

Review Article

THE AKT PATHWAY AND SATELLITE CELL ACTIVATION IN SKELETAL MUSCLE MASS REGULATION

Santi Sofiyanti^{1,4}, Hanna Goenawan^{2,3}, Ronny Lesmana^{2,3}, Vita Murniati Tarawan²¹Graduate School of Biomedical Sciences, Faculty of Medicine Universitas Padjadjaran, Bandung, Indonesia²Physiology Division, Department of Biomedical Sciences, Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia³Biology Activity Division, Central Laboratory, Universitas Padjadjaran, Bandung, Indonesia⁴Midwifery Department, Health Polytechnic, Ministry of Health, Bandung, Indonesia

ABSTRACT

Muscles have an important role as a regulator of glucose and triglyceride metabolism. Some researches show the correlation between skeletal muscle mass and metabolic diseases, such as diabetes. Skeletal muscle mass decrease occurs due to chronic illness or physiological process of aging, thus increasing the risk of metabolic diseases as well as motion difficulty in the elderly. Skeletal muscle mass depends on balanced protein synthesis and degradation, controlled through a variety of signal transduction pathways including the AKT. AKT or protein kinase B increases protein synthesis through the mTOR and GSK3 β and controls the degradation of proteins through FoxO transcription factors. Another factor that has an alleged role in the regulation of skeletal muscle is the satellite cells which provide remarkable ability to regenerate skeletal muscle. A comprehensive understanding of the biomolecular mechanism of muscle mass regulation is important to develop effective treatment or prevention of muscle atrophy in many cases, either caused by pathological conditions, such as chronic diseases, or the process of aging.

Keywords: Skeletal muscle mass; AKT pathway; satellite cells; human & health

Correspondence: Hanna Goenawan, Physiology Division, Department of Biomedical Science, Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia. Email: hanna@unpad.ac.id

How to cite: Sofiyanti, S., I oenawan, J ., Nesmana, T., (Tarawan, X. M. *4244+. The CMT Pathway and Satellite Eell Cctivation in Skeletal Muscle Mass Tegulation. *Holia Medica Indonesiana*, 7: *3+, 8: 695. <https://doi.org/10.24269/1fmi.v7.i3.35576>

pISSN:2355-8393 • eISSN: 2599-056x • doi: 10.20473/fmi.v58i1.13354 • Fol Med Indones. 2022;58: 68-73

• Submitted 22 Oct 2021 • Revised 21 Dec 2021 • Accepted 11 Jan 2021 • Published 5 March 2022

• Open access under CC-BY-NC-SA license • Available at <https://e-journal.unair.ac.id/FMI/>

Hii j iii j tur

1. CMT or protein kinase D increase protein synthesis and control the degradation of proteins.
4. Satellite cells was provided remarkable ability to regenerate skeletal muscle.
5. Stimulation of protein synthesis is effective therapy to maintain muscle mass, prevent muscle wasting to reduce risk sarcopenia and improve suality of life in the elderly.

INTRODUCTION

Skeletal muscle is a major part of the human body. Skeletal muscle has several important functions, such as maintaining posture, movement and regulating glucose and triglycerides metabolism. Skeletal muscle is the main organ for glucose deposition. Skeletal muscles convert glucose into energy to generate movement and activity. Decreasing of skeletal muscle mass has been connected to chronic diseases, such as diabetes mellitus (Collins et al. 2018, Hong et al. 2017). Insulin resistance in muscle is the main characteristic of metabolic diseases, such as obesity, type 2 diabetes (DM 2), and metabolic syndrome.

Skeletal muscle mass was thought to have a protective role against metabolic diseases (Lee et al. 2019).

The decrease of skeletal muscle mass also occurs physiologically in the aging process. Skeletal muscle mass decreases approximately 8% per decade up to 70 years and decreases 15% per decade after 70 years. Consequently, it leads to various problems in the elderly, such as immobility, fragility as well as increasing the risk of metabolic diseases (Gomes et al. 2017).

Skeletal muscle mass controlled by various molecular signaling pathways. Those signals regulate the balance of muscle protein synthesis and degradation. Altered muscle regulation resulted in muscle hypertrophy and atrophy. Signaling pathways regulate the growth, regeneration and regulation process of muscle mass since the embryonic period (Egerman & Glass 2014). Comprehensive understanding about the underlying molecular mechanism that regulates skeletal muscle mass is important as the basis for therapeutics research about sarcopenia in the aging population. The purpose of the therapeutic agent is to increase the mass and prevent muscle atrophy, thereby lessening the risk of various chronic diseases, reducing the burden of care and improving quality of life.

OVERVIEW

Skeletal muscle regulation and protein synthesis

Skeletal muscle has a specific structure for its typical function. Skeletal muscle fiber (myofibrils) are composed of proteins including actin, myosin, titin and other proteins that hold them together. These proteins are organized into myofilaments. A bundle of myofibril covered by epimysium forms a muscle fiber and bundles of muscle fibers constitute skeletal muscle tissue. About 80% of the muscle fiber is composed of proteins (Frontera & Ochala 2015). Muscle mass balance mechanisms are influenced by various factors, such as nutritional status, hormonal, physical activity, and injury or diseases (Cai et al. 2016).

Skeletal muscle hypertrophy is characterized by myofiber enlargement with no change in myofiber number or hyperplasia. Significant changes in skeletal muscle hypertrophy are the increases of protein synthesis, while muscle mass loss occurs through the protein degradation. Genetics regulation determines the physiology changes in muscle activity. Various signal transduction pathways influence protein synthesis and degradation, thus can control muscle growth (Moriya & Miyazaki 2018). An anabolic stimulus, such as Insulin Growth Factor (IGF) can trigger muscle hypertrophy, while myostatin and other growth factors, such as members of transforming growth factor (TGF- β) have an inhibitory effect on muscle growth (Egerman & Glass 2014).

Skeletal muscle increased through the AKT pathway

The muscle protein synthesis and degradation processes were controlled by several pathways of molecular signals, such as *phosphatidylinositol* 3-kinase (PI3K)/ AKT (serine/threonine-kinase)

pathway. Activation of AKT, also known as PKB (Protein Kinase B), mediated a variety of cellular functions including angiogenesis, metabolism, growth, proliferation, cells survival, protein synthesis, transcription, and apoptosis (Hemmings & Restuccia 2012).

PI3K/ AKT pathway mainly roled protein synthesis and inhibited protein degradation, so that AKT/ PKB was an important pathway in muscle hypertrophy. PI3K/ AKT pathway had been proven to be the main pathway of muscle hypertrophy. Studies using genetic approaches had shown expression of either PI3K or AKT induced muscle fiber hypertrophy both in vivo and in vitro (Cai et al. 2016, Hemmings & Restuccia 2012).

The AKT pathway worked through various channels by activation or inhibition of its effectors that could ultimately maintain the protein synthesis. AKT inhibited protein degradation by suppressing the transcription factor forkhead box O (FoxO) and stimulated protein synthesis through mechanistic target of rapamycin (mTOR) and glucose synthase kinase (GSK3 β). AKT could be activated by phosphorylation after a series of an intracellular signaling cascade that involved growth factors, such as IGF-1 and PI3K. PI3K is a lipid kinase that regulates the levels of phosphorylated *phosphatidylinositol* (PIP3) at plasma membrane. PIP3 acted as docking sites for the two kinases, such as phosphoinositide-dependent kinase 1 (PDK1) and AKT (Egerman & Glass 2014, Hemmings & Restuccia 2012).

PI3K activated AKT and mTOR, which regulated the metabolic activity of cellular biosynthesis supporters. mTOR regulates protein translation control through emultiple effectors. AKT indirectly affected mTOR by inhibiting protein tuberous sclerosis complex (TSC) 1 and 2 as a GTPase activating protein (GAP) that inactivated small G protein Ras (Rheb) which could activate the mTOR directly (Yoon 2017).

mTOR has two different complex biochemical structures that are bound to the raptor mTORC1 and mTORC2 bound by Rictor. mTORC1 is highly sensitive to rapamycin and regulates protein metabolism and autophagy. mTOR stimulation can initiate the process of protein translation and can be stimulated by some nutritional compounds, such as amino acids, insulin, growth factor and muscle contraction (Santos et al. 2017, Yoon 2017).

PI3K/ AKT activation mechanism

Muscle hypertrophy mechanism through activation of P13K/ AKT by IGF1 and insulin receptor IGF1 starts with ligand bound to its receptor located on the cell membrane. IGF 1 receptor is a tyrosine kinase receptor called as IGF1 (IGF1- R). This bonding caused phosphorylation of tyrosine kinase receptors which then formed a docking site to the insulin receptor substrate (IRS-1). Muscle hypertrophy signaling pathways through IRS- IGF1 was an important mediator for IGF signaling pathway (Andrade et al. 2017). Phosphorylated IRS acts as the mounting location to move and activate PI3K, which phosphorylated membrane phospholipids and generated phosphoinositide-3,4,5-triphosphate (PIP3) from phosphoinositide-4,5-bisphosphate (PIP2). The next cascade of autophosphorylation activated the AKT (Hemmings & Restuccia 2012).

In addition to initiating the synthesis of protein, AKT inhibited protein degradation by suppressed family transcription factor FoxO1, FoxO3, and muscle ring finger 1 (MuRF1). FoxO was an inhibitor of cell survival factor that stimulated the synthesis of proteins through mTOR and glycogen synthase kinase 3β (GSK3 β) (Egerman & Glass 2014). GSK3 β phosphorylation causes the release of a ribosomal translation inhibitor factor called eukaryotic translation initiation factor 2B (EIF2B) (Yoon 2017). Activation of mTOR could stimulate the activation of p70S6K that led to the ribosome s6 phosphorylation, so that the protein translation process could occur.

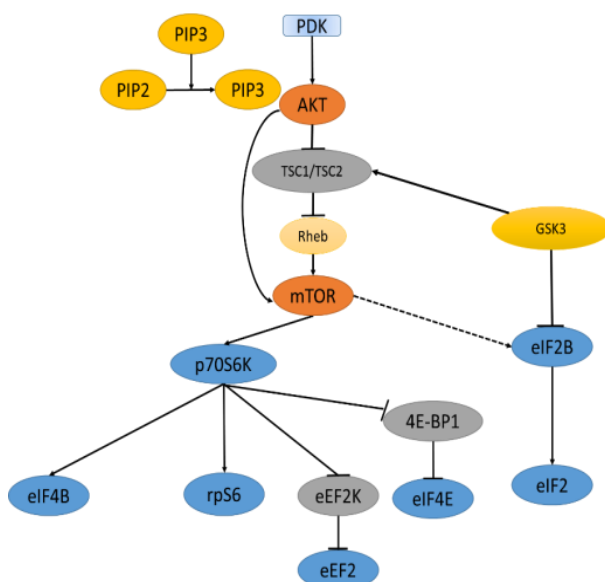


Figure 1. AKT pathway/ TSC2/ mTOR in protein synthesis (Favier et al. 2008)

Note: Activation of phosphatidylinositol-3-kinase (PI3K) led to phosphorylation of phosphatidylinositol-biphosphate (PIP2) into

phosphatidylinositol-triphosphate (PIP3) as the docking site membrane for two kinases: serine/ threonine kinase AKT (or protein kinase B) and protein kinase (PDK). AKT phosphorylated by PDK and thus enabled the translocation to the membrane. Once activated, AKT phosphorylates tuberous sclerosis complex (TSC) 2, which joined the TSC1 and caused the release of inhibition of the Ras homolog (Rheb).

Rheb directly activates mTOR which then stimulates ribosome biogenesis, mRNA initiation and elongation via the phosphorylation of the 70-kDa ribosomal protein, S6 kinase (p70S6K) and inhibit 4E-BP1 binding to eukaryotic initiation factor (eIF) 4E. In addition, Akt promotes protein translation via inhibition of glycogen synthase kinase (GSK) 3, which controls the activity of eIF2B. TSC2 enabled by GSK3 and inhibited by Akt.

Stimulation of serine 6 kinase 1 (S6K1) activity by mTORC1 could induce mRNA biogenesis processes, including transcription, elongation and protein translation on the ribosome. The ribosomal protein translation process is activated by the eukaryotic initiation factors (eIF4E), which in normal conditions bind to the binding protein 4E-BP1. Phosphorylation of 4E-BP1 by mTOR reduced the affinity of 4E-BP1 to eIF4E. The release of eIF4E from 4E-BP1 could initiate the translation process (Hemmings & Restuccia 2012). Therefore, the AKT-GSK-3β and AKT-mTOR pathway is important to increase protein synthesis associated with muscle hypertrophy.

Satellite cells functioned on muscle regeneration

Skeletal muscle has a great ability to adapt to the functional demands and regenerate after injury. This remarkable ability was regulated by satellite cell. Satellite cells are spindle-shaped mononucleus cells located under the basal lamina between fiber sarcolemma, and surrounded by mature muscle fibers. Satellite cells can only be recognized with an electron microscope and regarded as inactive sedentary myoblasts after the muscle differentiates. It mainly functioned to renew muscle fibers after trauma or in life after birth (Almeida et al. 2016).

Maintenance of muscle mass depends on the balance between protein synthesis and degradation. However, some studies revealed that satellite cells took a role in cell turnover, muscle growth and maintenance of muscle mass. Satellite cells played a role as the main donors of new nuclei, into myogenic precursor cells that are important for muscle growth and regeneration. Satellite cells contributed in the process of muscle hypertrophy in response to exercise and hormonal stimulation (Musarò 2014).

The involvement of satellite cells in muscle hypertrophy in adulthood is still debated, although theoretically the addition of new myonuclei caused by satellite cells and satellite cell fusion could occur during myofiber hypertrophy. A study conducted on



mouse models with AKT transgene showed muscle hypertrophy that was not accompanied by satellite cell proliferation (Blaauw et al. 2009). Physiologically, the satellite and myonuclei cells can undergo apoptosis during muscle atrophy, although myonuclear loss occurs in muscle atrophy is unclear. Increasing the size of myofiber can also occur in the cells regeneration process, but not in the postnatal hypertrophy phase. However, a different research showed that the body mechanism to maintain muscle mass needed the presence of the satellite cell in quiescent condition. A study using diphtheria toxins to diminished satellite cells showed a decrease in skeletal muscle mass (Sambasivan et al. 2011). Another study showed satellite cell proliferation and differentiation followed muscle hypertrophy induced by endurance and resistance exercise (Bazgir et al. 2017).

Satellite cell activation mechanism

In the off-state, satellite cells were in the G0 phase. In this condition, the satellite cells expressed myogenic factor 5 (Myf5). When an injury or other stimuli occurred, the silent satellite cells were activated, then proliferated and combined to form a new skeletal muscle fiber. The satellite cell activation was done through the transcription factor, paired box 7 (Pax7) (Musrò, 2014). The proliferation and myoblast Proliferative activity of IL-6 contributed to the growth of hypertrophic myofibers. IGF-1R signaling in cell regeneration and activation of satellite cells was highly important. A study conducted in vivo with heterozygote mice IGF-1R (IGF-1R +/-), showed a decrease in expression of MyoD and myogenin as a marker of proliferation and differentiation of satellite cells (Dong et al. 2013). IGF-1R activated satellite cells indirectly through the AKT pathway which had an important role in the myoblast differentiation process.

daughter fusion is marked by myogenic differentiation (MyoD) expression as a marker of active satellite cell proliferation. A small part of the satellite cells was then deactivated by downregulation of MyoD and was used as an independent regeneration of satellite cells, MyoD expression was induced in 24 hours on the satellite cell activation (Endo 2015, Musarò 2014).

Afterwards, satellite cells that have migrated to the injured site proliferated to produce a number of myoblasts needed for regeneration. Some growth factors and cytokines played an important role in this process, including HGF, FGF, IGF-1, LIF, and IL-6 released from injured muscle or infiltrated phagocytes (Musrò 2014). IGF-1 was produced by satellite cells, myofibers and liver and acted in autocrine, paracrine and endocrine systems. IGF-1 did pleiotropic functions, such as in the satellite cells or myoblasts proliferation, differentiation, maturation and hypertrophy during muscle regeneration. All these functions were mediated by IGF-1 receptor (IGF1R). IGF1 signaling - IGF1R activated the Ras-ERK pathway and signaling (Ras-) PI3K-AKT, so that it could process proliferation and differentiation. IL-6 secreted from myofibers also stimulated proliferation of satellite cells through activation of STAT3.

Some studies conducted in animal models of muscle atrophy showed satellite cell activation by an increase in the expression of MyoD through the AKT pathway (Hauersley et al. 2014). The similar result found in vitro experiment with black ginseng also showed an increase in the expression of MyoD and MHC through the AKT pathway (Lee et al. 2018). Another animal research using AKT1 KO mice treated by mechanical load indicated that the AKT pathway was important to induce satellite cell proliferation (Moriya & Miyazaki 2018).

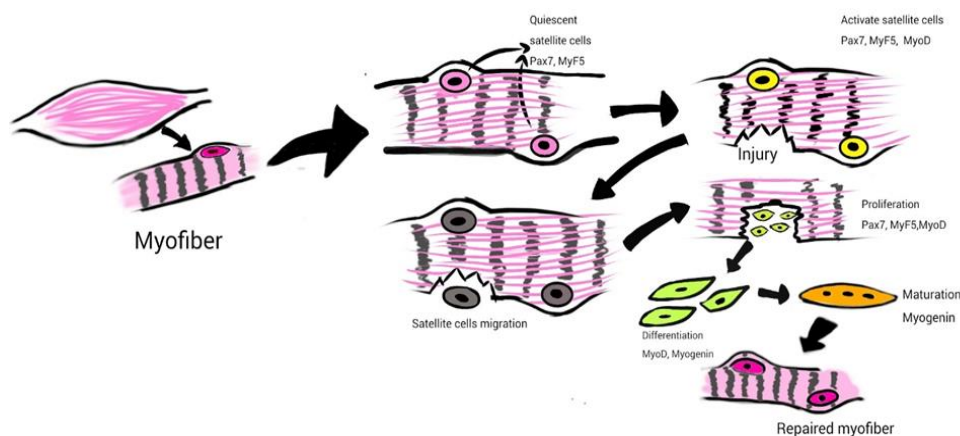


Figure 2. Skeletal muscle regeneration process (Endo 2015)

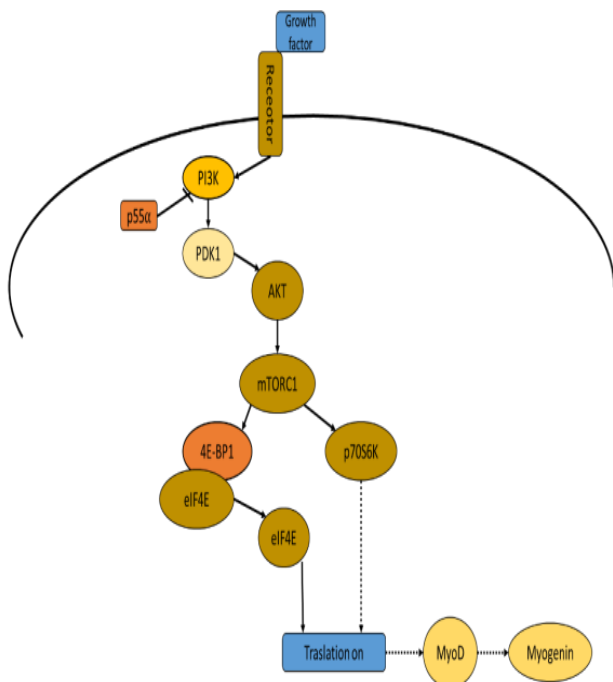


Figure 3. AKT signaling pathway in the activation of satellite cells (Hauersley et al. 2014)

Strength and limitation

The role of CMV and satellite cells in regulating skeletal muscle mass is explained, providing insight into the molecular mechanisms behind muscle atrophy and potential targets for treatment or prevention. The importance of understanding muscle mass regulation in both pathological conditions and aging is acknowledged, indicating the relevance of this topic for a wide range of individuals. The statement does not provide any specific research examples to support the correlation between skeletal muscle mass and metabolic diseases, making it difficult to assess the strength of the evidence presented.

CONCLUSION

AKT pathways regulated muscle mass by increasing protein synthesis through mTOR and GSK3 β . Other than the protein synthesis regulation, the AKT was also important to the activation of the satellite cells and suggested involvement in skeletal muscle hypertrophy. AKT pathway activation was done through a variety of growth factors that could be a ligand to activate the insulin receptor (IRS). The AKT pathway activation was mainly carried out through physical activity, especially resistance training or lifting weights. In addition, the intake of certain supplements had a potency to activate the AKT pathway, thus allegedly able to provide the same effects as physical activity to increase muscle mass

through the protein synthesis stimulation. This could be an effective therapeutic option for maintaining muscle mass and preventing muscle wasting, so that it can reduce the risk of sarcopenia and increase the quality of life in the elderly.

Acknowledgment

The authors would like to thank Physiology Division, Department of Biomedical Sciences, Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia.

Conflict of interest

None.

Funding disclosure

This project was supported by Hibah Internal UNPAD ALG to VMT to Hanna Goenawan with Grant number 1959/UN.6.3.1/PT.00/2021.

Author contribution

SS/conceptual and study design, XMV/collected data, TN/analysis data. J I was write the manuscript and final check.

REFERENCES

- Almeida, CF, Fernandes SA, Junior AR, et al (2016). Muscle satellite cells: Exploring the basic biology to rule them. *Stem Cells Int.* 2016, 1–14.
- Andrade GM, da Silveira JC, Perrini C, et al (2017). The role of the PI3K-Akt signaling pathway in the developmental competence of bovine oocytes. *PLoS One* 12, 1–15.
- Bazgir B, Fathi R, Valojerdi MR, et al (2017). Satellite cells contribution to exercise mediated muscle hypertrophy and repair. *Cell J.* 18, 473–484.
- Blaauw B, Canato M, Agatea L, et al (2009). Inducible activation of Akt increases skeletal muscle mass and force without satellite cell activation. *FASEB J* 23, 3896–3905.
- Cai X, Zhu C, Xu Y, et al (2016). Alpha-ketoglutarate promotes skeletal muscle hypertrophy and protein synthesis through Akt/mTOR signaling pathways. *Sci. Rep.* 6, 2–12.
- Collins KH, Herzog W, MacDonald GZ, et al (2018). Obesity, metabolic syndrome, and musculoskeletal disease: Common inflammatory pathways suggest a central role for loss of muscle integrity. *Front Physiol.* 9, 1–25.
- Dong YLR, Thomas SS, Dong YWXH, et al (2013). Interactions between p-Akt and Smad3 in injured muscles initiate myogenesis or fibrogenesis. *Am J Physiol Endocrinol Metab* 305, 367–375.

- Egerman MA, Glass DJ (2014). Signaling pathways controlling skeletal muscle mass. *Crit. Rev. Biochem. Mol. Biol.* 49, 59–68.
- Endo T (2015). Molecular mechanisms of skeletal muscle development, regeneration, and osteogenic conversion. *Bone* 80, 2–13.
- Favier FB, Benoit H, Freyssenet D (2008). Cellular and molecular events controlling skeletal muscle mass in response to altered use. *Pflugers Arch* 456, 587–600.
- Frontera WR, Ochala J (2015). Skeletal muscle: A brief review of structure and function. *Calcif Tissue Int* 96, 183–195.
- Gomes MJ, Martinez PF, Pagan LU, et al (2017). Skeletal muscle aging: influence of oxidative stress and physical exercise. *Oncotarget* 8, 20428–20440.
- Hauersley S, Vissing J, Krag TO (2014). Muscle atrophy reversed by growth factor activation of satellite cells in a mouse muscle atrophy model. *PLoS One* 9, 1–12.
- Hemmings BA, Restuccia DF (2012). PI3K-PKB/Akt pathway. *Cold Spring Harb. Perspect. Biol.* 4, 1–3.
- Hong, S., Chang, Y., Jung, H.-S., Yun, K.E., Shin, H., Ryu, S., 2017. Relative muscle mass and the risk of incident type 2 diabetes: A cohort study. *PLoS One* 12, 1–13.
- Lee MJ, Kim EH, Bae SJ, et al (2019). Protective role of skeletal muscle mass against progression from metabolically healthy to unhealthy phenotype. *Clin Endocrinol* 90, 102–113.
- Lee SY, Go GY, Vuong TA, et al (2018). Black ginseng activates Akt signaling, thereby enhancing myoblast differentiation and myotube growth. *J Ginseng Res* 42, 116–121.
- Moriya N, Miyazaki M (2018). Akt1 deficiency diminishes skeletal muscle hypertrophy by reducing satellite cell proliferation. *Am J Physiol Regul Integr Comp Physiol* 314, 741–751.
- Musarò A (2014). The basis of muscle regeneration. *Adv. Biol.* 2014, 1–16.
- Sambasivan R, Yao R, Kissenpfennig A, et al (2011). Pax7-expressing satellite cells are indispensable for adult skeletal muscle regeneration. *Development* 138, 3647–3656.
- Santos SDL, Garcia-Perez V, Hernández-Reséndiz S, et al (2017). ‘(-)-Epicatechin induces physiological cardiac growth by activation of the PI3K/Akt pathway in mice. *Mol Nutr Food Res* 61, 1–32.
- Yoon MS (2017). mTOR as a key regulator in maintaining skeletal muscle mass. *Front. Physiol* 8, 1–9.