PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Acute Effects of Breaking Up Prolonged Sitting on Fatigue and Cognition: A Pilot Study
AUTHORS	Wennberg, Patrik; Boraxbekk, Carl-Johan; Wheeler, Michael; Howard, Bethany; Dempsey, Paddy; Lambert, Gavin; Eikelis, Nina; Larsen, Robyn; Sethi, Parneet; Occleston, Jessica; Hernestål-Boman, Jenny; Ellis, Kathryn; Owen, Neville; Dunstan, David

VERSION 1 - REVIEW

REVIEWER	Stephanie Bonn Karolinska Institutet, Department of Medical Epidemiology and Biostatistics, Sweden
REVIEW RETURNED	14-Sep-2015

GENERAL COMMENTS	General comment This randomized two-condition crossover trail is, despite the main limitation of a small sample size, a well performed and interesting study that adds to the research field and our understanding of how prolonged sitting and breaking up such behavior may affect health. It is an important topic as sitting time and sedentary behaviors are increasingly common in society today. Please find some specific minor comments listed below. Minor comments 1. Abstract - Incomplete sentence on line 35. 2. What is the rationale for only including overweight and obese subjects in the study? 3. Methods section, second paragraph (Participants). Although the authors mention that recruitment of study participants was made from the general community it is not clear exactly how this recruitment was done. By public advertisement and self-selection of participants? Please clarify briefly. 4. I would appreciate if authors could include a short description of how raw accelerometer and inclinometer data was analyzed to generate variables of MVPA and sitting time.

REVIEWER	Ann Swartz University of Wisconsin-Milwaukee
REVIEW RETURNED	21-Sep-2015

GENERAL COMMENTS	REview Checklist #2: There is an incomplete sentence in the abstract (See below). #4: Information on the accerometer data processing is missing (see below). #6, 9, 10, 11: see below Overall Comments

	This study compares the acute effects of a short-bout physical activity intervention used to break up prolonged sitting with an uninterrupted sitting bout on self-reported fatigue, cognition, and neuroendocrine and cardio-metabolic biomarkers. This is an important question that will assist scientists in better understanding the detriments of prolonged sitting. The randomized design is a strength of this study. However, there are a number of methodological and data presentation issues that dampen the reviewers enthusiasm. This manuscript includes as number of outcomes that are not all addressed throughout the manuscript, which detracts from the main message of the paper. Abstract • The end of the “Interventions” section is not complete. “Fatigue and cognitive assessments, and” • It is not clear from the abstract whether both conditions were completed on the same day or different days. Strength and Limitations of this study • Add an “s” to “Strength” in the title of this section • Beneficial effects on glucose metabolism is mentioned here, but not in the abstract, and not presented in the results section of the paper. Further, the discussion mentions a lack of change in glucose levels. Since this is not the primary aim of the study, consider removing and replacing with new findings from this study. • The fourth bullet is not clear. Introduction • The addition of “fatigue” at the end of paragraph 1 seems like an add-on. It is not overtly clear why it was included at the end of the paragraph when the paragraph focuses on glucose and cognition. • It is important that the authors specify whether they are discussing cognitive fatigue, physical fatigue, or a more generalized fatigue. • The introduction could be strengthened to provide a stronger rationale for this study. For instance, the discussion of physical activity and cognition (paragraph 2 of introduction) needs to be related back to sedentary behavior, not solely focus on physical activity. Methods • Were there any participants who reported sitting for >5h/d or more MVPA than 150 min/wk, but objective data did not confirm? If so, how were these participants dealt with? From the data in table 1 it appears that these inclusion criteria were not met (MVPA averaged 32.7 minutes per day). • Page 6, lines 27-42. Please provide the reference for the published study on which you based your power calculations. • Page 8, lines 39-46. Please expand on how many participants visited the toilet (and how many times each) during the seated condition. This may be significant due to the sit-to-stand transitions and walking involved. Were there any differences in outcome measures between those who went to the toilet and those who did not? • Page 8, lines 46-52. Provide a rationale for the 3 minute LPA every 30 minutes intervention. Previous research has shown 2 minutes every 20 minutes to be effective. • Page 9, lines 3-5. The RPE data would fit better in the results section rather than the methods. Consider moving. • Page 11, lines 3-18. It is not clear why venous glucose was not measured, since it was drawn, and capillary finger pricks were used instead. Additionally, why use the CGMS when a cannula was used- why not draw blood from one source instead of three? Provide references supporting these method selections, and the superiority of the CGMS and capillary samples versus venous.

	• A Description of data analysis from the accelerometer and inclinometer is missing. Further, definitions of MVPA are missing (count cut point, bouts of 10 minutes or more, etc.). Results • Table 1. Did the authors rerun analyses with those individuals who met PA and SB inclusion criteria by objective assessment? If not, the authors are encouraged to do so. • Table 1. Why did the authors only report blood pressure medication use, why not also report glucose medications, since glucose was measured? Other medications of interest? • Page 12, lines 53-58. The following statement is not clear: "Physical activity levels did not significantly differ in the two days leading up to each experimental condition (sedentary, 32.6 • } 27.6 min/day, vs. active 32.5 □} 30.8 min/day; p=0.99)." Was there an active and sedentary group? Please clarify. • Was this study sufficiently powered? It would be useful to the reader if the authors would report the power of their results either in the results section, or in the discussion limitations section of the paper (page 17, lines 29-33). • Glucose values or changes in the glucose values are not reported in the results section. Discussion • Time effects on DOPA and DHPG are discussed in paragraph 3, but never revealed in the results section. • Page 15, last paragraph. Glucose results are discussed, but never revealed in the results section. • The discussions related to fatigue and cognition are well developed.

REVIEWER	Alan Batterham Teesside University, UK.
REVIEW RETURNED	23-Oct-2015

GENERAL COMMENTS	This paper reports the acute effects of breaking up sedentary time on measures of fatigue and cognition. The protocol is interesting and I thank you for the opportunity to read and review the manuscript. I have a number of comments/ concerns, outlined below, for the authors to consider. 1. The article title is a little misleading. First, with this sample size, I believe this is really a pilot study, and it should be explicitly labelled as such. Second, the title should emphasise that the effects are acute. 2. Abstract, line 35. "Fatigue and cognitive assessments, and.." The sentence just ends suddenly. Please fix. 3. Abstract, Results. Please report results such that the reader has some sense of the magnitude of the effects and their uncertainty – e.g. mean differences in the changes between conditions and confidence intervals. Just stating P=0.024 and 0.038 for the interaction is not very informative. 4. The study has limited impact, as the data are derived from a single bout of sedentary/ interrupted sedentary time. It is not possible to extrapolate these very acute effects to multiple days/ weeks/ months in the work environment. 5. The statement in the first bullet point under "Strengths and

	limitations of the study" (beneficial effects on glucose metabolism) does not appear to be a finding from the current study, but rather a general statement from the literature. This statement is therefore misleading. 6. The participant characteristics lack detail. Were these participants accustomed to long uninterrupted periods of sedentary time? The eligibility criterion is simply self-report of >5 hours/day of sitting, but we do not know whether the participants habitually sit for 7 hours uninterrupted. If not, as I suspect, then it is perhaps not surprising that they report greater 'fatigue' in the enforced continuous sitting condition. Please comment. 7. Importantly, given that it was the only variable for which you claim "significant" effects, "Fatigue" is not listed as an outcome in your clinical trial registration (ACTRN12613000137796). Item 24 of the CONSORT checklist is left blank, so I assume that no protocol is available in the public domain. Please clarify at precisely what stage did the fatigue VAS measure become the primary outcome. This is critical, as the emphasis of the report and its conclusions is on activity breaks as a fatigue countermeasure. 8. The sample size estimation section is flawed and, in any event, I think it is clear that this study is really a pilot. You state that the study was powered on glucose outcomes, as no information was available on the effect of sedentary behaviour on cognitive outcomes. However, the latter information is not required. What is needed is a sense of the minimum clinically/ practically important difference for the primary outcome and an idea of its variability and reliability. Moreover, the value that you define as a "clinically meaningful" difference in the change for glucose AUC is not provided, so the sample size estimations cannot be reproduced. If the study is properly labelled as a pilot then no formal sample size estimations are required. 9. Discussion: "We examined the effects of light-intensity walking breaks on acute fatigue, but the results may also have implications for persistent fatigue considering the increasing time spent sedentary in many countries [35], and the high prevalence of persistent fatigue [36]." Even with the use of the word "may", I am not convinced that the data/ literature allow you to make this claim. 10. Related to point 3, above, you state repeatedly that the sample size was likely too small to detect effects as statistically significant, but there is no indication of the size of the change that you would regard as clinically/ practically important.

VERSION 1 – AUTHOR RESPONSE

1.

We have removed text in the methods section relating to an inclinometer device, which appears here incorrectly as no inclinometer data is reported. Corrected text shown below.

(Methods: Diet and physical activity subsection, line 9)

Sitting time and MVPA were assessed with an accelerometer, Actigraph GT3X+ (ActiGraph LLC, Pensacola, FL, USA) which was worn on the hip during waking hours. The participants wore the accelerometer commencing from the familiarization visit until the end of the second experimental day. Wear time and activity type, duration, and intensity undertaken during any non-wear periods were recorded in activity diaries.

MVPA and sitting time were assessed with an accelerometer, Actigraph GT3X+ (ActiGraph LLC, Pensacola, FL, USA).

Removed:
'and sitting time with an inclinometer, ActivPAL3 (Pal Technologies Ltd., Glasgow, UK), in the 48 h prior to the first experimental condition and for the washout period.'

2.

A mistake was detected in how habitual moderate-to-vigorous physical activity and habitual sitting time were calculated. The incorrect values included the 48 hour restricted period in the analyses which was not habitual. The corrections changed the values slightly but do not change the conclusion. The original value for habitual moderate-to-vigorous physical activity, [32.7 (28.7)] and habitual sitting time, [10.0(1.8)] and the original values for moderate-to-vigorous physical activity during the 48 hour restricted period [32.6(27.6) before the sedentary condition, vs. 32.5(30.8) before the active condition; p=0.99] have been have been replaced with the values highlighted in yellow.

Table 1. Characteristics of the 19 participants in the study (10 men and 9 women).

BMI (kg/m^2)	31.5 (4.7)
Waist circumference (cm)	105.2 (12.4)
Completed university degree*	78.9
Taking antihypertensive medication*	47.4
Habitual moderate-to-vigorous physical activity (minutes/day)**	35.80 (30.86)
Habitual sitting time (h/day)**	9.82 (2.19)
Sleep time (h/night)*	7.4 (0.8)

Data are % or mean (SD).
*Assessed using self-reports.
**Assessed using activity monitors during the wash-out period between the two experimental days.

(Introduction: third paragraph, line 12)

The study had an explorative approach and a small sample size and was conducted as an evaluation of technical procedures and logistic feasibility of a full-scale study (clinical trial registration ACTRN12614000737639).

(Results: first paragraph, line 4)

Levels of MVPA in the 48 hours prior to each experimental condition did not differ significantly (36.29 ± 29.20 min/day before the sedentary condition vs. 30.18 ± 42.16 min/day before the active condition; p=0.607).

Further revisions of the text based on the relabelling of the study as a pilot study:

(Abstract: Conclusions subsection)
Fatigue levels corresponded with heart rate and neuroendocrine biomarker changes in uninterrupted sitting in this pilot study.

(Strengths and limitations: third bullet point)

- Given this is a pilot study, the sample size may have limited the ability to detect an effect from breaking up sitting in several of the outcome measures.

(Discussion: eighth paragraph, line 1)
First, given this is a pilot study, it was likely underpowered to detect an effect in cognitive performance (as well as several of the potential mediators).

(Discussion: ninth paragraph, line 8)
Thus, the role that reduced sedentary behaviour and walking breaks may play in the prevention and/or treatment of fatigue warrants further investigation in a full-scale study.

The following sentence has been added as a justification of the sample size:

(Methods: Participants, line 10)

In coherence with a previous trial with similar experimental design [1], 19 participants were recruited.

Reviewer #1

1.1. *Abstract - Incomplete sentence on line 35.*

Thank you for pointing this out.

(Abstract: “Interventions:”)

The incomplete sentence “Fatigue and cognitive assessments, and” has been erased.

1.2. *What is the rationale for only including overweight and obese subjects in the study?*

The inclusion of only overweight and obese subjects in the study was based on two distinct rationales. Individuals with overweight and obesity are one population group considered to be most susceptible to sedentary behaviour and its deleterious metabolic correlates (Dunstan DW, et al. The rising prevalence of diabetes and impaired glucose tolerance: the Australian Diabetes, Obesity and Lifestyle Study. Diabetes Care. 2002;25(5):829-34). In designing the current study we wanted to build on our experience from a recent experimental trial in overweight and obese adults, showing lowered postprandial glucose and insulin concentrations from regularly interrupting sitting time with short bouts of either light- or moderate-intensity walking when compared with prolonged sitting (Dunstan DW, et al. Breaking up prolonged sitting reduces postprandial glucose and insulin responses. Diabetes Care. 2012;35(5):976-83). As we wanted to limit the changes concerning study participants and study conditions from the previous to the current trial, we chose to include participants with similar

characteristics as in the previous trial. Also, considering the possibility of different acute response to regularly interrupting sitting time in individuals with different levels of adiposity and the limited number of participants (which constrains the possibilities for subgroup analysis), we saw an advantage in recruiting a group of participants that was not too heterogeneous regarding BMI.

1.3. *Methods section, second paragraph (Participants). Although the authors mention that recruitment of study participants was made from the general community it is not clear exactly how this recruitment was done. By public advertisement and self-selection of participants? Please clarify briefly.*

Participants were recruited through the use of study fliers and posters distributed throughout the Alfred Hospital and Baker IDI Heart and Diabetes Institute and affiliated clinics in Melbourne, Australia. Participants who had participated in previous studies or had registered their expression of interest in future studies were also contacted. We have added this text:

(Methods: Participants subsection)

Participants were recruited from the general community between May and August 2013 through the use of study fliers and posters or were contacted based on their previously registered expression of interest in future studies (Supplemental Figure).

1.4. *I would appreciate if authors could include a short description of how raw accelerometer and inclinometer data was analyzed to generate variables of MVPA and sitting time.*

Description added for handling of raw data.

(Methods: Diet and physical activity subsection, line 9)

Accelerometer data were downloaded using ActiLife 6.5.4 software and summarized using SAS 9.4 for each of the habitual days prior to the condition. Non-wear periods were deleted for analyses; these were periods with at least 60 minutes of zero counts per minute (cpm), allowing for up to two consecutive, one minute interruptions (count values between 1–49 cpm) per non-wear period [17]. Activity counts were categorised as sedentary (<100 cpm; predominantly sitting) [18], light-intensity activity (100-1951 cpm; typically gentle walking) [19], or MVPA (≥1952 cpm; typically at least brisk walking) [19]. Average of sitting time and MVPA were calculated for each individual across valid days (days where the monitor was worn for at least 600 mins).

New references

17. Winkler EA, Gardiner PA, Clark BK, et al. Identifying sedentary time using automated estimates of accelerometer wear time. Br J Sports Med 2012;**46**(6):436-42.
18. Healy GN, Dunstan DW, Salmon J, et al. Objectively measured light-intensity physical activity is independently associated with 2-h plasma glucose. Diabetes Care 2007;**30**(6):1384-9.
19. Freedson PS, Melanson E, Sirard J. Calibration of the Computer Science and Applications, Inc. accelerometer. Med Sci Sports Exerc 1998;**30**(5):777-81.

Reviewer #2

Abstract

2.1. *The end of the "Interventions" section is not complete. "Fatigue and cognitive assessments, and"*

Thank you for pointing this out (see revision in ***1.1.*** above).

2.2. *It is not clear from the abstract whether both conditions were completed on the same day or different days.*

Thank you for this comment. We have added this clarification:

(Abstract: Interventions subsection)

After an initial 2h period seated, participants consumed a meal-replacement beverage and completed (on two days separated by a 6 day wash-out period) each condition over the next 5h: uninterrupted sitting (sedentary condition) or sitting with 3min bouts of light-intensity walking every 30 min (active condition).

Strength and Limitations of this study
2.3. *Add an "s" to "Strength" in the title of this section*
2.4. *Beneficial effects on glucose metabolism is mentioned here, but not in the abstract, and not presented in the results section of the paper. Further, the discussion mentions a lack of change in glucose levels. Since this is not the primary aim of the study, consider removing and replacing with new findings from this study.*
2.5. *The fourth bullet is not clear.*

Thanks for these suggestions and comments. We realise that the first bullet point may be misleading and that the fourth bullet is unclear. We have made the following changes:

(Strengths and limitations of this study: Bullet points)

An "s" has been added to "Strength" in the title.
The first bullet point has been removed and replaced by a second bullet point:

- A randomised crossover study design was used.

The third bullet point has been rephrased:

- Given this is a pilot study, the sample size may have limited the ability to detect an effect from breaking up sitting in several of the outcome measures.

The fourth bullet point has been rephrased:

- The longer-term relevance of acute effects of breaking up prolonged sitting are uncertain.

Introduction
2.6. *The addition of "fatigue" at the end of paragraph 1 seems like an add-on. It is not overtly clear why it was included at the end of the paragraph when the paragraph focuses on glucose and cognition.*

Thank you for pointing this out. We agree that "fatigue" comes in a bit abrupt in the end of the paragraph. The word "fatigue" has been removed from the Introduction (first paragraph, line 13).

2.7. *It is important that the authors specify whether they are discussing cognitive fatigue, physical fatigue, or a more generalized fatigue.*

Thank you for this excellent suggestion. There are no clear definitions of fatigue, physical fatigue and mental fatigue, thus we realise the need to guide the reader and suggest the term 'general mental fatigue' to be used to this effect.

(Introduction: second paragraph, line 1)
Aside from the metabolic benefits, physical activity may mediate several putative pathways involved in general mental fatigue and cognition.

2.8. *The introduction could be strengthened to provide a stronger rationale for this study. For instance, the discussion of physical activity and cognition (paragraph 2 of introduction) needs to be related back to sedentary behavior, not solely focus on physical activity.*

We appreciate this suggestion from the reviewer. We have added the following sentence in order to strengthen the rationale for the study (and to relate back to sedentary behaviour):

(Introduction: second paragraph, line 9)

Some sedentary behaviours, such as TV viewing, have been linked to adverse long-term effects such as detrimental cognitive development in early childhood [11] and poorer cognitive function in older adults [12], but the mediating pathways are not well understood.

New references

11. Carson V, Kuzik N, Hunter S, et al. Systematic review of sedentary behavior and cognitive development in early childhood. Prev Med 2015;**78**:115-22.
12. Hamer M, Stamatakis E. Prospective study of sedentary behavior, risk of depression, and cognitive impairment. Med Sci Sports Exerc 2014;**46**(4):718-23.

Methods

2.9. *Were there any participants who reported sitting for >5h/d or more MVPA than 150 min/wk, but objective data did not confirm? If so, how were these participants dealt with? From the data in table 1 it appears that these inclusion criteria were not met (MVPA averaged 32.7 minutes per day).*

Yes, our objective data indicated that our participants, on average, may have been more physically active than they reported during screening. Such discrepancies between self-report and objective data are not surprising. The participants with MVPA > 30 minutes per day were included in our analyses. However, we realise that this may have dampened the effect of prolonged sitting on our outcomes and have added this as a limitation of the study:

(Discussion: eighth paragraph, line 4)

Third, our objective assessments indicated that the participants on average were engaged in MVPA > 30 min/day which may have dampened the effect of prolonged sitting during the trial.

2.10. *Page 6, lines 27-42. Please provide the reference for the published study on which you based your power calculations.*

We agree with the editor and reviewer #3 that the study should be framed as a pilot study. Therefore, we have deleted the text regarding power calculations:

(Methods: Participants subsection, line 10)

The section regarding power calculations has been replaced by the sentence:
Consistent with a previous trial with a similar experimental design [1], 19 participants were recruited.

2.11. *Page 8, lines 39-46. Please expand on how many participants visited the toilet (and how many times each) during the seated condition. This may be significant due to the sit-to-stand transitions and walking involved. Were there any differences in outcome measures between those who went to the toilet and those who did not?*

We have corrected expanded this section with more details on toilet visits. More participants visited the toilet and on more occasions in the sedentary condition compared to the active condition. A difference in the number of toilet visits between conditions was not surprising and could, at least partly, be explained by the physiological response to physical activity; a change (decrease) in renal blood flow and glomerular filtration rate (described in e.g. Poortmans JR, Ouchinsky M. Glomerular filtration rate and albumin excretion after maximal exercise in aging sedentary and active men. J Gerontol A Biol Sci Med Sci 2006;**61**(11):1181-5). We have re-run the analysis of fatigue changes stratified by participants who went to the toilet in either of the two conditions and those who did not. There was no significant difference between conditions when participants did not go to the toilet (p=0.487). Participants who went to the toilet showed an improvement in fatigue in the active condition (p=0.0438). However, as only 5 participants did not go to the toilet and the study is underpowered as it is, we are reluctant to present results based on subgroup analyses with risk of false positive results (type 1 error).

(Methods: Experimental procedure subsection, second paragraph, line 4)

During the sedentary condition 14 participants visited the toilet (8 participants on one occasion, 3 participants on two occasions, 2 participants on three occasions and 1 participant on four occasions) compared to the active condition where 8 participants visited the toilet only once.

2.12. *Page 8, lines 46-52. Provide a rationale for the 3 minute LPA every 30 minutes intervention. Previous research has shown 2 minutes every 20 minutes to be effective.*

Thank you for this suggestion. The change in the intervals was a pragmatic solution to avoid to interrupt the fatigue and cognitive testing sessions (total time for each session: 26±1 min) with a walking bout. We have added this clarification:

(Methods: Experimental procedure subsection: third paragraph, line 5)

Previous research has shown that a 2-min bout of light-intensity walking every 20 min lowered postprandial glucose and insulin concentrations [1], but the intervals were expanded in the current study to enable a complete session of fatigue and cognitive assessments during a single bout of sitting.

2.13. *Page 9, lines 3-5. The RPE data would fit better in the results section rather than the methods. Consider moving.*

We agree and have moved the RPE data to the results section:

(Results: second paragraph)

The mean rating of perceived exertion (RPE), assessed by the Borg RPE scale [17], for the walking bouts was 9.1 ± 2.0 (9 corresponds with “Very light” on the Borg RPE scale).

2.14. *Page 11, lines 3-18. It is not clear why venous glucose was not measured, since it was drawn, and capillary finger pricks were used instead. Additionally, why use the CGMS when a cannula was used- why not draw blood from one source instead of three? Provide references supporting these method selections, and the superiority of the CGMS and capillary samples versus venous.*

The main advantage with the CGMS is that the (interstitial) glucose measurement is repeated every 5 minute (in IPro2) and such frequent glucose testing using venous glucose or capillary finger pricks is difficult for practical reasons. Therefore, CGMS may identify trends that would not be noticed with venous and capillary testing. CGMS is also relatively non-invasive. One of the disadvantages with the

CGMS is that it must be calibrated using capillary finger pricks (and not venous glucose). We have added this clarification. Also, we have added two references regarding validity and reliability of CGMS:

(Methods: Continuous glucose monitoring subsection)
Glucose concentrations were recorded using an IPro2 (Medtronic) Continuous Glucose Monitoring System (CGMS) [28 29] during both experimental conditions. The CGMS sensor was inserted under the skin in the lower back, lateral to the mediocclavicular line between the iliac crest and the lowest rib and measured interstitial glucose concentrations every 5 min. Capillary finger-pricks (Optium Xceed) at 0h, 1h, and 4h were used for calibration in accordance with the manufacturer's instructions.

New references

28. Beck RW, Calhoun P, Kollman C. Use of continuous glucose monitoring as an outcome measure in clinical trials. Diabetes Technol Ther 2012;**14**(10):877-82.
29. Terada T, Loehr S, Guigard E, et al. Test-retest reliability of a continuous glucose monitoring system in individuals with type 2 diabetes. Diabetes Technol Ther 2014;**16**(8):491-8.

2.15. *A Description of data analysis from the accelerometer and inclinometer is missing. Further, definitions of MVPA are missing (count cut point, bouts of 10 minutes or more, etc.).*

Further description has been added for clarity. The changes in the box below also address (repeated here for sake of clarity) an issue raised by another reviewer (see **1.4**, above).

(Methods: Diet and physical activity subsection, line 9)

Accelerometer data were downloaded using ActiLife 6.5.4 software and summarized using SAS 9.4 for each of the habitual days prior to the condition. Non-wear periods were deleted for analyses; these were periods with at least 60 minutes of zero counts per minute (cpm), allowing for up to two consecutive, one minute interruptions (count values between 1–49 cpm) per non-wear period [17]. Activity counts were categorised as sedentary (<100 cpm; predominantly sitting) [18], light-intensity activity (100-1951 cpm; typically gentle walking) [19], or MVPA (≥1952 cpm; typically at least brisk walking) [19]. Average of sitting time and MVPA were calculated for each individual across valid days (days where the monitor was worn for at least 600 mins).

New references

17. Winkler EA, Gardiner PA, Clark BK, et al. Identifying sedentary time using automated estimates of accelerometer wear time. Br J Sports Med 2012;**46**(6):436-42.
18. Healy GN, Dunstan DW, Salmon J, et al. Objectively measured light-intensity physical activity is independently associated with 2-h plasma glucose. Diabetes Care 2007;**30**(6):1384-9.
19. Freedson PS, Melanson E, Sirard J. Calibration of the Computer Science and Applications, Inc. accelerometer. Med Sci Sports Exerc 1998;**30**(5):777-81.

Results

2.16. *Table 1. Did the authors rerun analyses with those individuals who met PA and SB inclusion criteria by objective assessment? If not, the authors are encouraged to do so.*

We did re-run the analyses with stratification based on participant meeting the criteria for physical activity or not and some statistically significant results came up, for example; participants who do not meet the criteria for physical activity show significantly better results in the episodic memory function test in the active condition compared to the sedentary condition (p=0.0348). Again, however, based on the same arguments as given under ***2.11***, we do not think that results based on subgroup analyses showed be presented in the manuscript.

2.17. *Table 1. Why did the authors only report blood pressure medication use, why not also report glucose medications, since glucose was measured? Other medications of interest?*

Antihypertensive medication was the only continuously used medication among participants. Current use of glucose-lowering, lipid-lowering, antidepressant or oral cortisone medication was an exclusion criteria and no other medications were used.

2.18. *Page 12, lines 53-58. The following statement is not clear: "Physical activity levels did not significantly differ in the two days leading up to each experimental condition (sedentary, 32.6 • } 27.6 min/day, vs. active 32.5 } 30.8 min/day; p=0.99)." Was there an active and sedentary group? Please clarify.*

Thank you for pointing this out. This was a comparison of physical activity before the sedentary condition and physical activity before the active condition. We have clarified this:

(Results: First paragraph, line 4)

Levels of MVPA in the 48 hours prior to each experimental condition did not differ significantly (36.29 ± 29.20 min/day before the sedentary condition vs. 30.18 ± 42.16 min/day before the active condition; p=0.607).

2.19. *Was this study sufficiently powered? It would be useful to the reader if the authors would report the power of their results either in the results section, or in the discussion limitations section of the paper (page 17, lines 29-33).*

The study is now adequately framed as a pilot study. Due to the study size, the study may have been underpowered. We have clarified this:

(Discussion: eighth paragraph, line 1)

First, given this is a pilot study, it was likely underpowered to detect an effect in cognitive performance (as well as several of the potential mediators).

2.20. *Glucose values or changes in the glucose values are not reported in the results section.*

Typically, results for comparisons of AUC have been reported (in e.g. Dunstan DW, Kingwell BA, Larsen R, et al. Breaking up prolonged sitting reduces postprandial glucose and insulin responses. Diabetes Care 2012;**35**(5):976-83). In the current manuscript we present results for the total AUC and the net incremental AUC (Results: second paragraph, line 14).

Fasting glucose and 2-hour glucose did not differ between conditions (p=0.251 and p=0.921, respectively).

We have further clarified the definitions of total AUC and the net incremental AUC:

(Methods: Continuous glucose monitoring subsection, line 6)

The total area under the glycaemic response curve (total AUC) and the net incremental AUC (net iAUC), includes all incremental area below the curve, including the area below the fasting concentration, was derived from the CGMS measurements.

Discussion

2.21. *Time effects on DOPA and DHPG are discussed in paragraph 3, but never revealed in the results section.*

The time effects on DOPA and DHPG was only observed in the sedentary condition and therefore we chose to present these findings in relation to the increased fatigue score in the sedentary condition: Results: third paragraph, line 12: "The changes in fatigue score over time correlated with a decrease in heart rate (0h to 4h: r_s=-0.60, p=0.007) and plasma level of DOPA (0h to 4h: r_s=-0.59, p=0.009) and an increase in plasma level of DHPG (0h to 4h: r_s=0.73, p<0.001; 0h to 7h: r_s=0.47, p=0.040) in the sedentary condition (but not in the active condition)."

2.22 *Page 15, last paragraph. Glucose results are discussed, but never revealed in the results section.*

Please see our response for ***2.20*** above.

2.23. *The discussions related to fatigue and cognition are well developed.*

Thank you for the positive assessment and the constructive comments.

Reviewer #3

3.1. *The article title is a little misleading. First, with this sample size, I believe this is really a pilot study, and it should be explicitly labelled as such. Second, the title should emphasise that the effects are acute.*

We agree and have changed the title accordingly: "Acute Effects of Breaking Up Prolonged Sitting on Fatigue and Cognition: A Pilot Study".

3.2. *Abstract, line 35. "Fatigue and cognitive assessments, and.." The sentence just ends suddenly. Please fix.*

Thank you for pointing this out (see revision in ***1.1.*** above).

3.3. *Abstract, Results. Please report results such that the reader has some sense of the magnitude of the effects and their uncertainty – e.g. mean differences in the changes between conditions and confidence intervals. Just stating P=0.024 and 0.038 for the interaction is not very informative.*

Thank you for this suggestion. We have added details on difference between conditions and confidence intervals:

(Abstract: Results subsection)

During the active condition fatigue levels were lower at 4h (-13.32 [95% CI -23.48 to -3.16]) and at 7h (-10.73 [95% CI -20.89 to -0.58]) compared to the sedentary condition. Heart rate was higher at 4h (4.47 [95% CI 8.37 to 0.58]) and at 7h (4.32 [95% CI 8.21 to 0.42]) during the active condition compared to the sedentary condition.

(Results, second paragraph, line 6)

During the active condition fatigue levels were lower at 4h (-13.32 [95% CI -23.48 to -3.16]) and at 7h (-10.73 [95% CI -20.89 to -0.58]) compared to the sedentary condition (see Figure 2). Heart rate was higher at 4h (4.47 [95% CI 8.37 to 0.58]) and at 7h (4.32 [95% CI 8.21 to 0.42]) during the active condition compared to the sedentary condition.

3.4. *The study has limited impact, as the data are derived from a single bout of sedentary/interrupted sedentary time. It is not possible to extrapolate these very acute effects to multiple days/ weeks/ months in the work environment.*

Thank you for pointing this out. We agree and have adjusted the text to limit the relevance for work environment.

(Abstract: Conclusions subsection)

The phrase “across the working day” has been erased from the sentence:
Interrupting prolonged sitting with light-intensity walking breaks may be an effective fatigue counter-measure acutely.

3.5. *The statement in the first bullet point under “Strengths and limitations of the study” (beneficial effects on glucose metabolism) does not appear to be a finding from the current study, but rather a general statement from the literature. This statement is therefore misleading.*
Thank you for highlighting this. We agree and the first bullet point has been removed, response below also addresses (repeated here for sake of clarity) the concern of another reviewer (see revision in ***2.3.-2.5.*** above).

(Strengths and limitations of this study: Bullet points)

An “s” has been added to “Strength” in the title.
The first bullet point has been removed and replaced by a second bullet point:

- A randomised crossover study design was used.

The third bullet point has been rephrased:

- Given this is a pilot study, the sample size may have limited the ability to detect an effect from breaking up sitting in several of the outcome measures.

The fourth bullet point has been rephrased:

- The longer-term relevance of acute effects of breaking up prolonged sitting are uncertain.

3.6. *The participant characteristics lack detail. Were these participants accustomed to long uninterrupted periods of sedentary time? The eligibility criterion is simply self-report of >5 hours/day of sitting, but we do not know whether the participants habitually sit for 7 hours uninterrupted. If not, as I suspect, then it is perhaps not surprising that they report greater ‘fatigue’ in the enforced continuous sitting condition. Please comment.*

Thank you, we agree it is important to consider whether the participants were accustomed to high amounts of sedentary time as this has implications for how the intervention is tolerated. Thus we have calculated the participant’s habitual moderate-to-vigorous physical activity and sitting time. We hope this will help clarify for the reader the type of behaviour the participants were accustomed to.

Table 1. Characteristics of the 19 participants in the study (10 men and 9 women).

BMI (kg/m^2)	31.5 (4.7)
Waist circumference (cm)	105.2 (12.4)
Completed university degree*	78.9
Taking antihypertensive medication*	47.4
Habitual moderate-to-vigorous physical activity (minutes/day)**	35.80 (30.86)
Habitual sitting time (h/day)**	9.82 (2.19)
Sleep time (h/night)*	7.4 (0.8)

Data are % or mean (SD).
*Assessed using self-reports.
**Assessed using activity monitors during the wash-out period between the two experimental days.

3.7. *Importantly, given that it was the only variable for which you claim "significant" effects, "Fatigue" is not listed as an outcome in your clinical trial registration (ACTRN12613000137796). Item 24 of the CONSORT checklist is left blank, so I assume that no protocol is available in the public domain. Please clarify at precisely what stage did the fatigue VAS measure become the primary outcome. This is critical, as the emphasis of the report and its conclusions is on activity breaks as a fatigue countermeasure.*

The background for the study was the obvious lack of studies on cognitive effects in the research field of sedentary behaviour – in contrast with the vast research on cognitive effects from physical activity. The trial was registered early in the development of the study to fit a tight time schedule for the study (a nine-month postdoc). However, in the previous trial on the effects on postprandial glucose and insulin concentrations from regularly interrupting sitting time with short bouts of either light- or moderate-intensity walking when compared with prolonged sitting (Dunstan DW, et al. Breaking up prolonged sitting reduces postprandial glucose and insulin responses. Diabetes Care. 2012;35(5):976-83) several of the participants anecdotally reported heightened fatigue. We therefore, after we had registered the trial but before the commencement of participants, decided to combine the objective cognitive tests with a subjective assessment in order to be able to capture a potential effect on fatigue in the trial. After contact with Prof Kathryn A. Lee at the University of California, San Francisco, who developed the VAS-F instrument, we decided to use the VAS-F score since it is probably more sensitive to longitudinal changes then other subjective fatigue scores. In order to limit the time for the cognitive and fatigue assessments we replaced one of the proposed cognitive tests (Corsi blocks) with the fatigue assessment.

3.8. *The sample size estimation section is flawed and, in any event, I think it is clear that this study is really a pilot. You state that the study was powered on glucose outcomes, as no information was available on the effect of sedentary behaviour on cognitive outcomes. However, the latter information is not required. What is needed is a sense of the minimum clinically/ practically important difference for the primary outcome and an idea of its variability and reliability. Moreover, the value that you define as a "clinically meaningful" difference in the change for glucose AUC is not provided, so the sample size estimations cannot be reproduced. If the study is properly labelled as a pilot then no formal sample size estimations are required.*

We agree and the study is now labelled as a pilot study (see revision in ***2.10.*** above).

3.9. *Discussion: "We examined the effects of light-intensity walking breaks on acute fatigue, but the results may also have implications for persistent fatigue considering the increasing time spent sedentary in many countries [35], and the high prevalence of persistent fatigue [36]." Even with the use of the word "may", I am not convinced that the data/ literature allow you to make this claim.*

We agree with the Reviewer and have adjusted the paragraph:

(Discussion: sixth paragraph, line 1)

In many countries, increasing time is spent sedentary [39] and there is high prevalence of persistent fatigue [40]. We examined the effects of light-intensity walking breaks on acute fatigue and whether these results may have implications for persistent fatigue is uncertain.

3.10. *Related to point 3, above, you state repeatedly that the sample size was likely too small to detect effects as statistically significant, but there is no indication of the size of the change that you would regard as clinically/ practically important.*

Thank you for pointing this out. There is no clear reference in the literature to a change in fatigue score that could be regarded as clinically or practically important. However, we think it is relevant to compare the change in fatigue score in the sedentary condition with the difference in fatigue score

that was recorded in a study of healthy individuals before and after one night's sleep in 75 healthy individuals in a study by Lee and colleagues (Lee KA, Hicks G, Nino-Murcia G. Validity and reliability of a scale to assess fatigue. Psychiatry Res 1991;**36**(3):291-8.).

We have added a comparison to the discussion:

(Discussion: first paragraph, line 8) As a comparison, the magnitude of change in fatigue score from 0h to 4h during uninterrupted sitting correspond to approximately 40 % of the difference in fatigue score recorded before and after one night's sleep in a study of 75 healthy individuals, aged 18-55 years [17].

VERSION 2 – REVIEW

REVIEWER	Stephanie Bonn Karolinska Institutet, Sweden
REVIEW RETURNED	17-Dec-2015

GENERAL COMMENTS	The authors have done a good job in responding to all the reviewers' comments. I have nothing further to add.

REVIEWER	Alan Batterham Teesside University, UK.
REVIEW RETURNED	14-Dec-2015

GENERAL COMMENTS	The authors have addressed all of my original concerns satisfactorily - thank you.