

SUPPLEMENTAL FIGURES AND TABLES

ESM Table 1. Additional participant characteristics

| Parameter | Mean \pm SD |
|--|------------------|
| Body weight (kg) | 80.1 \pm 9.2 |
| Waist circumference (cm) | 97 \pm 9 |
| Hip circumference (cm) | 110 \pm 8 |
| Fat mass (%) | 46.1 \pm 11.3 |
| Systolic blood pressure | 133 \pm 16 |
| Diastolic blood pressure | 85 \pm 9 |
| Physical activity level (Baecke score) | 6.55 \pm 0.95 |
| Habitual sitting + sleeping time (h) | 17.6 \pm 1.2 |
| Habitual standing time (h) | 4.3 \pm 1.2 |
| Habitual walking time (h) | 2.1 \pm 0.6 |
| Habitual steps/day (n) | 10141 \pm 3250 |
| Habitual cadence (steps/min) | 80 \pm 5 |

ESM Table 2. Medication of participants

| Medication | N |
|--------------------------------|---|
| Lipid lowering drugs (statins) | 2 |
| Blood pressure lowering drugs | 1 |
| Glaucoma medication | 1 |

ESM Table 3. Physical activity during activity regimes

| Parameter | SIT | SL | EXE | p-value | p-value SIT vs EXE | p-value SIT vs SL | p-value EXE vs SL |
|---------------------------------------|------------|-------------|------------|---------|--------------------|-------------------|-------------------|
| Sitting (h/day) | 13.6 ± 0.2 | 9.2 ± 0.2 | 12.5 ± 0.3 | <0.01* | <0.01* | <0.01* | <0.01* |
| Standing (h/day) | 1.3 ± 0.1 | 4.0 ± 0.1 | 1.2 ± 0.1 | <0.01* | 0.99 | <0.01* | <0.01* |
| Walking (h/day) | 1.0 ± 0.0 | 3.0 ± 0.1 | 1.0 ± 0.0 | <0.01* | 0.46 | <0.01* | <0.01* |
| Exercise (h/day) | - | - | 1.0 ± 0.1 | - | - | - | - |
| Sleeping (h/day) | 8.1 ± 0.1 | 7.9 ± 0.2 | 8.1 ± 0.1 | 0.28 | 0.99 | 0.83 | 0.29 |
| Steps/day (n) ¹ | 4878 ± 240 | 16875 ± 463 | 5082 ± 165 | <0.01* | 0.62 | <0.01* | <0.01* |
| Cadence (steps/min) ¹ | 85 ± 3 | 94 ± 2 | 86 ± 2 | <0.01* | 0.99 | <0.01* | <0.01* |
| Estimated EE ¹ (MET*h/day) | 32.0 ± 0.1 | 37.0 ± 0.2 | 36.6 ± 0.2 | <0.01* | <0.01* | <0.01* | 0.38 |

Abbreviations: SIT, sit regime; EXE, exercise regime; SL, sitting less regime. Data are expressed as mean ± SE. N=12, ¹n=11. *p<0.05.

ESM Table 4. Insulin sensitivity and substrate kinetics

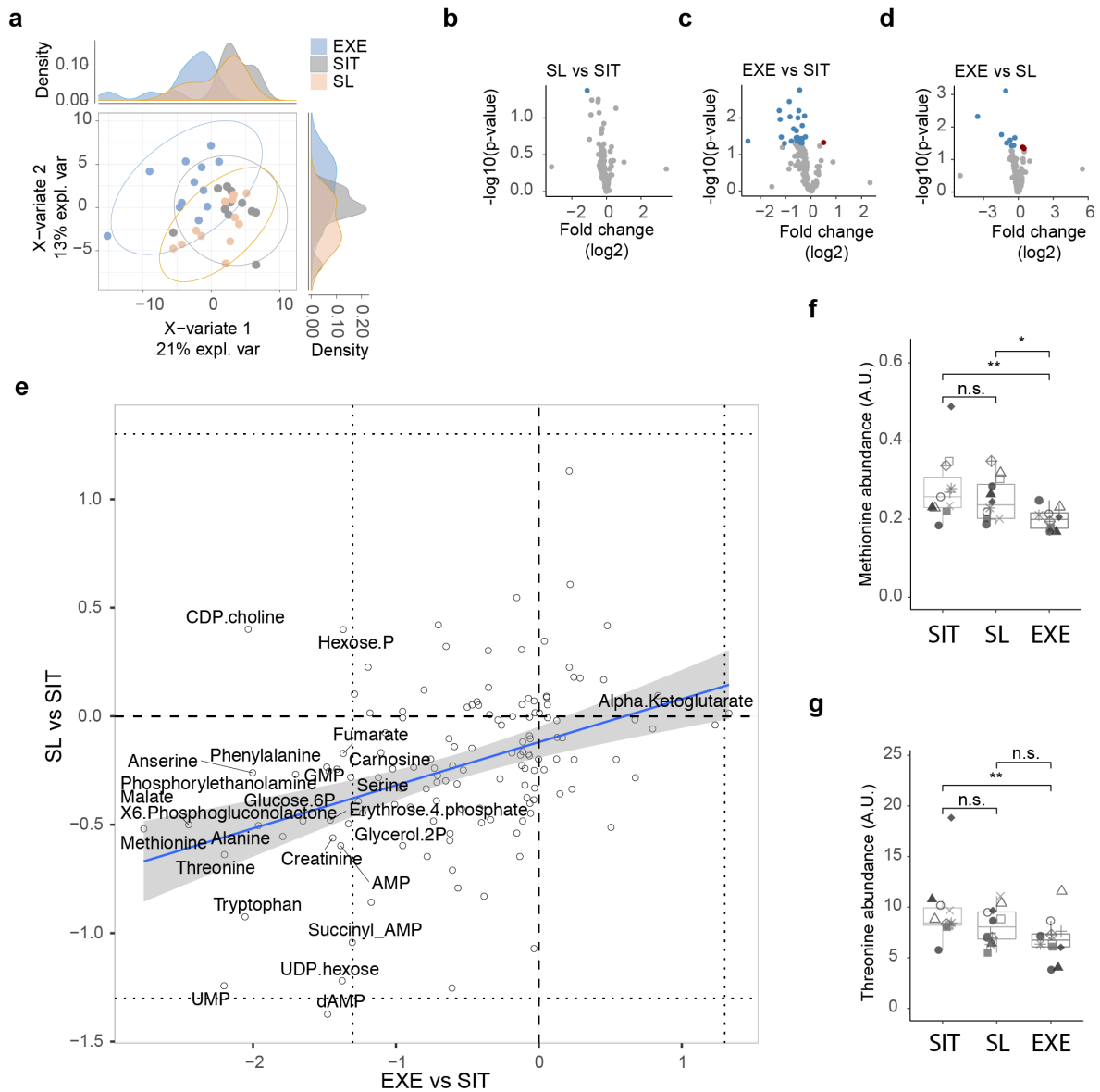
| Parameter | SIT | SL | EXE | p-value | p-value SIT vs EXE | p-value SIT vs SL | p-value EXE vs SL |
|---|-----------------|-----------------|-----------------|---------|--------------------|-------------------|-------------------|
| Ra ($\mu\text{mol/kg/min}$) | | | | | | | |
| Baseline | 7.6 \pm 0.4 | 8.4 \pm 0.5 | 7.3 \pm 0.5 | 0.21 | 0.99 | 0.46 | 0.31 |
| Low insulin ¹ | 11.9 \pm 1.3 | 11.9 \pm 0.7 | 11.7 \pm 1.0 | 0.73 | 0.99 | 0.99 | 0.99 |
| High insulin ² | 29.8 \pm 3.6 | 32.8 \pm 3.2 | 36.1 \pm 3.8 | <0.01** | 0.01* | 0.22 | 0.79 |
| Rd ($\mu\text{mol/kg/min}$) | | | | | | | |
| Baseline | 7.3 \pm 0.4 | 7.6 \pm 0.6 | 6.9 \pm 0.5 | 0.56 | 0.99 | 0.99 | 0.92 |
| Low insulin ¹ | 12.5 \pm 1.2 | 12.3 \pm 0.9 | 11.8 \pm 0.9 | 0.73 | 0.99 | 0.99 | 0.99 |
| High insulin ² | 29.4 \pm 3.7 | 33.1 \pm 3.2 | 35.2 \pm 3.8 | <0.01** | <0.01** | 0.03* | 0.99 |
| Delta baseline-low ¹ | 5.0 \pm 1.2 | 4.7 \pm 0.7 | 4.9 \pm 0.8 | 0.98 | 0.99 | 0.99 | 0.99 |
| Delta baseline-high ² | 20.7 \pm 3.7 | 25.5 \pm 2.8 | 28.2 \pm 3.3 | <0.01** | <0.01** | 0.08# | 0.99 |
| EGP ($\mu\text{mol/kg/min}$) | | | | | | | |
| Baseline | 7.6 \pm 0.4 | 8.4 \pm 0.5 | 7.3 \pm 0.5 | 0.21 | 0.99 | 0.46 | 0.31 |
| Low insulin ¹ | 2.0 \pm 0.2 | 2.3 \pm 0.2 | 2.3 \pm 0.2 | 0.16 | 0.60 | 0.17 | 0.99 |
| % suppression low ¹ | 73.6 \pm 3.7 | 72.1 \pm 2.9 | 69.2 \pm 3.7 | 0.98 | 0.99 | 0.99 | 0.99 |
| High insulin ² | 0.4 \pm 0.2 | 0.3 \pm 0.1 | 0.7 \pm 0.2 | 0.71 | 0.99 | 0.99 | 0.99 |
| % suppression high ² | 93.6 \pm 2.7 | 96.4 \pm 1.9 | 90.0 \pm 2.9 | 0.44 | 0.99 | 0.99 | 0.54 |
| NOGD ($\mu\text{mol/kg/min}$) | | | | | | | |
| Baseline ¹ | 2.6 \pm 0.6 | 4.0 \pm 0.7 | 3.0 \pm 0.6 | 0.63 | 0.99 | 0.99 | 0.86 |
| Low insulin ¹ | 3.9 \pm 1.2 | 4.9 \pm 1.0 | 4.3 \pm 0.9 | 0.63 | 0.86 | 0.99 | 0.99 |
| High insulin ² | 15.7 \pm 2.8 | 19.9 \pm 2.6 | 22.3 \pm 3.0 | <0.01** | <0.01** | 0.04* | 0.79 |
| Delta baseline-low ² | 1.0 \pm 1.7 | 1.0 \pm 0.7 | 1.0 \pm 0.8 | 0.60 | 0.99 | 0.79 | 0.99 |
| Delta baseline-high ³ | 12.2 \pm 2.9 | 15.0 \pm 2.6 | 18.5 \pm 2.6 | 0.01* | 0.01* | 0.10# | 0.99 |
| Carbohydrate oxidation ($\mu\text{mol/kg/min}$) | | | | | | | |
| Baseline ¹ | 5.0 \pm 0.6 | 4.2 \pm 0.7 | 4.5 \pm 1.0 | 0.24 | 0.46 | 0.13 | 0.91 |
| Low insulin ¹ | 8.6 \pm 0.6 | 7.5 \pm 0.6 | 7.5 \pm 0.8 | 0.35 | 0.41 | 0.86 | 0.99 |
| High insulin ² | 13.8 \pm 1.0 | 13.7 \pm 1.2 | 13.4 \pm 1.1 | 0.98 | 0.99 | 0.99 | 0.99 |
| Fat oxidation ($\mu\text{mol/kg/min}$) | | | | | | | |
| Baseline ¹ | 3.3 \pm 0.2 | 3.6 \pm 0.1 | 3.7 \pm 0.2 | 0.19 | 0.33 | 0.18 | 0.81 |
| Low insulin ¹ | 2.3 \pm 0.2 | 2.6 \pm 0.2 | 2.5 \pm 0.2 | 0.26 | 0.26 | 0.99 | 0.99 |
| High insulin ² | 1.1 \pm 0.2 | 1.3 \pm 0.2 | 1.3 \pm 0.2 | 0.51 | 0.99 | 0.72 | 0.99 |
| Respiratory exchange ratio | | | | | | | |
| Baseline ¹ | 0.78 \pm 0.01 | 0.76 \pm 0.01 | 0.76 \pm 0.01 | 0.16 | 0.59 | 0.10# | 0.99 |
| Low insulin ¹ | 0.84 \pm 0.01 | 0.82 \pm 0.01 | 0.82 \pm 0.01 | 0.20 | 0.63 | 0.22 | 0.99 |
| High insulin ² | 0.92 \pm 0.01 | 0.91 \pm 0.01 | 0.91 \pm 0.01 | 0.63 | 0.99 | 0.99 | 0.99 |
| Delta baseline-low ¹ | 0.07 \pm 0.01 | 0.06 \pm 0.01 | 0.07 \pm 0.01 | 0.66 | 0.99 | 0.99 | 0.99 |
| Delta baseline-high ² | 0.13 \pm 0.01 | 0.14 \pm 0.02 | 0.14 \pm 0.02 | 0.69 | 0.99 | 0.99 | 0.99 |

Abbreviations: SIT, sit regime; EXE, exercise regime; SL, sitting less regime; Ra, rate of appearance; Rd, rate of disappearance; EGP, endogenous glucose production; NOGD, non-oxidative glucose disposal Data are expressed as mean \pm SE. ¹n=11, ²n=10, ³n=9. ** p<0.01, *p<0.05, #p<0.10.

ESM Table 5. Ex vivo skeletal muscle mitochondrial respiratory capacity

| O ₂ flux (pmol/mg/s) | SIT | SL | EXE | p- value | p- value SIT vs EXE | p- value SIT vs SL | p- value EXE vs SL |
|------------------------------------|----------------|----------------|----------------|-------------|---------------------------------|-----------------------------|-----------------------------|
| MG3 | 37.5 \pm 2.3 | 35.8 \pm 2.2 | 38.5 \pm 2.7 | 0.42 | 0.99 | 0.69 | 0.78 |
| MO3 | 36.1 \pm 2.5 | 36.2 \pm 2.9 | 37.7 \pm 2.2 | 0.66 | 0.99 | 0.99 | 0.99 |
| MOG3 | 47.1 \pm 3.9 | 44.7 \pm 3.3 | 47.5 \pm 2.6 | 0.54 | 0.99 | 0.99 | 0.84 |
| MGS3 | 72.1 \pm 4.5 | 67.1 \pm 4.5 | 73.4 \pm 3.9 | 0.25 | 0.99 | 0.77 | 0.52 |
| MOGS3 | 72.0 \pm 6.1 | 68.0 \pm 5.0 | 71.9 \pm 3.2 | 0.58 | 0.99 | 0.99 | 0.99 |
| FCCP | 88.5 \pm 8.7 | 87.8 \pm 7.2 | 93.7 \pm 5.8 | 0.53 | 0.87 | 0.99 | 0.99 |
| Oligomycin | 24.5 \pm 1.8 | 23.0 \pm 2.1 | 23.8 \pm 1.5 | 0.53 | 0.99 | 0.88 | 0.99 |

Abbreviations: SIT, sit regime; EXE, exercise regime; SL, sitting less regime; MG3, malate + glutamate; MO3, malate + octanoyl carnitine + glutamate; MOG3, malate + octanoyl carnitine + glutamate; MGS3, malate + glutamate + succinate; MOGS3, malate + octanoyl carnitine + glutamate + succinate; FCCP, maximal FCCP-induced uncoupled respiration; Oligomycin, oligomycin induced respiration not coupled to ATP synthesis. Data are expressed as mean \pm SE in pmol per mg wet weight per second. N=11.



ESM Figure 1: Metabolomic effects of sitting, sitting less, and exercise in skeletal muscle: Regimes for sitting, sitting less, and exercise are denoted as SIT, SL, and EXE, respectively. **a)** Partial least squares discriminant analysis (PLS-DA) on the metabolomes of individuals in the sitting, sitting less, and exercise regimes, $n=11$, plotting the first component (X-variate 1) and second component (X-variate 2) of the PLS-DA. **b-d)** Volcano plots of fold change (x axis, log2 scale) versus p value (y axis, $-\log_{10}$ scale), for **b)** sitting less vs sit, **c)** exercise vs sit, and **d)** exercise vs sitting less. Reveals exercise to induce the greatest number of metabolic changes. **e)** Highlighted metabolites for plot comparing the significance of differences that exercise induces (compared to sitting), relative to what sitting less induces (compared to sitting). Units on the axes are p values in the $-\log_{10}$ scale. Directionality of induced changes are represented as either negative values (decreased) or positive values (increased). Pearson's $r = 0.393$, p value = $2e-06$ (pertains to main figure 3b). Further visualization of metabolites including **f)** methionine and **g)** threonine illustrates stepwise abundance levels in the sit, sitting less, and exercise regimes. For all panels: * $p < 0.05$, ** $p < 0.01$