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http://eprints.gla.ac.uk/113161/

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.

<table>
<thead>
<tr>
<th>Sequence</th>
<th>Period 1</th>
<th>Period 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 2</td>
</tr>
<tr>
<td>A</td>
<td>Sitting</td>
<td>Sitting</td>
</tr>
<tr>
<td>B</td>
<td>Standing</td>
<td>Sitting</td>
</tr>
<tr>
<td>C</td>
<td>Walking</td>
<td>Sitting</td>
</tr>
<tr>
<td>D</td>
<td>Sitting</td>
<td>Sitting</td>
</tr>
<tr>
<td>E</td>
<td>Standing</td>
<td>Sitting</td>
</tr>
<tr>
<td>F</td>
<td>Walking</td>
<td>Sitting</td>
</tr>
</tbody>
</table>
**Supplemental Figure 1.** Study protocol for treatment conditions

<table>
<thead>
<tr>
<th>Familiarisation visit</th>
<th>Day 1 Experimental condition</th>
<th>Day 2 Sitting protocol</th>
</tr>
</thead>
</table>
| Habitual physical activity | *Restrictive Period (no alcohol and MVPA 48 hrs before)  
| Record all food and drink consumed | *Restrictive Period  
| Consume identical amounts of the same food and drink as the previous evening | Habitual physical activity  
| *Restrictive Period (no alcohol and MVPA 48 hrs before)  
| Consume identical amounts as the previous experimental condition | *Restrictive Period  
| Consume identical amounts of the same food and drink as the previous evening |

<table>
<thead>
<tr>
<th>Day 1</th>
<th>Day 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental condition</td>
<td>Sitting protocol</td>
</tr>
<tr>
<td>Standardised meals</td>
<td>Standardised meals</td>
</tr>
<tr>
<td>Standardised meals</td>
<td>Standardised meals</td>
</tr>
</tbody>
</table>

Actigraph GT3X + worn
Actigraph GT3X + and activPAL worn
### Supplemental Table S2. Number of imputed values for each participant included across both experimental days

<table>
<thead>
<tr>
<th>Participant Number</th>
<th>Day 1: Missing values across both randomised experimental conditions (total scheduled samples = 22)</th>
<th>Day 2: Missing values across both randomised experimental conditions (total scheduled samples = 22)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glucose</td>
<td>Insulin</td>
</tr>
<tr>
<td>1</td>
<td>2 (9)</td>
<td>7 (32)</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>3 (14)</td>
</tr>
<tr>
<td>5</td>
<td>2 (9)</td>
<td>5 (23)</td>
</tr>
<tr>
<td>6</td>
<td>1 (5)</td>
<td>5 (23)</td>
</tr>
<tr>
<td>7</td>
<td>2 (9)</td>
<td>7 (32)</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td>2 (9)</td>
</tr>
<tr>
<td>9</td>
<td>3 (14)</td>
<td>6 (28)</td>
</tr>
<tr>
<td>10</td>
<td>1 (5)</td>
<td>2 (9)</td>
</tr>
<tr>
<td>11</td>
<td>0</td>
<td>2 (9)</td>
</tr>
<tr>
<td>12</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>13</td>
<td>0</td>
<td>4 (18)</td>
</tr>
<tr>
<td>14</td>
<td>1 (5)</td>
<td>4 (18)</td>
</tr>
<tr>
<td>15</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>16</td>
<td>3 (14)</td>
<td>3 (14)</td>
</tr>
<tr>
<td>17</td>
<td>2 (9)</td>
<td>3 (14)</td>
</tr>
<tr>
<td>18</td>
<td>1 (5)</td>
<td>3 (14)</td>
</tr>
<tr>
<td>19</td>
<td>1 (5)</td>
<td>3 (14)</td>
</tr>
<tr>
<td>20</td>
<td>0</td>
<td>2 (9)</td>
</tr>
<tr>
<td>21</td>
<td>0</td>
<td>5 (23)</td>
</tr>
<tr>
<td>22</td>
<td>1 (5)</td>
<td>5 (23)</td>
</tr>
</tbody>
</table>

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)

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

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)

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SEM</th>
<th>P-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sitting</td>
<td>Standing</td>
<td>Walking</td>
</tr>
<tr>
<td>Glucose (mmol/L·h)</td>
<td>4.8±0.6</td>
<td>3.9±0.8</td>
<td>4.0±0.7</td>
</tr>
<tr>
<td>Insulin (mU/L·h)</td>
<td>464.6±70.2</td>
<td>363.5±57.5</td>
<td>354.3±57.3</td>
</tr>
<tr>
<td>Triglycerides (mmol/L·h)</td>
<td>7.2±0.5</td>
<td>7.2±0.8</td>
<td>6.0±0.7</td>
</tr>
<tr>
<td>NEFA (mmol/L·h)</td>
<td>-1.5±0.2</td>
<td>-1.0±0.3</td>
<td>-1.0±0.3</td>
</tr>
</tbody>
</table>

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)

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sitting</td>
<td>Standing</td>
</tr>
<tr>
<td>Glucose (mmol/L • h)</td>
<td>46.5±1.2</td>
<td>44.0±1.1</td>
</tr>
<tr>
<td>Insulin (mU/L • h)</td>
<td>661.7±81.6</td>
<td>560.9±80.1</td>
</tr>
<tr>
<td>Triglycerides (mmol/L • h)</td>
<td>16.5±1.4</td>
<td>18.7±1.5</td>
</tr>
<tr>
<td>NEFA (mmol/L • h)</td>
<td>-2.5±0.2</td>
<td>-3.0±0.2</td>
</tr>
</tbody>
</table>

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)

<table>
<thead>
<tr>
<th>Glucose (mmol/L • h)</th>
<th>Mean ± SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sitting</td>
<td>44.9±0.9</td>
<td></td>
</tr>
<tr>
<td>Standing</td>
<td>43.9±1.0</td>
<td></td>
</tr>
<tr>
<td>Walking</td>
<td>43.7±1.0</td>
<td>0.102</td>
</tr>
<tr>
<td>Standing vs. sitting</td>
<td>0.082</td>
<td></td>
</tr>
<tr>
<td>Walking vs. sitting</td>
<td>0.009</td>
<td>0.033</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Insulin (mU/L • h)</th>
<th>Mean ± SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sitting</td>
<td>595.2±79.9</td>
<td></td>
</tr>
<tr>
<td>Standing</td>
<td>505.2±58.0</td>
<td></td>
</tr>
<tr>
<td>Walking</td>
<td>474.6±54.9</td>
<td>0.323</td>
</tr>
<tr>
<td>Standing vs. sitting</td>
<td>0.013</td>
<td></td>
</tr>
<tr>
<td>Walking vs. sitting</td>
<td>0.084</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Triglycerides (mmol/L • h)</th>
<th>Mean ± SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sitting</td>
<td>17.7±1.2</td>
<td></td>
</tr>
<tr>
<td>Standing</td>
<td>18.3±1.2</td>
<td></td>
</tr>
<tr>
<td>Walking</td>
<td>15.5±1.2</td>
<td>0.653</td>
</tr>
<tr>
<td>Standing vs. sitting</td>
<td>0.084</td>
<td></td>
</tr>
<tr>
<td>Walking vs. sitting</td>
<td>0.009</td>
<td>0.033</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>NEFA (mmol/L • h)</th>
<th>Mean ± SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sitting</td>
<td>-2.2±0.1</td>
<td></td>
</tr>
<tr>
<td>Standing</td>
<td>-2.6±0.2</td>
<td></td>
</tr>
<tr>
<td>Walking</td>
<td>-2.5±0.2</td>
<td>0.009</td>
</tr>
<tr>
<td>Standing vs. sitting</td>
<td>0.033</td>
<td></td>
</tr>
<tr>
<td>Walking vs. sitting</td>
<td>0.084</td>
<td></td>
</tr>
</tbody>
</table>

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)

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

Data presented as mean ± standard deviation
Supplemental Table S8. Physical activity data before and during each experimental condition (n = 22)

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

SD; standard deviation, MVPA; moderate-to-vigorous physical activity
**Supplemental Table S9. ActiPAL results during each experimental condition**

<table>
<thead>
<tr>
<th></th>
<th>Sitting</th>
<th>Standing</th>
<th>Walking</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1 - Sitting condition</td>
<td>479.7±4.3</td>
<td>1.5±0.7</td>
<td>0.8±0.1</td>
</tr>
<tr>
<td>Day 1 - Standing condition</td>
<td>419.1±6.9</td>
<td>62.9±2.2</td>
<td>1.4±0.2</td>
</tr>
<tr>
<td>Day 1 - Walking condition</td>
<td>412.7±7.3</td>
<td>4.8±0.8</td>
<td>64.2±0.3</td>
</tr>
<tr>
<td>Day 2 - Sitting condition</td>
<td>481.1±4.0</td>
<td>1.7±0.4</td>
<td>0.5±0.1</td>
</tr>
</tbody>
</table>

SD; standard deviation