

### Electronic Supplementary Material

Table S1. Inclusion and exclusion criteria

	STAND-UP	ACUTE	FIT2SIT	ARMING HEALTH
<b>INCLUSION</b>				
Aged 25-55 years			X	
Aged 50-75 years		X		
Aged 65-79 years	X			
Aged 30-75 years				X
Overweight or obese (BMI $\geq 27.5\text{kg/m}^2$ or $\geq 25\text{kg/m}^2$ if south Asian)		X		X
BMI $< 30\text{kg/m}^2$			X	
Screen detected impaired glucose regulation		X		X
Post-menopausal		X		
Able to walk	X	X	X	
Ability to communicate in and understand English and to provide informed consent.	X	X	X	X
<b>EXCLUSION</b>				
Undertaking regular purposeful exercise ( $\geq 150$ minutes of moderate or $\geq 75$ minutes of vigorous exercise per week)	X	X	X	X
Known type 2 diabetes	X	X	X	X
Known cardiovascular disease	X		X	X
Steroid use	X	X	X	X
Glucose lowering medication use	X	X	X	X
Regular smoking habit			X	X
Pregnancy			X	X
Psychotic illness	X	X	X	X
Psychological or neurological condition which limits participation in the study	X	X	X	X
Hormone replacement medication		X		

Table S2. Metabolic, demographic and anthropometric characteristics

	<b>ACUTE (n=22)</b>	<b>STAND-UP (n=60)</b>	<b>ARMING HEALTH (n=13)</b>	<b>FIT2SIT (n=34)</b>	<b>Overall (n=129)</b>
Age	66.7±4.8	70.0 ±4.9	66.5±6.0	40.0±9.0	63.6±13.6 [range: 25 to 79]
Sex (female)	22 (100)	29 (48)	7 (53.8)	18 (52.9)	76 (58.9)
BMI (kg/m <sup>2</sup> )	32.9±4.7	26.9±3.9	33.9±3.8	24.5±3.0	27.8±4.9 [range: 19.6 to 44.6]
Ethnicity (white European)	20 (90.9)	30 (50)	13 (100)	26 (76.5)	89 (69.0)
Fasting insulin (mU/L)	15.1±8.3	9.6±5.6	19.5±16.4	5.2±5.8	10.1±8.4 [range: 0.4 to 74.1]
Fasting glucose (mmol/L)	5.4±0.4	5.1±0.8	5.2±0.5	4.7±0.5	5.0±0.7 [range: 2.1 to 7.9]
HOMA-IR	3.6±2.1	2.2±1.5	4.6±3.9	1.0±1.2	2.4±2.0 [range: 0.1 to 17.1]
Non-diabetic hyperglycaemia	22 (100)	0 (0)	13 (100)	0 (0)	35 (27.1)
Data presented as mean ± SD and [range], median (interquartile range) or number (column percentage)					

Table S3. Stratified analysis for insulin and glucose responses during each treatment condition

		Insulin (mU/L)			Glucose (mmol/L)		
		Sitting	Standing	Light Physical Activity	Sitting	Standing	Light Physical Activity
BMI	BMI <27.2kg/m <sup>2</sup>	45.0 (40.2, 49.8)	53.6 (44.8, 62.5)	36.8 (33.6, 40.0)**	5.8 (5.5, 6.1)	5.9 (5.6, 6.2)	5.6 (5.3, 5.9)*
	BMI ≥27.2kg/m <sup>2</sup>	91.6 (76.9, 106.3)	96.4 (77.5, 115.3)	70.7 (59.5, 89.1)**	6.0 (5.7, 6.3)	5.8 (5.5, 6.1)	5.6 (5.3, 5.9)**
Sex	Male	68.7 (62.1, 75.3)	78.5 (67.4, 89.5)	56.6 (50.6, 62.5)**	5.9 (5.7, 6.1)	5.9 (5.5, 6.1)	5.8 (5.6, 6.0)
	Female	71.2 (64.4, 77.9)	73.4 (65.2, 81.7)	56.1 (50.5, 61.8)**	5.9 (5.6, 6.1)	5.8 (5.5, 6.1)	5.5 (5.3, 5.7)**
Ethnicity	White European	60.5 (54.3, 66.8)	63.7 (55.9, 71.4)	52.3 (47.0, 57.7)**	-	-	-
	South Asian	79.3 (71.3, 87.4)	88.3 (75.5, 101.0)	60.4 (53.2, 67.6)**	-	-	-

- Covariates to derive the estimated marginal means for sex and ethnicity were fixed at the following values: Age=63.3years; HOMA-IR=2.35; BMI=27.7kg/m<sup>2</sup>.  
- For BMI <27.2kg/m<sup>2</sup>, the covariates to derive the estimated marginal means were fixed at the following values: Age=61.4years; HOMA-IR=1.69; BMI=24.0 kg/m<sup>2</sup>.  
- For BMI ≥27.2kg/m<sup>2</sup>, the covariates to derive the estimated marginal means were fixed at the following values: Age=65.1years; HOMA-IR=3.00; BMI=31.4 kg/m<sup>2</sup>.  
\*\*p=<0.001, \*p=<0.05 compared to the prolonged sitting. Values displayed as time-averaged response (95% CI).

Table S4. Predicted insulin response stratified by sex, ethnic and BMI categories for a 60-year-old individual

Ethnicity	Age	Sex	BMI (kg/m <sup>2</sup> )	HOMA- IR	Treatment	Mean predicted insulin response (mU/L)
<b>South Asian</b>	60	Female	25	2.0	Sitting	70.7
<b>white European</b>	60	Female	25	2.0	Sitting	51.9
<b>South Asian</b>	60	Female	30	2.0	Sitting	80.5
<b>white European</b>	60	Female	30	2.0	Sitting	61.7
<b>South Asian</b>	60	Female	35	2.0	Sitting	90.3
<b>white European</b>	60	Female	35	2.0	Sitting	71.5
<b>South Asian</b>	60	Female	25	2.0	Physical activity	52.8
<b>white European</b>	60	Female	25	2.0	Physical activity	44.7
<b>South Asian</b>	60	Female	30	2.0	Physical activity	55.5
<b>white European</b>	60	Female	30	2.0	Physical activity	47.4
<b>South Asian</b>	60	Female	35	2.0	Physical activity	58.2
<b>white European</b>	60	Female	35	2.0	Physical activity	50.2
<b>South Asian</b>	60	Male	25	2.0	Sitting	68.3
<b>white European</b>	60	Male	25	2.0	Sitting	49.5
<b>South Asian</b>	60	Male	30	2.0	Sitting	78.0
<b>white European</b>	60	Male	30	2.0	Sitting	59.3
<b>South Asian</b>	60	Male	35	2.0	Sitting	87.8
<b>white European</b>	60	Male	35	2.0	Sitting	69.0
<b>South Asian</b>	60	Male	25	2.0	Physical activity	53.2
<b>white European</b>	60	Male	25	2.0	Physical activity	45.1
<b>South Asian</b>	60	Male	30	2.0	Physical activity	55.9
<b>white European</b>	60	Male	30	2.0	Physical activity	47.8
<b>South Asian</b>	60	Male	35	2.0	Physical activity	58.6
<b>white European</b>	60	Male	35	2.0	Physical activity	50.6

Table S5. Time-averaged area under the curve values (main effects) and outcome x interaction terms for insulin and glucose responses during each treatment condition – with the ARMING HEALTH participants removed (n=13)

Variable	Sitting	Standing	Light Physical Activity	Ethnicity x treatment	Sex x treatment	Age x treatment	BMI x treatment	HOMA-IR x treatment
Insulin (mU/L)	60.1 (55.1, 65.1)	66.6 (58.7, 74.5)	46.9 (43.0, 50.8)**	<b>&lt;0.001</b>	<b>0.056</b>	0.150	<b>0.001</b>	0.353
Glucose (mmol/L)	5.9 (5.7, 6.1)	5.8 (5.5, 6.1)	5.6 (5.4, 5.8)**	0.488	<b>0.008</b>	0.768	<b>0.001</b>	0.434

Covariates to derive the estimated marginal means are fixed at the following values: Age= 63.0years; HOMA-IR=2.15; BMI=27.1kg/m<sup>2</sup>. Values displayed as time-averaged response (95% CI).

\*\*p=≤0.001 compared to the prolonged sitting condition

Figure S1. Protocols for treatment conditions

