A Mouse Model of Creatine Transporter Deficiency Reveals Impaired Motor Function and Muscle Energy Metabolism.

Devashish Das*, Malte Stockebrand*, Ali Sasani* , Sönke Hornig, Irm Hermans-Borgmeyer, Hannah A. Lake, Dirk Isbrandt, Craig A. Lygate, Arend Heerschap, Axel Neu*, Chi-Un Choe*

Abstract

Energy supply for muscle contraction is provided by breakdown of adenosine triphosphate (ATP), which is replenished continuously by anaerobic glycolysis or oxidative phosphorylation. The (P)Cr system serves as rapid energy buffer to maintain constant ATP levels and mediates subcellular high energy phosphate transfer. Therefore, high (P)Cr levels are found in skeletal muscle in concentrations up to 20–40 mM. Defects of Cr synthesis or transport due to mutations in AGAT, GAMT, or SLC6A8 (CT1) genes are associated with Cr deficiency syndromes that affect muscle to various degrees. Previous studies on CT1-deficient mouse models have focused on the central nervous system. Therefore, we generated and characterized CT1-deficient mice with a focus on muscle physiology. Magnetic resonance spectroscopy experiments were performed at 11.7T to elucidate the high-energy phosphate metabolism in skeletal muscle of CT1-deficient mice. Our 31P-MRS data revealed an almost complete absence of phosphocreatine in skeletal muscle. Furthermore, using 31P -Saturation transfer MRS we can explain a reason for severely altered energy homeostasis as evident from strongly reduced phosphocreatine resulted in decreased ATP/Pi levels despite an increased inorganic phosphate to ATP flux.

Figure 1 Muscle energy metabolism. **(A)** Representative ^{31}P MR spectra of hind limb muscle in CT1$^{+/y}$ and CT1$^{-/y}$ mice (PCr, phosphocreatine; Pi, inorganic phosphate; PMEs, phosphomonoesters; ppm, parts per million). **(B)** PCr/P$_i$ ratios in CT1$^{+/y}$ and CT1$^{-/y}$ mice measured with ^{31}P MR spectroscopy (n = 4–5). **(C,D)** Saturation transfer ^{31}P MRS experiments on hind limb muscle of CT1$^{+/y}$ **(A)** and CT1$^{-/y}$ mice **(B)** to assess the Pi→ATP kinetics. The γATP peak is saturated (b) and this saturation is transferred to the PCr and Pi peaks due to the creatine kinase and ATPase activity (subtraction spectra c = a minus b). This effect was used to calculate the forward rate constant k_f. ATP to free phosphate ratios (ATP/Pi) **(E)**, k_f values **(F)**, and phosphate (Pi→ATP) flux **(G)** of hind limb muscle in CT1$^{+/y}$ and CT1$^{-/y}$ mice assessed with MR spectroscopy (n = 4–5) ($^{**}P < 0.01$; $^{***}P < 0.001$).