CROSSTALK

CrossTalk proposal: Skeletal muscle oxidative capacity is altered in patients with cystic fibrosis

Paula Rodriguez-Miguelez[1],
Melissa L. Erickson[2], Kevin K. McCully[2]
and Ryan A. Harris[1,3] (ID)

[1]*Georgia Prevention Institute, Department of Pediatrics, Augusta University, Augusta, GA, USA*

[2]*Department of Kinesiology, University of Georgia, Athens, GA, USA*

[3]*Sport and Exercise Science Research Institute, University of Ulster, Jordanstown, Northern Ireland, UK*

Email: ryharris@augusta.edu

Cystic fibrosis (CF) is an autosomal recessive disorder characterized by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, which functions as an ATP-gated anion channel. Pulmonary dysfunction is the most common clinical manifestation of CF; however, there are many other systemic consequences that contribute to the shortened life expectancy in this population. Exercise intolerance has been shown to predict mortality in patients with CF independent of lung function. Despite the clinical importance for assessing exercise capacity, the mechanisms which lead to exercise intolerance in CF have yet to be elucidated. Although evidence suggests that the impaired expression of CFTR in skeletal muscle may be associated with abnormalities in muscle oxygenation and muscle metabolism, there are recent studies that have described a preserved muscle metabolism in patients with CF. Considering the available data, support of a dysfunctional skeletal muscle oxidative metabolism in patients with CF is briefly discussed.

Patients with CF exhibit intrinsic skeletal muscle limitations

Numerous studies have described that patients with CF exhibit intrinsic skeletal muscle defects, independently of muscle mass, lung function, or physical activity status (de Meer *et al.* 1995; Moser *et al.* 2000; Sahlberg *et al.* 2005; Rosenthal *et al.* 2009; Troosters *et al.* 2009; Lamhonwah *et al.* 2010). In fact, even patients with CF who are competitive athletes show reductions in muscle strength and power compared to apparently healthy control athletes (Selvadurai *et al.* 2003). The manifestation of these skeletal muscle impairments in patients with CF has been closely related with the abnormal function of the CFTR in muscle tissue (Divangahi *et al.* 2009; Lamhonwah *et al.* 2010). The aforementioned studies have related a dysfunctional CFTR channel with an anomalous response to cell membrane polarization (Divangahi *et al.* 2009). In fact, CFTR channel dysfunction contributes to disturbed ion transport (Guo *et al.* 2014), thereby resulting in a reduction in action potential magnitude and impairment in skeletal muscle function.

Patients with CF exhibit mitochondrial dysfunction

Prevailing data in the literature have described how the impaired function of the CFTR protein in CF skeletal muscle is associated with different anomalies that are not observed in other respiratory pathologies (Divangahi *et al.* 2009; Lamhonwah *et al.* 2010; Wells *et al.* 2011). Among them, the existence of mitochondrial dysfunction has been confirmed in cells from patients with CF (Feigal & Shapiro, 1979; Shapiro *et al.* 1979; Feigal *et al.* 1982; Valdivieso *et al.* 2012). Such mitochondrial impairments have been related with alterations in mitochondrial morphology (i.e. mitochondrial shape) and mitochondrial energetic metabolism (i.e. electron transport chain) (Feigal & Shapiro, 1979; Shapiro, 1989; Antigny *et al.* 2009). Indeed, more than two decades ago, several studies described the existence of different contributors to mitochondrial dysfunctions in CF, including impairments in calcium homeostasis and the existence of an altered respiratory system (Feigal & Shapiro, 1979; Shapiro *et al.* 1979; Feigal *et al.* 1982; Shapiro, 1989). More recently, these same impairments were confirmed in CF (Antigny *et al.* 2009; Divangahi *et al.* 2009; Wells *et al.* 2011; Valdivieso *et al.* 2012) providing strong evidence for the existence of an impaired bioenergetics metabolism in this patient population.

Patients with CF exhibit reduced skeletal muscle oxidative capacity

There is compelling evidence at the cellular level that oxidative metabolism is diminished in CF. In this regard, several groups have described that patients with CF exhibit lower resting concentrations of ATP (Divangahi *et al.* 2009; Lamhonwah *et al.* 2010), which have been linked to a dysfunctional CFTR (Tu *et al.* 2010). Lower resting ATP coupled with significant reductions in ATP production during exercise (de Meer *et al.* 1995)

may compromise muscle function and contribute to exercise intolerance. In turn, ATP depletion may also impact the opening–closing cycle and the stability of the CFTR channel (Quinton & Reddy, 1992; Hwang & Kirk, 2013). In addition, mitochondrial complex I, a key respiratory chain protein involved in the synthesis of ATP, is positively regulated by the CFTR protein and its expression is reduced in CF (Shapiro *et al.* 1979; Dechecchi *et al.* 1988; Valdivieso *et al.* 2007; Valdivieso *et al.* 2012). Additionally, downregulation of the mitochondrial protein CISD1, a modulator of the oxidative capacity of the cell, has been also reported in CF (Taminelli *et al.* 2008).

Different methods have been employed to examine the functionality of mitochondria metabolism *in vivo*. ^{31}P magnetic resonance spectroscopy (^{31}P-MRS) has been widely used in different populations as a non-invasive technique to assess skeletal muscle oxidative capacity through monitoring the recovery rate after exercise of phosphocreatine (PCr). Evaluations of energetic metabolism with ^{31}P-MRS have documented abnormalities in the PCr recovery time of patients with CF compared to controls (de Meer *et al.* 1995; Wells *et al.* 2011). In addition, our group has used near infrared spectroscopy (NIRS) to assess the rate of recovery of skeletal muscle oxidative metabolism of the vastus lateralis in patients with CF (Erickson *et al.* 2015). The NIRS technology is methodologically similar to ^{31}P-MRS (Ryan *et al.* 2013), and validation studies have shown strong correlations between NIRS measured rate constants and ^{31}P-MRS rate constants as well as with state 3 respiratory rates from muscle biopsies (Ryan *et al.* 2014). NIRS offers an accessible, inexpensive and reproducible evaluation of mitochondrial capacity (a combination of mitochondrial function and mitochondrial number). Our findings indicate that patients with CF exhibit a 15% reduction in skeletal muscle oxidative metabolism compared with apparently healthy, demographically matched controls (Erickson *et al.* 2015). Although the assessment of mitochondrial metabolism with NIRS does not allow the differentiation between a reduction in mitochondrial number and/or mitochondrial dysfunction, our data support previous reports of impaired mitochondrial bioenergetics in patients with CF. It is important to note that, possible vascular manifestations may impact the supply of oxygen to the exercising muscles. Despite the existence of both conduit (Poore *et al.* 2013) and microvascular (Rodriguez-Miguelez *et al.* 2016) endothelial dysfunction in patients with CF, unpublished data from our lab indicates a similar NIRS response at rest providing evidence that NIRS is unaffected by vasculopathies.

The impact of disease severity on skeletal muscle function in patients with CF

Advancing age is often associated with disease severity and constitutes a key factor in the progression of the CF pathology. Reductions in muscle blood flow as well as changes in muscle fibre type towards an anaerobic metabolism have been closely associated with age-related exercise intolerance in other populations. Accordingly, our group identified a significant inverse relationship between age and skeletal muscle oxidative metabolism in patients with CF, which was not observed in control subjects (Erickson *et al.* 2015). Bearing in mind that muscle bioenergetics may be reduced in CF by approximately 2.5% per year and the impact that this drop may have on exercise capacity, it is important to consider the role of age in the evaluation of mitochondrial bioenergetics in this specific patient population.

Chronic inflammation, a common phenotype in patients with CF, has also been associated with mitochondrial dysfunction. In particular, the overexpression of the proinflammatory cytokine interleukin-1β, commonly elevated in patients with CF, seems to be involved in both modulation of CFTR expression (Cafferata *et al.* 2000) and reduction of the mitochondrial complex 1 activity (Lopez-Armada *et al.* 2006). Although no spontaneous inflammation in CF has been described previously (Becker *et al.* 2004; Stoltz *et al.* 2010), prevailing data indicate that an impaired CFTR protein upregulates the expression of nuclear factor κB, which is also directly involved in the reduction of oxidative metabolism with an overall reduction in cellular energy (Remels *et al.* 2013).

The elevated levels of reactive oxygen species (ROS), a common phenotype observed in patients with CF, also directly impact mitochondrial function. An excessive production of ROS promotes damage in the mitochondrial DNA and disturbs homeostasis within the electron transport chain homeostasis, thereby disrupting oxidative phosphorylation metabolism (Escames *et al.* 2012). In addition, CFTR knockout mice show a decrease in the expression of peroxiredoxin 6, an antioxidant enzyme also involved in the control of mitochondrial oxidative metabolism (Trudel *et al.* 2009). Oxidative damage and the associated mitochondrial dysfunction may also result in a disturbed autophagy mechanism: a natural regulatory process to recycle unnecessary or dysfunctional cellular components. In fact, CFTR dysfunction seems to alter autophagy regulation (Luciani *et al.* 2010), which appears to mediate an important role in the maintenance of mitochondrial oxidative metabolism (Guo *et al.* 2011).

In summary, data from multiple *in vitro* and *in vivo* studies provide compelling evidence to support the existence of mitochondrial dysfunction, impaired mitochondrial bioenergetics and altered skeletal muscle oxidative capacity in patients with cystic fibrosis. The dysfunctional CFTR as well as the elevated oxidative stress and systemic inflammation seem to contribute to the impaired muscle function, all of which may significantly impact exercise capacity and quality of life in CF.

Call for comments

Readers are invited to give their views on this and the accompanying CrossTalk articles in this issue by submitting a brief (250 word) comment. Comments may be submitted up to 6 weeks after publication of the article, at which point the discussion will close and the CrossTalk authors will be invited to submit a 'Last Word'. Please email your comment, including a title and a declaration of interest, to jphysiol@physoc.org. Comments will be moderated and accepted comments will be published online only as 'supporting information' to the original debate articles once discussion has closed.

References

Antigny F, Girardin N, Raveau D, Frieden M, Becq F & Vandebrouck C (2009). Dysfunction of mitochondria Ca^{2+} uptake in cystic fibrosis airway epithelial cells. *Mitochondrion* **9**, 232–241.

Becker MN, Sauer MS, Muhlebach MS, Hirsh AJ, Wu Q, Verghese MW & Randell SH (2004). Cytokine secretion by cystic fibrosis airway epithelial cells. *Am J Respir Crit Care Med* **169**, 645–653.

Cafferata EG, Gonzalez-Guerrico AM, Giordano L, Pivetta OH & Santa-Coloma TA (2000). Interleukin-1β regulates CFTR expression in human intestinal T84 cells. *Biochim Biophys Acta* **1500**, 241–248.

Dechecchi MC, Girella E, Cabrini G & Berton G (1988). The Km of NADH dehydrogenase is decreased in mitochondria of cystic fibrosis cells. *Enzyme* **40**, 45–50.

de Meer K, Jeneson JA, Gulmans VA, van der Laag J & Berger R (1995). Efficiency of oxidative work performance of skeletal muscle in patients with cystic fibrosis. *Thorax* **50**, 980–983.

Divangahi M, Balghi H, Danialou G, Comtois AS, Demoule A, Ernest S, Haston C, Robert R, Hanrahan JW, Radzioch D & Petrof BJ (2009). Lack of CFTR in skeletal muscle predisposes to muscle wasting and diaphragm muscle pump failure in cystic fibrosis mice. *PLoS Genet* **5**, e1000586.

Erickson ML, Seigler N, McKie KT, McCully KK & Harris RA (2015). Skeletal muscle oxidative capacity in patients with cystic fibrosis. *Exp Physiol* **100**, 545–552.

Escames G, Lopez LC, Garcia JA, Garcia-Corzo L, Ortiz F & Acuna-Castroviejo D (2012). Mitochondrial DNA and inflammatory diseases. *Hum Genet* **131**, 161–173.

Feigal RJ & Shapiro BL (1979). Mitochondrial calcium uptake and oxygen consumption in cystic fibrosis. *Nature* **278**, 276–277.

Feigal RJ, Tomczyk MS & Shapiro BL (1982). The calcium abnormality in cystic fibrosis mitochondria: relative role of respiration and ATP hydrolysis. *Life Sci* **30**, 93–98.

Guo JH, Chen H, Ruan YC, Zhang XL, Zhang XH, Fok KL, Tsang LL, Yu MK, Huang WQ, Sun X, Chung YW, Jiang X, Sohma Y & Chan HC (2014). Glucose-induced electrical activities and insulin secretion in pancreatic islet β-cells are modulated by CFTR. *Nat Commun* **5**, 4420.

Guo JY, Chen HY, Mathew R, Fan J, Strohecker AM, Karsli-Uzunbas G, Kamphorst JJ, Chen G, Lemons JM, Karantza V, Coller HA, Dipaola RS, Gelinas C, Rabinowitz JD & White E (2011). Activated Ras requires autophagy to maintain oxidative metabolism and tumorigenesis. *Genes Dev* **25**, 460–470.

Hwang TC & Kirk KL (2013). The CFTR ion channel: gating, regulation, and anion permeation. *Cold Spring Harb Perspect Med* **3**, a009498.

Lamhonwah AM, Bear CE, Huan LJ, Kim Chiaw P, Ackerley CA & Tein I (2010). Cystic fibrosis transmembrane conductance regulator in human muscle: Dysfunction causes abnormal metabolic recovery in exercise. *Ann Neurol* **67**, 802–808.

Lopez-Armada MJ, Carames B, Martin MA, Cillero-Pastor B, Lires-Dean M, Fuentes-Boquete I, Arenas J & Blanco FJ (2006). Mitochondrial activity is modulated by TNFα and IL-1β in normal human chondrocyte cells. *Osteoarthritis Cartilage* **14**, 1011–1022.

Luciani A, Villella VR, Esposito S, Brunetti-Pierri N, Medina D, Settembre C, Gavina M, Pulze L, Giardino I, Pettoello-Mantovani M, D'Apolito M, Guido S, Masliah E, Spencer B, Quaratino S, Raia V, Ballabio A & Maiuri L (2010). Defective CFTR induces aggresome formation and lung inflammation in cystic fibrosis through ROS-mediated autophagy inhibition. *Nat Cell Biol* **12**, 863–875.

Moser C, Tirakitsoontorn P, Nussbaum E, Newcomb R & Cooper DM (2000). Muscle size and cardiorespiratory response to exercise in cystic fibrosis. *Am J Respir Crit Care Med* **162**, 1823–1827.

Poore S, Berry B, Eidson D, McKie KT & Harris RA (2013). Evidence of vascular endothelial dysfunction in young patients with cystic fibrosis. *Chest* **143**, 939–945.

Quinton PM & Reddy MM (1992). Control of CFTR chloride conductance by ATP levels through non-hydrolytic binding. *Nature* **360**, 79–81.

Remels AH, Gosker HR, Bakker J, Guttridge DC, Schols AM & Langen RC (2013). Regulation of skeletal muscle oxidative phenotype by classical NF-κB signalling. *Biochim Biophys Acta* **1832**, 1313–1325.

Rodriguez-Miguelez P, Thomas J, Seigler N, Crandall R, McKie KT, Forseen C & Harris RA (2016). Evidence of microvascular dysfunction in patients with cystic fibrosis. *Am J Physiol Heart Circ Physiol* **310**, H1479–H1485.

Rosenthal M, Narang I, Edwards L & Bush A (2009). Non-invasive assessment of exercise performance in children with cystic fibrosis (CF) and non-cystic fibrosis bronchiectasis: is there a CF specific muscle defect? *Pediatr Pulmonol* **44**, 222–230.

Ryan TE, Brophy P, Lin CT, Hickner RC & Neufer PD (2014). Assessment of in vivo skeletal muscle mitochondrial respiratory capacity in humans by near-infrared spectroscopy: a comparison with *in situ* measurements. *J Physiol* **592**, 3231–3241.

Ryan TE, Southern WM, Reynolds MA & McCully KK (2013). A cross-validation of near-infrared spectroscopy measurements of skeletal muscle oxidative capacity with phosphorus magnetic resonance spectroscopy. *J Appl Physiol (1985)* **115**, 1757–1766.

Sahlberg ME, Svantesson U, Thomas EM & Strandvik B (2005). Muscular strength and function in patients with cystic fibrosis. *Chest* **127**, 1587–1592.

Selvadurai HC, Allen J, Sachinwalla T, Macauley J, Blimkie CJ & Van Asperen PP (2003). Muscle function and resting energy expenditure in female athletes with cystic fibrosis. *Am J Respir Crit Care Med* **168**, 1476–1480.

Shapiro BL (1989). Evidence for a mitochondrial lesion in cystic fibrosis. *Life Sci* **44**, 1327–1334.

Shapiro BL, Feigal RJ & Lam LF (1979). Mitochondrial NADH dehydrogenase in cystic fibrosis. *Proc Natl Acad Sci USA* **76**, 2979–2983.

Stoltz DA, Meyerholz DK, Pezzulo AA, Ramachandran S, Rogan MP, Davis GJ, Hanfland RA, Wohlford-Lenane C, Dohrn CL, Bartlett JA, Nelson GA 4th, Chang EH, Taft PJ, Ludwig PS, Estin M, Hornick EE, Launspach JL, Samuel M, Rokhlina T, Karp PH, Ostedgaard LS, Uc A, Starner TD, Horswill AR, Brogden KA, Prather RS, Richter SS, Shilyansky J, McCray PB Jr, Zabner J & Welsh MJ (2010). Cystic fibrosis pigs develop lung disease and exhibit defective bacterial eradication at birth. *Sci Transl Med* **2**, 29ra31.

Taminelli GL, Sotomayor V, Valdivieso AG, Teiber ML, Marin MC & Santa-Coloma TA (2008). CISD1 codifies a mitochondrial protein upregulated by the CFTR channel. *Biochem Biophys Res Commun* **365**, 856–862.

Troosters T, Langer D, Vrijsen B, Segers J, Wouters K, Janssens W, Gosselink R, Decramer M & Dupont L (2009). Skeletal muscle weakness, exercise tolerance and physical activity in adults with cystic fibrosis. *Eur Respir J* **33**, 99–106.

Trudel S, Kelly M, Fritsch J, Nguyen-Khoa T, Therond P, Couturier M, Dadlez M, Debski J, Touqui L, Vallee B, Ollero M, Edelman A & Brouillard F (2009). Peroxiredoxin 6 fails to limit phospholipid peroxidation in lung from Cftr-knockout mice subjected to oxidative challenge. *PLoS One* **4**, e6075.

Tu J, Le G & Ballard HJ (2010). Involvement of the cystic fibrosis transmembrane conductance regulator in the acidosis-induced efflux of ATP from rat skeletal muscle. *J Physiol* **588**, 4563–4578.

Valdivieso AG, Clauzure M, Marin MC, Taminelli GL, Massip Copiz MM, Sanchez F, Schulman G, Teiber ML & Santa-Coloma TA (2012). The mitochondrial complex I activity is reduced in cells with impaired cystic fibrosis transmembrane conductance regulator (CFTR) function. *PLoS One* **7**, e48059.

Valdivieso AG, Marcucci F, Taminelli G, Guerrico AG, Alvarez S, Teiber ML, Dankert MA & Santa-Coloma TA (2007). The expression of the mitochondrial gene MT-ND4 is downregulated in cystic fibrosis. *Biochem Biophys Res Commun* **356**, 805–809.

Wells GD, Wilkes DL, Schneiderman JE, Rayner T, Elmi M, Selvadurai H, Dell SD, Noseworthy MD, Ratjen F, Tein I & Coates AL (2011). Skeletal muscle metabolism in cystic fibrosis and primary ciliary dyskinesia. *Pediatr Res* **69**, 40–45.

Additional information

Competing interests

None declared.