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Henson, J., Davies, M. J., Bodicoat, D. H., Edwardson, C. L., Gill, J. M.R., Stensel, D. J., Tolfrey, K., Dunstan, D. W., Khunti, K., and Yates, T. (2015) Breaking up prolonged sitting with standing or walking attenuates the postprandial metabolic response in postmenopausal women: a randomized acute study. *Diabetes Care*

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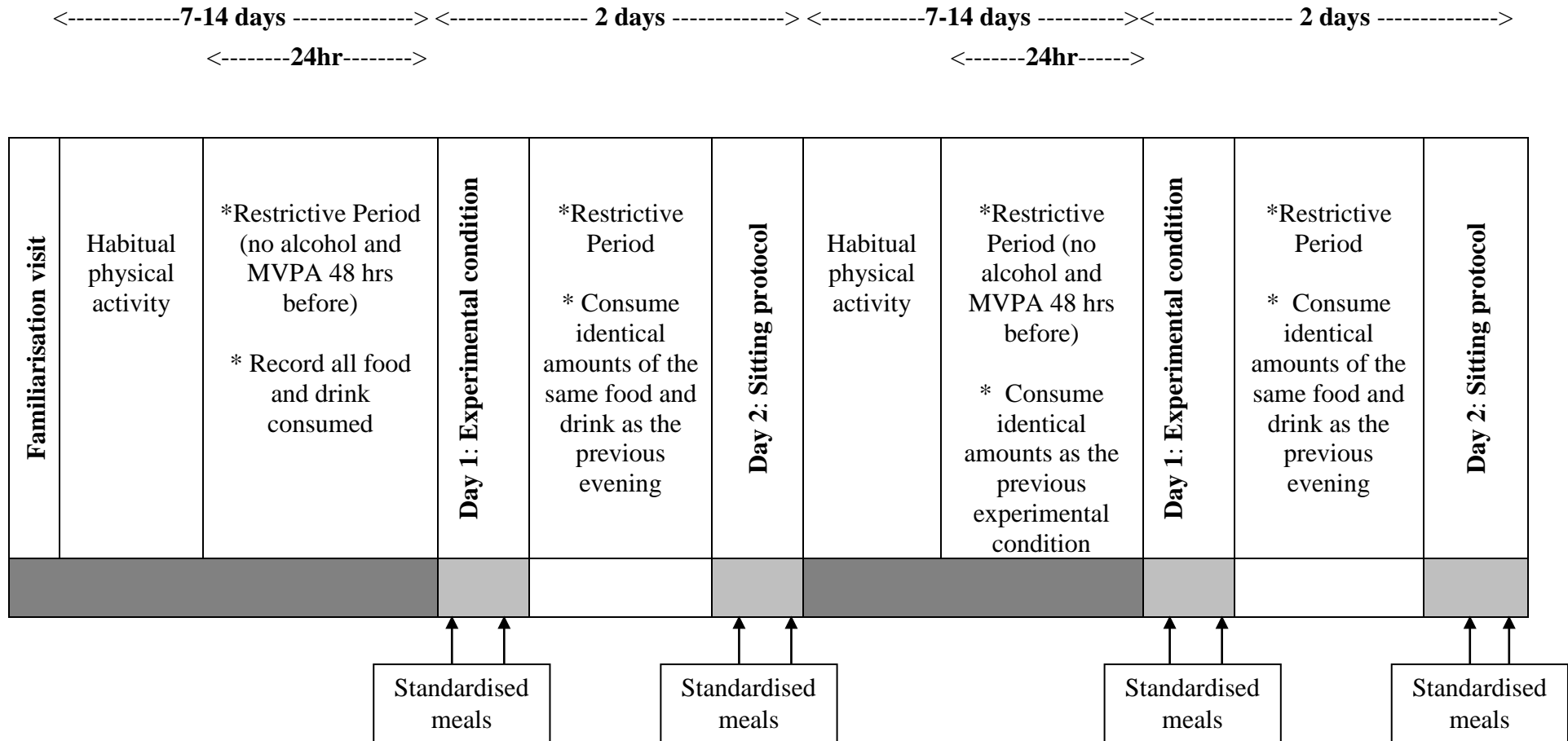
Deposited on: 9 December 2015

Online-Only Supplemental Material

Supplemental Table S1. Balanced incomplete block design sequence. Each participant was randomised to one of the sequences A-F.

Sequence	Period 1		Period 2	
	Day 1	Day 2	Day 1	Day 2
A	Sitting	Sitting	Standing	Sitting
B	Standing	Sitting	Walking	Sitting
C	Walking	Sitting	Sitting	Sitting
D	Sitting	Sitting	Walking	Sitting
E	Standing	Sitting	Sitting	Sitting
F	Walking	Sitting	Standing	Sitting

Supplemental Figure 1. Study protocol for treatment conditions



Actigraph GT3X+ worn
 Actigraph GT3X+ and activPAL worn

Supplemental Table S2. Number of imputed values for each participant included across both experimental days

Participant Number	Day 1: Missing values across both randomised experimental conditions (total scheduled samples = 22)				Day 2: Missing values across both randomised experimental conditions (total scheduled samples = 22)			
	Glucose	Insulin	Triglycerides	NEFA	Glucose	Insulin	Triglycerides	NEFA
1	2 (9)	7 (32)	6 (28)	7 (32)				
2	0	0	1 (5)	2 (9)	0	1 (5)	4 (18)	6 (28)
3	0	0	2 (9)	2 (9)	4 (18)	5 (23)	5 (23)	7 (32)
4	0	3 (14)	3 (14)	3 (14)	1 (5)	1 (5)	1 (5)	2 (9)
5	2 (9)	5 (23)	3 (14)	4 (18)				
6	1 (5)	5 (23)	3 (14)	1 (5)	1 (5)	1 (5)	2 (9)	1 (5)
7	2 (9)	7 (32)	2 (9)	3 (14)	0	1 (5)	1 (5)	2 (9)
8	0	2 (9)	1 (5)	3 (14)	2 (9)	2 (9)	2 (9)	3 (14)
9	3 (14)	6 (28)	6 (28)	6 (28)	0	2 (9)	2 (9)	1 (5)
10	1 (5)	2 (9)	1 (5)	2 (9)	0	0	0	1 (5)
11	0	2 (9)	0	0	0	0	0	2 (9)
12	0	0	0	0				
13	0	4 (18)	3 (14)	8 (36)	3 (14)	3 (14)	4 (18)	4 (18)
14	1 (5)	4 (18)	4 (18)	4 (18)	4 (18)	5 (23)	5 (23)	5 (23)
15	0	0	1 (5)	8 (36)				
16	3 (14)	3 (14)	3 (14)	4 (18)	4 (18)	4 (18)	4 (18)	4 (18)
17	2 (9)	3 (14)	5 (23)	5 (23)	4 (18)	5 (23)	5 (23)	4 (18)
18	1 (5)	3 (14)	2 (9)	2 (9)	3 (14)	4 (18)	4 (18)	4 (18)
19	1 (5)	3 (14)	1 (5)	1 (5)	2 (9)	2 (9)	2 (9)	2 (9)
20	0	2 (9)	0	0				
21	0	5 (23)	2 (9)	2 (9)	1 (5)	1 (5)	2 (9)	3 (14)
22	1 (5)	5 (23)	1 (5)	1 (5)	1 (5)	2 (9)	2 (9)	4 (18)

Data presented as number (percentage of total available samples)

Supplemental Table S3. Incremental area under the curve (iAUC) results on day 1 for biochemical measurements for each experimental condition (n = 22)

	Mean \pm SEM			P-value	
	Sitting	Standing	Walking	Standing vs. sitting	Walking vs. sitting
Glucose (mmol/L•h)	5.3 \pm 0.8	3.5 \pm 0.8	3.8 \pm 0.7	0.022	0.009
Insulin (mU/L•h)	548.2 \pm 71.8	437.2 \pm 73.5	347.9 \pm 78.7	0.045	0.008
Triglycerides (mmol/L•h)	5.6 \pm 0.7	6.2 \pm 0.8	6.1 \pm 0.8	0.493	0.673
NEFA (mmol/L•h)	-1.5 \pm 0.2	-1.0 \pm 0.2	-0.8 \pm 0.2	0.024	0.003

SEM; standard error of the mean, NEFA; non-esterified fatty acids

Supplemental Table S4. Incremental area under the curve (iAUC) results on day 2 for biochemical measurements for each experimental condition (n = 17)

	Mean ± SEM			P-value	
	Sitting	Standing	Walking	Standing vs. sitting	Walking vs. sitting
Glucose (mmol/L•h)	4.8±0.6	3.9±0.8	4.0±0.7	0.039	0.027
Insulin (mU/L•h)	464.6±70.2	363.5±57.5	354.3±57.3	0.325	0.038
Triglycerides (mmol/L•h)	7.2±0.5	7.2±0.8	6.0±0.7	0.603	0.077
NEFA (mmol/L•h)	-1.5±0.2	-1.0±0.3	-1.0±0.3	0.161	0.144

SEM; standard error of the mean, NEFA; non-esterified fatty acids

Supplemental Table S5 Total area under the curve (AUC) results on day 1 for biochemical measurements for each experimental condition (n = 22)

	Mean \pm SEM			P-value	
	Sitting	Standing	Walking	Standing vs. sitting	Walking vs. sitting
Glucose (mmol/L•h)	46.5 \pm 1.2	44.0 \pm 1.1	44.1 \pm 1.1	0.004	<0.001
Insulin (mU/L•h)	661.7 \pm 81.6	560.9 \pm 80.1	475.2 \pm 85.9	0.048	0.018
Triglycerides (mmol/L•h)	16.5 \pm 1.4	18.7 \pm 1.5	17.8 \pm 1.5	0.641	0.571
NEFA (mmol/L•h)	-2.5 \pm 0.2	-3.0 \pm 0.2	-2.9 \pm 0.2	0.019	0.046

SEM; standard error of the mean, NEFA; non-esterified fatty acids

Supplemental Table S6. Total area under the curve (AUC) results on day 2 for biochemical measurements for each experimental condition (n = 17)

	Mean \pm SEM			P-value	
	Sitting	Standing	Walking	Standing vs. sitting	Walking vs. sitting
Glucose (mmol/L•h)	44.9 \pm 0.9	43.9 \pm 1.0	43.7 \pm 1.0	0.102	0.082
Insulin (mU/L•h)	595.2 \pm 79.9	505.2 \pm 58.0	474.6 \pm 54.9	0.323	0.013
Triglycerides (mmol/L•h)	17.7 \pm 1.2	18.3 \pm 1.2	15.5 \pm 1.2	0.653	0.084
NEFA (mmol/L•h)	-2.2 \pm 0.1	-2.6 \pm 0.2	-2.5 \pm 0.2	0.009	0.033

SEM; standard error of the mean, NEFA; non-esterified fatty acids

Supplemental Table S7. Fasting biochemical values for each experimental condition (Day 1 and Day 2)

	Day 1	Day 2	P value (Day 1 vs. Day 2)
<u>Glucose (mmol/L) – Sitting</u>	<u>5.4 ± 0.4</u>	<u>5.5 ± 0.4</u>	<u>0.878</u>
<u>Glucose (mmol/L) - Standing</u>	<u>5.5 ± 0.4</u>	<u>5.6 ± 0.4</u>	<u>0.902</u>
<u>Glucose (mmol/L) - Walking</u>	<u>5.4 ± 0.4</u>	<u>5.5 ± 0.3</u>	<u>0.898</u>
<u>Insulin (mu/L) - Sitting</u>	<u>15.6 ± 9.6</u>	<u>17.4 ± 11.1</u>	<u>0.471</u>
<u>Insulin (mu/L) - Standing</u>	<u>16.0 ± 8.6</u>	<u>20.1 ± 11.8</u>	<u>0.183</u>
<u>Insulin (mu/L) - Walking</u>	<u>16.4 ± 5.6</u>	<u>16.8 ± 10.7</u>	<u>0.823</u>
<u>NEFA (mmol/L) - Sitting</u>	<u>0.55 ± 0.16</u>	<u>0.49 ± 0.13</u>	<u>0.163</u>
<u>NEFA (mmol/L) – Standing</u>	<u>0.53 ± 0.13</u>	<u>0.48 ± 0.15</u>	<u>0.219</u>
<u>NEFA (mmol/L) –Walking</u>	<u>0.51 ± 0.11</u>	<u>0.47 ± 0.09</u>	<u>0.378</u>
<u>Triglycerides (mmol/L) – Sitting</u>	<u>1.54 ± 0.49</u>	<u>1.39 ± 0.36</u>	<u>0.363</u>
<u>Triglycerides (mmol/L) – Standing</u>	<u>1.66 ± 0.47</u>	<u>1.47 ± 0.37</u>	<u>0.121</u>
<u>Triglycerides (mmol/L) - Walking</u>	<u>1.56 ± 0.47</u>	<u>1.27 ± 0.43</u>	<u>0.093</u>

Data presented as mean ± standard deviation

Supplemental Table S8. Physical activity data before and during each experimental condition (n = 22)

Experimental regimens	Mean (range)	Mean \pm SD				
	Days accelerometer worn	Wear-time (Minutes)	Sedentary time (Minutes)	Light activity (Minutes)	MVPA (Minutes)	Steps
Familiarisation visit	6 (4-7)	831 \pm 85	594 \pm 80	218 \pm 52	19 \pm 10	5024 \pm 2021
7 days prior to experimental condition 1	5 (4-6)	821 \pm 89	569 \pm 91	234 \pm 59	18 \pm 14	4731 \pm 2667
2 days prior to experimental condition 1	2	826 \pm 87	597 \pm 98	225 \pm 56	4 \pm 3	3399 \pm 2027
7 days prior to experimental condition 2	5 (4-6)	829 \pm 86	588 \pm 91	223 \pm 54	18 \pm 9	4822 \pm 1659
2 days prior to experimental condition 2	2	838 \pm 102	600 \pm 108	233 \pm 62	5 \pm 2	3612 \pm 1983
Day 1 - Sitting Condition	1	478 \pm 27	475 \pm 11	3 \pm 1	Nil	121 \pm 50
Day 1 - Standing Condition	1	475 \pm 11	448 \pm 13	27 \pm 5	Nil	197 \pm 64
Day 1 - Walking Condition	1	477 \pm 9	415 \pm 9	41 \pm 11	21 \pm 13	4818 \pm 1550
Day 2 – Sitting Condition	1	480 \pm 11	476 \pm 11	4 \pm 1	Nil	116 \pm 45

SD; standard deviation, MVPA; moderate-to-vigorous physical activity

Supplemental Table S9. ActivPAL results during each experimental condition

	Minutes \pm SD		
	Sitting	Standing	Walking
Day 1 – Sitting condition	479.7 \pm 4.3	1.5 \pm 0.7	0.8 \pm 0.1
Day 1 – Standing condition	419.1 \pm 6.9	62.9 \pm 2.2	1.4 \pm 0.2
Day 1 - Walking condition	412.7 \pm 7.3	4.8 \pm 0.8	64.2 \pm 0.3
Day 2 – Sitting condition	481.1 \pm 4.0	1.7 \pm 0.4	0.5 \pm 0.1

SD; standard deviation