

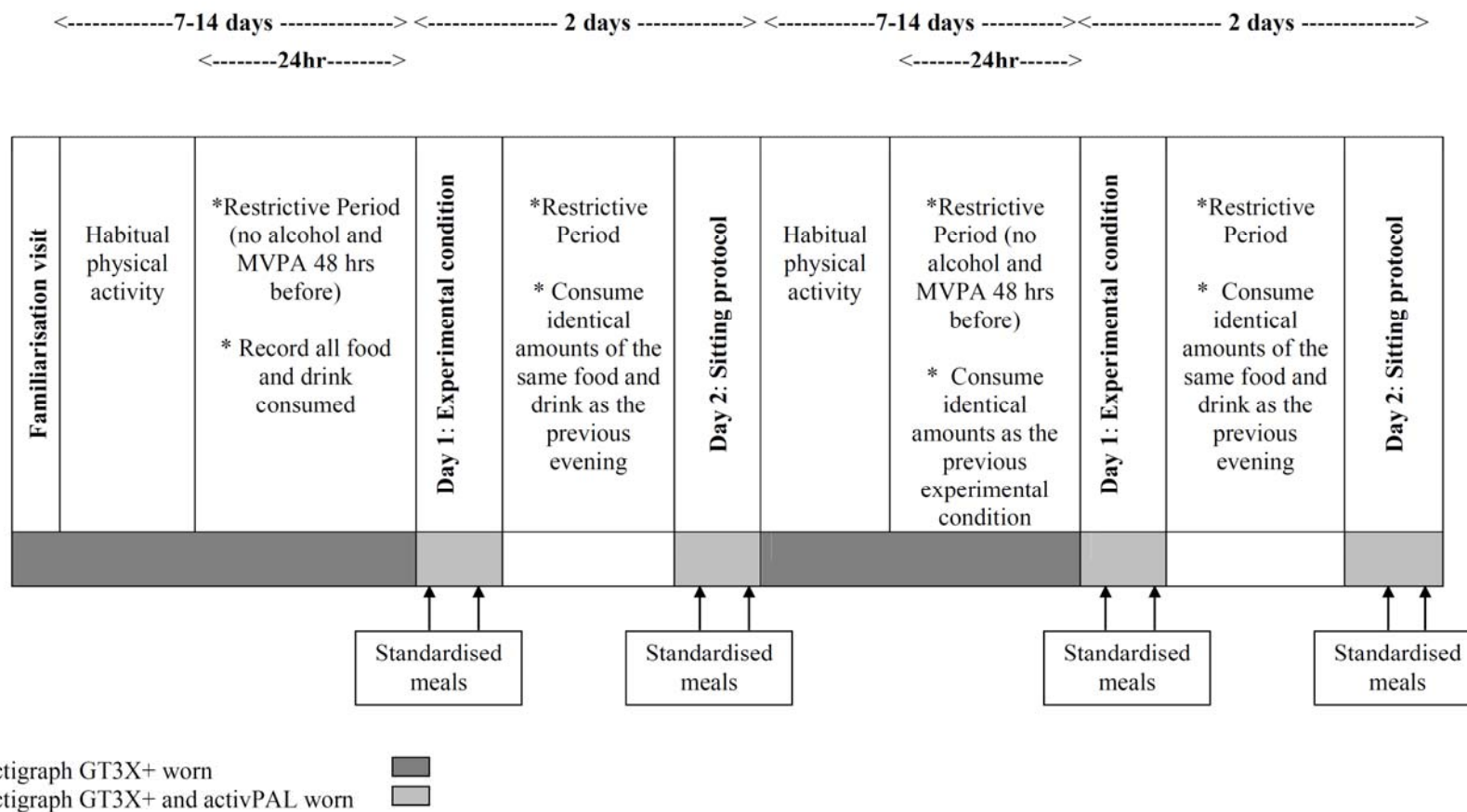
SUPPLEMENTARY DATA

Supplementary 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

SUPPLEMENTARY DATA

Supplementary Figure 1. Study protocol for treatment conditions



SUPPLEMENTARY DATA

Supplementary 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)

SUPPLEMENTARY DATA

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

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

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

SUPPLEMENTARY DATA

Supplementary 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

SUPPLEMENTARY DATA

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

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

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

SUPPLEMENTARY DATA

Supplementary Table S6. Total area under the curve (AUC) 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)	44.9±0.9	43.9±1.0	43.7±1.0	0.102	0.082
Insulin (mU/L•h)	595.2±79.9	505.2±58.0	474.6±54.9	0.323	0.013
Triglycerides (mmol/L•h)	17.7±1.2	18.3±1.2	15.5±1.2	0.653	0.084
NEFA (mmol/L•h)	-2.2±0.1	-2.6±0.2	-2.5±0.2	0.009	0.033

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

SUPPLEMENTARY DATA

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

	<u>Day 1</u>	<u>Day 2</u>	<u>P value</u> <u>(Day 1 vs. Day 2)</u>
<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>
<hr/>			
<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>
<hr/>			
<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>
<hr/>			
<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

SUPPLEMENTARY DATA

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

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

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

SUPPLEMENTARY DATA

Supplementary Table S9. ActivPAL results during each experimental condition

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

SD; standard deviation