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High-intensity, but not moderate-intensity, exercise increases post-exercise rate of fat oxidation in type 2 diabetics

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Ref.: Ms. No. JCTRes-D-16-00004
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Dear Prof. Vieira Browne,

Reviewers have now commented on your paper. You will see that they are advising that you revise your manuscript. If you are prepared to undertake the work required, I would be pleased to reconsider my decision.

For your guidance, reviewers' comments are appended below.

If you decide to revise the work, please submit a list of changes or a rebuttal against each point which is being raised when you resubmit your work.

Your revision is due by May 01, 2016.

To submit a revision, go to http://jctres.edmgr.com/ and log in as an Author. You will see a menu item call Submission Needing Revision. You will find your submission record there.

Yours sincerely

Rowan van Golen
Associate Editor
Journal of Clinical and Translational Research

******Reviewers' comments******

Reviewer #1: Well done but needs to reference statements made and make sure they don't lose the reader. More figures would really help the reader keep track of all the results. Several times they mention one thing but then mention another, consistency will also help the reader stay on track throughout the manuscript.

Reviewer #2: The authors have sought to investigate the effects of 20 min bouts of high intensity vs moderate intensity cycling on CHO and fat oxidation during and after the exercise bouts in older participants with type 2 diabetes. While keeping the exercise duration the same, when a bout is performed at the heavy intensity domain (i.e. S120) while the other bout is performed at the moderate intensity domain (i.e. S80) it is expected that the S120 will induce higher absolute oxidations and rates of oxidation of CHO and fat, and it is also expected to induce higher rates of post-exercise fat oxidation (EPOC).
This reviewer would have been more enthusiastic if, for instance, a bout of high intensity interval training was compared with the continuous exercise bout.

Other points to be addressed:
Abstract
L7-8: the background is unclear. Suggest to re-write it for clarity
L18: describe the 'control' condition.
Materials and methods:
P3-4 Sample: how many were on pharmacological regime, and which class of drugs? How many only treated by diet? How many hypertensives (if they were allowed)?
P5: Experimental sessions: It would have been useful to collect lactate samples to confirm exercise intensity domains. Also, blood glucose samples would have been helpful. Specify if you used the same metalyzer as for the MIT for gas analyses.It would have been more appropriate to calculate the relative intensity of the heavy exercise bout based on both, workload at lactate threshold and peak exercise (rather than 120% of LT).
Results:
P8, L50: again, how many were on oral hypoglycemic drugs?

There is additional documentation related to this decision letter. To access the file(s), please click

the link below. You may also login to the system and click the 'View Attachments' link in the Action column.

Authors' rebuttal:

Date: 4-May-2016

Ref. Revised version of the manuscript (Ms. No. JCTRes-D-16-00004): "High-intensity, but not moderate-intensity, exercise increases post-exercise rate of fat oxidation in type 2 diabetics".

Dear Editor,

We would like to thank you for this review process. Below you can find the responses to the comments raised by the reviewers. Also, we have made changes in the manuscript in order to address the reviewer's and editor's suggestions and make it clearer for the readers. Each comment is followed by the respective reply and any eventual change made in the manuscript highlighted in yellow.

Sincerely,

The authors

Reviewer #1:

Well done but needs to reference statements made and make sure they don't lose the reader. More figures would really help the reader keep track of all the results. Several times they mention one thing but then mention another, consistency will also help the reader stay on track throughout the manuscript.

Whenever you are performing human subjects research (especially in a patient population) it requires a large effort. The authors should be commended on completing an arduous study such as this.

1. **Abstract and Manuscript: The word "better" (Abstract, line9 and Manuscript and page 3, line 1) is used in the manuscript when it is not appropriate. The authors should consider an alternative that actual tells the reader why it is better.**

Reply: Dear reviewer, thank you for helping us to improve this manuscript. As suggested, we replace the word "better".

Abstract, line 9: "Aerobic exercise is recommended for glycemic and weight control in type 2 diabetes (T2D), but exercise intensity that increase post-exercise fat oxidation has not been established yet."

Page 3, line 1: "Although moderate-intensity exercise can be beneficial for glycemic control, the optimal exercise intensity for maximize post-exercise fat oxidation in individuals with T2D has not been established yet."

2. **Abstract: The authors haven't mentioned the results of the fat oxidation rates during exercise.**

Reply: Again, we appreciate the effort to improve our manuscript. As requested, the data for fat oxidation was included in the abstract.

"The rate of CHO oxidation during exercise was significantly higher during S120 in relation to S80 and SC (18.2±5.6 *vs.* 9.5±2.7 *vs.* 1.1±0.4 mg·min^{-1}), the absolute rate of fat oxidation was significantly higher in S120 compared to S80 and SC during exercise (13.5±3.3, 9.5±2.2, and 0.7±0.2 mg·min^{-1}, respectively, $p<0.05$)."

3. **Abstract and Manuscript: The authors should consider not making strong statements without providing a reference for backing it up with actual data.**

Reply: Sorry about that. All statements are now accompanied by a reference.

4. **Manuscript: The authors need to remember that with each mention of fat or CHO oxidation they need to be specific to the time that they are referring to, exercise, post exercise or rest. It is easy for the reader to lose track when talking about repeated instances**

Reply: Sorry again. The manuscript was entirely revised in order to meet the request of the reviewer.

5. **Manuscript: What were the participants instructed to do in the 24 hours to each testing session?**

Reply: we appreciate the effort to improve our manuscript.

"All medication was washed-out for 24h prior to the initial screening visit and the three subsequent sessions. The individuals were also asked to avoid physical exercises and alcoholic or caffeinated drinks for 24h prior to each visit to the laboratory."

6. **Manuscript: Why wasn't fasting insulin, insulin resistance, insulin sensitivity, an oral glucose tolerance test or glucose considered to accompany the findings in this manuscript, it would help tell the story more for this patient population.**

Reply: Unfortunately, we were not able to carry out the measurements suggested above. On the other hand, all participants were recruited from a Public Hospital at Taguatinga, and all had been previously assessed in standard tests conducted by endocrinologists and cardiologists. In addition, as suggested by the Expert Committee on the Diagnosis and Diabetes Mellitus [1] classification, the use of diabetes medications is sufficient for the diagnosis of the condition in question.

7. **Manuscript: The authors should clarify oxygen consumption at maximal or peak effort as both are mentioned.**

Reply: Sorry. The correct term is VO_2peak. We carried out corrections throughout the manuscript.

8. **Manuscript: What were the criteria for reaching volitional exhaustion (page 5, line 0)?**

Reply: Sorry for the absence of this information. The below sentence was inserted in the methods section.

"The following criteria were used to determine whether participants achieved maximal effort: respiratory-exchange ratio (RER) ≥1.1, HR> 90% maximum predicted by age and RPE >17 [2]."

9. **Manuscript: Did the participants use a facemask or mouthpiece with nose clip? Was the same used for resting, exercise and post exercise?**

Reply: Sorry about that. The gas collection procedures were conducted using a facemask. As requested, this information was included in the manuscript (page 5).

10. **Manuscript: BP (page 5, line 26) was mentioned in the methods although no results were presented nor was it discussed in the discussion. Also, lactate was not mentioned in the results section even though it was measured throughout. RPE was mentioned in the statistical analysis section but were no results were presented.**

Reply: Sorry about that. All variables cited by the reviewer were used only for monitoring internal workload. However, as requested by reviser, we inserted the blood lactate values, rate-pressure product (SBP × HR) and rate perceived exertion (Table 1).

Table 1. Mean values for VO_2, blood lactate, % maximal HR, and rate-pressure product (SBP × HR) and RPE during the exercise and control condition (CON, 80%LT and 120%LT).

	CON	80%LT	120%LT
VO_2 (mL·kg^{-1}·min^{-1})	2.9 ± 0.4	12.9 ± 2.6[a]	17.6 ± 3.0[a,b]
Blood Lactate (µM)	1.3 ± 0.5	2.8 ± 0.9	5.2 ± 1.5[a]
HRmax (%)	46.5± 6.9	77.5± 7.1	92.6± 11.1
Rate-pressure product	7632.1 ± 1184.3	15803.3 ± 1989.7[a]	17898.0 ± 2723.1[a]
RPE (score)		11.0 ± 1.3	13.0 ± 0.6[b]

HRmax, maximal heart rate; RPE, rating of perceived exertion; SBP, systolic blood pressure; CON, control session; 120%LT, exercise condition at 120% of lactate threshold; 80%LT, exercise condition at 80% of lactate threshold; VO_2, oxygen consumption. [a] $p \leq 0.05$ to CON; [b] $p \leq 0.05$ to 80%LT.

11. **Manuscript: Very little information is provided as to what participants were instructed to do following the exercise sessions for 45 minutes (page 5, section 2.5). Were participants allowed to use the bathroom? Did they read or participate in any activities or were they instructed just to sit or lay?**

Reply: Forgive us again in the absence of this information. Yes, before starting the recovery period was allowed volunteers to go to the bathroom (5 minutes). However, it is noteworthy that none of them used this period. Furthermore, it was not allowed any other activity in that period. We recognize that recent studies have allowed some kind of reading during that period. On the other hand, visual inputs can change positively or negatively the autonomic nervous activity and, in turn, metbolic activity [3–5]. So, therefore, in order to try to isolate the effect of exercise, we do not allow any kind of activity in this period.

The following sentence was inserted to try to better explain what happened during the recovery period.

"In order to try to isolate the effect of exercise on metabolic activity, individuals were prevented from receiving any visual stimulus in the recovery period. Therefore, no activity of reading, for example, was allowed during this period. Thus, individuals were instructed to remain seated until the 45th minute."

12. **Manuscript: Figure 1. may need the X axis labeled as time.**

Reply: Figure was corrected.

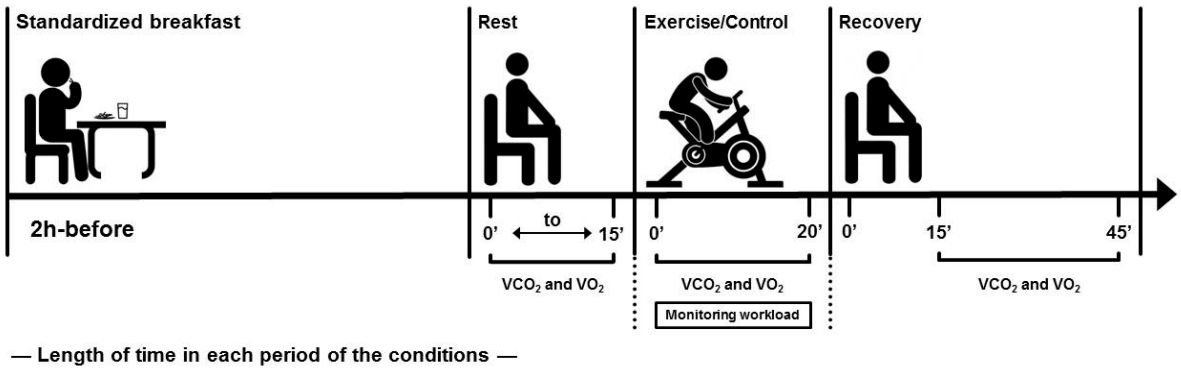

Figure 1. Summary of experimental design.

13. **Manuscript: RER only has an acronym, please define for the reader (page 6, line 60).**

Reply: As requested, it was inserted the definition of RER.

"The following criteria were used to determine whether participants achieved maximal effort: respiratory-exchange ratio (RER) ≥1.1, heart rate > 90% maximum predicted by age and RPE >17 [2]."

14. **Manuscript: The authors should consider more figures (RER, lactate, or other variables at rest, and during both exercise sessions).**

Reply: We agree of the reviewer. Figures with RER and lactate were inserted as follows:

Results

The description of effort of the experimental conditions (80%LT, 120%LT, and CON) is presented in Table 1. VO_2 was significantly higher in 120%LT compared to 80%LT and CON and when comparing 80%LT to CON. Blood lactate was higher only in 120%LT compared to CON. Rate-pressure

product was higher in 120%LT and 80%LT compared to CON. RPE was higher in 120%LT compared to 80%LT, validating that both exercise intensities were different.

In addition, Figure 2 shows the RER and blood lactate at each 5 min during exercise and control conditions. The RER of 80%LT and 120%LT conditions was higher than CON, but 120%LT was even greater than 80%LT in minutes 5th and 10th (Figure 2A). Similarly, blood lactate of 80%LT and 120%LT conditions was higher than CON, but 120%LT was even greater than 80 at all times (5' – 20') (Figure 2B).

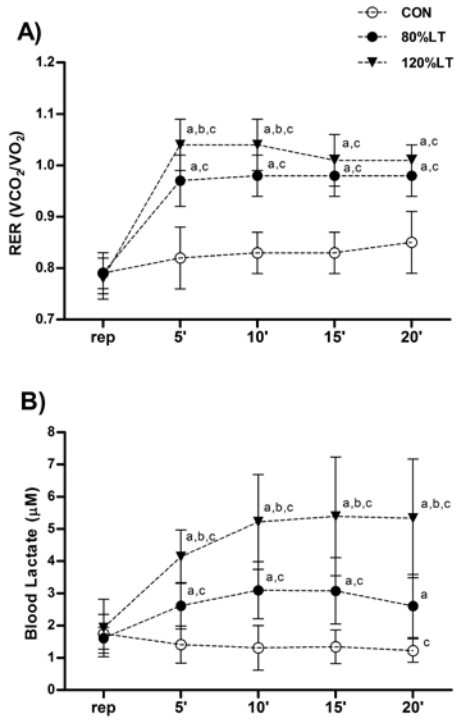

Figure 2. Respiratory-exchange ratio (VCO_2/VO_2) (A) and blood lactate (B) at each 5 min during intervention (5' – 20') for the exercise and control conditions. Values are mean (±SD). CON, control session; 120%LT, exercise at 120% of lactate threshold; 80%LT, exercise at 80% of lactate threshold. [a] $p<0.05$ at the same time to CON; [b] $p<0.05$ at the same time to 80%LT; [c] $p<0.05$ to rest at the same session.

15. Manuscript: How was body composition variables measured (page 4, line 54)?

Reply: Sorry about that. All procedures were described in the methods section.

2.3 Anthropometric Measurements

Body mass index (BMI) was calculated considering the ratio of body mass (Toledo 2096 PP) in kilograms and height in meters (stadiometer SECA® 214, USA) raised to the second power ($kg \cdot m^{-2}$). The percentage body fat was estimated from the technical skinfold, wherein the body density was calculated using the seven folds protocol suggested by Jackson and Pollock [6], collected at each point in rotational sequence, the right side of the body, and logged a mean value of three measurements. The measurements were performed by a single examiner, using a skinfold caliper (Lange, Cambridge Scientific Instruments, Cambridge, Maryland, USA). After calculating the body density, it was converted into fat percentage using the equation proposed by Siri [7].

16. **Manuscript: How was HRmax% measured? During the maximal (peak) exercise test or calculated?**

Reply: HRmax% was measured during incremental test. This information was inserted in manuscript (Methods).

"Maximal heart rate, lactate threshold and VO_2peak were determined during maximal incremental test (MIT)."

17. **Manuscript: Figure 3. Could use more labeling for exercise or recovery on the actual figure and not just in the description.**

Reply: Figure was corrected.

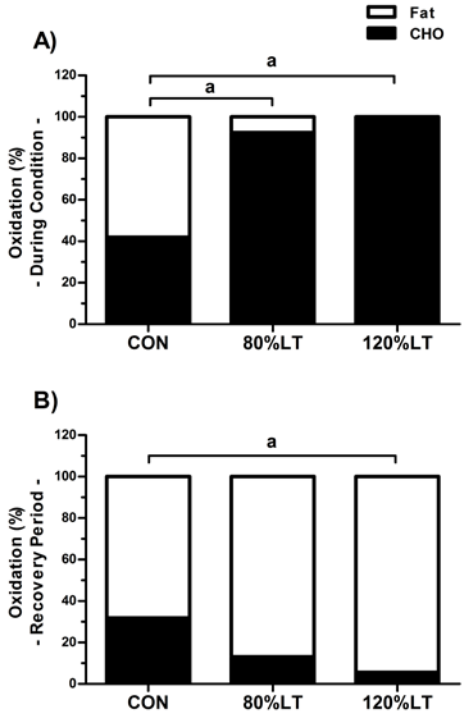

Figure 3. Mean percentage of carbohydrate (CHO) and fat oxidation during exercise and control conditions (A), and at the recovery period (B). CON, control session. 80%LT, exercise condition at 80% of lactate threshold. 120%LT, exercise condition at 120% of lactate threshold. [a] $p \leq 0.05$ *vs.* CON.

18. **Manuscript: The authors discuss that the exercise intermittently may be effective but doesn't provide a reference (page 12, line 57).**

Reply: In fact, it is an inference based on our findings. What is justified, since in both sessions tested was observed positive effects on the oxidation of substrates in the investigated populations.

19. **Manuscript: (Awkward sentence, page 13, line 6) For both intensities, the percentage of carbohydrate used after exercise as energy substrate was significantly higher than a control condition, demonstrating that carbohydrate utilization during exercise is not impaired in T2D individuals, mostly because of the different glucose uptake mechanism induced by muscle contraction.**

Reply: Ok. The sentence was rephrased.

"Maximal exercise and 90% of LT promoted high percentage of carbohydrate utilization than a rest condition, demonstrating that carbohydrate utilization during exercise is not impaired in T2D individuals. In other words, the glucose uptake mechanism independent of insulin appears to remain preserved in this population."

20. **Manuscript: Although indirect calorimetry is the gold standard for estimating energy expenditure, its results can be affected by the high production of non-metabolized CO_2, which induces an increase in ventilatory responses. (page 14, lines 6-11) should have a reference.**

Reply: The reference was inserted as requested.

"Simões HG, Moreira SR, Moffatt RJ, Campbell CSG. [Methods to identify the anaerobic threshold for type-2 diabetic and non-diabetic subjects]. Arq Bras Cardiol 2010; 94: 71–8."

Reviewer #2:

The authors have sought to investigate the effects of 20 min bouts of high intensity vs moderate intensity cycling on CHO and fat oxidation during and after the exercise bouts in older participants with type 2 diabetes. While keeping the exercise duration the same, when a bout is performed at the heavy intensity domain (i.e. S120) while the other bout is performed at the moderate intensity domain (i.e. S80) it is expected that the S120 will induce higher absolute oxidations and rates of oxidation of CHO and fat, and it is also expected to induce higher rates of post-exercise fat oxidation (EPOC).

1. **This reviewer would have been more enthusiastic if, for instance, a bout of high intensity interval training was compared with the continuous exercise bout.**

Reply: We agree with the reviewer. On the other hand, as the available literature on the effects of exercise on oxidation of substrates is still scarce, we consider that, for now, evaluate the continuous exercise relevant enough. However, we encourage further studies to test different models of exercise, including intermittent exercise.

2. **Abstract. L7-8: the background is unclear. Suggest to re-write it for clarity.**

Reply: The Background section was rephrased.

"Background: Aerobic exercise is recommended for glycemic and weight control in type 2 diabetes (T2D), but exercise intensity that increase post-exercise fat oxidation has not been established yet. It is expected that high-intensity exercise induce higher absolute oxidations and rates of oxidation of CHO (during) and fat (after) in normoglycemic, but in hyperglycemic it is unclear."

3. **Abstract. L18: describe the 'control' condition.**

Reply: The control condition was described in the abstract as requested.

"3) 20 minute of control session (CON) – no exercise was performed and the individuals remained seated during the whole time."

4. **Materials and methods. P3-4 Sample: how many were on pharmacological regime, and which class of drugs? How many only treated by diet? How many hypertensives (if they were allowed)?**

Reply: All used one (1) type of pharmacological regime (Sulfonylureas, Metformin, Glibenclamide + Metformin, Glimepiride, Pioglitazone Hydrochloride) and food intake control. Furthermore, four (4) were under use of antidiuretic (Chlorpropamide). That information are in session "methods".

"All individuals were under medical and nutritional treatment, using one (1) oral hypoglycemic medication (Sulfonylureas, Metformin, Glibenclamide + Metformin, Glimepiride, Pioglitazone Hydrochloride) and/or food intake control. Furthermore, four (4) were under use of antidiuretic (Chlorpropamide)."

5. **Materials and methods. P5: Experimental sessions: It would have been useful to collect lactate samples to confirm exercise intensity domains. Also, blood glucose samples would have been helpful. Specify if you used the same metalyzer as for the MIT for gas analyses. It would have been more appropriate to calculate the relative intensity of the heavy exercise bout based on both, workload at lactate threshold and peak exercise rather than 120% of LT.**

Reply: Yes, we use the same gas analyzer for all sessions. We agree with the reviewer. However, due to the lack of studies investigating the effects of exercise on the substrates oxidation rate in diabetic subjects, we consider appropriate test first relative intensities to the anaerobic threshold.

6. **Results. P8, L50: again, how many were on oral hypoglycemic drugs?**

Reply: All subject used 1 (one) hypoglycemic drugs like Sulfonylureas, Metformin, Glibenclamide + Metformin, Glimepiride, Pioglitazone Hydrochloride. Furthermore, four (4) were under use of antidiuretic (Chlorpropamide), but washout of 24-hour was carried out.

References

1. Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Report of the expert committee on the diagnosis and classification of diabetes mellitus. Diabetes Care. 2003;26 Suppl 1: S5–20.
2. American College of Sports Medicine. ACSM's guidelines for exercise testing and prescription. Philadelphia, PA: Lippincott Williams & Wilkins; 2006.
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4. Eisenberg N, Fabes R, Bustamante D, Mathy R, Miller P, Lindholm E. Differentiation of vicariously induced emotional reactions in children. Dev Psychol. 1988;24: 237–46.
5. Kroenke CH, Epel E, Adler N, Bush NR, Obradovic J, Lin J, et al. Autonomic and adrenocortical reactivity and buccal cell telomere length in kindergarten children. Psychosom Med. 2011;73: 533–40. doi:10.1097/PSY.0b013e318229acfc
6. Jackson AS, Pollock ML, Ward A. Generalized equations for predicting body density of women. Med Sci Sports Exerc. 1980;12: 175–81.
7. Siri W. Body composition from fluid spaces and density. Analysis of methods. In: Brozek J, Henschel A, editors. Techniques for Measuring Body Composition. Washington, DC: National Academy of Sciences, National Research Council; 1961. pp. 223–44.

2nd editorial decision:

Date: 4-May-2016
Complete accept: 16-May-2016

Ref.: Ms. No. JCTRes-D-16-00004R1
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Dear Prof. Vieira Browne,

I am pleased to inform you that your manuscript has been accepted for publication in the Journal of Clinical and Translational Research.

Thank you for submitting your work to JCTR.

Kindest regards,

Rowan van Golen
Associate Editor
Journal of Clinical and Translational Research