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Supplemental information

A potent physiological method to magnify and sustain soleus oxidative metabolism improves glucose and lipid regulation

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Table S1. Demographics, N=25 (13 females and 12 males), related to Table 4.

EXPERIMENT I, N=10		
	Mean ± SD	Range
Age (years)	37.7 ± 10.9	24 – 53
Gender		5 F / 5 M
Height (m)	1.71 ± 0.07	1.63 – 1.86
Weight (kg)	79.5 ± 18.7	56.6 – 111.8
BMI (kg/m^2)	26.8 ± 4.6	21.2 – 33.1
Percent body fat	37.6 ± 6.9	23.6 – 45.1
Sitting time (hr/day)	10.8 ± 2.2	6.1 – 13.4
Stepping time (hr/day)	1.30 ± 0.52	0.73 – 2.17
Steps per day	6,196 ± 2,820	3,247 – 11,243
Standing time (hr/day)	4.09 ± 1.65	2.49 – 7.78
VO_2max (mL/kg/min)	30 ± 9	20 – 45

EXPERIMENT II, N=15		
N=15 (SED, SPU1)	Mean ± SD	Range
Age (years)	54.3 ± 19.0	22 – 82
Gender		8 F / 7 M
Height (m)	1.68 ± 0.09	1.56 – 1.88
Weight (kg)	81.0 ± 21.1	48.1 – 122.4
BMI (kg/m^2)	28.4 ± 6.7	19.7 – 42.9
Percent body fat	33.7 ± 10.7	18.9 – 51.9
Sitting time (hr/day)	10.6 ± 2.0	6.7 – 13.9
Stepping time (hr/day)	1.38 ± 0.51	0.6 – 2.4
Steps per day	6,025 ± 2,433	2,061 – 10,843
Standing time (hr/day)	4.29 ± 1.40	2.5 – 7.1
N=10 (SED, SPU1, SPU2)	Mean ± SD	Range
Age (years)	52.8 ± 18.3	24 – 82
Gender		6 F / 4 M
Height (m)	1.67 ± 0.07	1.57 – 1.79
Weight (kg)	78.9 ± 16.3	56.5 – 108.8
BMI (kg/m^2)	28.4 ± 5.8	20.4 – 36.0
Percent body fat	35.2 ± 10.1	18.9 – 44.9
Sitting time (hr/day)	10.3 ± 1.9	6.7 – 13.1
Stepping time (hr/day)	1.36 ± 0.37	0.9 – 2.1
Steps per day	5,939 ± 1,607	4,434 – 9,800
Standing time (hr/day)	4.5 ± 1.6	2.5 – 7.1

This N=10 subgroup in Experiment II are the individuals from the above 15 who also participated in SPU2.

Table S2. Total body energy expenditure (kcal/min), related to Tables 1 and 2.

Experiment I	t1	t2	Mean
Control	1.18 ± 0.05	1.21 ± 0.06	1.20 ± 0.05
	t1	t2	Mean
SPU	2.70 ± 0.19	2.71 ± 0.21	2.71 ± 0.20

Experiment II	t1	t2	t3	Mean
Control	1.14 ± 0.06	1.18 ± 0.06	1.11 ± 0.06	1.15 ± 0.06
	t1	t2	t3	Mean
SPU1	1.78 ± 0.09	1.73 ± 0.09	1.73 ± 0.08	1.75 ± 0.09
	t1	t2	t3	Mean
SPU2	2.33 ± 0.16	2.21 ± 0.12	2.17 ± 0.13	2.24 ± 0.13

Mean ± SEM. There was not a significant difference between any time period *within* a test day. However, between test day differences were all significant ($P<0.0001$). In Experiment I, t1 was the average energy expenditure in the period before the first biopsy and t2 was the period between the first and second biopsy. In Experiment II, t1, t2, and t3 were the respective rates of energy expenditure in the 1st, 2nd and 3rd hours of the OGTT. Differences between conditions were determined by a mixed effects model followed by Tukey's multiple comparison tests.

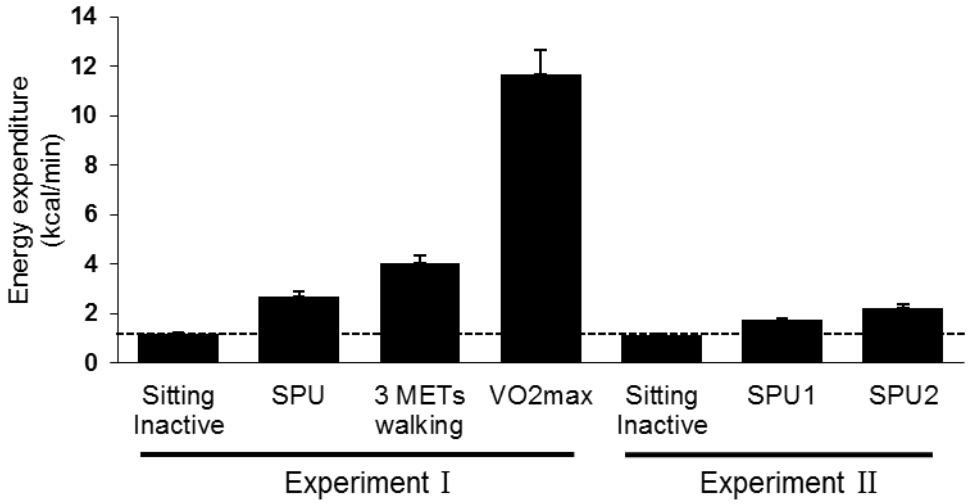


Figure S1. Total body energy expenditure, related to Figure 2, and Tables 1 and 2. An SPU is by design isolated contractile activity of a small muscle mass when sitting to induce a significant, yet subtle increase in total energy expenditure. Individuals in Experiment I performed SPUs in addition to completing treadmill exercise testing to compare the energy demands with a large muscle mass activity involving compound movements. Differences between conditions within each experiment were determined by a mixed effects model followed by Tukey's multiple comparison tests. Both walking and running at VO2max expectedly had a greater whole body energy demand per minute than SPU contractions ($P=5x10^{-5}$ and $P=1x10^{-5}$, respectively). In Experiment II, the rate of energy expenditure when sitting inactive vs SPU1 ($P=2x10^{-8}$) and vs SPU2 ($P=4x10^{-6}$) are shown. The energy expenditure of SPU contractions in Experiment I was 55 and 21% greater than that of SPU1 and SPU2 of Experiment II (P=0.0007 and 0.064, respectively; unpaired *t*-test). All 3 levels of SPU contractions were significantly different from sitting inactive (see Tables 1 and 2 for more statistics). Broken horizontal line denotes sitting inactive resting energy expenditure. Mean ± SEM bars.

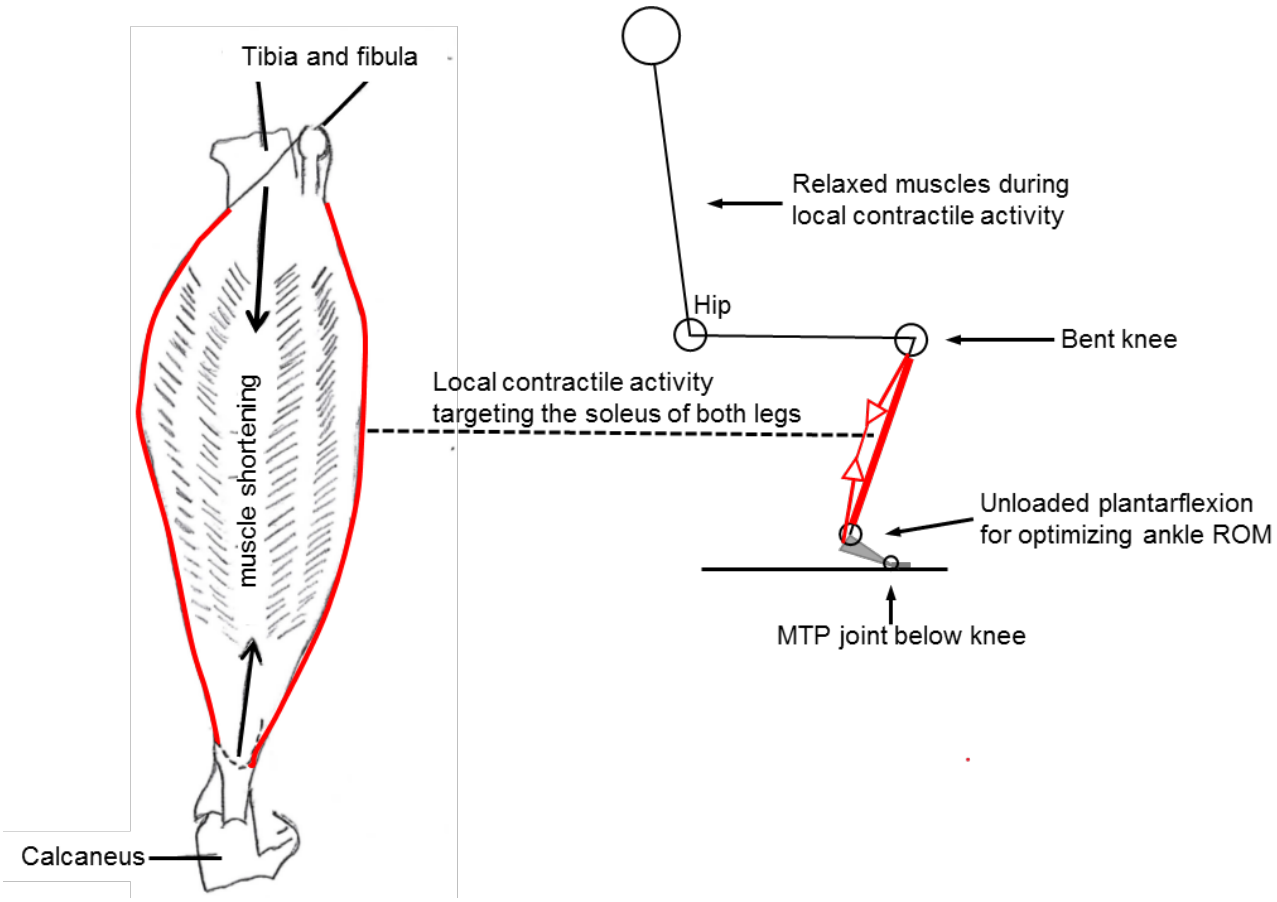
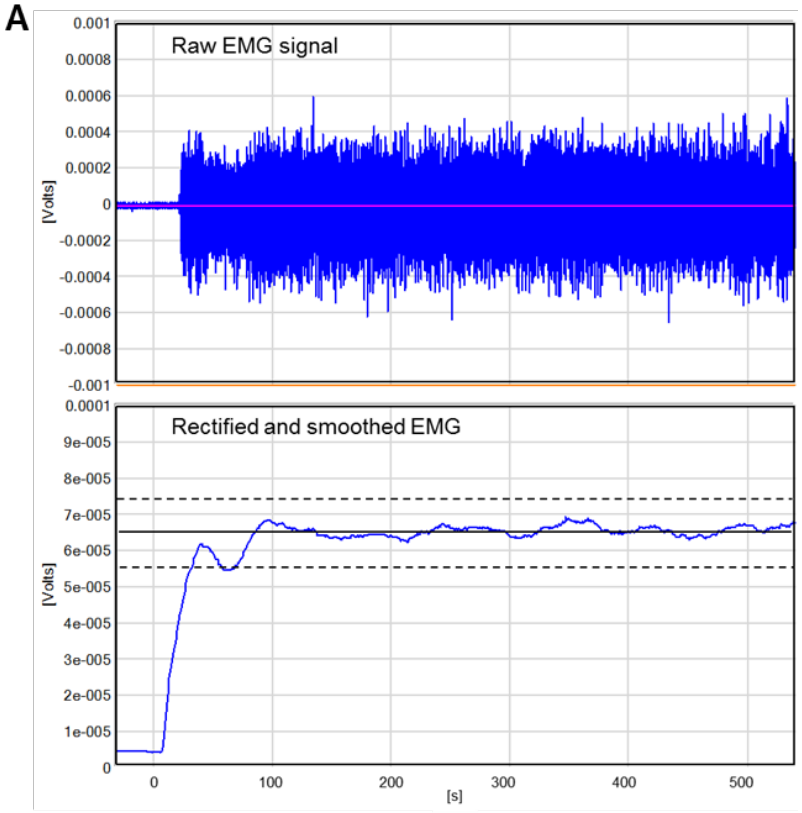


Figure S2. Illustration of SPU contractions, related to STAR Methods. This method focused on prolonged isolated contractile activity during sitting. It involved unloaded contractions (no more than the weight of the leg) in order to enhance the range of motion and minimize fatigue or other adverse responses like soreness or cramping. These seated contractions targeted the soleus, a slow oxidative muscle (with a mass of ~1 kg as determined directly with MRI measurements). The knee was bent with the metatarsophalangeal joint (MTP joint) below the knee to minimize involvement of the gastrocnemius synergists and accentuate the soleus contribution. There was a gravity-induced passive leg lowering (i.e. soleus muscle lengthening) between each SPU contraction. Volunteers sat comfortably, with or without shoes, and with the chair height and back rest individualized in order to maintain a relaxed posture for the resting musculature. Real-time EMG was used to teach the method and provide feedback for setting the desired level of metabolic activity.



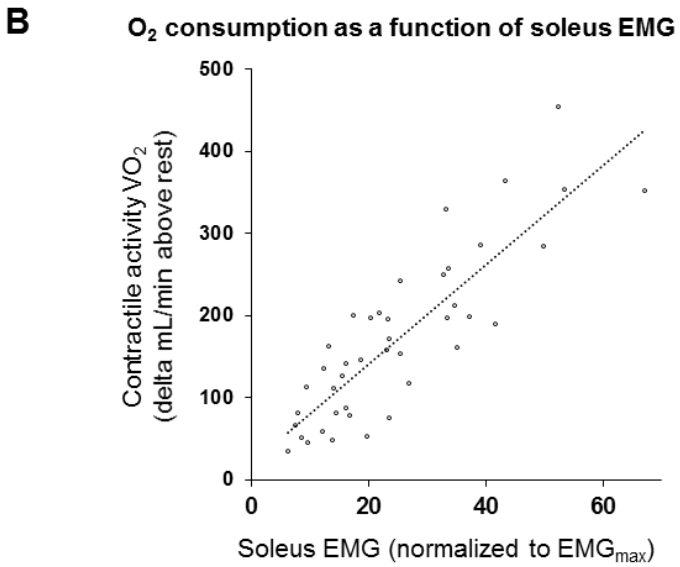


Figure S3. Smoothed EMG signal for biofeedback and linear relationship of soleus activation to VO2, related to a methodological validation test as described in the STAR Methods. (**A**) The raw EMG signal collected at 2000 Hz (top panel) was rectified (root mean square) and smoothed in the panel below it to display feedback of the soleus activation during SPU contractions. This real time feedback was displayed on a large computer screen to help participants maintain a steady local soleus intensity. When viewed over ~500 seconds as above, each of the individual EMG spikes cannot be discerned (please see Figure S4 for more precise results over a shorter window of time to visualize individual EMG bursts). (**B**) Soleus activation was highly related to the steady-state increase in oxygen consumption above the normal resting metabolic rate when sitting ($r=0.86$; $P=4x10^{-13}$) in a preliminary experiment in which 10 individuals were tested during graded SPU contractile activity.

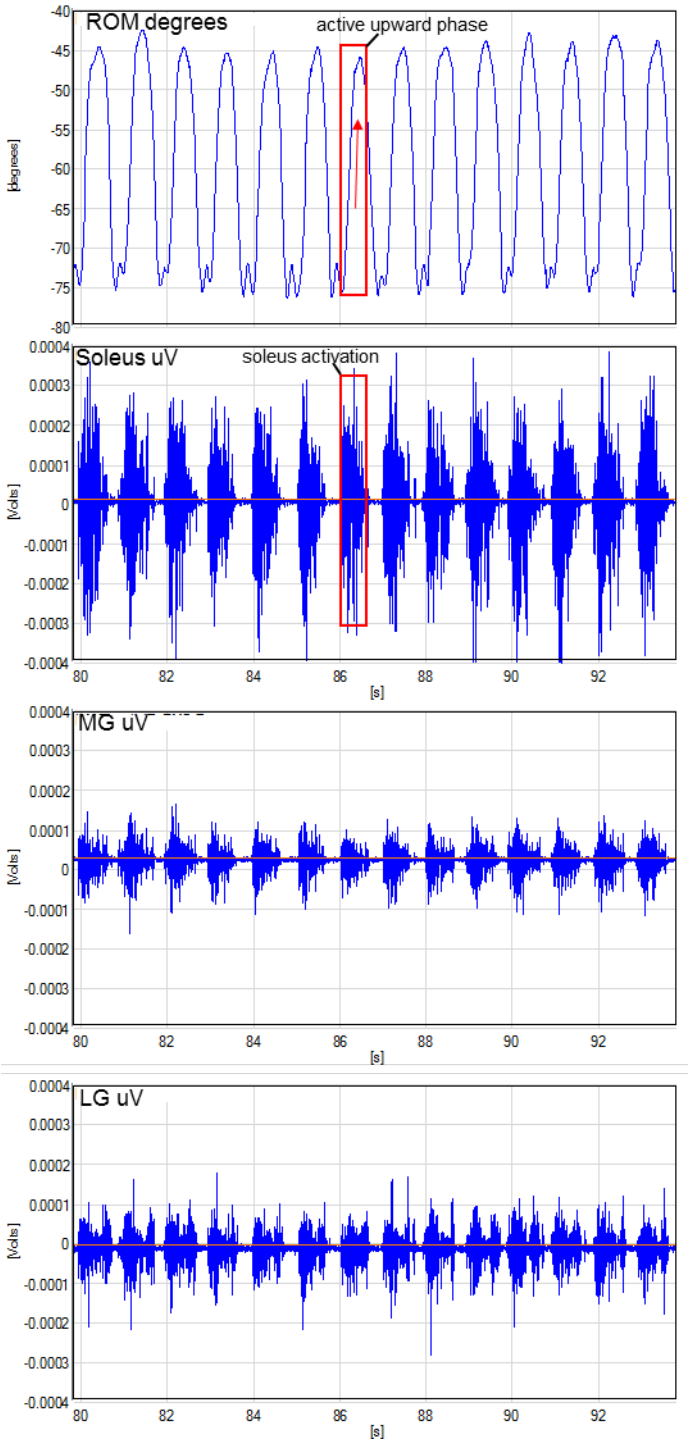


Figure S4. Ankle angular motion during SPU contractions (isolated plantarflexion when sitting) and EMG of the soleus and the accessory triceps surae muscles, related to STAR Methods. Range of motion (ROM) of the ankle was measured in this individual in a methodological validation test to illustrate that the EMG on-time (soleus activation) coincides with the "concentric shortening phase" during this particular type of plantarflexion. This participant wore Delsys EMG sensors over the soleus and medial and lateral gastrocnemius (MG and LG) muscles as well as ankle strain gauge goniometers. The raw EMG was collected at 2000 Hz.

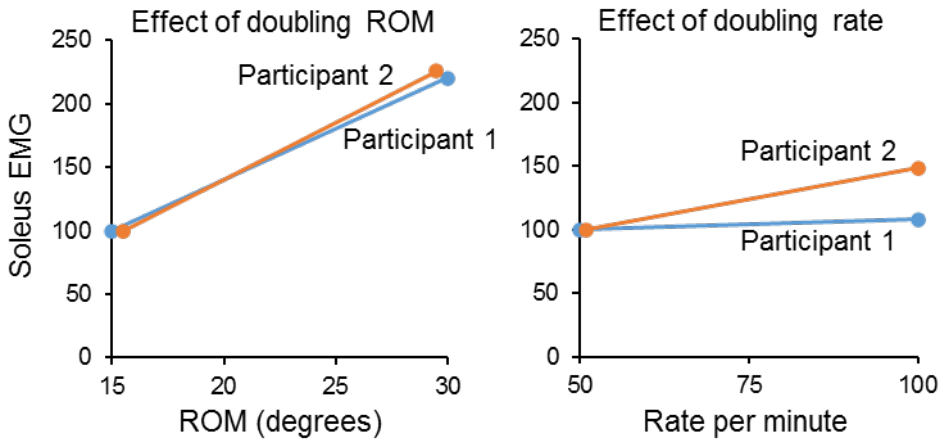


Figure S5. The effect of range of motion (ROM) at a fixed rate and the effect of rate (at a fixed ROM), related to methodological validation test in STAR Methods. While the impact of raising the plantarflexion ROM was predictable and proportional to soleus EMG (left panel), raising the rate was not proportional (right panel). In the left panel the rate was fixed at 50 contractions/min. In the right panel, the ROM was fixed at 30 degrees. Results from 2 representative examples.

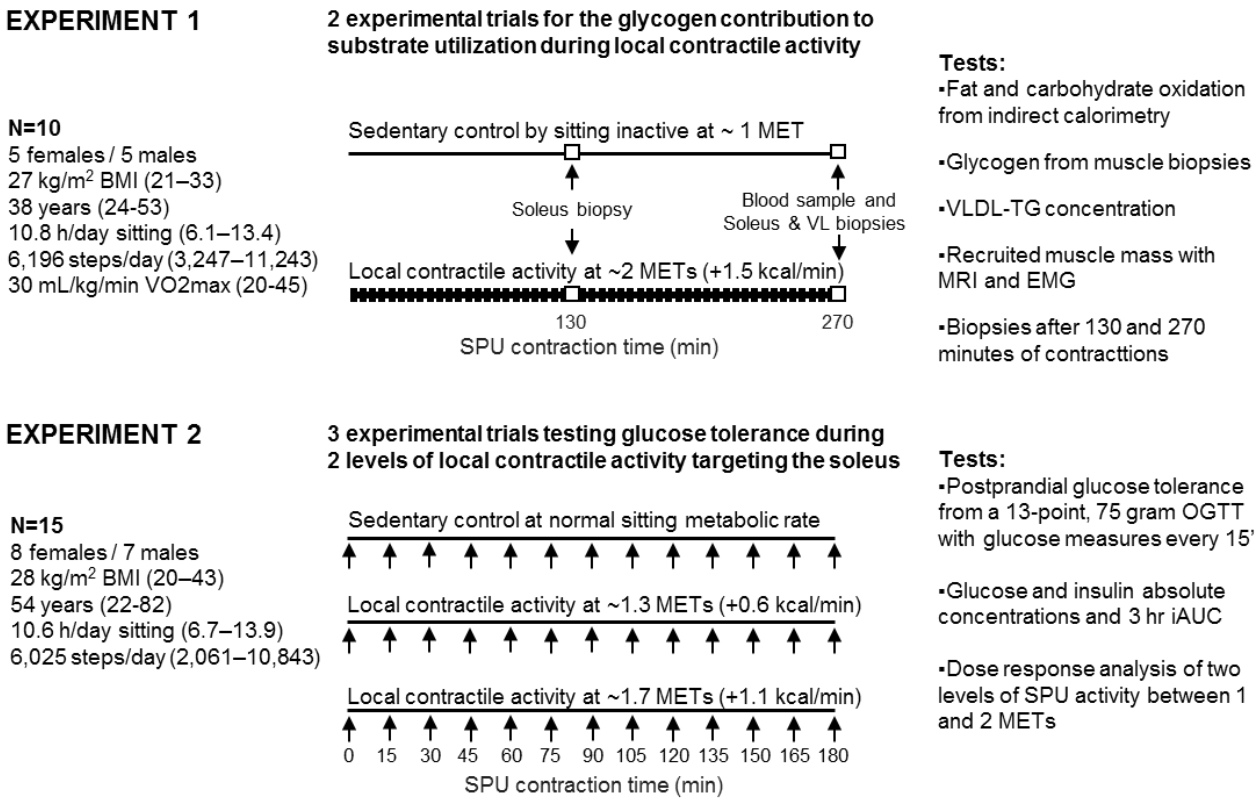


Figure S6. Experimental overview, related to STAR Methods. Two experiments were performed to understand the metabolic responses to isolated plantarflexion targeting local soleus contractile activity (SPU contractions). Both studies enrolled an equal number of male and female participants with a wide diversity of BMI, age, sedentary behavior, and habitual daily steps. See also Table S1 for participant demographics. Each participant served as their own control in this randomized cross-over design by sitting inactive at the normally low resting metabolic rate. Experiment I tested a single level of activity energy expenditure (~2 METs or 1.5 kcal/min) above the resting level of energy expenditure in 10 participants. Experiment II involved the responses to one low level of SPU contractions (~1.3 METs or 0.6 kcal/min above resting) compared to when sitting at a normal metabolic rate in all 15 participants, and also a second level (~1.7 METs or 1.1 kcal/min above resting) in 10 of those 15 volunteers (in random order).

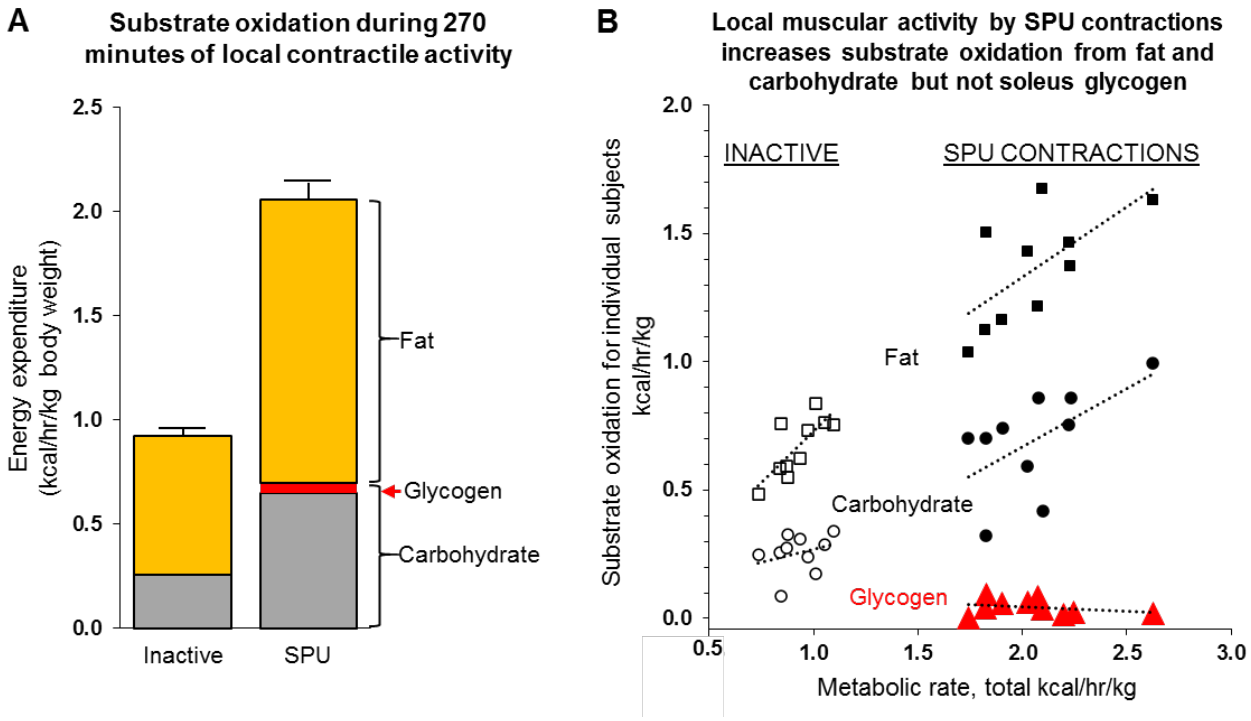


Figure S7. Total substrate oxidation in Experiment I, related to Figures 1 and S8. (**A**) Total energy expenditure and the contribution of substrates fueling oxidative metabolism. Fat oxidation ($P=5x10^{-7}$) and total carbohydrate oxidation (P=0.0001) were both significantly increased (paired *t*-tests comparing SPU contractions to sitting inactive in 10 individuals). Mean ± SEM bars for energy expenditure. (**B**) Individual responses. There was not a relationship between glycogen use to energy expenditure over this narrow range, as there was for the other substrates.

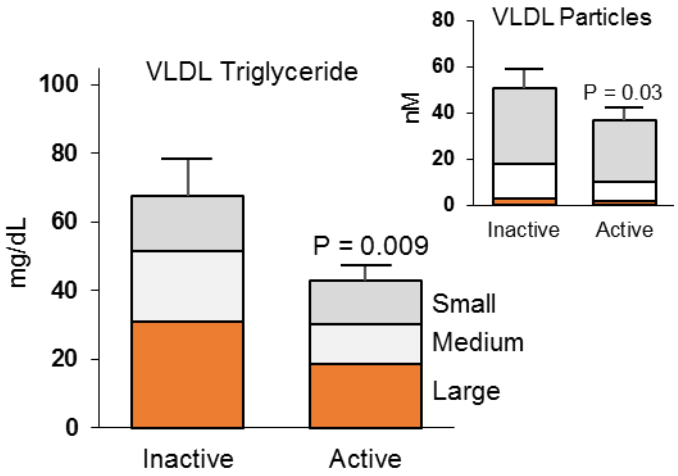


Figure S8. VLDL triglyceride responses to SPU contractions in Experiment I, related to Table 1 and Figure S7. The three subfractions of VLDL determined by nuclear magnetic resonance spectroscopy are shown. P-values from paired *t*-test analyses. Mean ± SEM.

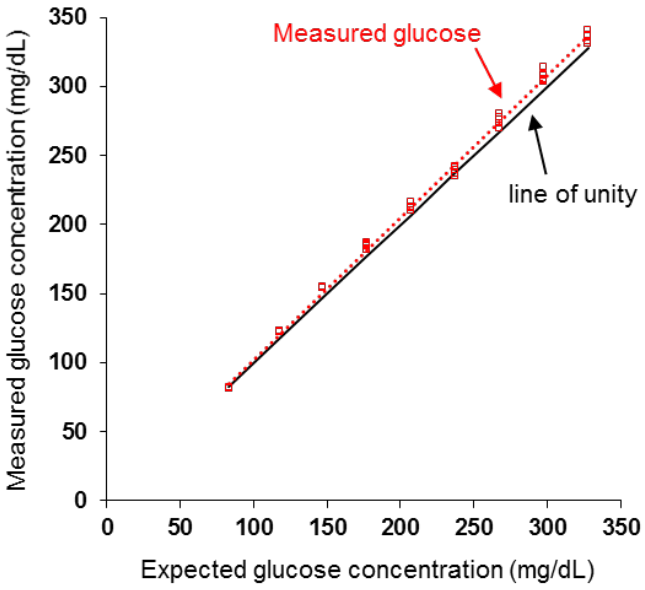


Figure S9. A preliminary methodological examination of the relationship between measured and expected glucose concentrations, related to STAR Methods. Whole blood in a series of test tubes was spiked with known amounts of glucose to test the accuracy of glucose measurements from the glucometer in a preliminary control experiment. The measured (Y-axis) and expected glucose concentrations (x-axis) were in close agreement over 9 concentrations and replicated in 6 samples. The 6 replicates at each concentration are often difficult to see because of overlapping values at the same points in the graph due to a low coefficient of variation (1.3%).

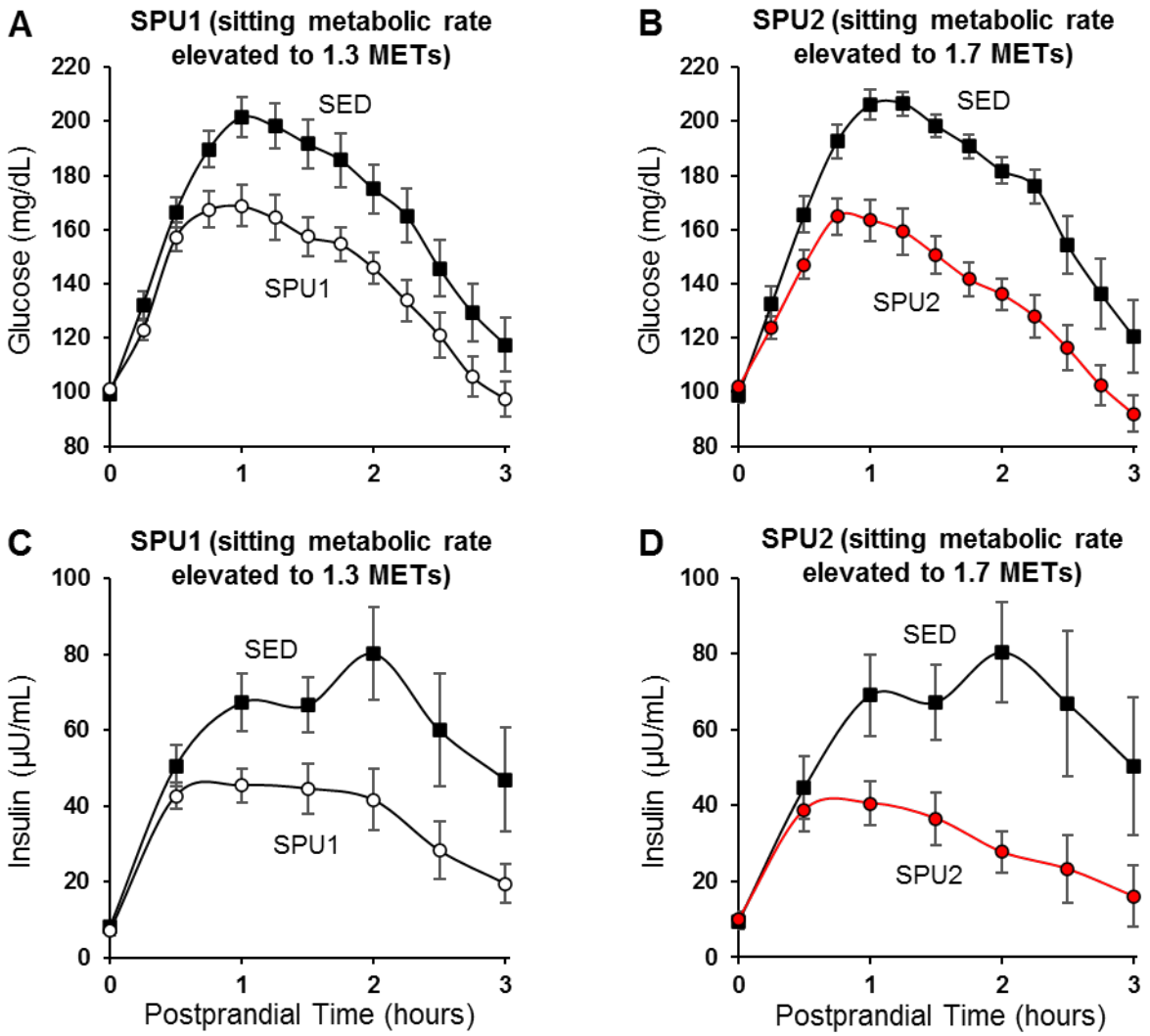


Figure S10. Time course of glucose and insulin responses during the sedentary control and SPU conditions in Experiment II, related to Table 3 and Figure 3. (**A**) Effects of SPU1 on glucose. (**B**) Effects of SPU2 on glucose. (**C**) Effects of SPU1 on insulin. (**D**) Effects of SPU2 on insulin. The statistics are provided in Figure 3 and Table 3. METs during sitting inactive (SED) averaged 0.86 ± 0.05 (Table 2). Mean ± SEM.