, we would like to provide more information about the 250mn PA. The amount per week of physical activity outside school will be obtain during the screening visit by a member of the research team and will be confirmed thanks to the physical activity questionnaire: IPAQ. We did choose 250 minutes as it correspond to 4 hours. We wanted to recruit adolescent that are not too much active. The threshold of 250 min per week as be chosen as it correspond to about 2-3 PA session per week, which is usually used in similar studies.

- Are the inclusion criteria of the longitudinal study the same than those of the cross-sectional study? This is on page 5 and it was not clear to me.
In accordance with the reviewer's comment, a clarification has been made in the manuscript. Page 5 & 11

- Any reason by which the power calculations for the CS are based on a 80% power and those of the LS based on a 90% power?
As both sample size have been calculated based on two existing research, one cross-sectional, one longitudinal, the power calculation vary.

- At some point the authors mention that the LS will last 40 weeks, but it seems it will be 42 weeks, right? Please correct this.
In accordance with the reviewer's comment, we changed the LS to 42 weeks.

- Methods: Maturation; the authors mention that in addition to Tanner stages, blood analyses will be performed. Maybe you could briefly mention here which biomarkers/hormones will be used to measure maturation.
In accordance with the reviewer's comment, we detailed the hormones in the manuscript.

- Physical activity: Is there any chance you could measure PA using objective devices (i.e. accelerometers). This could be used as a confounding variable in your analyses (maybe Vitamin D as well). As it is, you are going to collect PA using questionnaires but previous studies have shown that overweight/obese adolescents tend to overestimate their PA levels.
We would like to thank you the reviewer for highlighting this point. Indeed, it would have be interesting to have an objective measure of PA. We choose to not assess PA with devices such as accelerometers devices as it is complicated in an organizational point of view. Indeed, for the LS the physical activity program is part of the institution program and not conducted by our team and regarding the CS asking participant to come twice is not convenient and increase the risk of "drop-out".

- DXA: Please check all you have in brackets when explain DXA in the methods. It seems there are too many DXA's.

In accordance with the reviewer's comment, a clarification has been made in the manuscript regarding DXA devices.

VERSION 2 – REVIEW

REVIEWER	Rachel Duckham Deakin University
REVIEW RETURNED	21-May-2016

GENERAL COMMENTS	I would like to commend the authors for the work put into addressing the comments raised by the reviewers for the manuscript entitled "Cross-sectional and longitudinal study protocols of the "AIposity and Bone metabolism: effects of eXercise induced weight loss in obese adolescents" (ADIBOX) project". Although the manuscript is improved there are still some aspects of the manuscript, which require further consideration to strengthen this work. Introduction Page 30 line 16: The impact of obesity on bone metabolism is gaining the attention. Gaining the attention of what? Methods Page32 line 28: A citation after similar studies is required. Page 35 Line 20: Remove sentence starting "As the pQCT 2000 was created to measure tibial ……" this is not needed. Page35 Line31: remove "scanners pictures" Page 35 Line 15 add manufactures between pQCT and software. Page 35 Line 16 remove the sentence beginning "for each scan…." This sentence is not needed. Page35 Line42: The authors have added that raidial and polar distribution will be measured with image J. Image J is a software package it can estimate/ calculate distribution but it is not a device that measures distribution. The authors need to consider the wording. Also it is advised that further information is added for this particular estimate such as (see below which is to aid the authors it should not be added word for word into the manuscript) to make it clear to the reader what and how is actually being estimated: "Polar distribution (cortical bone mineral mass; mg) and radial distribution (radial vBMD; mg/cm3) of the tibia will be estimated using Image J as described previously (Rantalainen 2011). Briefly, a threshold of xxx mg/cm3 for the xx% site and xx mg/cm3 for the xx% and xx% sites with a XxX median filtering of the image will be used to differentiate the cortical bone from the surrounding soft-tissue and bone marrow. To eliminate partial volume effects, the outermost and innermost layers of cortical pixels will be excluded from the analysis. Bones between individuals will be aligned according to xxxxxxxx and calculated polar distribution by subdividing the tibia cortex into xxxx sectors around its centre of mass with the average bone mass estimated for each sector. Radial distribution will be estimated by subdividing the cortex into three concentric rings (endocortical, midcortical and pericortical) by first removing all pixels below the threshold of each site from the image and dividing the remaining cortical bone into three concentric circles with the same thickness. " page37 Line 27: "Similarly to the previous study" are the authors referring to the cross sectional study if so be state " Similar to the

	cross sectional study, obese girls …. Page38 Line 8: Authors are excluding those whose compliance is less than 80%. It is advised that an explanation is given to justify this, as this a protocol paper. Page 39 Line 49: two training sessions per week for what duration. Will the investigators note what other activities are engaged in and for what duration. In terms of bone health engaging in swimming will have very different adaptations than ball sports. Page 40 Line 7: What will the battery of tests include? Page 40 Line 10: The authors discuss that the program is a 42 week program with 4 testing time points T0 (baseline) T1, T2, T3 of which will be 12 weeks (3 months) apart or in line with school holidays. If testing occurs ever 12 weeks then this would be a 36-week program what will happen to the last 6 weeks? Authors need to clarify the wording in this section to make this section clearer. Page 40 Line 18: How will the paediatrician determine the participant is or is not suitable to be enrolled in the study? Page 40 Line 20: “complete it” complete what? It may be better to word as follows: “ A paediatrician will meet the participants, prior to the beginning of the study to ensure the suitability of the adolescents to complete the intervention. Page 40 Line 25: “Will be gathered” gathered how, by questionnaire or interview? Page 40 Line55: add questionnaire after existing habits if this is a questionnaire. Page 40 Line 24: Please mention if for both the cross sectional and longitudinal study if the voxel size, the slice thickness and the scanning speed will be consistent and if the similar manufacturers software package (Stratec Medical, Pforzheim, Germany) version will be used in conjunction a similar edge detection and thresholding steps will be used to acquire densitometric and structural parameters of bone and soft tissue. Overall comment: It is advised that the authors read over the manuscript with fine detail there are a number or incorrect spellings, missing words, word phasing and gramma issues throughout the entire resubmitted manuscript which I have not individually highlighted.

REVIEWER	Dr. Luis Gracia-Marco University of Exeter (UK)
REVIEW RETURNED	21-May-2016

GENERAL COMMENTS	I am satisfied with the way you answered to my comments. Congratulations and I hope the study goes well.

VERSION 2 – AUTHOR RESPONSE

Reviewing: 1
Comments to the Author

★ Reviewer number 1:
Introduction
The impact of obesity on bone metabolism is gaining the attention. Gaining the attention of what?
In accordance with the reviewer’s comment, we completed this sentence.

Methods
A citation after similar studies is required
We would like to thank you the reviewer's comment and reworded and added citation to this sentence.

Remove sentence starting "as the pQCT…"
In accordance with the reviewer comments we removed this sentence.

Remove "scanners pictures"
In accordance with the reviewer comments we removed "scanners pictures".

Add manufactures between pQCT and software.
In accordance with the reviewer comments we added manufactures.

Remove the sentence beginning "for each scan"
In accordance with the reviewer comments we removed it.

The authors have added the radial and polar distribution but this is not a device that measure distribution. The authors need to consider the wording. Also it is advised that further information is added for this particular estimate…
In accordance with the reviewer's comments we reworded the sentence and provide further information as requested.

"Similarly to the previous study" are the authors referring to the cross sectional study ?
In accordance with the reviewer's comments we clarified this sentence and replace previous by cross sectional.

Authors are excluding those whose compliance is less than 80%. It is advised that an explanation is given to justify this.
In accordance with the reviewer's comments we explained how the compliance threshold has been set.

Two training sessions per week for what duration. Will the investigators note what other activities are engaged in and for what duration. In terms of bone health engaging in swimming will have very different adaptations than ball sports.
We would like to thank you the reviewer for this comment. The recreational session offered by the institution are about 2 hours depending on the activity. We do know the type of activity however, for logistical reasons we are unable to know the exact time of activity that adolescents are engaged in.

What will the battery of tests include?
We would like to thank you the reviewer for addressing this point. The battery of tests are those described below.

The authors discuss that the program is a 42 week program with 4 testing time points T0 (baseline) T1, T2, T3 of which will be 12 weeks (3 months) apart or in line with school holidays. If testing occurs ever 12 weeks then this would be a 36-week program what will happen to the last 6 weeks? Authors need to clarify the wording in this section to make this section clearer.
We would like to thank you the reviewer for highlighting this point as it can be confusing for the reader. We reworded this paragraph to make it clearer. As stated in the article testing point is every 14 weeks (42 weeks in total) which match with the school holiday period and the bone remodelling cycle as a bone remodelling cycle is about 12 weeks (3months).

How will the paediatrician determine the participant is or is not suitable to be enrolled in the study?
We would like to thank you the reviewer for addressing this point. The paediatrician will determine if the participant can or cannot take part in the study regarding our inclusion and exclusion criteria detailed in the article.

"Complete it" complete what?
We reworded this sentence as suggested by the reviewer.

"Will be gathered" gathered how, questionnaire or interview?
In accordance with the reviewer's comments we explained how we gathered the information.

Add questionnaire after existing habits if it is a questionnaire.
In accordance with the reviewer's comments we added questionnaire after existing habits.

Please mention if for both CS and LS if the voxel size, the slice thickness and the scanning speed will be consistent and if the similar manufacturers software package version will be used in conjunction a similar edge detection and thresholding steps will be used to acquire densitometric and structural parameters of bone and soft tissue.
In accordance with the reviewer comment's we did answer to this. Indeed both device will use the same set up and the manufacturer's software package.

It is advice that the authors read over the manuscript with fine detail there are a number or incorrect spellings, missing words, word phrasing and gramma issues throughout the entire resubmitted manuscript which I have not individually highlighted.
In accordance with the reviewer's comments the manuscript has been re-read.

VERSION 3 – REVIEW

REVIEWER	Dr Rachel Duckham Deakin University, Melbourne, Australia
REVIEW RETURNED	12-Jul-2016

GENERAL COMMENTS	The authors have improved this manuscript and it is to a standard required for publication.