Author's response to reviews

Title: Physical fitness among urban and rural Ecuadorian adolescents and its association with blood lipids: a cross sectional study

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Response letter to Reviewers

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Version: 2

Dear Editor,

Dear reviewer,

On behalf of all authors, I would like to thank the reviewers for their constructive comments that have helped us to considerably improve our article. We apologize for the delay in submitting this revised version and hope this has not caused an inconvenience. All comments and suggestions were addressed and the text was revised accordingly. Detailed responses to the reviewers’ comments are provided below. The original text fragments copied from the old version are in bold, while corrections and insertions are underline.

Thank you for considering our revised work for publication in BMC Pediatrics.

Sincerely
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Leading author of the paper

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Reviewer # 1

Background

First paragraph

1. You mention that dyslipidemia was higher in rural group compared to urban. However there is no discussion on the reasons.

   ANSWER: We agree that it is important to mention the reason why dyslipidemia was higher in the rural group compared to the urban group. We briefly discuss what could explain such differences in the rural group compared to the urban group.

   CORRECTION IN THE TEXT: Unexpectedly, a previous analysis showed that dietary intake was weakly associated with plasma lipid (Ochoa–Aviles unpublished data). Therefore, it was hypothesized that an association of blood lipids with physical fitness is probable, and is a dimension of analysis that could further be explored (Lines 41-43)

2. Also it is not clear how rural or urban are related to lifestyle.

   ANSWER: The components of lifestyle like dietary intake, the physical activity behaviors and physical fitness can be influenced by the availability of health services, number of specialist medical doctors, sport facilities, recreational areas, ways of transportation, safety issues, food availability and formal education among others. All these environmental factors differ considerably in rural compared to urban areas. This argument was introduced in the revised introduction in order to clarify how rural or urban conditions are related to lifestyle:

   CORRECTION IN THE TEXT: To the author's knowledge no studies thus far have assessed associations of blood lipid levels with a similar variety of fitness components (speed, muscular strength endurance, cardio-respiratory endurance, flexibility and balance) according to residential location (rural vs. urban). This is surprising considering incidence of cardiovascular risk factors is known to vary along with environmental factors, such as location of residence (urban vs. rural areas)[15]. Rural areas differ considerably to urban areas, i.e. in terms of available health services, medical specialists [15], sport facilities or recreational areas [16], transportation (traffic and means of transport), safety issues [17], food availability [4] and formal education, among others [15]. (Lines: 48-55)
3. You mention that mention “type of residence” as environmental factors. It is not clear what you mean by “type of residence. Do you mean “location” of the residence?

ANSWER: The “residential location” is indeed more appropriate to refer to for the area where a person lives. We changed “type of residence” into “residential location” throughout the manuscript.

Second paragraph

4. You mention that CV risk factors are inversely associated with physical activity. Do you mean that CV risk factors are inversely associated with “increased levels” of physical activity?

ANSWER: We agree with the suggestion and changed this sentence as follows:

CORRECTION IN THE TEXT: Current evidence indicates that the development of non-communicable disease starts early in life [2] and is associated with poor physical fitness, low physical activity levels [3] and inadequate diet [4]. (Lines: 30-32)

5. Also, you mention that physical fitness is better associated with CV risk factors than physical activity levels but there is no further discussion on why physical fitness is better associated CV risk factors than physical activity levels.

ANSWER: We elaborated on the associations of physical fitness and physical activity with CV risk factors as follows:

CORRECTION IN THE TEXT: Physical fitness, in contrast to physical activity, is stable over several months within an individual [6] (Lines: 33-34)

6. You mention that estimates of physical fitness and its association with the risk factors for CVD are rare in low and middle income countries but there are no references for this statement or any studies presented.

ANSWER: We added the references in the revised version.

CORRECTION IN THE TEXT: There are few studies that have assessed physical fitness [9-13] and its association with cardiovascular risk factors in low- and middle- income countries [14] (Lines 44-45)

References:


7. Description of the fitness tests that are available including the EUROFIT, advantages and disadvantages of these tests should also be described.

ANSWER: As suggested, the EUROFIT items were now described in the text of the methods. As a detailed description of the EUROFIT items could lead to a long text we refer to the EUROFIT manual where a detailed description of all items can be found. Other test batteries were mentioned in the text. There we stated that the advantages and disadvantages of the EUROFIT test battery in comparison to other assessment tools. We added the advantages and disadvantages of this test battery. The text was changed as follows:

CORRECTION IN THE TEXT: EUROFIT is a valid method to evaluate fitness components [25], it offers advantages over other objective methods such as AAPHERD, CAHPER and Canadian as it assesses health-related fitness [25, 26]. Furthermore, this test is easy to apply and can be performed in large groups, and requires few materials. A potential disadvantage of EUROFIT could be that scoring might be considered subjective, since practice and motivation levels can influence the score attained [20].

In each school the EUROFIT [20] test battery was used to assess different dimensions of physical fitness with nine tests: cardio-respiratory endurance (shuttle run 20m measured in laps), strength (handgrip measured in kilogram-force and vertical jump measured in centimeter), muscular endurance (bent arm hang measured in seconds and sit-ups measured in the number of sit-ups/30 seconds), speed (shuttle run 10x5 m measured in seconds and plate tapping as time needed to complete 25 cycles), flexibility (sit and reach measured in centimeter) and balance.
The flamingo balance measured as the number of tries needed to keep balance for the duration of one minute. (Lines: 107-118)

8. The research question is not well defined.
ANSWER: We clarified the research questions as follows:
This study has two objectives: i) to assess the physical fitness in a group of urban and rural Ecuadorian adolescents and ii) to analyze the associations of physical fitness and lipid profile in adolescents according to residential location. (Lines: 56-58)

Methods

First paragraph

9. The writing is very hard to understand. What do you mean by “between both two areas”? What do you mean by “the latter area”? Are you referring to Nabon? How about Cuenca?
ANSWER: The writing of the complete manuscript was revised. The sentence you refer to currently reads:
Data from the National Institute of Statistics in Ecuador indicate that the estimated prevalence of poverty is substantially higher in Nabón compared to Cuenca (93% vs. 2% respectively) [18]. (Lines 65-67)

Second paragraph

10. What do you mean by “771 school going adolescents aged”
ANSWER: We clarified this statement in the text:
CORRECTION IN THE TEXT: This cross-sectional study involved 773 students between the ages of 10 to 16 years old (Figure 1). (Line 68)

11. Under Ethical approval heading, you mention that adolescents with muscle or bone injuries... were excluded from the study” Do you mean that you exclusion criteria included adolescents with muscle or bone injuries...? Or were they initially included and later excluded because they were injured...? If so, could you please provide the number of adolescents excluded for each reason?
ANSWER: As suggested, on line 86-90 we clarified that the exclusion criteria for the study were: concomitant chronic disease interfering with participants’ normal diet and physical activity, or physical disability or pregnancy. We corrected the flowchart in which we included the number of
adolescents excluded for each reason. In the text we provided a detailed description of participants excluded for these different reasons.

CORRECTION IN THE TEXT: Overall, adolescents were excluded from the sampling if they had reported a concomitant chronic disease that interfered with their normal diet and physical activity, had physical disabilities or were pregnant. In the assessment of physical fitness, adolescents with chronic muscle pain or bone fractures were not able to perform any of the tests (Figure 1). (Ethical approval: in lines 86-90)

CORRECTION IN THE TEXT: A total of 125 (16.2%) adolescents did not perform the fitness tests, most of them declined to participate (n=91), or had otherwise experienced bone /muscle injury (n=18) or had changed schools (n=13) (figure 1). (Lines: 123-125)

12. Under physical fitness heading, how were reliability, repeatability and reproducibility assessed?

ANSWER: In our sample we did not assess reliability, repeatability and reproducibility of the EUROFIT items as this was evaluated previously in similar populations eg. Mexican adolescents and we have no reason to assume validity would be different in our study population. The results showed a good validity and a moderate to high reliability. We added the references in line 101-102.

CORRECTION IN THE TEXT: The reliability and validity of fitness tests in adolescents has been widely documented [11, 21-24]. (Lines 106-107)

13. It is not clear what is the unit of the EUROFIT shuttle run test. Is it laps / time? Do high the numbers indicate better results?

ANSWER: We addressed this comment in lines 113, 118-120. We clarified that the units for the 20m shuttle run is laps. In addition, we stated that the higher scores in the EUROFIT tests indicate a higher level of physical fitness, except for the shuttle run 10x5 m test, plate tapping and flamingo balance, for which lower scores indicated higher levels of physical fitness.

CORRECTION IN THE TEXT: cardio-respiratory endurance (shuttle run 20m measured in laps)...

High scores indicate higher levels of physical fitness, apart from the shuttle run 10x5 m, plate tapping and flamingo balance, for which lower scores indicate a higher level of fitness. (Lines: 113-120)

14. Also, you have different numbers in figure 1 for the adolescents that refused to complete one or more test items.
ANSWER: We corrected the numerical inconsistencies in lines 123-125 and revised in figure 1 (please, refer to the comment 11 to see the changes in the figure 1).

CORRECTION IN THE TEXT: A total of 125 (16.2%) adolescents did not perform the fitness tests, most of them declined to participate (n=91), or had otherwise experienced bone/muscle injury (n=18) or had changed schools (n=13) (figure 1). (Lines: 123-125)

15. Under UBN heading, you mention that the method classifies the household as “poor” when one or more deficiencies in urban services is reported. Wouldn’t urban services be the same for all people living in the same area? Shouldn’t this be used to assess UBN of school rather than household?

ANSWER: We clarified our approach. Services are not the same for all people living in the same area. In Ecuador, there are quite large disparities in services between and within both the rural and urban areas. Differences resulting from national and international migration, variable household income and unequal allocation of government budget, are some of the reasons for these disparities. This makes the UBN a relevant instrument for official use at national level in Ecuador. To enhance comparability of our findings with national data we have adopted this method. To clarify this point, we provide a few examples of services that tend to differ within rural or urban areas. In response to the last question, this method evaluates household characteristics it was not considered suitable to assess school characteristics.

CORRECTION IN THE TEXT: We adopted this method to enhance comparability of our findings with national data. The method classifies a household as “poor” when one or more deficiencies in access to education, health, nutrition, housing, urban services (electricity, potable water or waste recollection) and employment is reported. (Lines 137-140)

16. You mention that two interview-administered 24h dietary recall were used to estimate food intake. Did the people who administered the interview-administered 24h dietary recall had training? Were they nutritionists?

ANSWER: We added a description of the training and composition of the team in charge of the measurements.

CORRECTION IN THE TEXT: Prior to data collection, medical doctors, nutritionists and health professionals were trained for three full days to assess outcomes: anthropometrics, physical fitness, unsatisfied basic needs and 24 hour recall questionnaires. A manual with standardized
procedures was developed for the purpose of the study and used during the training. Two biochemists were in charge of collecting and analyzing blood samples. (Lines: 92-96)

17. *How were reliability, repeatability and reproducibility assessed?*

**ANSWER:** The complete methodology for the 24-hour recall was reported elsewhere (Ochoa-Aviles unpublished data). Therefore, in the revised version we only included results of analysis evaluating associations of food intake with other parameters (ie. plasma lipid and fitness tests), and we did not include the reliability, repeatability and reproducibility of the 24-hour recall. Nevertheless, we acknowledge that the assessment of reliability, repeatability and reproducibility is important to control due to the possible errors that may occur in a 24-hour recall such us omitting foods, adding foods, incorrect estimation of food weights and day-to-day variation. For that reason, we followed a rigorous approach to assess food intake as described by (Gibson, 2008) and clarified this in the text.

**The procedures used to assess the dietary intake were in line with the recommendations of current literature [29]** (Lines: 147-148)

**Reference:**


18. *What are the references for the blood lipid profile measurements?*

**ANSWER:** We added the reference for the method used for blood lipid analysis in lines 158-163.

**CORRECTION IN THE TEXT:** Serum total cholesterol ... and triglycerides were analyzed by a calorimetric enzymatic method [30] ... . High-density lipoprotein cholesterol (HDL) ... [31]. The Friedewald formula was used to calculate low-density lipoprotein cholesterol (LDL) [32]. (Lines: 158-163)

**References**

19. **The methods are not appropriately described.**

**ANSWER:** We revised the methods, and in the current version we described the training and composition of the team in charge of taking measurements. We clarified the exclusion criteria. We added the appropriate references for validity and repeatability for EUROFIT test. We described the EUROFIT scoring. We also included the references for the methodology used to analyze blood lipid profile (cfr comments 10 to 18).

**Are the data sound?**

20. 1) **The results for the interview-administered 24h dietary recall are not presented. Are the discussion and conclusions well balanced and adequately supported by the data?**

**ANSWER:** Thank you for the comment. The main objective of the paper was to assess physical fitness and its association with blood lipids. In this context, total daily energy intake was only used in this study to adjust the association between blood lipids and physical fitness. We added the total energy intake of the children in both the rural and urban area to ensure clarity and revised the text as follows:

**CORRECTION IN THE TEXT:** The association found in this study between blood lipids and fitness was adjusted for BMI and total energy intake, as these factors have previously been found associated with blood lipids [4, 7]. Mean energy intake was not significantly different (P=0.08) between urban (1863±181 kcal/day) and rural (1766±153 kcal/day) adolescents. (Ochoa-Avilés unpublished data). In our sample, the relationship of different blood lipid parameters with each of the EUROFIT tests according to residential location was generally weak and non-significant. (Discussion: lines 318-324)

21. **On page 12, some reasons for the findings are mentioned. For example the application of school physical education programs could be different in rural and urban. Couldn’t this have being investigated as the schools were approached to participate in the study?**

**ANSWER:** Unfortunately, we did not measure differences in the application of the physical education program and can only rely on observations while performing the study. We added text in order to clarify this aspect.

**CORRECTION IN THE TEXT:** Thirdly, we observed that urban schools had specialized physical education teachers in their physical education programs, while these kinds of specialized teachers were virtually absent in rural areas. (Lines: 299-301)
a) Some sections are just a repetition of what was presented in the results section. For example on page 13, the first paragraph about the dyslipidemia and its association with the tests; b) It is not discussed what is the association between energy intake and physical fitness with BMI. c) In addition no data on energy intake is reported or discussed.

ANSWER: Thank you for the comment. We have split the comment in three parts and addressed them in the following paragraphs:

ANSWER a): This paragraph was revised. One section of the original paragraph was integrated in the results. And the other part of the paragraph was used to discuss the association between blood lipid profiles and fitness. The was changed as follows:

CORRECTION IN THE TEXT: In the urban area there was an inverse association of bent-arm-hang and handgrip with cholesterol and LDL. In the rural area the adolescents who reached the Healthy Fitness Zone according to the FITNESSGRAM standards had significantly lower cholesterol and LDL levels. (Results: lines 245-248)

These studies have reported that increased cardiorespiratory fitness and muscular strength are associated with favorable lipid profile in adolescence [7, 24, 38, 57]. These associations were partially confirmed in our study. Total cholesterol and triglycerides were negatively associated with muscular strength in the urban area whilst in the rural population these lipids were negatively associated with cardiorespiratory fitness. (Discussion: lines 311-315)

ANSWER b): In the present manuscript we only focus on physical fitness among urban and rural adolescents and its association with blood lipid profiles. The association between blood lipids and fitness was adjusted by BMI and total energy intake as these two factors can influence the blood lipid profile. It is not our aim to describe associations between BMI and energy intake with blood lipid profiles. We discussed why we used the BMI and energy intakes to adjust the associations between the fitness test and blood lipids as follows:

CORRECTION IN THE TEXT: The association found in this study between blood lipids and fitness was adjusted for BMI and total energy intake, as these factors have previously been found associated with blood lipids [4, 7]. Mean energy intake was not significantly different (P=0.08) between urban (1863±181 kcal/day) and rural (1766±153 kcal/day) adolescents. (Ochoa-Avilés unpublished data). In our sample, the relationship of different blood lipid parameters with each of the EUROFIT tests according to residential location was generally weak and non-significant. (Lines 318-324)
ANSWER c): We kindly refer to the previous answer and comment #20. Briefly, detailed results about the food intake are reported elsewhere. The article that reported the associations between blood lipids and the dietary intake in urban and rural areas did not find important significant associations in our sample. In the present manuscript energy intake was used to adjust the associations between blood lipids and physical fitness.

Is the writing acceptable?

23. No writing is hard to understand.

ANSWER: The writing of the complete manuscript was revised.

Level of interest: An article of limited interest
Quality of written English: Not suitable for publication unless extensively edited
Statistical review: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests:
I declare that I have no competing interests.
Reviewer # 2

Major revisions

1. **Page 3: Strengthen the rationale and state the hypotheses tested.** This phrase is too vague, “estimates of physical fitness and its association with the risk factors ... are rare for low and middle income countries.” Clarify and expand upon the intended meaning of “rare.” If there are other studies performed in low/middle-income countries the authors should briefly summarize the findings and the limitations in those studies. What are the gaps in knowledge that the existing studies have not yet addressed? State specifically how this study addresses a novel question. The authors need to provide some additional statements about the study rationale here. Is there a reason to expect that the association between fitness and lipids would be different in high versus low income countries or rural versus urban children? Is there existing evidence on this topic or is this a novel question? Revising to include some of this background rationale will help the reader understand why the study was performed and why it has value. Stating the hypothesis will also help frame the specific question that was investigated.

**ANSWER:** We appreciate this comment and clarified the research questions. We redrafted the complete introduction in order to accommodate the reviewer’s comment. Please refer to the revised manuscript.

2. **Page 3: “A recent study ... reported a high prevalence of cardiovascular risk factors” Please clarify, high prevalence compared to what norms?**

**ANSWER:** In the revised version we clarify this statement and reported the actual figures of prevalence of cardiovascular risk factors.

**CORRECTION IN THE TEXT:** A recent study in a group of urban and rural Ecuadorian adolescents [8] reported that dyslipidemia, abdominal obesity and overweight were prevalent in 34.2%, 19.7% and 18.0% of the population. (Introduction: lines 37-39)

3. **Results: The dietary recall data were not included, nor was there any discussion of how those data were used to interpret the outcomes. This is an important oversight.**

**ANSWER:** Similar comment was mentioned by the reviewer #1 (comment 20). The results of the food intake in our sample were reported in a separate article which is currently under revision. The article analyzed dietary intake and its association with blood lipid profiles and the article did not
find many important associations. In the present manuscript focuses on physical fitness among urban and rural adolescents and its association with blood lipid profiles. The association between the blood lipid profile and fitness was adjusted for BMI and total energy intake as these two factors can influence the blood lipid profile. We added the results of the food intake data (total energy per day) and clarified why we used BMI and energy intakes to adjust the associations between fitness test and blood lipids in lines 317-324.

CORRECTION IN THE TEXT: We report that differences in blood lipid profile among urban and rural adolescents are not explained by differences in physical fitness, even after adjusting for BMI and total energy intake. The association found in this study between blood lipids and fitness was adjusted for BMI and total energy intake, as these factors have previously been found associated with blood lipids [4, 7]. Mean energy intake was not significantly different (P=0.08) between urban (1863±181 kcal/day) and rural (1766±153 kcal/day) adolescents. (Ochoa-Avilés unpublished data). In our sample, the relationship of different blood lipid parameters with each of the EUROFIT tests according to residential location was generally weak and non-significant. (Discussion: lines 317-324)

4. Page 13: The first paragraph is redundant. The statements here mostly repeat what was presented in the results and prior parts of the discussion. Remove this paragraph or revise to integrate the current data set with prior literature, either here or in expanded sections elsewhere.

ANSWER: This paragraph was revised as suggested. The first part of the original paragraph was integrated in the results. While the last part was used to discuss the association between lipids and fitness.

CORRECTION IN THE TEXT: In the urban area there was an inverse association of bent-arm-hang and handgrip with cholesterol and LDL. In the rural area the adolescents who reached the Healthy Fitness Zone according to the FITNESSGRAM standards had significantly lower cholesterol and LDL levels. (Results: lines 245-248)

These studies have reported that higher cardiorespiratory fitness and muscular strength are associated with favorable lipid profiles in adolescence [7, 24, 38, 57]. These associations were partially confirmed in our study. Total cholesterol and triglycerides were negatively associated with muscular strength in the urban area, whilst in the rural population these lipids were negatively associated with cardiorespiratory fitness. (Discussion: lines 311-315)
Previous research indicates that such low fitness levels can linger on into adulthood [39] where low cardiorespiratory fitness [40] or low muscular strength [41] is associated with increased mortality risk. (Discussion: lines 265-267)

5. Page 13: The second paragraph is overly speculative and unclear. To invoke epigenetic differences to explain the lipid variation isn’t plausible as written and must be better supported or removed. What about other lifestyle factors like daily physical activity or diet? Are there any potential racial/ethnic differences between the rural and urban populations?

**ANSWER:** We appreciate this comment and improved the discussion accordingly. As suggested we removed the epigenetic difference as a possible explanation of differences in blood lipid profiles. In addition, we expanded the discussion and included the physical activity. In addition we reviewed the literature and added that the differences in body fat distribution could explain the differences in blood lipid profiles.

To address the reviewer comment we revised the discussion in lines 325-340 as is shown below:

**CORRECTION IN THE TEXT:**

Another possible explanation for the differences in blood lipid profile among urban and rural adolescents may be the differences in moderate to vigorous physical activity [58], or body fat distribution [59]. Physical activity and fitness have been found independently associated with certain blood lipid levels among children and adolescents [6]. For example, the favorable TG and HDL levels are inversely associated with moderate to vigorous physical activity, independent of time spent sedentary [58] and fitness [6]. In our sample, the time spent on moderate to vigorous physical activity could be longer in urban adolescents compared to rural adolescents because of differences in the availability of sport facilities and organized group sports, detailed earlier in this discussion. In addition, qualitative research performed in adolescents from Cuenca and Nabon has shown that the rural adolescents felt an inability to perform physical activity in contrast to the urban adolescents (Van Royen unpublished data). This fact could lead to differences in physical activity levels between urban and rural adolescents, as self-efficacy is an important determinant of physical activity in adolescence [60]. On the other hand, total cholesterol, LDL, HDL and TG also have been associated with fat distribution measured by skin-fold thickness. Lean adolescents, as determined using this measurement system, have been found to have healthier blood lipid profiles compared to their heavier peers [61]. However, skin-fold thickness was not a parameter measured in the present study. (Discussion in lines 325-340)
In addition, a study about the genetic differences in the Ecuadorian population concluded that there were small genetic differences between urban population (mestizos) and rural populations (amerindian populations) compared to the differences with the European population. Regarding the ethnic differences, a National Demographic Survey showed that 32% of Nabon's population was believed indigenous in contrast to 2% of the Cuencan population. We did not include the racial or ethnic differences as a possible explanation for the differences in blood lipid profiles, since the formal studies found small differences in genetic variation among urban and rural populations. Furthermore, as the Ecuadorian population is ethnically quite diverse, the sense of belonging to a particular ethnic group is subjective and the result of any investigation into ethnicity is also subjective and cannot be considered an objective measure.

6. **In the discussion please comment on the potential study limitations:**

6.1.a) Has the Eurofit test been validated in Ecuadorian children or other similar populations? 6.1.b) Is there any evidence to support that they should have the same range of outcomes as the other nationalities of children cited on page 11 (Refs 16-23)?

**ANSWER:** Thanks for the comment. We split the comment in two parts and addressed them in the following paragraph:

6.1.a) The EUROFIT battery has not been validated in Ecuadorian adolescents, as this was evaluated previously in a similar population of Mexican adolescents. The result showed a good validity and we had no reason to assume that the method would not be valid in our study population. We added the corresponding references as follows:

**CORRECTION IN THE TEXT:** Physical fitness was measured using the EUROFIT [20] test battery, which is considered a valid and standardized test for adolescents [21]. The reliability and validity of fitness tests in adolescents has been widely documented [11, 21-24]. (Methods: lines 105-107)

6.1.b) There is no evidence that suggests Ecuadorian adolescents should have the same range of outcomes as the other nationalities of children cited on lines 269-281. With these comparisons we attempt to put our results in the context of current literature. These studies are comparable to our study, as they had included urban and rural adolescents and used some EUROFIT tests. We only found one study performed in a similar population, this study was carried out in urban and rural Mexican adolescents and used some items of EUROFIT battery. We included this as a limitation of our study on lines 342-357
CORRECTION IN THE TEXT: There are a few limitations of this study. Finally, our results could be compared with only one other study in a low- and middle-income country, which hinders comparison of our findings with previous data in similar populations. (Discussion: 342-357)

To clarify the fitness comparisons on lines 269-281 we added lines 281-286, where we state that there are some factors i.e. levels of moderate to vigorous physical activity and genetic factors than can influence physical fitness. These factors can potentially vary between regions. Thus, when we compared our fitness results with those from similar population, for example Colombian or Mexican adolescents, these differences in fitness are small. However, differences in fitness become larger when we compare our result with European populations where there are major differences in the environmental and genetic factors.

CORRECTION IN THE TEXT: The favorable fitness scores in European compared to Ecuadorian adolescents may be a reflection of the favorable environmental conditions for physical activity found in Europe [46], as well as a longer tradition of health promotion programs [47], and differences in genetic factors [48, 49]. This hypothesis may be reinforced by the fact that our results were similar when compared to those from Mexican [11] and Colombian [13] studies, which have similar environmental and genetic patterns to those of Ecuador [48]. (Discussion: lines 281-286)

6.2) Do the authors have any data on the reliability of these tests in their sample of children? When performing fitness tests there is always a potential concern about familiarity with the tasks, confidence in performance, and motivation to give the best effort. It is not uncommon for fitness test outcomes to improve during repeat testing (learning effect). The authors should comment on this aspect of their study.

ANSWER: The reliability of the EUROFIT battery has not been assessed in Ecuadorian adolescents. However, the reliability of EUROFIT tests have been assessed in Mexican adolescents and these have shown a moderate to high reliability. We added the corresponding references in lines 106-107 (please, refer to the comment 6.1.a). In addition, we followed the EUROFIT guidelines during the survey. According to the manual the adolescents were not allowed to practice the test before the final measurement, and were encouraged to perform the best they could every time. As such, we included this point as a limitation in lines 342-349.

CORRECTION IN THE TEXT: There are a few limitations of this study. … Fourth, reliability and validity of EUROFIT were not done in our sample. Although, EUROFIT has showed good validity in previous studies performed in the region[11]. We followed the EUROFIT guidelines in order to
avoid source of bias, such as learning effect, or low motivation of adolescents to do their best performance during each test [20]. (Limitations on lines: 342-349)

7. English grammar and usage should be reviewed throughout.
   ANSWER: We reviewed the grammar of the whole manuscript.

Minor revisions

8. Abstract: The final concluding sentence is out of place. The theme of the paper is about exploring the association between fitness and lipids. Since there wasn’t a significant association between those two outcomes the low fitness scores are a secondary finding. It would be better to move the last sentence to an earlier place, or even better to remove it, so that it isn’t the final statement. Instead keep the focus on the paper’s theme (fitness and lipids).
   ANSWER: We agree with this comment and we modified the text of the abstract in order to be consistent with the objectives. Additionally, we realized that the objectives of the manuscript were not clear in the introduction and clarified them (cfr comment 1). As suggested, the abstract was modified:
   CORRECTION IN THE TEXT: Conclusions: Physical fitness, in our sample of Ecuadorian adolescents, was generally poor. Urban adolescents had better physical fitness and blood lipid profiles than rural adolescents. The differences in fitness did not explain those in blood lipid profile between urban and rural adolescents. (Abstract: lines: 18-20)

9. Page 3: Awkward phrasing: “by reducing modifiable risk factors such as a diet and lifestyle” Need to specify what components of diet and lifestyle should be changed.
   ANSWER: We reorganized the introduction and this sentence was clarified. The objective of this sentence was to introduce the inverse association between the incidence of non-communicable diseases and poor physical fitness, or low physical activity levels.

10. Page 3: Awkward phrasing: “environmental factors such as type residence” Do the authors mean “such as location of residence?”
    ANSWER: We changed “type of residence” into “residential location” throughout the manuscript as suggested by reviewer 1 to ensure clarity.
11. **Page 11:** Awkward phrasing: “adolescents who were identified in the health risk showed worse” Perhaps it should be revised to “adolescents with one or more health risks for dyslipidemia…”

**ANSWER:** The comment was addressed in line 256. The sentence currently reads:

**CORRECTION IN THE TEXT:** Furthermore, adolescents who had a low aerobic capacity as defined by the FITNESSGRAM had lower scores for physical tests, such as speed-agility, flexibility, muscle strength-endurance and balance. (Discussion: lines: 256-256)

12. **Page 14:** Conclusion, first line—specifically state what is meant by “a high proportion.” (Provide the actual number).

**ANSWER:** We clarified this sentence and provide the actual figure.

**CORRECTION IN THE TEXT:** The results from our study suggest that 59% of Ecuadorian adolescents have poor physical fitness. (Line: 363)

Level of interest: An article of limited interest

Quality of written English: Not suitable for publication unless extensively edited

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:

I declare that I have no competing interests
Reviewer # 3

This study is a cross sectional study assessing physical fitness and its association to blood lipid profile comparing rural and urban adolescents in a low and middle income country. It is a well-written manuscript. Appropriate design and methods were used to answer the objective and these are thoroughly described. Relevant and important confounders were identified and used in analysis.

The differences found in associations between rural and urban adolescents are interesting as is the comparison of physical fitness with adolescents from other countries.

Attention to the following might strengthen the manuscript. Major Compulsory Revisions:

1. Please clarify the interpretation of Table 2.

The title says “association between physical fitness and BMI.....stratified by residence”. The second column is for interaction between BMI and residence. How is that to be interpreted? Should only those with significant interactions (p <0.10) be stratified? Or how should the reader read this interaction into the results?

ANSWER: We agree that the interpretation of table 2 was not clear. The revised version currently reports the beta percentages and p-values for the whole sample only when the interaction terms were not significant (P>0.10). Otherwise we stratified these results for urban and rural groups. We also changed the interpretation of table 2 as follows:

CORRECTION IN THE TEXT: The associations between fitness and BMI are shown in table 2. The interaction in terms of BMI-residence was significant for speed shuttle run, plate tapping, sit up, vertical jump, bent arm hang and the proportion adolescents who reached the Healthy Fitness Zone. In the total sample, BMI was significantly associated with low performance on the 20m shuttle test and flamingo, and with high performance on hang grip (p<0.01 for all tests). When the associations between the fitness tests and BMI were analyzed according to residential location, the results showed that the proportion of adolescents that reach the Healthy Fitness Zone in both urban and rural areas decreased significantly as mean BMI increased. In addition, in both rural and urban areas the improved scores the performance on the speed shuttle run and longer duration of bent arm hang were significant, and inversely associated with BMI. In both areas, the associations between BMI with plate tapping and vertical jump test were not significant. The only difference, when considering residential location, was the association
between the sit up test and BMI which was only significant in urban adolescents. (Results: lines 224-235)

2. A lot of associations were tested. (i.e. table 4: 9x5=45 associations). In statistics, the Bonferroni correction is a method used to counteract the problem of multiple comparisons. Has this been considered by the authors?

ANSWER: We agree that the Bonferroni correction is a method used to counteract the problem of multiple testing. As this study is exploratory and tests new associations, we propose not to control for multiple questions in our result tables. This would inform and guide future research in the issues explored. However, we agree that correcting for multiple testing should be dealt with and discuss the implications on our findings when applying a Bonferroni correction (using an adjusted p-value of 0.005)

CORRECTION IN THE TEXT: As this study was exploratory and not confirmatory, we did not adjust for multiple testing[36]. Nevertheless, we also report our results on associations between blood lipid profiles and EUROFIT tests after applying a Bonferroni correction using an adjusted p-value of 0.005. (Methods: lines 192-195)

Although, after the Bonferroni correction only the association between cholesterol levels and the adolescents who reached the Healthy Fitness Zone according to the FITNESSGRAM standards remained significant. (Results: lines 248-250)

Reference:

Minor Essential Revisions:

3. Please state the participants (N=648) in the study as a percentage of the eligible (result section page 9, first line)

ANSWER: We addressed this comment as follows:

CORRECTION IN THE TEXT: In this study data from 648 adolescents were analyzed [83.3% of total sample]. (Results: lines 198)
4. Please clarify some details on the exclusion criteria: a time interval for bone injuries (for the last year? or at any time?), what kind of concomitant disease? You probably asked for some specific ones? (Page 5 paragraph 2, Ethical approval)

ANSWER: The reviewer #1 (comment 11) had a similar comment. As suggested we clarified the exclusion criteria for the study, number of adolescents excluded for each reason and provided a detailed description of participants excluded in figure 1 is provided below.

CORRECTION IN THE TEXT: Overall, adolescents were excluded from the sampling if they had reported a concomitant chronic disease that interfered with their normal diet and physical activity, had physical disabilities or were pregnant. In the assessment of physical fitness, adolescents with chronic muscle pain or bone fractures were not able to perform any of the tests (Figure 1). (Lines: 86-90)

CORRECTION IN THE TEXT: A total of 125 (16.2%) adolescents did not perform the fitness tests, most of them declined to participate (n=91), or had otherwise experienced bone/muscle injury (n=18) or had changed schools (n=13) (figure 1). (Methods: lines: 123-125)
5. It is a little unclear on page 10, paragraph 1, line 3 whether the association is negative or positive? Please clarify.

**ANSWER:** We revised this paragraph and clarified e the directions of associations (we kindly refer to comment number 1 for changes in the manuscript).

6. Same page and paragraph, end of line 3: please clarify what analysis is referred to in the sentence “When the analysis was stratified for place of residence, no major differences were noticed between
urban and rural areas”. And also, please clarify on what variables no major differences were noticed.
ANSWER: We clarified the findings of this analysis. (cfr see also comment 1 for changes in the text).

7. In the discussion (paragraph 2, page 13) possible explanations for the not found significant association between physical fitness and blood lipid profile is discussed in a relevant way. However it might be interesting to also include some lines on the fact that physical activity and cardiorespiratory fitness are differently and independently associated to blood lipid profiles? (i.e. ref: Ekelund U, 2007). Maybe some data on physical activity and sedentary levels would have explained some of the associations?
ANSWER: We thank the reviewer for this comment. In the revised version we expanded possible explanations for the no significant associations between blood lipid profile and physical fitness, we stated that physical activity and fitness are separately and independently associated with HDL and triglycerides and we discussed the association between blood lipid profiles and moderate to vigorous physical activity (Lines 327 - 336). Unfortunately, we didn't measure physical activity in our study and we stated the lack of physical activity data as a limitation of our study in line 344.
CORRECTION IN THE TEXT: Another possible explanation for the differences in blood lipid profile among urban and rural adolescents may be the differences in moderate to vigorous physical activity [58], or body fat distribution [59]. Physical activity and fitness have been found independently associated with certain blood lipid levels among children and adolescents [6]. For example, the favorable TG and HDL levels are inversely associated with moderate to vigorous physical activity, independent of time spent sedentary [58] and fitness [6]. In our sample, the time spent on moderate to vigorous physical activity could be longer in urban adolescents compared to rural adolescents because of differences in the availability of sport facilities and organized group sports, detailed earlier in this discussion. In addition, qualitative research performed in adolescents from Cuenca and Nabon has shown that rural adolescents felt an inability to perform physical activity in contrast to the urban adolescents (Van Royen unpublished data). This fact could lead to differences in physical activity levels between urban and rural adolescents, as self-efficacy is an important determinant of physical activity in adolescence [60]. On the other hand, total cholesterol, LDL, HDL and TG also have been associated with fat distribution measured by skin-fold thickness. Lean adolescents, as determined using this measurement system, have been found to have healthier blood lipid
profiles compared to their heavier peers [61]. However, skin-fold thickness was not a parameter measured in the present study. (Lines 325-340)

There are a few limitations of this study. Firstly the cross-sectional nature of this study allows us to only establish associations and not causality. Secondly, we did not measure important variables associated to blood lipids such as physical activity... (Limitations: lines 342-344)

8. As the FITNESSGRAM was used to identify healthy and unhealthy children in the sample it would be appropriate to present these prevalence in table 1 or 2? And it could be very interesting to see some subgroup analyses on the association of these healthy/unhealthy adolescents stratified by residence.

ANSWER: We agree that the result from FITNESSGRAM should be presented in the tables and added them accordingly. We also included these results in the text as follows:

CORRECTION IN THE TEXT: The associations between fitness and BMI are shown in table 2. The interaction in terms of BMI-residence was significant for... and the proportion adolescents who reached the Healthy Fitness Zone. ... When the associations between the fitness tests and BMI were analyzed according to residential location, the results showed that the proportion of adolescents that reach the Healthy Fitness Zone in both urban and rural areas decreased significantly as mean BMI increased...

The interaction terms of residence x physical fitness.... In addition, the association between cholesterol / LDL with the proportion of adolescents who reached the Healthy Fitness Zone was significantly different between urban and rural adolescents (Table 3).

The associations between the physical fitness tests and blood lipid profile were weak (Table 4).... In the rural area, adolescents who reached the Healthy Fitness Zone according to the FITNESSGRAM standards had significantly lower cholesterol and LDL levels. Although, after the Bonferroni correction only the association between cholesterol levels and the adolescents who reached the Healthy Fitness Zone according to the FITNESSGRAM standards remained significant. (Results: lines 224-250)

9. Some limitations on using FITNESSGRAM and a field-test for cardiorespiratory fitness (Eurofit – shuttle run) to express health or not, could be mentioned in the discussion.

ANSWER: A limitation of FITNESSGRAM standards is that the estimation of VO2max from the result of the 20m shuttle run is highly dependent on the equation used. A previous study (Boiarskaia, 2011)
has tested the degree of agreement between various equations used to estimate VO\(_{2\text{max}}\) and the actual the VO\(_{2\text{max}}\). In the present study, we used the equation that shows the highest agreement. In addition, measurements of the 20m shuttle run could be influenced by the temperature and weather conditions during the test. In Cuenca and Nabon, however, the average temperature and altitude are similar. In addition, participant’s motivation, feeling of support and familiarity with the test could also influence the scoring. For this purpose, we adhered to the guidelines for administer the EUROFIT tests. Accordingly, in each school and during test all adolescents were encouraged to do their best and were not allowed to practice the test on beforehand. We also included these limitations in the text

CORRECTION IN THE TEXT: **Measurements of the 20m shuttle run could be influenced by the temperature and weather conditions during the test.** In Cuenca and Nabon, however, the average temperature and altitude are similar. In addition, the estimation of VO\(_{2\text{max}}\) from the FITNESSGRAM standards of the 20m shuttle run is known to vary with the equation used. A previous study [28] has tested the degree of agreement between various equations used to estimate VO\(_{2\text{max}}\) and the actual the VO\(_{2\text{max}}\). In the present study, we used the equation that shows the highest agreement. (Lines: 350-355)

References:

10. **Please provide a few lines on your opinion on external validity/ generalisability of your results in the discussion section.**

ANSWER: We added the generalization of our results in lines 357-360.

CORRECTION IN THE TEXT: **The trial included adolescents from high altitude urban and rural areas of Ecuador that are characterized by mixed mestizo (in urban area) and Amerindian (in rural area) ethnicities [49]. The external validity of our findings is hence limited to urban and rural schools in the regions that share these characteristics [62]**
11. In the perspectives (page 14, last paragraph) it is stated that additional studies are needed to clarify the higher occurrence of unfavorable blood lipids among rural participants. Please suggest which kind of studies that are needed?

ANSWER: We agree that the conclusion should be more specific and in lines 369-372 we added a list of the suggested studies.

CORRECTION IN THE TEXT: Additional studies are needed to clarify the frequent occurrence of unfavorable blood lipid profiles among rural participants. Such studies might explore associations with physical activity levels, body fat distribution, risk factors at early ages, familial hypercholesterolemia and ethnic differences.

Discretionary Revisions:

12. The comparison of physical fitness with adolescents from other countries is very interesting and I wonder if the authors have any plausible explanations on why Ecuadorian adolescents should perform worse than adolescents from other comparable countries? (Page 11 bottom to page 12 top).

ANSWER: We agree and therefore we added some explanations on why Ecuadorian adolescents may have exhibited lower performance than other groups of adolescents. We do acknowledge that these explanations are speculative.

CORRECTION IN THE TEXT: The favorable fitness scores in European as compared to Ecuadorian adolescents may be a reflection of the favorable environmental conditions for physical activity found in Europe [46], as well as a longer tradition of health promotion programs [47], and genetic factors [48, 49]. This hypothesis may be reinforced by the fact that our results were similar when compared to those from Mexican [11] and Colombian [13] studies, which have similar environmental and genetic patterns to those of Ecuador [48]. (Lines: 281-286)

13. In the flow chart, figure 1, it would be good to present the number of students in the number of schools selected (30 schools, n? students) as it switches to number of students in the bottom of the flow chart (there is at “t” too much in the right box “lost during the study”)

ANSWER: we thank the reviewer for the comment since it improves the presentation of figure 1. We added the number of the students on the flowchart (1) and revised the typing error. Please, refer to the comment 4 to see the changes in the figure 1.
Quality of written English: acceptable (but some typing errors occur)

Statistical review: No, the manuscript does not need to be seen by a statistician (statistics are assessed and found appropriate)

Level of interest: An article of importance in its field

Quality of written English: Needs some language corrections before being published

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I declare that I have no competing interest