

## Supplementary digital content 1: Additional details of EMG analysis procedures

### *Muscle inactivity and activity patterns after sedentary-time targeted RCT*

This supplement provides additional results and describes further details of EMG analysis procedures.

**SUPPLEMENTARY TABLE 1.** Group, gender and domain specific EMG inactivity and activity values at baseline.

	Intervention			Control			All
	All (n=24)	Female (n=15)	Male (n=9)	All (n=24)	Female (n=13)	Male (n=11)	n=48
<b>Recording time</b>							
Total	11.7±1.2	11.3±1.1	12.4±1.0	11.9±1.1	12.2±1.1*	11.6±1.0	11.8±1.1
Work	5.8±1.3	5.5±1.4	6.2±0.9	6.1±1.2	6.0±1.2	6.2±1.3	5.9±1.2
Commute	0.9±0.4	1.0±0.5	0.9±0.3	0.9±0.5	1.0±0.6	0.9±0.3	0.9±0.4
Leisure	5.1±1.2	5.0±1.1	5.3±1.5	4.9±1.3	5.1±1.2	4.6±1.5	5.0±1.3
<b>EMG inactivity</b>							
Muscle inactivity time (%)							
Total	69.1±8.5	67.2±8.8	72.3±7.3	69.2±13.4	67.5±13.4	71.2±13.7	69.1±11.1
Work	79.7±8.9	78.4±9.4	81.8±8.1	77.6±12.6	77.9±11.4	77.2±14.4	78.6±10.8
Commute	48.0±21.1	43.3±20.5	56.0±20.7	40.5±22.0	40.0±20.8	41.2±24.4	44.3±21.6
Leisure	59.1±11.9	58.0±11.6	61.0±12.8	64.1±18.6	61.9±19.6	66.6±17.9	61.6±15.6
Sum of the 5 longest muscle inactivity periods (min)							
Total	35.6±14.8	32.9±11.6	40.0±18.8	37.9±17.4	40.5±21.7	34.8±10.4	36.7±16.0
Work	29.7±13.1	27.8±10.7	32.9±16.6	33.6±18.5	37.9±22.6	28.6±11.1	31.7±16.0
Commute	8.5±6.5	8.0±7.2	9.3±5.5	7.2±7.1	7.2±6.7	7.1±7.9	7.8±6.8
Leisure	26.4±13.9	23.4±11.5	31.5±16.6	24.7±9.9	22.9±9.0	26.7±11.0	25.6±12.0
<b>EMG activity</b>							
Light muscle activity time (%)							
Total	22.2±7.9	23.2±7.9	20.4±8.1	21.7±11.9	24.4±12.7	18.5±10.4	21.9±10
Work	15.6±7.7	16.4±8	14.2±7.6	16.8±10.7	17.7±10.6	15.8±11.2	16.2±9.3
Commute	35.5±15.8	37.3±13.4	32.6±19.8	30.0±11.6	32.0±13.3	27.5±9.2	32.8±14
Leisure	29.1±11.6	29.7±11.3	28.1±12.6	25.8±15.4	29.6±17.7	21.4±11.4	27.5±13.6

Moderate muscle activity time (%)								
Total	7.1±3.2	7.7±2.4	6.3±4.2	7.6±3.3	7.5±3.5	7.9±3.3	7.4±3.2	
Work	4.0±2.1	4.3±2.1	3.4±1.9	5.0±3.2	4.1±1.6	6.0±4.3	4.5±2.7	
Commute	14.1±8.4	16.5±9.2	10.1±5.2	24.1±14.5*	24.6±16	23.5±13.4*	19.1±12.8	
Leisure	9.4±4.7	9.7±3.4	8.9±6.6	8.1±4.3	7.9±4.7	8.2±4.1	8.7±4.5	
Vigorous muscle activity time (%)								
Total	1.6±2.2	1.9±2.7	1.1±1.0	1.5±2.9	0.7±1	2.4±4.1	1.5±2.6	
Work	0.8±1.2	0.9±1.4	0.6±0.6	0.6±0.8	0.3±0.3	1.0±1.0	0.7±1.0	
Commute	2.3±3.3	2.9±4.1	1.3±1.0	5.4±6.6	3.4±4.7	7.8±7.8	3.9±5.4	
Leisure	2.4±3.5	2.7±4.1	1.9±2.3	2.0±6.4	0.5±0.4	3.8±9.4	2.2±5.1	
EMG amplitude (%EMG <sub>MVC</sub> )								
Total	2.5±1.3	3.0±1.4	1.7±0.9	2.2±1.8	2.0±1.1*	2.4±2.4	2.4±1.6	
Work	1.6±0.9	1.8±1.0	1.1±0.6	1.4±0.6	1.3±0.6	1.4±0.6	1.5±0.8	
Commute	4.2±2.6	5.2±2.8	2.6±1.3	5.2±3.2	5.4±3.7	5.0±2.6*	4.7±2.9	
Leisure	3.3±1.9	3.8±2.0	2.5±1.3	2.7±4.0	2.0±1.1**	3.6±5.9**	3.0±3.1	
No of bursts per minute								
Total	22.5±14.9	24±16.2	19.9±13.0	24.3±15.2	28.8±18.1	19±8.9	23.4±14.9	
Work	19±14.5	18.8±13.1	19.5±17.5	19.8±14.5	22.7±17.7	16.5±9.1	19.4±14.3	
Commute	26.2±16.9	29.0±20.0	21.6±8.8	36.3±30.9	43.8±39.5	27.4±12.6	31.3±25.1	
Leisure	27±18.9	30.1±22.2	21.8±11	27.5±16.8	33.2±19.2	20.9±10.9	27.2±17.7	

\* denotes to  $p < .05$  and \*\* to  $p < .01$  within genders between groups or between group means.

**Signal pre-processing.** The recording electronic module contains signal amplifiers, microprocessor with firmware, data memory and PC interface. In the module, the EMG signal is measured in its raw form with a sampling frequency of 1000 Hz and a frequency band of 50 Hz – 200 Hz (-3dB). The raw EMG signal was first rectified and then averaged over 100 ms non-overlapping intervals. The averaged data was stored in the memory of the module from which the data was downloaded to a PC using the specifically designed HeiMo PC-software (Myontec Ltd, Kuopio, Finland).

**Artefact removal.** The rectified EMG signal was visually evaluated for occasional artefacts (e.g. toilet visits when electrodes were displaced, short-term movement artefacts), which were manually

removed in MegaWin software (Mega Electronics Ltd., Kuopio, Finland). These occasional artefacts usually appeared simultaneously in all channels, and the corresponding data period was deleted from every channel. In case the artefact was longer than 30 min, the particular channel was removed from the analysis. These cases were probably caused by improper function of the measurement device or impedance problems between skin and electrodes due to lose contact of the particular electrodes. The effect of artifact on other channels was carefully evaluated, and only channels that contained physiological data were included in the analysis.

All channels were included in the analysis from 20 subjects (intervention  $n=9$ , control  $n=11$ ). In 12 subjects analysis included three channels (intervention  $n=6$ , control  $n=6$ ), in 10 subjects two channels (intervention  $n=5$ , control  $n=5$ ), and in 9 subjects only one channel was included (intervention  $n=4$ , control  $n=5$ ). From all channels removed, 29.5% were clean and were excluded to be able to compare the same muscle groups between the days. The effect of channel removal on EMG variables was carefully evaluated. Although omitting channels affected outcome measures, it should be noted that for both measurement days, the same channels were included in the analysis (e.g. if first day had only 1 hamstring and 1 quadriceps channel, those channels were used for analysis also on the second day).

**Effect of channel removal.** To take into account the possibility that results may vary depending on the number of muscle groups removed due to artefact, we investigated the differences between the averaged EMG containing all four channels, and the averaged EMG from which either one or more channels were removed. This was done with data including all four channels from 13 subjects (Supplementary table 2). The results seemed to be most sensitive on removal of hamstring muscles, but for example lack of both quadriceps muscle groups did not change inactivity time significantly if both

hamstring muscle groups were included. To make the comparison between the two measurement days possible, only comparable channels from each day were included in the analysis.

**SUPPLEMENTARY TABLE 2.** Percent difference in variables of averaged EMG from different constitution of channels compared to average EMG from all channels. N=13.

No of quadriceps channels	No of hamstring channels	Total no of channels	Inactivity (%)	Average EMG (uV)	Aver of 5 longest inact. periods (min)	Number of bursts	Aver duration of bursts (s)
2	2	4	Ref.	Ref.	Ref.	Ref.	Ref.
1	2	3	-0,1±4,0	12,6±7,4***	-2,2±6,0	4,1±5,9	0,3±12,8***
2	1	3	5,6±4,8**	-6,4±7,6**	6,4±9,8*	13,0±10,2***	-16,8±9,0***
1	1	2	5,9±5,5**	5,2±6,7***	9,0±10,3**	22,8±16,1***	-24,6±11,3
0	2	2	0,8±8,9	33,6±12,8***	-4,8±11,0	9,5±10,6	-6,3±18,7***
2	0	2	20,3±13,1***	-23,9±13,1***	22,3±18,7***	10,0±24,2	-40,8±12,1***
1	0	1	24,5±14,6***	-20,1±13,3**	36,3±22,0***	18,8±34,0	-53,8±10,0***
0	1	1	8,0±10,3*	38,4±12,2***	9,1±19,8	28,7±25,1**	-32,6±16,7***

Ref=reference data, \* denotes to  $p<.05$ , \*\* to  $p<.01$  and \*\*\* to  $p<.001$ .

**Channel averaging.** In order to determine the complete inactivity and activity periods from quadriceps and hamstring muscles, the channels from four muscle groups were averaged after normalizing the data (Supplementary digital content 2: Supplementary figure 1A).

**Matlab analysis.** After artefact removal and channel averaging the data was ran through a custom made Matlab algorithm (MATLAB, MathWorks, Massachusetts), from which the final results were obtained. The signal baseline sometimes drifted and the drift was corrected for by using a 5 minute moving window throughout the entire recording time (see next paragraph). After the baseline correction the algorithm calculated the final results.

**The minimum filter window length.** After channel averaging any fluctuations in signal baseline were corrected by searching a minimum value from a 5 minute window and by subtracting this value from a preceding data point. This was repeated systematically throughout the recording period (Supplementary digital content 2: Supplementary figure 1B). The length of the filter window was selected according to pilot analysis in laboratory conditions and comparison of daily data analysed by alternative filter lengths. The laboratory tests included a variety of controlled activities from low to high intensities that lasted up to 1 minute, and uncontrolled periods of standing and ambulatory activities that lasted several minutes. According to pilot analysis in 5 subjects, the longest continuous burst duration was on average  $88 \pm 16$ s. For example, during a task which included a short period of sitting and 3 minutes of standing and ambulating while talking to phone, the longest continuous burst duration was  $17 \pm 9$  seconds, although burst time was  $71 \pm 17\%$  of the measurement time. This can be taken as illustration of the intermittent nature of EMG even while maintaining a posture. The choice of 5 minute window may of course affect real physiological data in long-term static muscle activations, but these were assumed to be rare during normal daily life.

We also tested the effect of different minimum filter window lengths on daily data. The most sensitive variable on minimum filter was the longest continuous burst duration, but other variables seemed not to be affected considerably when changing the minimum filter window length (Supplementary table 3). The 5 minute window was considered not to shorten physiological muscle activity periods, but to effectively correct for possibly fluctuating baseline.

**Repeatability.** Paired t-tests were used to assess for systematic differences in laboratory tests between the days. Day-to-day reliability in laboratory tests was evaluated with intra-class correlation coefficients (ICC), coefficient of variation (CoV) and limits of agreement (LoA).

In the laboratory measurements, the maximal voluntary contraction increased significantly in post measurement ( $P<.001$ ), but EMG/force -relationship assessed during maximal voluntary contraction remained the same. The intra-class correlation coefficient revealed high to moderate repeatability of the measured variables (Supplementary table 4). The poorer repeatability of submaximal laboratory tests could be explained by the fact that some subjects reported being unfamiliar with testing conditions, e.g. walking and running on treadmill during the first measurements. Therefore, the results from the second laboratory tests were used to categorize the data of both measurement days.

**SUPPLEMENTARY TABLE 3.** Results from pilot analysis including 42 days from 21 subjects analyzed by different minimum filter window lengths.

<b>Filter window</b>	<b>Inactivity(%)</b>	<b>Average EMG (<math>\mu</math>V)</b>	<b>Bursts/min</b>	<b>Longest burst (min)</b>	<b>Longest inact. period (min)</b>
No filter	15,1 $\pm$ 23,4	12,1 $\pm$ 6,7	14,9 $\pm$ 26,0	404,3 $\pm$ 330,8	2,3 $\pm$ 3,7
60min	63,0 $\pm$ 10,7	7,5 $\pm$ 5,9	22,8 $\pm$ 17,1	7,7 $\pm$ 10,1	9,9 $\pm$ 5,6
30min	64,0 $\pm$ 10,6	7,3 $\pm$ 5,8	22,8 $\pm$ 16,5	6,3 $\pm$ 8,2	10,2 $\pm$ 5,7
10min	65,1 $\pm$ 10,3	7,1 $\pm$ 5,5	22,8 $\pm$ 15,5	4,0 $\pm$ 3,1	10,4 $\pm$ 5,8
5min	65,7 $\pm$ 10,1	6,9 $\pm$ 5,3	22,9 $\pm$ 15,2	3,1 $\pm$ 1,9	10,4 $\pm$ 5,8
3min	66,1 $\pm$ 10,0	6,8 $\pm$ 5,2	23,0 $\pm$ 15,0	2,3 $\pm$ 1,1	10,5 $\pm$ 5,8
1min	67,3 $\pm$ 9,6	6,5 $\pm$ 4,8	23,9 $\pm$ 15,1	1,2 $\pm$ 0,4	10,5 $\pm$ 5,8

**SUPPLEMENTARY TABLE 4.** Repeatability of maximal voluntary contraction, EMG/force - relationship (MVC), walking at 5 km/h and running at 10 km/h between pre and post measurements. N=43.

	Pre	Post	%Diff	ICC (95% CI)	Mean CoV (%)	LoA
<b>MVC</b>						
MVC Extension (kg)	78.2±23.2	82.7±23.4	6.6±11.2***	0.95 (0.91-0.97)	10.6	2.08-6.51
MVC flexion (kg)	46.6±18.0	48.4±17.0	6.8±13.2***	0.96 (0.93-0.98)	12.1	0.81-3.70
EMG/force quadriceps (MVC)	3.9±1.5	3.8±1.5	-1.2±17.1	0.87 (0.76-0.93)	19.5	-0.37-0.18
EMG/force hamstrings (MVC)	6.9±3.1	7.1±3.5	4.5±28.2	0.85 (0.74-0.91)	30.3	-0.43-0.68
<b>Submaximal</b>						
90% of Standing (%EMG <sub>MVC</sub> )	1.6±0.9	1.5±0.9	-4.5±44.4	0.70 (0.51-0.82)	62.0	-0.38-0.05
Walking 5 km/h (%EMG <sub>MVC</sub> )	8.1±3.3	7.3±3.3	-7.5±23.1**	0.79 (0.64-0.88)	27.8	-1.45--0.08
Running 10 km/h (%EMG <sub>MVC</sub> )	24.1±9.5	22.4±9.3	-5.2±20.3*	0.81 (0.67-0.89)	24.4	-3.53-0.08

\* denotes to p<.05, \*\* to p<.01 and \*\*\* to p<.001.