



Original article

Using complex II as an emerging therapeutic target for the treatment of muscle lesions

Kefeng Li, Wei Han, Ying Zhao, Yujuan Qi, Yadan Li, Jun Bi, Yi Wang, Guijun Dong*

College of Sports and Health, Shandong Sport University, Jinan 250102, China



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ABSTRACT

After patients has been trapped into skeletal muscle injury, hypoxic and dysfunctional mitochondria brings about a crisis in energy supply that severely disrupts the repair of skeletal muscle. This study aims to elucidate injury-induced adaptations in the mitochondria and provide statistics for the role of complex II in instilling cells with energy under hypoxic conditions. Fifty-six male Wistar rats were divided into control, 12 h, 2 d, 5 d, 7 d, 10 d, 15 d, and 30 d postinjury groups. Contusion injury was made via an instrumented drop-mass technique delivering single impact to the posterior surface of the gastrocnemius of one limb of the rats. ROS levels, loss of mitochondrial membrane potential (MMP), activities of marker enzymes (miCK, LDH, and ALP), and activities of complexes I–III were determined. Our findings reveal that the first 2 d postinjury, especially at 12 h, is the period with most severe oxidative stress. After injury, the activities of mitochondrial complexes I–III display different behaviors based on time and various energy production mechanisms. Our results highlight that complex II participates in electron transport in the acute phase of blunt trauma. We proposed that CII could be a therapeutic target in muscle lesions.

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1. Introduction

Skeletal muscle lesions are the most commonplace traumas in sports, accounting for 55% in all injuries (Delos et al., 2013). Among all the muscle injuries, contusions or strains account for 90%. Other scholars have determined three phases on the basis of histopathologic changes after muscle injury: (1) destruction (postinjury in the week 1), (2) repair (postinjury in the week 2–6), and (3) remodeling (postinjury in the week 7 and afterwards) (Järvinen et al., 2014, 2005). The destruction stage witnesses the formation of myofibers and local blood vessels rupture, and a hematoma. These events further bring about inflammatory responses and a decrease in metabolic activities (Tsai et al., 2018). The treatment in the initial phase immediately after injury is a key to the full outcome of recovery (Hardy et al., 2016). Reducing blood loss and inflammation are the primary goals for emergency treatment on postinjury.

However, energy, which is another essential element for cellular life, activity, and metabolism, has yet to be fully investigated.

Oxygen is key for classical mitochondria to realize ATP synthesis. Electrons of NADH and succinate are transferred to ubiquinone through complex I (CI) and complex II (CII) in the respiratory chain, respectively. Oxygen deprivation rapidly halts mitochondrial ATP production because CI can be deactivated through producing superoxide in acute hypoxia (Hernansanz-Agustín et al., 2017). During the destruction period, ischemia causes oxygen deficiency, hence resulting in energy stress of cells. Thus, respiratory adaptation is needed to address this energy deficiency.

The mitochondria of some eukaryotes, such as protists and metazoans, can produce ATP without any oxygen, which shows the anaerobic function of mitochondria (Tielens et al., 2002). CII is at the intersection of two essential pathways: OXPHOS and TCA cycle. As an oxygen sensor (Baysal et al., 2000) and key regulator of respiratory and metabolic activities, it responds to changing inflammation-linked pathologies and abnormalities (Hollinshead and Tennant, 2016; Rasheed and Tarjan, 2018; Bezawork-Geleta et al., 2018). Under harsh conditions, such as hypoxia, CII shows an increased activity, which is stimulated by reactive oxygen species (ROS) (Acín-Pérez et al., 2014; Tretter et al., 2016; Tropeano et al., 2018). Therefore, it can be concluded that CII may be the major source for the remodeling of mitochondrial energy systems in anaerobic energy metabolism (Bezawork-Geleta et al., 2017).

* Corresponding author.

E-mail address: donggj@163.com (G. Dong).

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In addition to energy stress, mitochondrial dysfunction is also manifested in mitochondrial membrane potential breakdown in injured muscle tissues (Boekema and Braun, 2007). Then, proteins are released from the intermembrane space (Ge et al., 2013). Eventually, ATP-related disorders are caused. Multiple adverse effects increase ROS levels and aggravate mitochondrial dysfunction, thereby causing severe oxidative stress (Serra et al., 2018). Here, the changes of postinjury mitochondrial oxidative damage and respiratory dysfunction based on the time courses were investigated to show the important role of CII in response to energy stress and oxidative stress at the acute stage of postinjury.

2. Materials and methods

2.1. Animals

After 1 week of environmental acclimatization, fifty-six male Wistar rats (body mass = 231.5 ± 22.7 g) were randomly divided into either the control group ($n = 7$) or the lesioned group ($n = 49$). The lesioned rats were subject to standard contusion in the skeletal muscle and then randomly divided into seven subgroups, that is, 12 h, 2 d, 5 d, 7 d, 10 d, 15 d, and 30 d group after lesion, respectively. Gastrocnemius specimens were collected at different time points ($n = 7$ per group). The experimental protocol has been approved by the Animal Care and Use Committee of Shandong Sport University and follows the principles of laboratory animal care (NIH publication No. 86-23, revised 1985).

2.2. Muscle trauma model

We developed a muscle trauma model based on the method of Crisco et al. (1994) with few modifications. The rats were anesthetized through intraperitoneal injection of ketamine (50 mg/kg; i.p.) and xilazine (10 mg/kg; i.p.). Then, they were placed in a prone position. Their right hind limbs were exposed to induced contusion. A mass of 0.336 kg was dropped down from a height of 40 cm to directly ram the skin covering the mid-abdomen in the right gastrocnemius muscle (radius of 5.0 mm). Hence, the impact kinetic energy of 1.317 J was delivered. To ensure standardization, the control rats that were not subject to muscle trauma were also anesthetized.

2.3. Assessment on ROS levels

ROS assay kits were purchased from a bioengineering institute (Nanjing Jiancheng, China) to determine the intracellular ROS levels. The free radical activity of ROS in total was measured through using dichlorofluorescein diacetate (DCFH-DA) as a fluorescent probe. 500 μ L of ice cold PBS were used to wash muscle tissues (5 mg). At the same time, insoluble substances were removed via centrifuging at 10,000g for 5 min. A kit of Coomassie brilliant blue protein was used to determine the total protein content in the supernatant (Nanjing Jiancheng, China). The supernatant of all of the samples were treated with 10 mM DCFH-DA and then incubated at 37 °C for 30 min. A fluorescent microplate reader was used for determining fluorescence at 480 nm excitation and 530 nm emission. The amount of ROS was calculated based on the ratio of fluorescence intensity and protein content (OD/ μ g).

2.4. Isolation of skeletal muscle mitochondria

The animals were sacrificed through cervical dislocation. The right gastrocnemius muscle was removed. Fat and connective tissue were trimmed. The mitochondria were separated based on the instructions (mitochondrial isolation kit, Nanjing Jiancheng,

China). In brief, damaged muscles were resuspended with lysis buffer. In addition, they were homogenized with a hand-held borosilicate glass homogenizer. The homogenate was centrifuged at 800g for 5 min at 4 °C. The supernatant was then added to ice-cold medium A with a ratio of 1:1 and recentrifuged at 15,000g for 10 min at 4 °C. The pellet of the mitochondria was resuspended in a wash buffer and then centrifuged at 15,000g for 10 min at 4 °C. As a result, pellet of the mitochondria was suspended in a small volume of storage buffer.

2.5. Measurement of MMP

The MMP of the purified mitochondria was quantified in a JC-1 MMP assay kit and a fluorescence microplate. The mitochondria were incubated with JC-1. Right after the incubation, it was immediately measured by a microplate reader. The 590 nm/530 nm ratio was therefore obtained.

2.6. Biochemical assays

In the study, mitochondrial protein extraction kits were purchased from Nanjing Jiancheng Bioengineering Institute (China). Mitochondrial protein extraction kits were used for assessing the total protein in the mitochondria and calculating the activities of other enzymes. Commercial alkaline phosphatase (ALP), mitochondrial creatine kinase (miCK), lactate dehydrogenase (LDH). Mitochondrial respiratory chain enzymes (CI, CII, and CIII) assay kits were provided by Nanjing Jiancheng (China).

2.7. Statistical analysis

We used SPSS 18.0 (IBM, Chicago, IL, USA) to analyze the data. One-way ANOVA was adopted for determining statistical significance. The LSD multiple comparison test was used to examine the differences among groups. Data were displayed as mean \pm SD, and significant differences were considered at $P < 0.05$.

3. Results

3.1. Changes in mitochondrial ROS levels postinjury

ROS production induced by oxidative stress causes cell death by oxidizing many important proteins, leading to mitochondrial dysfunction and cell death (Avery, 2011; Cheong et al., 2016). To investigate the changes in post-traumatic muscle oxidative damage based on the time course, the research determined ROS levels at different time points by using DCFH-DA. The ROS level significantly increased as early as 12 h after injury. At 2, 5, and 7 d, the ROS levels decreased compared with that at 12 h, but still significantly increased than those of control rats. At 10 d, the baseline levels recovered (Fig. 1). These results suggested that after blunt trauma, the most severe periods of oxidative stress occurred on the first 7 d postinjury, and oxidative stress in the muscles was significantly alleviated 10 d postinjury.

3.2. Time course of changes in mitochondrial membrane potential postinjury

Excessive ROS impairs mitochondrial membrane, so there is possibility of arousing mitochondrial dysfunction (Karbowski and Neutzner, 2012). Mitochondrial dysfunction includes a decrease in MMP (Kim et al., 2013; Cheong et al., 2016). MMP is essential for mitochondrial homeostasis during oxidative phosphorylation. To investigate the time course of mitochondrial dysfunction, the research determined MMP at different time points. As early as 12

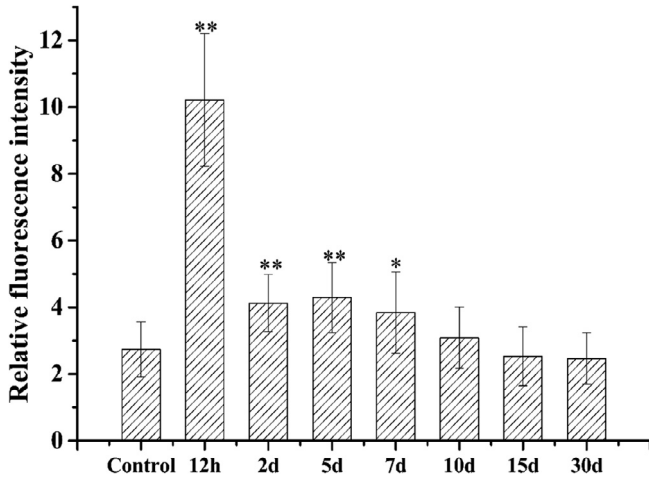


Fig. 1. Relative levels of intracellular ROS after injury. * $P < 0.05$, ** $P < 0.01$, $n = 7$.

h after injury, the lowest level of significant loss in MMP was observed. At 2 d, 5 d and 7 d, MMP increased compared with that at 12 h but still significantly lower than the level of control ($P < 0.01$). At 10 d, MMP was still significantly lower than that of control, but the loss was alleviated ($P < 0.05$). Then, at 15 d, the baseline level recovered (Fig. 2).

3.3. Biochemical parameters of skeletal muscle damage and mitochondrial antioxidant status

CK, LDH, and ALP are indicators for muscle damage and mitochondrial functions (Malaguti et al., 2013). Fig. 3 shows the changes in the activities of miCK, LDH, and ALP after lesion within 30 d. The miCK activity significantly increased during the period of 30 days with the observable highest activity at 2 d. At 2 d, the value was 13.96-fold higher than that of the control (0.26 ± 0.126 U/mg prot vs. 3.821 ± 1.212 U/mg prot, respectively, $P < 0.01$; Fig. 3A). Generally, its activity initially peaked at 2 d, and then dramatically decreased to a low ebb from 7 d to 10 d. Then, the activity increased again from 15 d to 30 d. The LDH activity remarkably enhanced at 12 h after injury (Fig. 3B). The significant increase of ALP activity was observed at 12 h and 2 d (Fig. 3C) respectively.

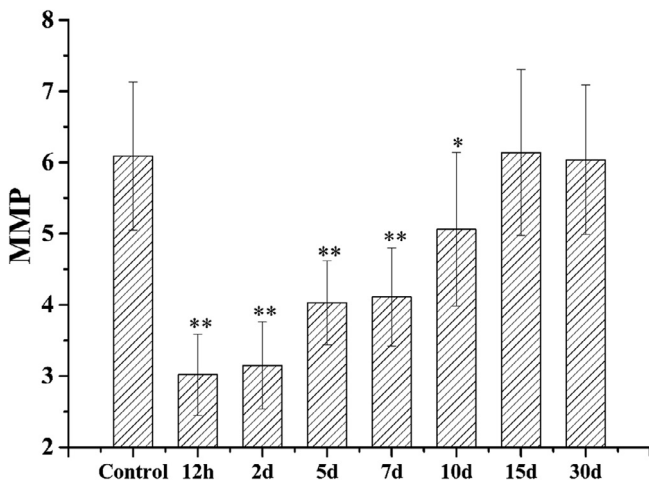


Fig. 2. Mitochondrial membrane potential (MMP) after injury. * $P < 0.05$, ** $P < 0.01$, $n = 7$.

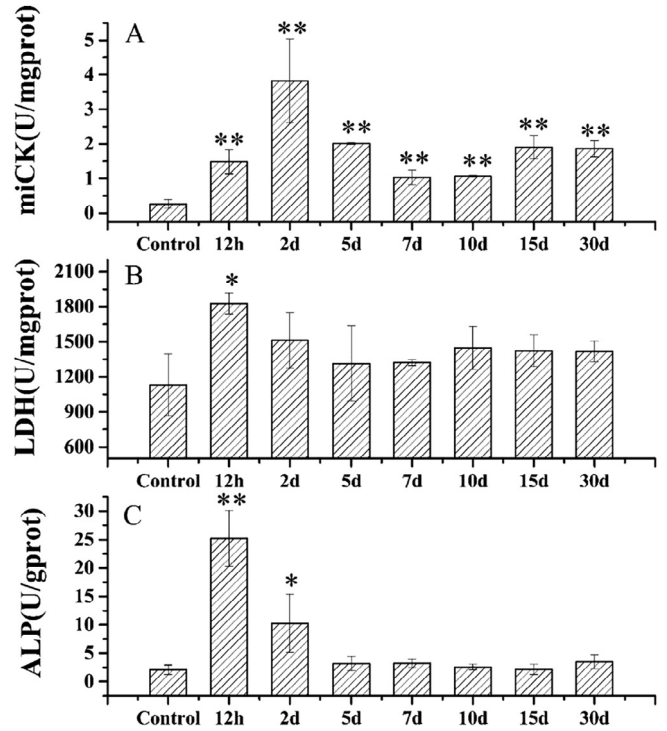


Fig. 3. The changes in the activities of different enzymes in the mitochondria after injury: (A) miCK; (B) LDH; and (C) ALP. * $P < 0.05$, ** $P < 0.01$, $n = 7$.

3.4. Activity of mitochondrial respiratory chain complexes

We analyzed the activities of CI-III after muscle blunt traumatic injury (Fig. 4). The activity of CI did not change at 12 h and 2 d postinjury. Its activity significantly decreased at 5 d and significantly increased at 10 d. The activity of CII significantly decreased as early as 12 h and significantly increased at 2 d. The activity of CIII significantly increased at 2 d, gradually decreased thereafter, reached a secondary climax at 15 d, and dramatically decreased.

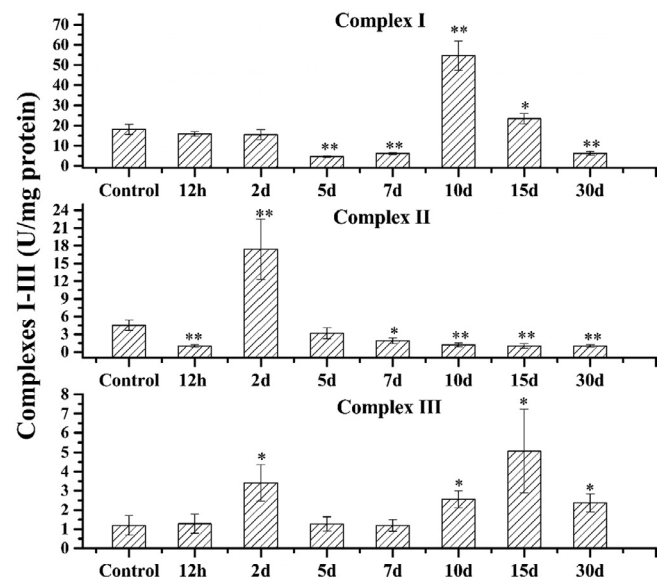


Fig. 4. Activity changes in CI-III in the skeletal muscle after injury (30 days) based on different periods. * $P < 0.05$, ** $P < 0.01$, $n = 7$.

4. Discussion

Muscle contusion, the main cause for morbidity from sports-related injuries, ranks second, following the strain. It corresponds to a structural damage of muscle cells along with mitochondrial dysfunction (Puntel et al., 2011). Mitochondria are powerhouse organelles for ATP. During this process, CI, CIII, and CIV extract and pump protons from the mitochondrial matrix to the intermembrane space. As a result, between the intermembrane space and the matrix, a proton gradient develops. As the energy stored as an electrochemical gradient of protons drives, ATP synthesizes. Oxidative phosphorylation drives ATP production by mitochondria to maintain skeletal muscle homeostasis. In an injured muscle, mitochondria is dysfunctional and thereby causes ATP-related disorders. Tissue repair, affected by ATP production, is a dynamic interactive process of at least 4 weeks. Therefore, in addition to short-term or early-stage postinjury studies (Singh et al., 2006; Gilmer et al., 2009; Hernansanz-Agustín, et al., 2017), follow-up studies in the long term of the research are necessary for revealing the functional characteristics of mitochondria at different stages of injury. Hardy et al. (2016) have demonstrated that the initial phase right after the infliction of injury is critical and would further bring about the overall outcome of regeneration. However, the underlying reason is rarely known. In the study, changes based on time courses in postinjury mitochondrial oxidative damage and respiratory dysfunction were investigated. This study attempts to explain why the initial phase following injury could affect the subsequent remodeling from the perspective of energy supply.

Mitochondrial dysfunction in contusion-lesioned muscle is similar to that in ischemia-reperfusion (IR) injury of skeletal muscles. It is characterized by reduced blood flow as a common circulatory disorder (Puntel et al., 2011, 2013). Under the physiological conditions of hypoxia, the oxygen delivered to the injured area is far from meeting the needs of mitochondrial oxidation in postischemic muscle, which makes mitochondrial ETS in a more reduced state. This leads to an increase in electron leakage of ETS that in turn reacts with residual molecular oxygen to form ROS and increase oxidative stress (Sudheesh et al., 2013). The ROS levels in our study suggested that the severe periods of oxidative stress after blunt trauma were observed on the first 7 d. Importantly, the most severe oxidative stress time occurred on the first 2 days after injury, especially at 12 h. The worst loss of MMP and the lowest levels of ROS were at 12 h postinjury. The miCK, ALP, and LDH activities peaked at 2 d, 12 h, and 12 h, respectively, indicating that the levels of mitochondrial antioxidant defenses were upregulated. These results suggested that 2 d and 12 h were the critical periods of postinjury.

After injury occurred, the activities of mitochondrial complexes I–III displayed different behaviors based on different periods. In our research, the marked increase in the activity of CII was observed at 2 d. The activity of CI significantly increased during 10–15 d. Additionally, the activity of CIII significantly increased at 2 and 10–30 d, respectively. Its significant increase at 2 d might come from the significant increase in the activity of CII. The significant improvement of activity at 10–30 d might be related to the significant increase in the activity of CI. These results indicated that CII was the place where electrons enter the ETS at the early stage of injury, such as 2 d postinjury. However, CI performed electron transfer at the following stage of injury. ROS can trigger the phosphorylation of CII so as to increase its activity (Acín-Pérez et al., 2014). Therefore, cumulative stress from ROS induced an explosive increase in the activity of CII at 2 d and strengthened CII-driven respiration. These results show that CII plays a predominant role in injury-induced oxidative stress during a muscle blunt traumatic injury. Also, according to the results, electrons are transferred to the ETS mainly through CII in this period.

CII is the second entrance in the electron transport chain. More importantly, the electron transport process in CII is not coupled to proton translocation across the inner mitochondrial membrane (Boekema and Braun, 2007). Therefore, the contribution of CII to mitochondrial electrochemical gradient (Δp) is less than that of CI, indicating that CII was unsusceptible to changes in Δp . Δp , which comprises the pH gradient (ΔpH) and MMP, is crucial to controlling energy transduction. In the present study, MMP significantly declined within 7 d after injury ($P < 0.01$). Therefore, we hypothesized that CII played a key role in electron transfer and attenuated CI-driven respiration in the early period of postinjury. At 10 d, MMP increased based on the level at 12 h. With vascular remodeling and mitochondrial repair, the electrochemical gradient is reconstructed, CI plays a major function in electron transfer and CII is deactivated.

CII may have two forms, anaerobic and aerobic ones so as to accommodate electron and proton transport under different oxygen conditions (Cecchini et al., 2003). Under hypoxic conditions, CII plays an important role in anaerobic energy metabolism. CII also acts as a potential target for several complicated conditions, such as cancer, IR injury and inflammation, and shows possible clinical relevance (Bezawork-Geleta et al., 2017). CII oxidizes succinate to fumarate and transfers electrons from succinate to ubiquinone. Some treatments related to CII and CII-dependent respiration intermediates are applied to deal with pathologies and abnormalities. For example, small molecules can enhance CII so as to better maintain the energy homeostasis of cells (Kluckova et al., 2013). On the basis of our results, we proposed that CII participated in electron transport at the early stage of muscle injury. Mechanisms for enhancing the activity of CII are crucial to muscle injury treatment. CII is a flavoenzyme functionally dependent on biologically active flavin adenine dinucleotide (FAD), which is derived from the dietary component riboflavin (Udhayabanu et al., 2017). Riboflavin or FAD supplements have been efficiently used for some inheritable diseases (Yatsyshyn et al., 2014). On the basis of our results, we further proposed that riboflavin or FAD supplementation at the early stage of postinjury might improve the function of CII, promote the recovery of an injured muscle, and shorten the course of a disease. However, it still remains to be explored about the stress response mechanism of CII postinjury. As such, further studies are needed to explore the details of the pharmacological and clinical effects of CII.

5. Conclusion

Skeletal muscle contusion leads to ischemia and hypoxia, accompanied by oxidative stress and mitochondrial dysfunction. The data from the research revealed that the most severe oxidative stress covered the first 2 days postinjury, especially at 12 h. CI was deactivated due to oxygen deficiency, superoxide production, and mitochondrial ultrastructural destruction. However, these harsh conditions greatly upregulated the energy metabolism functions of CII. In late postinjury, with mitochondrial repair, the electrochemical gradient was reconstructed so that CI performed a major electron transfer function. The time-course changes in CI–CIII activities following a muscle blunt traumatic injury in rats had an implication for the role of CII in early postinjury in terms of electron transfer. Then, it is proposed that CII could be a therapeutic target in muscle lesions. In addition, the pharmacological and clinical effects of CII need to be further studied.

Data availability

The data used to support the findings of this study are available from the corresponding author upon request.

Declaration of Competing Interest

The authors declare no conflicts of interest.

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References

- Acín-Pérez, R., Carrascoso, I., Baixauli, F., Roche-Molina, M., Latorre-Pellicer, A., Fernández-Silva, P., Mittelbrunn, M., Sanchez-Madrid, F., Pérez-Martos, A., Lowell, C.A., Manfredi, G., Enriquez, J.A., 2014. ROS-triggered phosphorylation of complex II by FGR kinase regulates cellular adaptation to fuel use. *Cell Metab.* 19, 1020–1033. <https://doi.org/10.1016/j.cmet.2014.04.015>.
- Avery, S.V., 2011. Molecular targets of oxidative stress. *Biochem. J.* 434, 201–210. <https://doi.org/10.1042/Bj20101695>.
- Baysal, B.E., Ferrell, R.E., Willett-Brozick, J.E., Lawrence, E.C., Myssiorek, D., Bosch, A., van der Mey, A., Taschner, P.E., Rubinstein, W.S., Myers, E.N., Richard, C.W., Cornelisse, C.J., Devilee, P., Devlin, B., 2000. Mutations in SDHD, a mitochondrial complex II gene, in hereditary paraganglioma. *Science* 287, 848–851.
- Bezawork-Geleta, A., Rohlena, J., Dong, L., Pacak, K., Neuzil, J., 2017. Mitochondrial complex II: at the crossroads. *Trends Biochem. Sci.* 42, 312–325. <https://doi.org/10.1016/j.tibs.2017.01.003>.
- Bezawork-Geleta, A., Wen, H., Dong, L., Yan, B., Vider, J., Boukalova, S., Krobava, L., Vanova, K., Zabalova, R., Sobol, M., Hozak, P., Novais, S.M., Caisova, V., Abaffy, P., Naraine, R., Pang, Y., Zaw, T., Zhang, P., Sindelka, R., Kubista, M., Zuryn, S., Molloy, M.P., Berridge, M.V., Pacak, K., Rohlena, J., Park, S., Neuzil, J., 2018. Alternative assembly of respiratory complex II connects energy stress to metabolic checkpoints. *Nat. Commun.* 9, 2221. <https://doi.org/10.1038/s41467-018-04603-z>.
- Boekema, E.J., Braun, H.P., 2007. Supramolecular structure of the mitochondrial oxidative phosphorylation system. *J. Biol. Chem.* 282, 1–4. <https://doi.org/10.1074/jbc.R600031200>.
- Cecchini, G., Maklashina, E., Yankovskaya, V., Iverson, T.M., Iwata, S., 2003. Variation in proton donor/acceptor pathways in succinate: quinone oxidoreductases. *FEBS Lett.* 545, 31–38.
- Cheong, C.U., Yeh, C.S., Hsieh, Y.W., Lee, Y.R., Lin, M.Y., Chen, C.Y., Lee, C.H., 2016. Protective effects of costunolide against hydrogen peroxide-induced injury in PC12 cells. *Molecules* 21. <https://doi.org/10.3390/molecules21070898>, pii: E898.
- Crisco, J.J., Jokl, P., Heinen, G.T., Connell, M.D., Panjabi, M.M., 1994. A muscle contusion injury model: biomechanics physiology and histology. *Am. J. Sports Med.* 22, 702–710. <https://doi.org/10.1177/036354659402200521>.
- Delos, D., Maak, T.G., Rodeo, S.A., 2013. Muscle injuries in athletes: enhancing recovery through scientific understanding and novel therapies. *Sports Health* 5, 346–352. <https://doi.org/10.1177/1941738113480934>.
- Ge, X., Dong, G., Wang, Y., Li, K., Pan, W., 2013. The proteome variation of rat skeletal muscle induced by repeated eccentric exercise. *China Sport Sci.* 33, 41–49.
- Gilmer, L.K., Roberts, K.N., Joy, K., Sullivan, P.G., Scheff, S.W., 2009. Early mitochondrial dysfunction after cortical contusion injury. *J. Neurotrauma* 26 (8), 1271–1280. <https://doi.org/10.1089/neu.2008.0857>.
- Hardy, D., Besnard, A., Latil, M., Jouvion, G., Briand, D., Thépenier, C., Pascal, Q., Guguin, A., Gayraud-Morel, B., Cavaillon, J.M., Tajbakhsh, S., Rocheteau, P., Chrétien, F., 2016. Comparative study of injury models for studying muscle regeneration in mice. *PLoS One* 11, e0147198. <https://doi.org/10.1371/journal.pone.0147198>.
- Hernansanz-Agustín, P., Ramos, E., Navarro, E., Parada, E., Sánchez-López, N., Peláez-Aguado, L., Cabrera-García, J.D., Tello, D., Buendia, I., Marina, A., Egea, J., López, M.G., Bogdanova, A., Martínez-Ruiz, A., 2017. Mitochondrial complex I deactivation is related to superoxide production in acute hypoxia. *Redox Biol.* 1040–1051. <https://doi.org/10.1016/j.redox.2017.04.025>.
- Hollinshead, K.E., Tennant, D.A., 2016. Mitochondrial metabolic remodeling in response to genetic and environmental perturbations. *Wiley Interdiscip. Rev. Syst. Biol. Med.* 8, 272–285. <https://doi.org/10.1002/wsbm.1334>.
- Järvinen, T.A., Järvinen, T.L., Kääriäinen, M., Kalimo, H., Järvinen, M., 2005. Muscle injuries: biology and treatment. *Am. J. Sports Med.* 33, 745–764. <https://doi.org/10.1177/0363546505274714>.
- Järvinen, T.A., Järvinen, M., Kalimo, H., 2014. Regeneration of injured skeletal muscle after the injury. *Muscles Ligaments Tendons J.* 3, 337–345.
- Karbowski, M., Neutzner, A., 2012. Neurodegeneration as a consequence of failed mitochondrial maintenance. *Acta Neuropathol.* 123, 157–171. <https://doi.org/10.1007/s00401-011-0921-0>.
- Kim, K.Y., Cho, H.J., Yu, S.N., Kim, S.H., Yu, H.S., Park, Y.M., Mirkheshti, N., Kim, S.Y., Song, C.S., Chatterjee, B., Ahn, S.C., 2013. Interplay of reactive oxygen species, intracellular Ca²⁺ and mitochondrial homeostasis in the apoptosis of prostate cancer cells by deoxydopodophyllotoxin. *J. Cell Biochem.* 114, 1124–1134. <https://doi.org/10.1002/jcb.24455>.
- Kluckova, K., Bezawork-Geleta, A., Rohlena, J., Dong, L., Neuzil, J., 2013. Mitochondrial complex II, a novel target for anti-cancer agents. *Biochim. Biophys. Acta* 1827, 552–564. <https://doi.org/10.1016/j.bbabi.2012.10.015>.
- Malaguti, M., Angeloni, C., Hrelia, S., 2013. Polyphenols in exercise performance and prevention of exercise-induced muscle damage. *Oxid. Med. Cell Longev.* 825928. <https://doi.org/10.1155/2013/825928>.
- Puntel, G.O., Carvalho, N.R., Amaral, G.P., Lobato, L.D., Silveira, S.O., Daubermann, M. F., Barbosa, N.V., Rocha, J.B., Soares, F.A., 2011. Therapeutic cold: an effective kind to modulate the oxidative damage resulting of a skeletal muscle contusion. *Free Radic. Res.* 45, 125–138. <https://doi.org/10.3109/10715762.2010.517252>.
- Puntel, G.O., Carvalho, N.R., Dobrachinski, F., Salgueiro, A.C., Puntel, R.L., Folmer, V., Barbosa, N.B., Royes, L.F., Rocha, J.B., Soares, F.A., 2013. Cryotherapy reduces skeletal muscle damage after ischemia/reperfusion in rats. *J. Anat.* 222, 223–230. <https://doi.org/10.1111/joa.12009>.
- Rasheed, M.R.H.A., Tarjan, G., 2018. Succinate dehydrogenase complex: an updated review. *Arch. Pathol. Lab. Med.* 142, 1564–1570. <https://doi.org/10.5858/arpa.2017-0285-RS>.
- Serra, A.J., Prokić, M.D., Vasconsuelo, A., Pinto, J.R., 2018. Oxidative stress in muscle diseases: current and future therapy. *Oxid. Med. Cell Longev.* 6439138. <https://doi.org/10.1155/2018/6439138>.
- Singh, I.N., Sullivan, P.G., Deng, Y., Mbye, L.H., Hall, E.D., 2006. Time course of post-traumatic mitochondrial oxidative damage and dysfunction in a mouse model of focal traumatic brain injury: implications for neuroprotective therapy. *J. Cereb. Blood Flow Metab.* 26, 1407–1418. <https://doi.org/10.1038/sj.jcbfm.9600297>.
- Sudheesh, N.P., Ajith, T.A., Janardhanan, K.K., 2013. Ganoderma lucidum ameliorate mitochondrial damage in isoproterenol-induced myocardial infarction in rats by enhancing the activities of TCA cycle enzymes and respiratory chain complexes. *Int. J. Cardiol.* 165, 117–125. <https://doi.org/10.1016/j.ijcard.2011.07.103>.
- Tretter, L., Patocs, A., Chinopoulos, C., 2016. Succinate, an intermediate in metabolism, signal transduction, ROS, hypoxia, and tumorigenesis. *Biochim. Biophys. Acta* 1857, 1086–1101. <https://doi.org/10.1016/j.bbabi.2016.03.012>.
- Tielens, A.G., Rotte, C., van Hellemond, J.J., Martin, W., 2002. Mitochondria as we don't know them. *Trends Biochem. Sci.* 27, 564–572.
- Tropeano, C.V., Fiori, J., Carelli, V., Caporali, L., Daldal, F., Ghelli, A.M., Rugolo, M., 2018. Complex II phosphorylation is triggered by unbalanced redox homeostasis in cells lacking complex III. *Biochim. Biophys. Acta Bioenerg.* 1859, 182–190. <https://doi.org/10.1016/j.bbabi.2017.12.003>.
- Tsai, W.C., Yu, T.Y., Chang, G.J., Lin, L.P., Lin, M.S., Pang, J.S., 2018. Platelet-rich plasma releasate promotes regeneration and decreases inflammation and apoptosis of injured skeletal muscle. *Am. J. Sports Med.* 46, 1980–1986. <https://doi.org/10.1177/0363546518771076>.
- Udhayabanu, T., Manole, A., Rajeshwari, M., Varalakshmi, P., Houlden, H., Ashokkumar, B., 2017. Riboflavin responsive mitochondrial dysfunction in neurodegenerative diseases. *J. Clin. Med.* 6. <https://doi.org/10.3390/jcm6050052>, pii: E52.
- Yatsyshyn, V.Y., Fedorovych, D.V., Sibirny, A.A., 2014. Metabolic and bioprocess engineering of the yeast *Candida famata* for FAD production. *J. Ind. Microbiol. Biotechnol.* 41, 823–835. <https://doi.org/10.1007/s10295-014-1422-7>.