J Muscle Res Cell Motil (2015) 36:297–300
DOI 10.1007/s10974-015-9427-z

NEWS AND VIEWS

The extended, dynamic mitochondrial reticulum in skeletal muscle and the creatine kinase (CK)/phosphocreatine (PCr) shuttle are working hand in hand for optimal energy provision

Theo Wallimann[1]

Received: 8 August 2015 / Accepted: 14 October 2015 / Published online: 20 October 2015

The existence of a mitochondrial reticulum or network was postulated already in 1986 (Kirkwood et al. 1986) and the effect of endurance training on the fine structure of this very reticulum was documented in the following year (Kirkwood et al. 1987). A decade later the concept of mitochondrial networks in skeletal muscle definitely gained support by improved techniques, using a detailed ultra-structural analysis of ultrathin cryo-sections of human muscle by electron microscopy (Ogata and Yamasaki 1997). Now, in their letter on "*Mitochondrial reticulum for cellular energy distribution in muscle*" the authors Glancy et al. with Balaban (Nature 523:617–620, 2015) presented very elegant and technically brilliant work corroborating the existence of a mitochondrial reticulum network inside skeletal muscle fibers. In addition, these authors provide new evidence that this mitochondrial reticulum network provides a conductive pathway for energy distribution and helps to minimize diffusion distances for metabolites such as ATP. The authors propose that mitochondrial membrane potential conduction via this mitochondrial reticulum is the dominant pathway for energy distribution in skeletal muscle and that the phospho-creatine (PCr)/kinase creatine kinase (CK) shuttle (Bessman and Geiger 1981; Wallimann et al. 1992) would not be critical for normal muscle function. In their discussion it is stated, however, that CK would probably still provide a significant evolutionary advantage, justifying the retention or development of the system (Glancy et al. 2015). As a matter of fact, evolutionarily the phosphagen kinase system dates back several hundred millions of years to early metazoan CK (Uda et al. 2012) or even bacteria with arginine kinase (AK) (Suzuki et al. 2013).

To support the interpretation of their data, the authors argue that the skeletal muscle phenotypes of CK knock-out or creatine(Cr)-deficiency mice are small with nearly normal muscle function and that only modest adaptations are seen in these transgenic animals.

Unfortunately, both statements are incorrect. First, at a closer look, the phenotype of the CK double knock-out mouse is physiologically relevant in terms of muscle force and relaxation (Steeghs et al. 1997), and CK injection into CK-deficient skeletal muscle fibers restores contractile function and calcium handling (Dahlstedt et al. 2003) that are both distinctly disturbed in the CK double knock-outs lacking expression of cytosolic muscle MM-CK as well as mitochondrial mtCK (Steeghs et al. 1997; 1998). Even skeletal muscles of mice that are deficient only in cytosolic MM-CK clearly lack muscle burst activity (van Deursen et al. 1993) and down-regulated expression of MM-CK in skeletal muscle of transgenic mice leads to a correspondingly lowered ability to perform muscle contractile burst activity that closely correlates with the level of MM-CK expression (Van Deursen et al. 1994).

Second, the adaptation changes observed in skeletal muscle due to abrogation of the CK system are astonishing, e.g. the mitochondrial content in fast-type glycolytic muscles (normally devoid of intra-myofibrillar mitochondria) of these transgenic animals is highly up-regulated and the appearance of these transformed muscles lacking CK rather resemble oxidative insect endurance flight muscles with a very high mitochondrial content and a vast propensity of intra-myofibrillar mitochondria (van Deursen et al. 1993, see Fig. 2; and Steeghs et al. 1997, see Fig. 4) with a corresponding mitochondrial network. Most relevant with respect to muscle pathology is the observed formation

✉ Theo Wallimann
theo.wallimann@cell.biol.ethz.ch

[1] Department of Biology, ETH Zürich, Zürich, Switzerland

of tubular aggregates with participation of a vastly expanded sarcoplasmic reticulum (SR) in the MM-CK/mtCK double knock-outs (Novotová et al. 2002). Thus, by expanding the sarcoplasmic reticulum membrane surface, the MM-CK/mtCK double k.o. animals obviously can compensate for their difficulties in calcium re-uptake by the energetically demanding SR Ca^{2+}-ATPase for muscle relaxation (Steeghs et al. 1997). Based on specific data with myotubes from these CK k.o. animals, the CK system was found to be essential for optimal refill of the SR Ca2+ store in skeletal muscle (de Groof et al. 2002). In normal muscle, besides being specifically located at the sarcomeric M-band, a significant proportion of MM-CK is also located in propinquity to the SR Ca^{2+}-pump, with CK at these locations maintaining a high ATP/ADP ratio and thus guaranteeing a thermodynamically efficient function (ΔG of ATP hydrolysis is kept high) of the myofibrillar actomyosin Mg^{2+}-ATPase and the Ca^{2+}-ATPase (Wallimann et al. 2007).

Thus, the various CK knock-outs obviously display distinctive and remarkable structural and metabolic adaptation strategies, together with an additionally increased glycogen storage capacity, that clearly serve the purpose of improving energy supply and reducing diffusion distances for energy provision from mitochondria to the myofibrils and the sarcoplasmic reticulum. This likely is the explanation for the seemingly relatively "mild" skeletal muscle phenotype, which nevertheless is physiologically significant, especially at higher work load (Steeghs et al. 1998: Dahlstedt et al. 2003).

On the other hand, knocking out the second of the two Cr synthesis enzymes (guanidino acetic acid amino transferase or GAMT), to create a transgenic mouse with "Cr-deficiency" (Lygate et al. 2013), had the disadvantage that guanidine acetic acid (GAA), the precursor of creatine is accumulated in the muscles. GAA then is phosphorylated by CK to provide GAA-P. Thus, the seemingly mild skeletal muscle phenotype observed in GAMT k.o. mice can be explained by compensatory utilization of GAA-P (instead of PCr) as a functional high-energy phosphagen, albeit with a lower efficiency compared to PCr (Kan et al. 2004). On the other hand, the phenotype of L-arginine:glycine amidinotransferase (AGAT), the enzyme involved in the first step of endogenous Cr biosynthesis, where no GAA is formed, displays a strong neurological but also a noticeable skeletal muscle phenotype in mice (Choe et al. 2013), as well as in humans (Schulze 2003). AGAT-deficient patients present with myopathy (Nouioua et al. 2013), neurological dysfunctions, as well as with developmental and speech delay (Schulze 2003), clearly indicating that Cr is critical for normal muscle and brain function (Wallimann et al. 2011). Astonishingly, the pathological symptoms can largely be reduced or reversed by a simple nutritional Cr supplementation at early stages of this inherited Cr-deficiency disorder (Nouioua et al. 2013), again indicating that Cr is needed for normal body function. Thus, neither CK nor Cr is a disposable enzyme or metabolite, respectively (Taegtmeyer and Ingwall 2013). Although neither deletion of the genes coding for the CK isoenzymes nor of those for endogenous Cr synthesis display a lethal phenotype, CK and Cr/PCr are physiologically important for muscle performance especially at high workload and, in the long-term, for thermodynamically most efficient utilization of energy (ATP) by maintaining the ΔG of ATP hydrolysis high at those subcellular locations where ATP is needed by ATPases at work (Wallimann et al. 2007). A small reduction in overall energy costs (work done per ATP hydrolyzed) facilitated by the CK/PCr system may be evolutionarily crucial for survival, e.g. to escape a predator (Selinger et al. 2015). This can be exemplified by the fact that by increasing the PCr pool in muscle as a result of Cr supplementation, an established standard regimen utilized by athletes, leads to a significant increase in muscle force and high-intensity exercise performance that were decisive in the past for winning many gold medals in athletic competitions (Volek and Rawson 2004).

Finally, and most importantly, by extending the mitochondrial reticulum between the myofibrils, the CK system, with mitochondrial CK (mtCK), sandwiched between inner and outer mitochondrial membranes and linked to the adenine nucleotide carrier (ANT) and voltage-dependent anion carrier (VDAC) (Schlattner et al. 1998; Guzun et al. 2012), will also be part of this mitochondrial reticulum that is anchored to the cytoskeleton via VDAC and the mitochondrial interactosome (Guzun et al. 2012). That means, wherever the mitochondrial reticulum network is extending to, mtCK and with it, a PCr-circuit terminal will be positioned at the same sites or metabolic microcompartments (Saks et al. 2008), where a reliable energy provision in the form of PCr is mostly needed to locally regenerate ATP necessary for energy-dependent processes in muscle, e.g. actomyosin ATPase and calcium pump ATPase (Wallimann et al. 2011). At the same time, the presence of mtCK between inner and outer mitochondrial membrane is an important mediator of the extramitochondrial physiological environment and critical for regulation of oxidative phosphorylation itself (Saks et al. 2000).

Thus, although this extended, elegant work on the mitochondrial reticulum provides a novel but not an exclusive aspect for energy provision in muscle, it certainly does not refute the physiological importance of the PCr-shuttle, a message subtly insinuated at by the authors. Rather, by extending the mitochondrial reticulum into the vicinity of those places in the myofibres, where energy for muscle contraction and relaxation is urgently needed (Saks et al. 2014), energy provision via the PCr-circuit would also be

optimized topologically by the dynamic mitochondrial reticulum. Thus, mtCK bound to mitochondrial membranes would also be where the reticulum sends out its fine membrane structures. At the same time, the conductive pathway via the mitochondrial reticulum, proposed by the authors to act as a conductive system, would then work hand-in-hand with the PCr/CK shuttle and not against it. That is to say, mtCK within the mitochondrial reticulum is topologically positioned in close propinquity to those subcellular places where PCr is actually needed. PCr produced by mtCK can then be used by cytosolic MM-CK bound to sarcomeric M- and I-bands, sarcoplasmic reticulum and plasma membranes for most efficient in situ ATP regeneration. By this mechanism, ATP-requiring reactions are supplied directly with ATP at a subcellular level and the maintenance of a high local ATP/ADP ratio even at high work load is always warranted (Wallimann et al. 2007).

References

Bessman SP, Geiger PJ (1981) Transport of energy in muscle: the phosphorylcreatine shuttle. Science 211(4481):448–452

Choe CU, Nabuurs C, Stockebrand MC, Neu A, Nunes P, Morellini F, Sauter K, Schillemeit S, Hermans-Borgmeyer I, Marescau B, Heerschap A, Isbrandt D (2013) L-arginine:glycine amidinotransferase deficiency protects from metabolic syndrome. Hum Mol Genet 22(1):110–123

Dahlstedt AJ, Katz A, Tavi P, Westerblad H (2003) Creatine kinase injection restores contractile function in creatine-kinase-deficient mouse skeletal muscle fibres. J Physiol 547(Pt 2):395–403

de Groof AJ, Fransen JA, Errington RJ, Willems PH, Wieringa B, Koopman WJ (2002) The creatine kinase system is essential for optimal refill of the sarcoplasmic reticulum Ca2+ store in skeletal muscle. J Biol Chem 277(7):5275–5284

Glancy B, Hartnell LM, Malide D, Yu ZX, Combs CA, Connelly PS, Subramaniam S, Balaban RS (2015) Mitochondrial reticulum for cellular energy distribution in muscle. Nature 523(7562): 617–620

Guzun R, Gonzalez-Granillo M, Karu-Varikmaa M, Grichine A, Usson Y, Kaambre T, Guerrero-Roesch K, Kuznetsov A, Schlattner U, Saks V (2012) Regulation of respiration in muscle cells in vivo by VDAC through interaction with the cytoskeleton and MtCK within Mitochondrial Interactosome. Biochim Biophys Acta 1818(6):1545–1554

Kan HE, Renema WK, Isbrandt D, Heerschap A (2004) Phosphorylated guanidinoacetate partly compensates for the lack of phosphocreatine in skeletal muscle of mice lacking guanidinoacetate methyltransferase. J Physiol 560(Pt 1):219–229

Kirkwood SP, Munn EA, Brooks GA (1986) Mitochondrial reticulum in limb skeletal muscle. Am J Physiol 251(3 Pt 1):C395–C402

Kirkwood SP, Packer L, Brooks GA (1987) Effects of endurance training on a mitochondrial reticulum in limb skeletal muscle. Arch Biochem Biophys 255(1):80–88

Lygate CA, Aksentijevic D, Dawson D, ten Hove M, Phillips D, de Bono JP, Medway DJ, Sebag-Montefiore L, Hunyor I, Channon KM, Clarke K, Zervou S, Watkins H, Balaban RS, Neubauer S (2013) Living without creatine: unchanged exercise capacity and response to chronic myocardial infarction in creatine-deficient mice. Circ Res 112(6):945–955

Nouioua S, Cheillan D, Zaouidi S, Salomons GS, Amedjout N, Kessaci F, Boulahdour N, Hamadouche T, Tazir M (2013) Creatine deficiency syndrome. A treatable myopathy due to arginine-glycine amidinotransferase (AGAT) deficiency. Neuromuscul Disord 23(8):670–674

Novotová M, Zahradník I, Brochier G, Pavlovicová M, Bigard X, Ventura-Clapier R (2002) Joint participation of mitochondria and sarcoplasmic reticulum in the formation of tubular aggregates in gastrocnemius muscle of CK-/- mice. Eur J Cell Biol 81(2):101–106

Ogata T, Yamasaki Y (1997) Ultra-high-resolution scanning electron microscopy of mitochondria and sarcoplasmic reticulum arrangement in human red, white, and intermediate muscle fibers. Anat Rec 248(2):214–223

Saks VA, Kongas O, Vendelin M, Kay L (2000) Role of the creatine/phosphocreatine system in the regulation of mitochondrial respiration. Acta Physiol Scand 168(4):635–641

Saks V, Beraud N, Wallimann T (2008) Metabolic compartmentation—a system level property of muscle cells: real problems of diffusion in living cells. Int J Mol Sci 9(5):751–767

Saks VA, Schlattner U, Tokarska-Schlattner M, Wallimann T, Bagur R, Zorman S, Pelosse M, Dos Santos P, Boucher F, Kaambre T, Guzun R (2014) Systems level regulation of cardiac energy fluxes via metabolic cycles: role of creatine, phosphotransfer pathways, and AMPK signaling. In: Miguel AA, Valdur S, Uwe S (eds) Systems biology of metabolic and signaling networks. Springer series of biophysics, vol 16. Springer, New York, pp 261–320

Schlattner U, Forstner M, Eder M, Stachowiak O, Fritz-Wolf K, Wallimann T (1998) Functional aspects of the X-ray structure of mitochondrial creatine kinase: a molecular physiology approach. Mol Cell Biochem 184(1–2):125–140

Schulze A (2003) Creatine deficiency syndromes. Mol Cell Biochem 244(1–2):143–150

Selinger JC, O'Connor SM, Wong JD, Donelan JM (2015) Humans can continuously optimize energetic cost during walking. Curr Biol 25:2452

Steeghs K, Benders A, Oerlemans F, de Haan A, Heerschap A, Ruitenbeek W, Jost C, van Deursen J, Perryman B, Pette D, Brückwilder M, Koudijs J, Jap P, Veerkamp J, Wieringa B (1997) Altered Ca2+ responses in muscles with combined mitochondrial and cytosolic creatine kinase deficiencies. Cell 89(1):93–103

Steeghs K, Oerlemans F, de Haan A, Heerschap A, Verdoodt L, de Bie M, Ruitenbeek W, Benders A, Jost C, van Deursen J, Tullson P, Terjung R, Jap P, Jacob W, Pette D, Wieringa B (1998) Cytoarchitectural and metabolic adaptations in muscles with mitochondrial and cytosolic creatine kinase deficiencies. Mol Cell Biochem 184(1–2):183–194

Suzuki T, Soga S, Inoue M, Uda K (2013) Characterization and origin of bacterial arginine kinases. Int J Biol Macromol 57:273–277

Taegtmeyer H, Ingwall JS (2013) Creatine–a dispensable metabolite? Circ Res 112(6):878–880

Uda K, Ellington WR, Suzuki T (2012) A diverse array of creatine kinase and arginine kinase isoform genes is present in the starlet sea anemone *Nematostella vectensis*, a cnidarian model system for studying developmental evolution. Gene 497(2):214–227

van Deursen J, Heerschap A, Oerlemans F, Ruitenbeek W, Jap P, ter Laak H, Wieringa B (1993) Skeletal muscles of mice deficient in muscle creatine kinase lack burst activity. Cell 74(4):621–631

van Deursen J, Ruitenbeek W, Heerschap A, Jap P, ter Laak H, Wieringa B (1994) Creatine kinase (CK) in skeletal muscle energy metabolism: a study of mouse mutants with graded reduction in muscle CK expression. Proc Natl Acad Sci U S A 91(19):9091–9095

Volek JS, Rawson ES (2004) Scientific basis and practical aspects of creatine supplementation for athletes. Nutrition 20(7–8):609–614

Wallimann T, Wyss M, Brdiczka D, Nicolay K, Eppenberger HM (1992) Intracellular compartmentation, structure and function of creatine kinase isoenzymes in tissues with high and fluctuating energy demands: the 'phosphocreatine circuit' for cellular energy homeostasis. Biochem J 281(Pt 1):21–40

Wallimann T, Tokarska-Schlattner M, Neumann D, Epand RM, Epand RF, Andres RH, Widmer HR, Hornemann T, Saks VA, Agarkova I, Schlattner U (2007) Molecular system bioenergetics. In: Saks VA (eds) Energy for life. WILEY-VCH Publishing GmbH & Co. KGaA, Weinheim, pp. 195–264 ISBN: 978-3-527-31787-5

Wallimann T, Tokarska-Schlattner M, Schlattner U (2011) The creatine kinase system and pleiotropic effects of creatine. Amino Acids 40(5):1271–1296