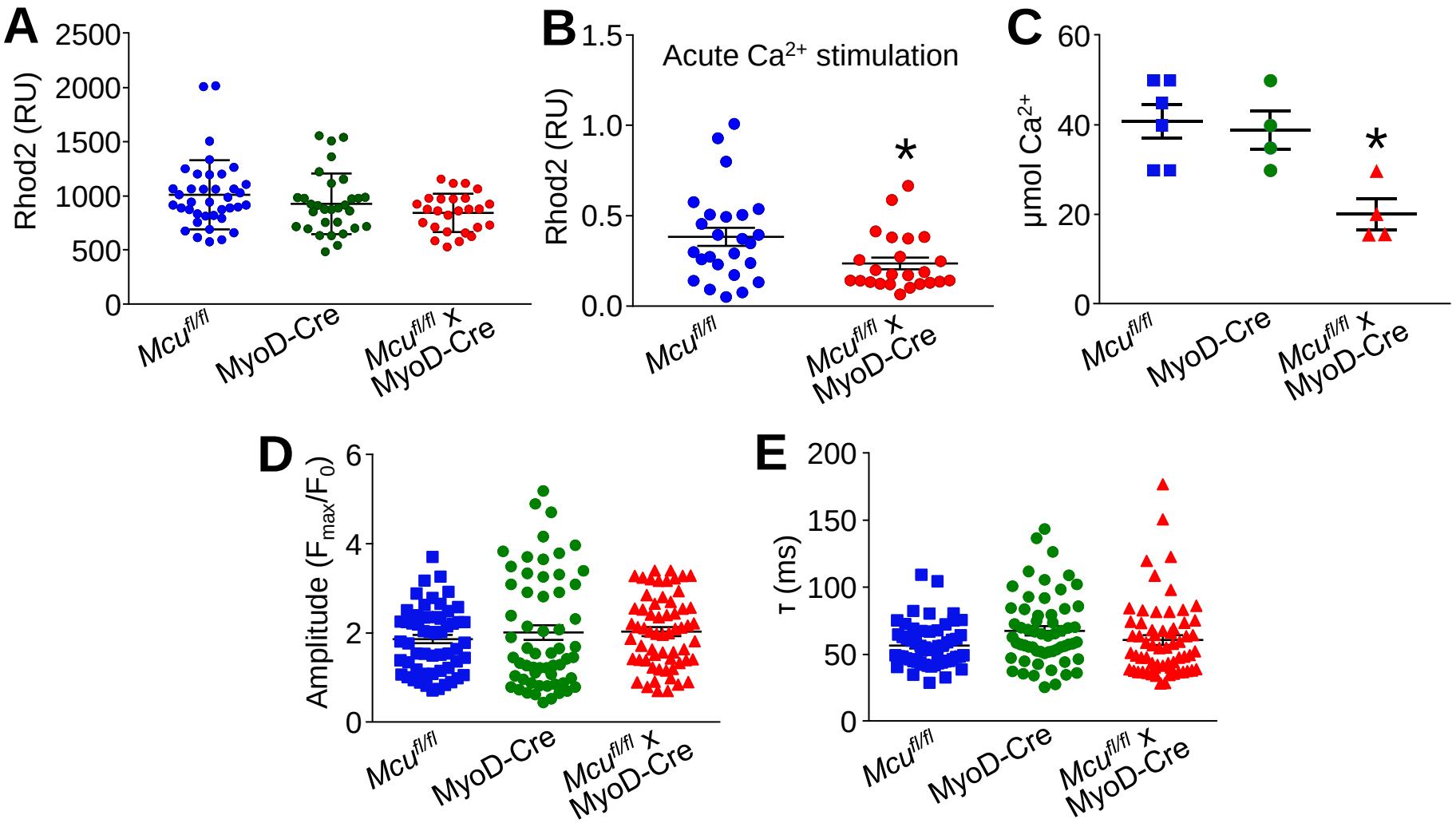
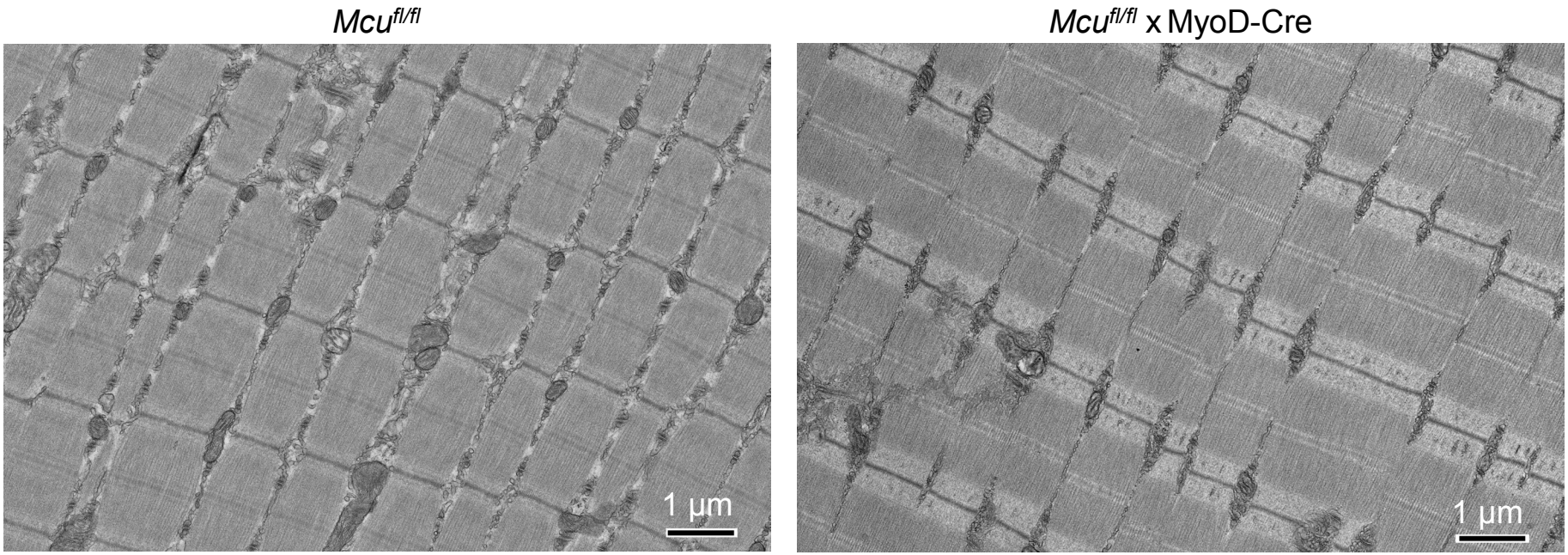
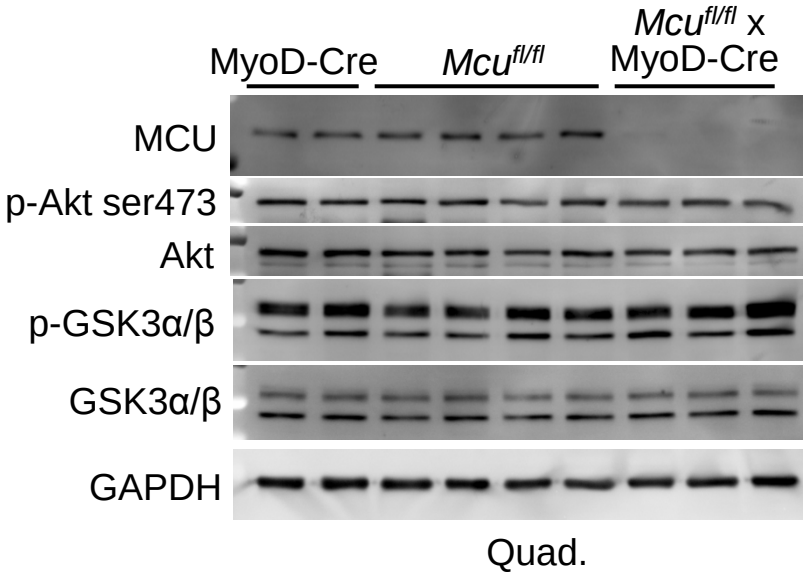


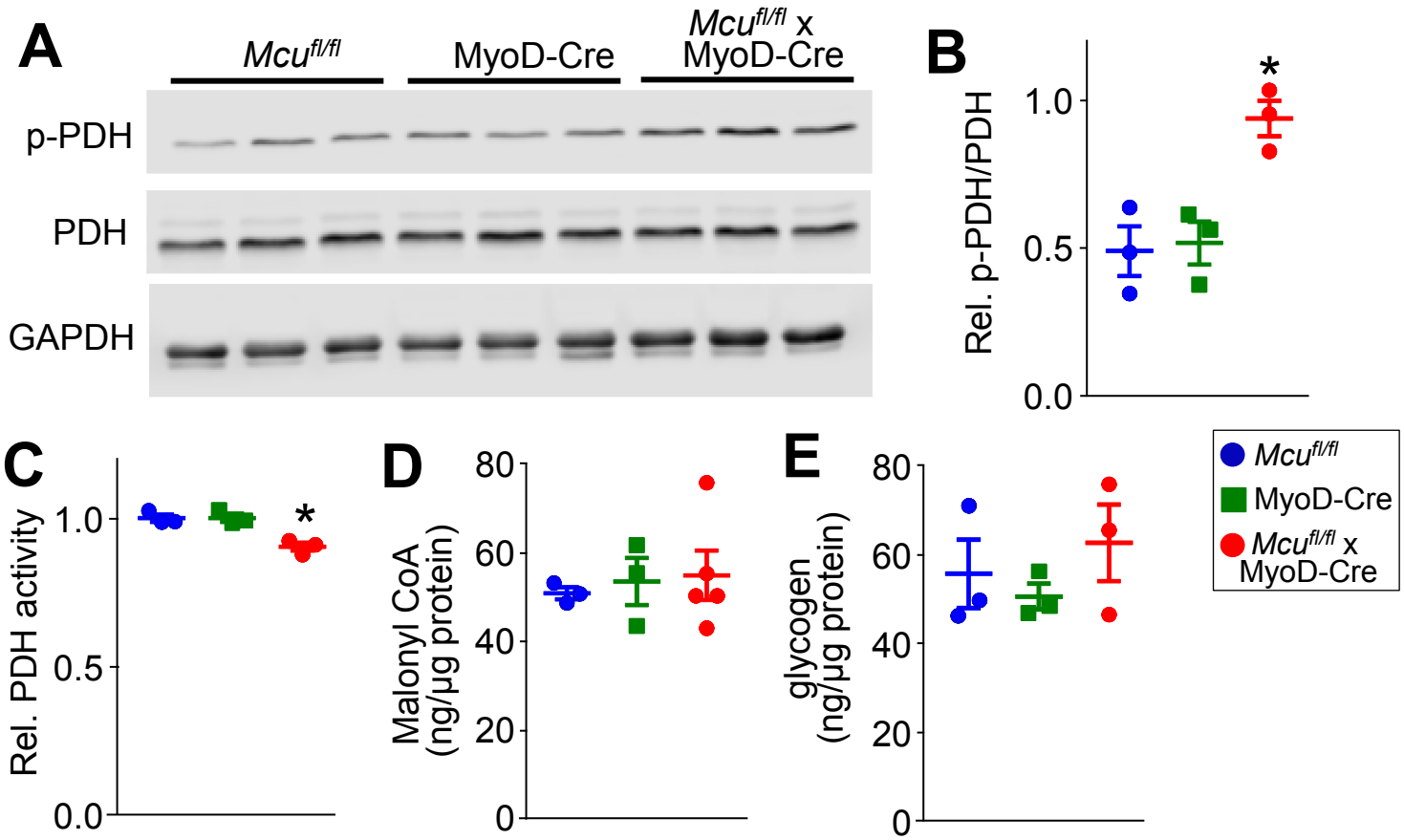
Supplemental Figure 1. (**A**) Baseline mitochondrial Ca^{2+} measurement with Rhod2 florescence (relative units, RU) in intact primary FDB myofibers; *Mcu*$^{fl/fl}$ n = 32 fibers from 6 mice/isolations; MyoD-Cre n = 20 cells from 3 mice/isolations; *Mcu*$^{fl/fl\text{-}MyoD\text{-}Cre}$ n = 25 cells from 5 mice/isolations. (**B**) Peak amplitude of average axial mitochondrial Rhod2 fluorescence (in relative units, RU) to measure Ca^{2+} uptake in saponin permeabilized FDB myofibers challenged with an acute bolus of 20 µM Ca^{2+} (from Figure 1D). Fibers were harvested from *Mcu*$^{fl/fl\text{-}MyoD\text{-}Cre}$ (n = 24 cells from 4 isolations/mice) and *Mcu*$^{fl/fl}$ (n = 24 cells from 4 isolations/mice) mice. Data are plotted as mean ± SEM. * p ≤ 0.05. (**C**) Quantification of mitochondrial Ca^{2+} capacity prior to onset of permeability transition (MyoD-Cre, *n* = 4 and *MCU*$^{fl/fl}$, *n* = 6) or uniporter complex inhibition (*Mcu*$^{fl/fl\text{-}MyoD\text{-}Cre}$, *n* = 4). One-way ANOVA with Dunnett's multiple comparisons test was used to analyze groups. * P<0.05 versus MyoD-Cre control. (**D** and **E**) Fluo-4AM loaded FDB myofibers stimulated at 1 Hz to measure maximal amplitude of the intracellular Ca^{2+} transient (**D**) and total Ca^{2+} clearance time (**E**). Number of samples are shown in the scatter plots. Data are plotted as mean ± SEM. * p ≤ 0.05. Students 2-tailed t-test or one-way ANOVA with Dunnett's multiple comparisons test used for statistical analysis.



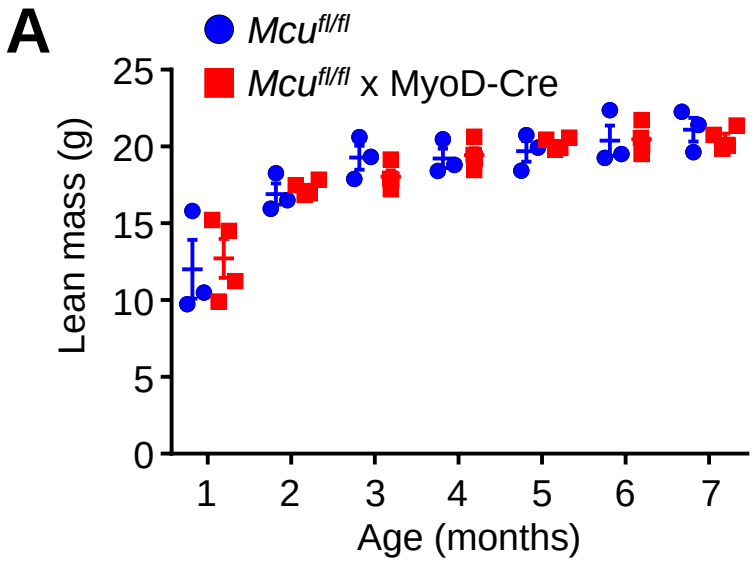
Supplemental Figure 2. Representative electron micrographs from *Mcu*$^{fl/fl}$ and *Mcu*$^{fl/fl\text{-}MyoD\text{-}Cre}$ quadriceps muscle sections. The ultrastructure of the muscle myofilaments and mitochondria appear similar between the 2 genotypes of mice.

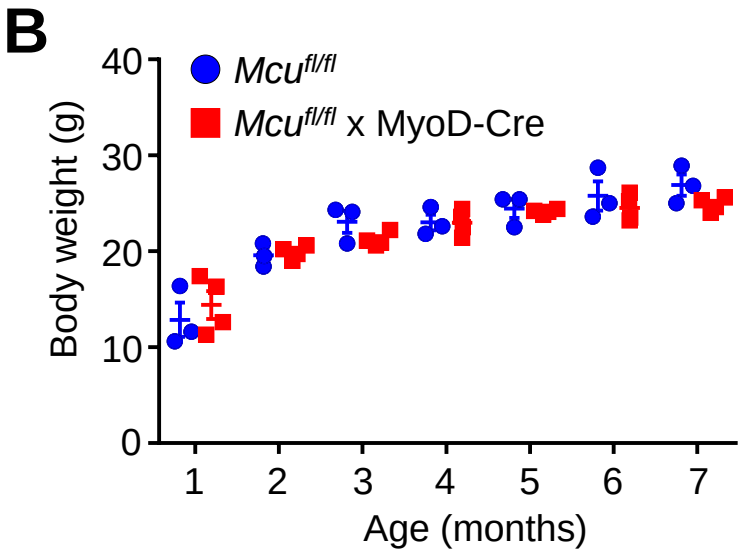


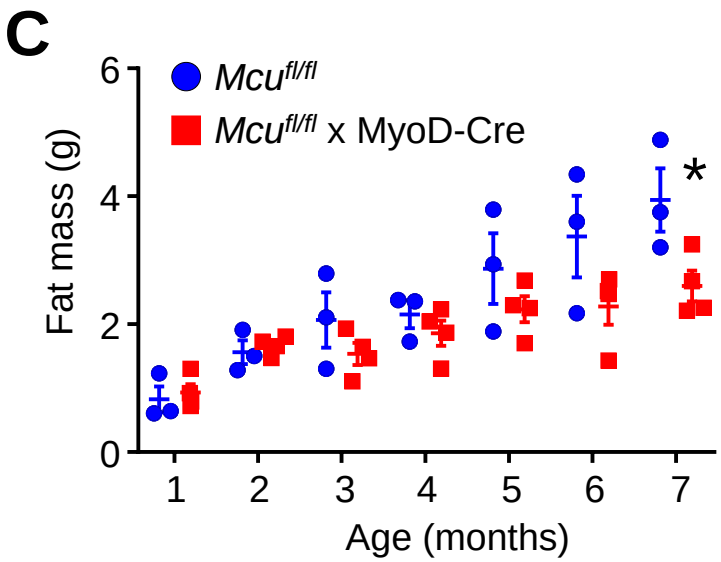
Supplemental Figure 3. Analyses of Akt and GSKα/β kinase activation. Western blot analyses of MCU, phosphorylated Akt (p-Akt Ser473), total Akt, phosphorylated GSKα/β and total GSKα/β from quadriceps (Quad) protein lysates from 4 month-old mice of the indicated genotypes of mice. GAPDH was used as a protein loading control. Except for the loss of MCU protein in the skeletal muscle-specific *Mcu*-deleted mice, there were no statistical differences in protein expression or phosphorylation between the groups.



Supplemental Figure 4. (**A**) Western blots for the indicated proteins from the indicated genotypes of mice at rest from quadriceps muscle. (**B**) Quantitation of phosphorylated-PDH/PDH ratio from 3 independent experiments as shown in A. (**C**) Relative PDH enzymatic activity from resting quadriceps from the indicated genotypes of mice. (**D** and **E**) Quantitation of quadriceps malonyl CoA (**D**) and glycogen (**E**) content in quadriceps of the indicated genotypes of mice at rest. Scatter plots show individual values and sample number and means ± SEM. *P<0.05 versus *Mcu*$^{fl/fl}$ or MyoD-Cre. One-way ANOVA with Dunnett's multiple comparisons test used for statistical analysis.







Supplemental Figure 5. MRI analyses of total body composition with aging in mice. (**A**) Lean body mass, (**B**) total body mass, and (**C**) total fat mass in the indicated genotypes of mice. Values represented as mean ± SEM. *P<0.05 versus *Mcu*$^{fl/fl}$ control. N= 3 *Mcu*$^{fl/fl}$ and N= 4 *Mcu*$^{fl/fl\text{-}MyoD\text{-}Cre}$ mice analyzed. While total body weight was not significantly different between the 2 groups, fat mass was significantly ~1.3 g less in the *Mcu* deleted mice, although this was not enough to impact total body weight.

Supplementary Table*. Metabolites in exercised muscle from *Mcu*$^{fl/fl\text{-}MyoD\text{-}Cre}$ (deleted) versus wildtype MyoD-Cre controls

Metabolites	Mcu wildtype (N=3)	Mcu-MyoD deleted (N=3)	P value
AMP	38.49 ± 7.24 mM/mg	80.95 ± 12.5 mM/mg	0.0071
Carnosine	43.74 ± 17.7 mM/mg	104.2 ± 20.0 mM/mg	0.0173
Choline	3.799 ± 1.27 mM/mg	7.626 ± 1.94 mM/mg	0.0464
Citrate	6.383 ± 1.66 mM/mg	12.13 ± 2.94 mM/mg	0.0421
Creatine	349.6 ± 40.6 mM/mg	799.1 ± 244 mM/mg	0.0346
Glutamine	19.08 ± 1.62 mM/mg	48.99 ± 12.0 mM/mg	0.0129
Glycine	55.60 ± 11.6 mM/mg	107.1 ± 22.4 mM/mg	0.0241
Lactate	147.6 ± 21.6 mM/mg	330.8 ± 31.6 mM/mg	0.0011
Niacinamide	1.613 ± 0.08 mM/mg	4.651 ± 1.29 mM/mg	0.0154
O-Phosphocholine	2.724 ± 1.80 mM/mg	7.557 ± 1.97 mM/mg	0.0353
Phenylalanine	1.375 ± 0.27 mM/mg	2.887 ± 0.55 mM/mg	0.0129
Taurine	639.1 ± 115 mM/mg	1270 ± 357 mM/mg	0.0437
Trimethylamine	0.659 ± 0.07 mM/mg	1.521 ± 0.51 mM/mg	0.0465
Valine	4.595 ± 0.82 mM/mg	8.390 ± 1.62 mM/mg	0.0226

*Quadriceps muscle was harvested 2 min after exhaustion and muscles were snap frozen in liquid nitrogen and metabolite extraction was performed for acquisition of one--dimensional 1H NMR spectra using a Bruker Avance II 600 MHz spectrometer (see Methods). Data are presented as Mean ± SD.