Author's response to reviews

Title: Extremely short duration high intensity training substantially improves insulin action in young healthy males

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Author's response to reviews: see over
Reviewer 1: Athanasios Jamurtas

General Comments:

1. The authors examined whether there are any differences in the glycemic control between the second and third day following cessation of the training program. This is not listed as one of the purposes of the study and should be clearly defined.
   - **We have now clarified this in the Methods section.**

2. Furthermore, it is not listed in the statistics section that a correlation was run between changes in the biochemical measures and performance.
   - **We have added this.**

3. In addition, the authors should separate the effects of HIT on healthy and insulin resistant individuals.
   - **We have performed our study using sedentary healthy individuals first, as the protocol was immediately applicable to this group and in terms of disease prevention, improving insulin action in young and middle aged healthy subjects is critical important. We believe there is currently insufficient information available to confidently predict whether a similar training program would improve insulin sensitivity to a smaller or larger extent in insulin resistant individuals (we suspect it would provide a larger benefit) and this will require further research.**

Specific Comments:

Abstract

4. P. 2, ln 10. Replace “or” with “and”
   - **We have rewritten the Methods section of the abstract to improve clarity**

Introduction

   - **We have amended this.**

Methods

6. P.5. Indicate the CV values of the biochemical measurements
   - **We have added these values.**

7. P. 6, under time trials: Explain why there were two self paced cycling time trials performed?
   - **In self-paced time-trials a familiarization trial is required to avoid familiarization effects (see Jeukendrup et al., MSSE, 28(2):266-70, 1996). We have clarified this in the revised document.**

8. Furthermore, I do not understand what you mean with the phrase “The linear factor …….rate at 90 rpm”. Was this a submaximal test? I thought that the time trials were an all out efforts. Please elaborate.
   - **To be able to do a self-paced performance trial on a cycle ergometer, the intensity has to increase with increasing pedal frequencies. On an electrically braked ergometer (like the Lode we used) this can be achieved with the linear mode function. The linear factor can be set to ensure all subjects will be able to perform the test at similar pedal frequencies of between ~70-100 rpm. We used information from the VO_{2}max test to calculate the linear factor (L) as \( L = \frac{\text{Power Output (W)}}{\text{rpm}^2} \) to achieve a power output corresponding to 75% of the subject’s maximal power output if the subject cycled at 90 rpm. With higher pedal frequencies higher power outputs were achieved (i.e.: the subjects could pace themselves to achieve the fastest time to complete the 250 kJ time trial).**
   - We have added this information.

10. P. 6. Indicate whether any soreness was felt by the subjects following this type of training.
    - Although we did not measure any indices of muscle soreness following training, no subjects made any complaints about it, and Wingate performance during training sessions was not impaired at the second session compared to the first session.

11. P. 7, ln 1. Indicate sooner the reason behind the performance of the OGTT two or three days after the last training session. It is not revealed until the discussion!
    - We have added this information.

12. P. 7. Indicate what kind of correlation was run.
    - We have added this information.

13. Indicate the accumulated time of an exercise session.
    - We have added this information.

Results
14. P. 8, Last and last but one lines. Present data as (Fig 1C; 0 min: 350+36 μmol.l-1 v 60 min: 255+48 μmol.l-1, P<0.01) and (Fig 1C; 0 min: 290+39 μmol.l-1 v 60 min: 153+17 μmol.l-1, P<0.01).
    - We have amended this.

15. P. 9, In 13-14. “Changes in performance … other changes”. Indicate which other changes
    - We have added this information.

16. P.9, In 17. Include insulin values in the brackets.
    - Peak insulin values have been added.

Discussion
17. The authors should make an attempt to separate the effects of high intensity training on insulin resistant and healthy individuals.
    - See our response to point 3.

18. P. 10, ln 4. Change the word “high” from italics to normal.
    - We have changed this.

19. The authors discuss the role NEFA play on insulin resistance. They should also discuss the possible role the fat intermediates (ceramides, diacylglycerol) might play on insulin resistance and how this factor might be modified by HIT.
    - We have not measured changes in these fat intermediates. Although we would have been happy to add a discussion on their potential role, other reviewers have requested a shorter Discussion with less speculation, so we feel we cannot accommodate this request.

    - We have made this change.

    - Has been amended.

22. The authors should also discuss the limitations of the study.
    - Has been included.
References
23. References appear incomplete (i.e. #2, #10, #15). References #45 and #46 do not appear on the manuscript!!!
   • We have made these changes.

Table 1.
24. Include resting insulin levels.
   • We have removed the table as it did not improve clarity. Resting values for glucose, insulin, and NEFAs are mentioned in the Results section and can be seen in the figure.

25. Move “values …SD” from the bottom of the table into brackets at the end of the title
   • We have removed the table.

Figure
26. Please clarify the legend!!!
   • The legend has been amended.

27. Plasma insulin levels at 120 min are messing
   • We have only measured insulin at 0, 60, and 90 minutes; we have now clarified this in the Methods section.
Reviewer 2: John J Nolan

28. The matching of subjects is questionable.
   - The study design was not intended to have matched experimental and control groups. As we compared pre- and post-training values, the subjects in the HIT-group acted as their own controls and in that sense were perfectly matched.
   - We deemed it highly unlikely that glycemic control would change substantially over a 3-week period without an intervention, and the 2nd group was included to provide some additional test-retest data for glucose AUC to estimate typical 'random' within-subject variation present in OGTT measurements. Therefore we included a no-training group to approximate this issue. We agree that on reflection that the term 'control group' may have been confusing, and we have changed this in the revised document.

29. In Table 1, there is a typographical error for height in the Training group - however they are 9-10 kg heavier than the control group.
   - We have removed the table (see above). Although the mean weight for the no-training group is 9 kg higher than for the HIT-group this difference is not significant as both groups included subjects with a wide range of body-weights. Of course, randomly allocating subjects to one of two groups does not necessarily lead to a good match between the groups for all variables. However, we don't know of any reason why using this group, with the same weight range, for looking at OGTT variation, would be compromised.

30. Fasting insulin data were not provided.
   - We have removed the table.

31. The OGTT results for the control group should also have been provided, for glucose, insulin and NEFA, as in Figure 1.
   - We have amended the Methods and Results sections to more clearly indicate the purpose of the control group.

32. The fasting NEFA at baseline looks of borderline significance (and probably not significant) in Fig 1 C.
   - On the basis of the reviewers comments we have altered the statistical analysis of the data. Using a two-way repeated measures ANOVA with Student Newman Keuls post hoc testing the P-value for the 17% difference between pre- and post-training NEFA levels is only now of borderline significance (P=0.058).

33. The study overall should be reviewed by a statistician.
   - Statistical analyses have now been reviewed and altered.

32. No details are given for diet during the study. There is a strong possibility for a study effect - in that the intervention group are in an active study (with exercise) and the control group have no intervention. Dietary changes could have a significant impact
   - We are aware of the potential for life-style and/or dietary changes to affect the results of this type of study (as discussed by Grossman and Gibala, J Appl Physiol 99:2473-2475, 2005), and therefore we thoroughly instructed our subjects not to change their normal lifestyle or diet throughout the duration of the study. We have stressed this point in the Methods section of the revised document.

33. Insulin sensitivity was measured by the Cederholm model, which is not in common use. More commonly used models such as HOMA, OGIS, QUICKI, Matsuda should be tested for the same data.
   - As HOMA and QUICKI are based on fasting glucose and insulin concentrations these measures were unchanged in this study (fasting levels of glucose and
insulin were unchanged following HIT). The improvement following HIT is in how quickly the blood glucose is being utilized, which makes the Cederholm the ideal sensitivity index. Further, the Cederholm is more specific for peripheral tissue changes which are where any improvements following exercise training are likely to occur. Matsuda is very similar to the Cederholm calculation and also shows a 26% improvement in insulin sensitivity following HIT. Using OGIS will decrease the magnitude of the response as it is a whole body measure and post exercise improvements will be found mainly in the peripheral tissues and will be masked at the whole body level.

34. The Discussion is too long, with too much speculation.
   - We have amended the discussion but feel there is some key literature data that helps us speculate on the underlying mechanisms that may be leading to such dramatic changes in glycemic control. One can’t get away from the fact that the physiological effect we have found is remarkable and this merits discussion within the context of the vast literature. Overall the manuscript is now concise.

35. Much of the literature cited is out of date. Many recent relevant studies have not been cited.
   - We don’t recognize the issue the reviewer refers too and feel this might reflect the use of the term “high intensity training”. Our study is a new model and not aerobic in nature (during the protocol).
   - Many recent studies use the terms “high intensity training” when in fact they are referring to simply conventional albeit intense, aerobic training. This is vastly different from our study (in terms of duration and calorie consumption). Furthermore, if the reviewer could specify which of the out-of-date studies we cited have been performed in a less than optimal fashion we will be happy to remove them from the manuscript. We have mostly limited comparisons with the literature to studies using OGTTs to evaluate the effects of training, and omitted studies using alternative measures like euglycaemic hyperinsulinaemic clamps, as results of these studies would be difficult to compare with ours (a pharmacological hyperinsulinaemic clamp is non-physiological and unlikely to reflect a situation relevant for most insulin resistant subjects)
Reviewer 3: LABROS S SIDOSSIS

36. In the title, abstract, and everywhere else in the manuscript, the authors should make clear that they refer to “interval” exercise.
   • We have made these changes.

Abstract, P2
36. l.11: A second time trial? When was the first done? Refer to that along with the baseline VO2max test.
   • We have changed this section to avoid confusion.

37. l.17: low case p-value
   • We have checked case of p-values for consistency throughout the manuscript.

38. Do the authors really believe that this high-intensity exercise regime could be applied to sedentary populations or the obese and insulin-resistant subjects? I mean, 4-6 Wingate tests with 4 min of rest in between?
   • Yes, we believe that some form of HIT will be useful for the subjects mentioned. Indeed, we know that Gibala et al have recently completed a study in middle-aged subjects without any problems. Although it is fair to say that for many sedentary individuals and/or patients the full protocol may be too strenuous to embark on, we show that only 2 weeks of training consisting of a total of 15 minutes of exercise is sufficient to substantially improve glycemic control in sedentary healthy individuals. Thus, this type of training is clearly very potent. It is, therefore, not unlikely that individuals with poor glycemic control may benefit from similar training programs involving fewer or less strenuous sprints, perhaps performed over several more weeks.
   • We would also like to point out that we make the point that as a tool for prevention in young and healthy middle aged subjects, this approach may be ideal, as it is time efficient. Preventing the induction of insulin resistance in 20-40 yr olds is as important as treating the later stages of the disease.

Introduction
39. P3, l.10-12: “Furthermore, as we do not understand the precise mechanisms which link physical activity and a reduced risk of developing CVD or T2D the scientific basis for current health guides can be challenged[4].” – First, add a space between the end of the sentence and the reference and delete the space in between the reference and the period. My main concern with this sentence is that it is not necessary to understand the mechanisms linking exercise to reduced disease risk for making recommendations; knowing how much exercise, what kind, what frequency, etc., affects morbidity and mortality usually suffices. The mechanisms between these relationships are good to know, but certainly not a prerequisite.
   • In principal we agree with the reviewer and we have altered the concerning sentence to take this discussion into account.
   • It would be good to have a thorough understanding of the mechanisms linking exercise to reduced disease risk, so that one can base exercise recommendations on the few training programs which have been tested. If we can identify biomarkers that when measured tell us what type of training to give a subject, that would be even better. Typically such a biomarker will relate to a molecular measure. Our current findings demonstrate how little we know about which exercise programs to prescribe. Knowledge of mechanisms is not a prerequisite to prescribe just any type of exercise, but in our opinion it is going to be useful to prescribe the optimal type of exercise for any individual.

40. P3, l.20: ref.7 does not compare different intensities, ref.8 found what the authors assert only in obese but not in diabetic patients, ref.9 did find what the authors assert, but it should be taken into account here that the higher intensity training was also of higher energy expenditure than the
low intensity training. Braun (J Appl Physiol 1995;78:300) found no effect of intensity provided that total energy expenditure of training be the same. Hence only the study by Kang (ref.6) and only in obese subjects supports a key role for intensity independently of duration/energy expenditure. The authors need to revise this sentence.

- **Reference #7 was incorrect thanks for spotting this; we have replaced it with the correct reference. Furthermore we have revised the sentence to reflect the uncertainty about the effects of intensity on glycemic control.**

**Methods**

41. P4, subjects: how did you randomize subjects in a group of 16 and a group of 9, instead of two approximately equal groups? Where there any dropouts from the control group? Please explain.

- **Please see our response to comment 28.**

42. P6, time trials: why did you choose the fastest time out of the two trials, and not the mean of the two which would be more accurate anyway?

- **Please see our response to comment 7. The first trial was used to familiarize the subjects with the protocol, as the first time round it can be difficult to perform the time trial at the optimal intensity throughout the trial (it was self-paced). In support of this, 19 out of our 25 subjects performed better in the 2nd trial. According to Jeukendrup et al. (1996), a single familiarization trial should be sufficient to prevent any further familiarization effects. To use the mean of the two trials would mean we would get a poorer estimate of the subjects’ pre-training performance than by using the better of the two trials.**

43. P7, post-training assessment: why was the second OGTT performed either 2 or 3 days later? How many subjects in each case?

- **We have rewritten this section to improve clarity.**

45. P7, post-training assessment: why did the authors not perform a post-training VO2max test? This would inform about the efficacy of training much better than the self-selected time trials.

- **Burgomaster et al. (J Appl Physiol 100:2041-2047, 2006) have previously demonstrated that this type of training does not improve aerobic capacity, whereas aerobic performance does improve (we have also recently shown that these endpoints are easy to dissociate)

- **We included the time trials to demonstrate that our program resulted in similar adaptations to those observed in the studies by Burgomaster et al.**

46. P7, Calculations and statistical analysis: did the authors consider the possibility that OGTT-derived indices of insulin sensitivity are not optimized for higher-than-normal values? See Niakaris (J Sports Sci 2005;23:1065) and references therein.

- **The subjects used in our study did not have higher than normal baseline insulin sensitivity, and values for VO2peak were similar to those in the control group in the reference provided. Moreover, by comparing pre- to post-training values our subjects acted as their own controls, so any bias in the calculation should be negated.**

47. P7, Calculations and statistical analysis: the authors should analyze their data with two way ANOVA for repeated measures, to include the between-subject factor (training vs control). Unless they assume that responses in the training and control groups were different (i.e. significant interaction) a priori and thus only use paired analysis?

- **Please see our response to comment 28. The control group was not a control for the exercise training study but rather a control for variability in OGTT-response over time. The training group acted as their own controls, as we compared pre-to
post-training changes. We have probably used the term control in a clumsy manner.

Results
48. P7-9, glucose, insulin and NEFA responses: include a statement that fasting plasma glucose and insulin concentrations were not affected by training (from what I can tell from Figure 1, at least), like you do for NEFA.
   - We have made this change.

49. Also, report insulin response to OGTT for the control group.
   - Neither the glucose nor NEFA AUC changed over the 2 weeks in the control group. Therefore it is unlikely that the insulin AUC will have changed but we do not have this data. The measurement of NEFAs and glucose allows us to be pretty confident that the changes observed in the training group are as a result of training and not down to fluctuations in OGTT response.

50. And, since fasting NEFA were different, calculate the “incremental” (“decremental” in this case) AUC and compare this pre and post to document whether NEFA were reduced “to a greater extent” after training.
   - Incremental NEFA AUC has been added. The magnitude of the drop is similar despite a smaller increase in insulin post-training. This suggests a training-induced increase in adipocytes insulin sensitivity.

51. P9, physiological considerations: what about insulin response 2 vs. 3 days after training? By “improvement” you mean reduction? In this sense, it is interesting that the responses after 3 days were somewhat further improved than after 2 days.
   - Yes, we have changed ‘improvement’ to reduction. The difference between 2 days and 3 days was not significant, but the fact that the response after 3 days was not lower was encouraging as it suggests that we are not dealing with an acute effect of the last training session rather than a training effect.

52. Table 1: Height for the training group is 1.3 meters?
   - We have removed the table.

53. Legend to Figure 1: use just one symbol for differences between time-points and one for differences between pre/post, P<0.05.
   - This has been amended.

54. Figure 1: Shouldn’t the units for AUC (all three subpanels) be mol x min / liter? Also, y-axis on top panels says “concentration” while the other two don’t. The legend to bottom panel doesn’t show the “pre/post” indication (probably covered under the AUC subpanel).
   - This has been amended.

55. I would like to see the same figure with data from the control group – add Figure 2.
   - We have amended the Methods and Results sections to more clearly indicate the purpose of the control group.

Discussion
56. P10, first half of second paragraph: A single bout of exercise affects insulin sensitivity for at least 48 hours into recovery (see the review by Magkos & Sidossis. European Endocrinology 2008;4:22-25). Hence it is not clear what the contribution of the last bout of training is in this study, where OGTT was performed 2 and 3 days after the last bout.
   - Unfortunately we don’t have access to this review article. We do not believe that we are seeing the effect of the last bout of exercise as there is no difference between the 48h and 72h OGTT response. This suggests that the response of an
acute bout of HIT will last less than 48h, or much longer than 72h. The latter possibility seems unlikely.

57. And, your ref. 32 (Hughes) found a reduction in glucose (but not insulin) response to OGTT given 72 hours after the last bout of training, so your statement that glucose AUC is reduced in previous studies only when assessed within 24 hours is not entirely correct. The authors should modify this section accordingly, and perhaps consider subject characteristics (e.g. lean or obese or insulin-resistant, etc.) in these comparisons.
   • This has been amended.

58. P10, second half of second paragraph: How did you calculate the energy expenditure of the training program? It seems to me that 45 + 55 = 100 kcal for a total of 30 Wingate tests, i.e., ~3 kcal per Wingate test (!), is extremely low.
   • This was, indeed written incorrectly, sorry. The external work was equivalent to ~3.5 kcal per test (average power output of just under 500 W), but with an estimated efficiency of ~20% this should have produced values of ~225 kcal for week 1 and ~275 kcal for week 2. We have changed this in the revised document.

59. P11, last line of second paragraph: what other mechanisms relevant to HIT may be responsible? You speculate on this in the following paragraph (glycogen etc.), but the study by Hughes (ref. 32) involves continuous aerobic exercise, not HIT, and they just failed to find a correlation between the increase in GLUT4 and the increase in insulin-mediated glucose disposal; yet both increased.
   • This reference demonstrates that it is more than GLUT4 concentration that is important for insulin sensitivity in the muscle (of course we know this from other studies where flux is regulated down-stream). This reference is important in light of the finding of increased GLUT4 by Gibala following HIT. We have now added a more speculative section on the role of insulin signaling proteins as well as glycogen turnover, but have kept it brief to avoid excessive speculation.

60. P12, second paragraph: The authors suggest that the lowering of fasting plasma NEFA from 350 to 290 umol/l in this study could be mediating the observed responses to the OGTT, and cite studies with exogenously-induced suppression (acipimox) or increase (intralipid) in plasma NEFA concentration to support their assertion. However in the latter cases, changes in NEFA were extreme (e.g., ~75% decrease in ref. 40, and 5-fold increase in ref. 41) and thus not comparable to the 17% decrease in fasting plasma NEFA concentration in this study.
   • In the interest of being concise but complete we commented on the change in plasma NEFA only as a possible mechanism. This is a popular theory for explaining insulin resistance and therefore we only proposed a possible link between NEFA concentration and insulin sensitivity but felt unable to fully discuss the role of NEFA in insulin resistance. We agree, that reducing from a high level down to ~300 umol/l is more likely to have a large effect than the small change we observed, however the long term benefit of this reduction cannot be ignored...

61. Also, along with ref. 42, the authors should consider many other studies (e.g., Horowitz, Am J Physiol Endocrinol Metab 2000;279:E348 - Sial, Am J Physiol 1998;274:E785) that found no significant changes in fasting NEFA concentration and rate of appearance in plasma 36-72 hours after the last training session. The authors should revise this paragraph to reflect these uncertainties.
   • Has been amended.

62. Minor Essential Revisions
   Abstract, P2
   l.17: low case p-value
   • We have checked case of p-values for consistency throughout the manuscript.
Introduction
63. P4, l.2: change “aimed” to “aiming”
   • We have added commas to improve clarity.

Discussion
64. P10, l.3: delete “the” before “low compliance”
   • We have made this change.

65. P11, l.1: change “observe” to “observed”
   • We have made this change.

References
66. 1 & 10 – Association AD is American Diabetes Association? (Insert a comma after “American Diabetes Association” in your reference manager software so that it formats correctly!). 2 – Association AH is American Heart Association? “In” where? 4 – citation details are missing. 15 – JAMA, not Jama. 16 – Abbreviate journal name.
   • We have made these changes.
Reviewer 4: Heikki Kainulainen

Minor essential revisions:
67. Page 5, NEFA determination: The authors should increase the number of replicates used in the NEFA assay.
   - We measured NEFA levels in duplicate for each sample. We believe that with a CV of 8% for the used assay this should give reasonable confidence that we have made an accurate determination. We are not able to carry out further analysis (which would be subject to additional storage time issues).

Discretionary revisions:
68. Introduction, first sentences: The authors present data of the prevalence of T2D and economical burden in USA. Maybe it would be more relevant to present world-wide data.
   - We would, but current world-wide data was hard to find. The type II diabetes epidemic is currently still discussed in the context of the Western world, and data for the USA are provided as representative values. We do agree that it would be better to have detailed data from all major countries.