

# **O-GlcNAcylation as a regulator of the functional and structural properties of the sarcomere in skeletal muscle: an update review**

**Running title: O-GlcNAc and skeletal muscle functions**

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## ABSTRACT

Although the O-GlcNAcylation process was discovered in 1984, its potential role in the physiology and pathophysiology of skeletal muscle only emerged twenty years later. An increasing number of publications strongly support a key role of O-GlcNAcylation in the modulation of important cellular processes which are essential for skeletal muscle functions. Indeed, over a thousand of O-GlcNAcylated proteins have been identified within skeletal muscle since 2004, which belong to various classes of proteins, including sarcomeric proteins.

In this review, we focused on these myofibrillar proteins, including contractile and structural proteins. Because of the modification of motor and regulatory proteins, the regulatory myosin light chain (MLC2) is related to several reports that support a key role of O-GlcNAcylation in the fine modulation of calcium activation parameters of skeletal muscle fibers, depending on muscle phenotype and muscle work. In addition, another key function of O-GlcNAcylation has recently emerged in the regulation of organization and reorganization of the sarcomere. All together, this data supports a key role of O-GlcNAcylation in the homeostasis of sarcomeric cytoskeleton, known to be disturbed in many related muscle disorders.

## INTRODUCTION

Skeletal muscle is recognized to be an intricate and highly complex machinery whose function is to generate force through muscle contraction.<sup>1</sup> Indeed, the efficient work of skeletal muscle is allowed by specialized myofibrils resulting from end to end arrangement of sarcomeres. The sarcomere is the functional unit of skeletal muscle (and in larger extent of striated muscles) and results from the strict organization of extremely sophisticated macromolecular protein complexes within a cytoskeletal network, termed nowadays “sarcomeric cytoskeleton”.<sup>1-3</sup> The precise and conserved localization of constitutive proteins of these complexes is highly regulated, and leads to regular arrangement of the thin and thick filaments predominantly composed of actin and myosin; these two contractile proteins transiently interact during the cross-bridge cycles, leading to filament sliding and so generating force.<sup>4</sup> Among actin and myosin which drive the mechanism of contraction, additional proteins regulate the process; in particular, tropomyosin coordinates the activation of thin filament, and the troponin complex modulates actomyosin cross-bridge formation. In addition to the thin and thick filaments, a third filament, composed of the giant protein titin, contributes to sarcomere elasticity and completes its organization through the constitution of a genuine scaffold which permits the anchoring of several proteins and regulation of their sarcomeric localization. Titin filament is essential to muscle function ensuring not only the modulation of mechanical elasticity properties, but also the modulation of intracellular signaling pathways.<sup>5</sup>

While the contractile apparatus has to be maintained in this semi-crystalline organization to ensure its work, the sarcomeric cytoskeleton is neither passive nor static, but outstandingly dynamic. As consequence, all the components of this cytoskeletal framework need to be strictly maintained in an equilibrium in terms of protein-turnover, assembly and maintenance.<sup>1,6-8</sup> It is worth to note that phosphorylation regulates some aspects of this structural homeostasis, in particular through the modulation of protein-protein interactions at two nodal points of the sarcomere: M-band and Z-disk. Phosphorylation is involved in the formation and maintenance of the structural interactome, as it was demonstrated for telethonin,<sup>9</sup> myomesin,<sup>10,11</sup> or desmin.<sup>12</sup> Interestingly, changes of protein phosphorylation were associated to skeletal muscle disorders, such as for desmin filaments<sup>12</sup> or MyBPC

in mouse dystrophic muscles.<sup>13</sup> Phosphorylation is also involved in the modulation of skeletal muscle contraction in particular through the modification of regulatory myosin light chain which provides a molecular memory of contraction.<sup>14,15</sup>

In addition, another post-translational modification emerged in the last fifteen years which is crucial in the physiology and physiopathology of skeletal muscle. This post-translational modification, termed O-GlcNAcylation (O-N-acetyl- $\beta$ -D-glucosaminylation), corresponds to an atypical glycosylation since a unique monosaccharide, the N-acetyl-D-glucosamine, is linked on hydroxyl group of serine and threonine amino acids of a protein through a  $\beta$ -linkage<sup>16</sup> (for recent reviews<sup>17–19</sup>). In addition, O-GlcNAcylation is highly dynamic and reversible (such as phosphorylation) because of a unique pair of antagonist enzymes: the OGT or O-GlcNAc transferase, which transfers the monosaccharide onto a protein,<sup>20,21</sup> and the OGA or O-GlcNAcase, which removes it.<sup>22,23</sup> In the overall skeletal muscle cell, over a thousand of O-GlcNAc-modified proteins have been identified since 2004.<sup>24–29</sup> The nature of these proteins is diverse, including contractile, sarcolemmal, structural and cytoskeletal proteins, which are involved in sarcomeric cytoarchitecture; an updated and summarized schematic cartoon of the O-GlcNAcylated skeletal muscle proteome is presented figure 1. The nature of O-GlcNAcylated proteins is even more diverse, including mitochondrial proteins, enzymes, transcription factors and signaling proteins as well. Thus, akin to phosphorylation,<sup>30–33</sup> O-GlcNAcylation could play a significant role in sarcomere regulation as well as in various physiological functions of skeletal muscle. This review discusses the updated involvements of O-GlcNAcylation in the modulation of contractile and structural properties of skeletal muscle, as well as its potential repercussion in a physio-pathological context.

## **O-GLCNACYLATION: A MODULATOR OF CALCIUM ACTIVATION PROPERTIES OF THE SARCOMERE IN SKELETAL MUSCLE**

### **A first story with evidences**

Many O-GlcNAc-modified contractile proteins as well as regulatory proteins of the contraction have been identified within the sarcomere of skeletal muscle including actin, myosins, myosin light chains (the essential MLC or MLC1, and the regulatory MLC or MLC2), and tropomyosins, troponins T and I, respectively (Figure 1). In the view of a large number of contractile and regulatory proteins bearing O-GlcNAc moiety, our lab focused on the involvement of O-GlcNAcylation in skeletal muscle contractility and successfully highlighted O-GlcNAcylation as a modulator of calcium activation properties of the sarcomere using skinned skeletal muscle fibers. Measurement of relative tension developed by the fiber depending on various calcium concentrations (tension/pCa relationship) led to determine four parameters (i) the calcium sensitivity of sarcomere from pCa threshold for fiber tension activation; (ii) the affinity of sarcomere for calcium from pCa<sub>50</sub> for 50% of maximal developed tension; and (iii) the cooperativity between the different regulatory proteins (troponins and tropomyosins) within the thin filaments from the slope of the curve (Hill coefficient  $n_H$ ) (Figure 2).<sup>34</sup> The maximal tension  $P_0$  developed by the fiber, induced by a saturating calcium concentration (pCa 4.2), had been consequently determined as well (Figure 2).<sup>34</sup>

In a first approach, fibers from slow-twitch rat soleus were incubated with N-acetyl-D-glucosamine (GlcNAc) in excess to impair potential O-GlcNAc-dependent protein-protein interactions.<sup>26</sup> Compared to control conditions, T/pCa relationship shifted toward lower pCa values (Figure 2). The pCa threshold and pCa<sub>50</sub> value were significantly reduced, meaning that calcium sensitivity and affinity of sarcomere were decreased respectively. Hill coefficient ( $n_H$ ) and  $P_0$  value were not significantly altered (Figure 2). Interestingly, when GlcNAc was then removed from the solution, the modulated effects were fully reversible and returned to control values.<sup>26</sup> These results were also reproduced on human skeletal muscle fibers, and confirmed the reversible decrease of sarcomere sensitivity and affinity to calcium.<sup>35</sup> Additional experiments were done on rat soleus fibers after an increase of global O-GlcNAc level by using PUGNAc and Thiamet-G, both potent inhibitors of O-

GlcNAcase (OGA).<sup>25</sup> Unlike the excess GlcNAc conditions, T/pCa relationship was shifted to higher pCa values (Figure 2). Indeed, pCa<sub>50</sub> value was significantly increased (but not pCa threshold value) meaning that calcium affinity of sarcomere was increased, whereas Hill coefficient ( $n_H$ ) and P<sub>0</sub> value were not significantly altered (Figure 2).<sup>25</sup> This first story with evidences revealed that calcium activation properties of sarcomere were modulated according to O-GlcNAcylation. However, the exact mechanism(s) involved in the modulation of calcium activation parameters through O-GlcNAcylation is (are) still not well understood and need to be clarified.

In this last study, the regulatory myosin light chain sMLC2 was the only slow fiber isoform presenting a significant modulation and increase of O-GlcNAcylation in slow-twitch skinned rat soleus treated with PUGNAc (the rest of proteins showing a modulation of O-GlcNAcylation belonged to fast isoforms and were then excluded from the study). Thus, sMLC2 was proposed to be one of the candidate proteins which could be involved in the regulation of calcium activation parameters of skeletal muscle fibers through its O-GlcNAcylation (for review<sup>36</sup>).<sup>25</sup>

### **The regulatory myosin light chain (MLC2): a candidate protein involved in this mechanism**

Calcium release from sarcoplasmic reticulum is essential for skeletal muscle to modulate actomyosin interactions and contraction. Calcium binds troponin C that changes the conformational state of tropomyosin and troponin complex to provide free binding sites on actin filaments to myosin motor domain and assists actomyosin interactions (for reviews<sup>4,30</sup>).<sup>37-40</sup> If calcium is essential to trigger the sliding theory of actomyosin filaments, it can also modulate the contraction in another way. Indeed, through the increase of cytoplasmic calcium concentration, four calcium ions bind calmodulin and initiate its interaction with Myosin Light Chain Kinase (MLCK).<sup>41</sup> This Ca<sup>2+</sup>/calmodulin-dependent serine-threonine protein kinase is thus activated, and exposes its catalytic domain to phosphorylate the disordered N-terminal region of sMLC2 at Ser14 (or Ser15 for fMLC2) (for review<sup>14</sup>). The MLC2 non-covalently surrounds the neck domain of myosin and provides it a mechanical support.<sup>42,43</sup> Although non-essential for contraction, phosphorylation of regulatory light chains enhances sarcomere

contractility, especially by increasing the calcium sensitivity.<sup>44,45</sup> This raise of calcium sensitivity is proportional to ATPase activity of actomyosin and suggests that MLC2 phosphorylation enhances the number of actomyosin cross-bridges.<sup>46</sup> More precisely, some studies revealed that addition of negative charge from phosphate leads to position shift of myosin heads through electrostatic repulsive forces between MLC2 and myosin heavy chains. The myosin motor domain is moved toward actin thin filaments axially and proximally which facilitates cross-bridges formation.<sup>47–49</sup> From a kinetic point of view, MLC2 phosphorylation is slower than contraction and would be a biochemical memory to enhance muscle mechanical functions during prolonged or repetitive activity, and modulate muscle fatigue.<sup>14</sup> This molecular mechanism is also reversible through myosin phosphatase target subunit 2 (MYPT2)<sup>50</sup> and protein phosphatase 1 (PP1)<sup>50,51</sup> that recognizes and dephosphorylates MLC2, respectively.

Interestingly, MLC2 has been identified as O-GlcNAcylated in skeletal muscle.<sup>26</sup> While the O-GlcNAcylation site is not precisely localized to date in skeletal muscle, it is mapped in cardiac muscle tissue at Ser15.<sup>52</sup> Interestingly, this potential O-GlcNAcylated site on slow skeletal isoform is located to the only site of phosphorylation at Ser14 on sMLC2.<sup>41,52</sup> Thus, O-GlcNAcylation would play a significant role in calcium activation properties of sarcomere including a close interplay with phosphorylation in skeletal muscle. In this way, an original study on rat soleus showed that phosphorylation and O-GlcNAcylation were mutually exclusive on sMLC2.<sup>53</sup> This fine interplay was reinforced by the interaction of enzymes involved in O-GlcNAcylation and phosphorylation of MLC2, *i.e.* the pairs MLCK/MYPT2/PP1 and OGT/OGA, within a multienzymatic complex around the Z-line of the sarcomere (Figure 3); it was the first report showing that OGT and OGA presented a preferential localization at the Z-line in striated muscle and in less extent to the I-band.<sup>53</sup> To support the key role of this multienzymatic complex in the modulation of O-GlcNAcylation/phosphorylation interplay in the fine regulation of sMLC2 activity, it was demonstrated that the co-localization of MLCK and OGA changed in disuse conditions, in correlation with changes in the level of O-GlcNAcylation and phosphorylation on sMLC2 (this specific point will be discussed later in this review).<sup>53</sup> However, the exact role and molecular mechanism of O-GlcNAcylation on MLC2 is still unknown. Its involvement in the modulation of electrostatic forces between MLC2 and MHC seems unlikely, since O-GlcNAc is

a non-charged moiety. One hypothesis would be that O-GlcNAc moiety causes a steric hindrance between MLC2 and MHC since O-GlcNAc gets its large size due to its Stokes radius which is four to five times larger than a phosphate.<sup>54</sup> Taken together, this data strongly suggests that MLC2 is one of the protein candidates to explain the role of O-GlcNAcylation as a modulator of contractile activity in skeletal muscle.

### **The dynamic of O-GlcNAcylation/phosphorylation interplay on MLC2 varies according to activity pattern of skeletal muscle**

**(i) Impact of exercise on O-GlcNAcylation/phosphorylation of MLC2.** Two recent studies revealed the impact of exercise on the interplay between phosphorylation and O-GlcNAcylation on rat MLC2, considering a treadmill running through a single acute exercise (exercise exhaustion) or a long-term exercise (6-weeks training).<sup>55,56</sup>

After a single acute exercise, whereas the global O-GlcNAcylation level remained unchanged in both slow-twitch soleus and fast-twitch EDL,<sup>56</sup> a fine modulation of O-GlcNAcylation was observed on some specific myofilament proteins.<sup>55</sup> Indeed, in the soleus, the O-GlcNAc level of sMLC2 tended to slightly increase ( $p=0.07$ ), while its phosphorylation significantly decreased.<sup>55</sup> The modulation of sMLC2 phosphorylation and O-GlcNAcylation was reversed and similar to control after 24h recovery.<sup>55</sup> After this acute exercise, expression of MLCK was not altered in the total extract, whereas its expression in the myofilament fraction was decreased (Figure 4). The modulation of sMLC2 and MLCK seemed to be swift since a 100 sec *in situ* exercise protocol (fatiguing shortening contractions) in the soleus showed both a decrease of sMLC2 phosphorylation and MLCK expression in myofilament fraction.<sup>55</sup> It reveals a quick responding system, and might suggest changes of MLCK localization by dissociating the enzyme from the myofilament to reduce the amount of available MLCK near sMLC2. As already described above, MLCK can be localized at the sarcomere within a multienzymatic complex including OGA and OGT (Figure 3).<sup>53</sup> However, after acute exercise, expression of O-GlcNAc processing enzymes OGT/OGA was not altered in the total nor in myofilament fraction, and rather suggested that



enzyme activity could change to finely modulate the O-GlcNAcylation level in the soleus.<sup>55</sup> Regarding EDL, no changes of fMLC2 phosphorylation/O-GlcNAcylation balance or regulating enzyme expressions were observed.<sup>55</sup> This data reinforces the paradigm of a differential modulation of fatigue through MLC2, and *in fine* the calcium sensitivity of sarcomere, between slow and fast-twitch skeletal muscles (Figure 4) (for review<sup>57</sup>).<sup>58,59</sup> The role of O-GlcNAcylation in this mechanism is still unclear and might be complex, since unlike the soleus and EDL, the global O-GlcNAcylation level of fast-twitch white gastrocnemius was significantly increased following a single acute exercise in rat; however post-translational modifications of MLC2 were not investigated (Figure 4).<sup>60</sup> Such complexity was also displayed at resting conditions since O-GlcNAcylation and phosphorylation level of MLC2 were higher in EDL compared with soleus, on slow as well as on fast isoforms; there was also a discrepancy between the two type of muscles in the expression of proteins involved in both post-translational modifications.<sup>56</sup>

Unlike a single acute exercise, a long-term training program on rat led to an increase of global O-GlcNAcylation level in the total extract of soleus and EDL, but remained unaltered in the myofilament fraction (Figure 4).<sup>56</sup> In the soleus, phosphorylation of sMLC2 decreased while its O-GlcNAcylation was not modified; the fMLC2 post-translational modifications in EDL were not provided. Finally, in the soleus, expression of MLCK was decreased in the myofilament fraction and in the total extract as well (Figure 4).<sup>55</sup> Contrary to the acute exercise and a potential relocalization of MLCK, the involvement of transcriptional mechanisms was also suggested for the regulation of MLCK expression in a long-term exercise. Interestingly, OGA expression was decreased in the total extract but not in the myofilament fraction, which could explain the increase of global O-GlcNAc level in soleus. In contrast, expression of the regulating enzymes was not altered in the EDL (Figure 4).<sup>55</sup>

Thus, these O-GlcNAc adaptations following skeletal muscle activity seemed to be fully different according to exercise protocol as well as skeletal muscle fiber type. Using the “MLC2 case”, first concepts can be assumed since a single acute exercise might involve local short-term O-GlcNAc-related adaptations on localization and protein activity. In another way, a long-term exercise might involve additional global long-term O-GlcNAc-related adaptations on gene expressions (Figure 4). The differences observed between muscle types might also be partly explained by a differential metabolism

linked to O-GlcNAcylation. Indeed, the modulation of the O-GlcNAc pattern and the O-GlcNAc processing enzymes is also completely different between both of these muscle fiber type at resting conditions.<sup>55,61</sup> It is well known that the metabolism and stress response between fast-twitch glycolytic muscles and slow-twitch oxidative muscles are different; this could suggest a differential modulation of the O-GlcNAcylation process with differential consequences on cellular functions during basal and exercise conditions (for recent review<sup>62</sup>).

**(ii) Impact of disuse on O-GlcNAcylation/phosphorylation of MLC2.** In a model of disuse atrophy (rat model of hindlimb unloading (HU)), it was shown that global O-GlcNAcylation level decreased in the soleus (whereas it increased in EDL),<sup>61</sup> while specific variations were investigated on proteins of interest, in particular MLC2. Following 14-days HU, sMLC2 O-GlcNAcylation decreased while its phosphorylation increased (Figure 5).<sup>53</sup> This modulation of these post-translational modifications was totally reversed and returned to control values after 14-days reloading.<sup>53</sup> Interestingly, both post-translational modifications were mutually exclusive on sMLC2, and the multienzymatic complex involved in O-GlcNAc and phospho-MLC2 modifications, seemed to be partially reorganized at the Z-line where the colocalization between the MLCK and OGA was increased (Figure 3).<sup>53</sup> Interestingly, in the cardiac tissue of diabetic STZ-rats and diabetic human patient where global O-GlcNAcylation level is increased and sarcomere calcium sensitivity is decreased, OGT and OGA localization pattern appeared to be redistributed along the sarcomere. This observation was associated with an increase of OGA interactions with  $\alpha$ -actin,  $\alpha$ -tropomyosin, and MLC1, whereas its interaction with OGT was unaltered.<sup>63</sup> In the context of skeletal muscle disuse, we can likely assume that potential fine relocalizations and changes of interactions might happen between enzymes regulating O-GlcNAc- and phospho-MLC2 modifications (Figure 3). A second level of regulation showed that OGT and OGA activities were respectively increased and decreased in atrophied soleus following HU period (Figure 5).<sup>61</sup> A potential redistribution of enzymes within the sarcomere associated to a change of activity might result to a change of O-GlcNAcylation/phosphorylation stoichiometry in some compartments within the sarcomere, and explain the fine regulation of PTMs of some key myofilament proteins, including MLC2.

Such further investigations could reinforce the close interplay concept between phosphorylation and O-GlcNAcylation to modulate MLC2 functions, as well as contractile properties in atrophied soleus regardless of muscle mass loss.

In human, sMLC2 was also altered following 60-days Bed-Rest.<sup>64</sup> Indeed, MLC2 O-GlcNAcylation decreased while its phosphorylation increased (Figure 5),<sup>64</sup> which was associated to impairment of sarcomeric calcium properties.<sup>65</sup> These changes were accompanied by slow-to-fast transitions in Myosin Heavy and Light chains, as well as troponin C and T.<sup>64</sup> The modulation of muscle phenotype and MLC2 modifications was fully reversed following a resistance training program and aerobic exercises during Bed-Rest while branched chain amino acids supplementation was not successful to counteract the atrophy phenotype nor the MLC2 modifications (Figure 5).<sup>64</sup> Unfortunately, the modification of O-GlcNAcylation and phosphorylation pattern on MLC2 were not investigated in human EDL following Bed-Rest. It would have been interesting to compare the molecular response of the EDL, since the modulation after exercise seems to be dependent of muscle phenotype.

It is worth noting that several data suggested that phosphorylation of MLC2 was closely associated to increase of calcium sensitivity of muscle fibers.<sup>14,44,45</sup> However, disuse and aging are closely associated to decrease of calcium sensitivity<sup>66</sup> whereas MLC2 phosphorylation increases,<sup>67,68</sup> thus, the decrease of calcium sensitivity during HU might not only result from variation of phosphorylation. While it was demonstrated that O-GlcNAcylation could be involved in the modulation of calcium activation parameters in skeletal muscle,<sup>25,26,35</sup> potentially *via* the regulatory Myosin Light Chain MLC2, it is reasonable to hypothesize that MLC2's O-GlcNAcylation changes could be associated to changes in calcium sensitivity observed during functional atrophy. As support to this hypothesis, decrease of sMLC2 O-GlcNAcylation was observed in rat HU and human Bed-Rest, which may explain the decrease of calcium sensitivity throughout functional atrophy of soleus. From this view, changes of O-GlcNAcylation of sMLC2 seem to be associated with contractile dysfunction whereas phosphorylation changes associated to muscle plasticity could be rather associated with phenotypic changes. More studies need to be done to precisely define the role the O-GlcNAcylation/phosphorylation balance of MLC2 and how it works on skeletal contractility as well as on muscle phenotype changes.

Indeed, decrease of MLC2 O-GlcNAcylation has been shown to be associated with slow-to-fast transitions in human soleus after Bed-Rest as previously described. It has also been shown that a global increase of O-GlcNAcylation in skeletal muscle occurs during aging,<sup>69</sup> associated to sarcopenia and slow-to-fast changes; however, O-GlcNAcylation of MLC2 has not been studied. From this second view, it would be worth to investigate this context, regardless of phosphorylation.

### **Other candidate proteins to explain the modulation of the calcium properties of skeletal muscle through O-GlcNAcylation?**

Although MLC2 could explain the modulation of calcium activation properties by O-GlcNAcylation not only in skeletal muscle but also in cardiac muscle,<sup>52</sup> the involvement of other contractile or regulatory proteins cannot be excluded.

For instance, troponins I and T have been identified as being O-GlcNAcyated in skeletal and cardiac muscle.<sup>25,52</sup> Phosphorylation on Ser23 and 24 of troponin I through PKA (Protein Kinase A) is known to decrease the calcium sensitivity in cardiac cells;<sup>70</sup> however, cardiac skinned fibers exposed to free GlcNAc in excess also showed a decrease in calcium sensitivity without affecting phosphorylation on Ser23/24 of troponin I.<sup>52</sup> Moreover, in myectomy samples from patients affected by hypertrophic obstructive cardiomyopathy due to troponin T abnormality, level of TnI Ser23/24 phosphorylation was decreased and not associated to any alteration of calcium sensitivity.<sup>71</sup> In another way, troponin I is also described to be O-GlcNAcyated or phosphorylated on Ser150 through PAK3 (Serine/Threonine-protein kinase PAK3); the phosphorylation at that site increased the calcium sensitivity,<sup>72,73</sup> but the role of O-GlcNAc moiety on this amino acid residue has not been yet investigated.

In another study, a dynamism between O-GlcNAcylation and phosphorylation was recently described on cardiac troponin T from ischemic heart cells where contractile properties are altered.<sup>74</sup> However, in our model of slow fibers exposed to OGA inhibitors,<sup>25</sup> even if fast isoforms TnT and TnI showed an increase of their O-GlcNAcylation level, they are not expressed in slow-twitch soleus; so, TnT O-GlcNAcylation could not explain the modulation of the calcium activation properties of the sarcomere

in this context.<sup>25</sup> It is worth noting that many sarcomeric proteins, including troponins, contain intrinsically disordered regions,<sup>75,76</sup> that might serve as crucial regulators for different cellular mechanisms.<sup>77</sup> O-GlcNAcylation preferentially occurs in secondary structures such as loop and disorganized regions instead of  $\alpha$ -helix or  $\beta$ -helix peptides.<sup>78</sup> Thus, more O-GlcNAc sites might be expected in this class of proteins, such as troponins or others, known to be involved in calcium properties of sarcomere. In STZ-rat heart, MHC,  $\alpha$ -sarcomeric actin and  $\alpha$ -tropomyosin also displayed an increase of O-GlcNAcylation on some identified sites.<sup>63</sup> Although the phenotype was associated with a decrease of sarcomere calcium sensitivity, these specific O-GlcNAc changes have not yet been investigated related to calcium sensitivity. To elucidate the role of O-GlcNAcylation at local level, precise O-GlcNAc mapping site identification on these proteins of interest is required. Thus, sMLC2 is to date the only actor described in the skeletal muscle that could modulate the calcium activation properties of sarcomere through O-GlcNAcylation. However, its exact molecular mechanism, as well as its close interplay with phosphorylation, especially through the action and the regulation of multienzymatic complex within the sarcomere Z-line, needs to be investigated in further studies. Moreover, most of the contractile and regulatory proteins previously cited are involved in the length-dependent activation of sarcomere. Since the global O-GlcNAcylation level impacts the morphometry of sarcomere (*i.e.* sarcomere length discussed later in this review) in skeletal muscle cells,<sup>28</sup> the potential effect of O-GlcNAcylation in this mechanism associated to calcium properties of sarcomere should be investigated to bring new insights in the regulation of striated muscle contractility.

## **EMERGENCE OF A NEW ROLE OF O-GLCNACYLATION IN THE SARCOMERE STRUCTURE OF SKELETAL MUSCLE**

It is well known that skeletal muscle, characterized by its striated appearance, has an incredible architecture, essential for muscle functions including contraction. This structure is due to the accurate and fine organization of actin and myosin myofilaments within the sarcomere (for reviews<sup>2,3,79,80</sup>). Although highly dynamic,<sup>8</sup> this sarcomeric organization is maintained through multiple protein-protein interactions within an intricate sarcomeric cytoskeleton network, including structural proteins (for

reviews<sup>2,3,81</sup>). In particular, the Z-line<sup>82,83</sup> and M-band<sup>84</sup> are described as nodal points and hotspots for several dynamic interactions between different types of proteins (Figure 1). Some studies revealed that post-translational modifications such as phosphorylation can modulate protein-protein interactions known to be crucial for the sarcomere structure. Indeed, telethonin phosphorylation on its C-terminal domain seems to be required for sarcomere assembly.<sup>9</sup> Moreover, other studies reported that phosphorylation plays a significant role in titin-myomesin<sup>10</sup> and ZASP-myotilin interactions.<sup>85</sup> Recent mass spectrometry analysis revealed that the Z-line of striated muscle is a hotspot for protein phosphorylation<sup>33</sup> and O-GlcNAcylation as well.<sup>29</sup> Indeed, O-GlcNAcylation also modifies many key structural proteins of the sarcomere, such as  $\alpha$ -actinin,  $\alpha$ B-crystallin, BAG3 (BCL2 Associated Athanogene 3), desmin, filamin-C, myomesin, myopalladin, plectin, titin, ZASP (Z-band Alternatively Spliced PDZ-motif), and so on (Figure 1).

In a recent study, we focused on the role of O-GlcNAcylation in sarcomere structure. Using Thiamet-G to inhibit O-GlcNAcase and increase the global O-GlcNAcylation level in C2C12 myotubes, the main data of this study was a linear modulation of the sarcomeric morphometry according to myofilament O-GlcNAc rate.<sup>28</sup> Indeed, the dark band and M-band widths increased, while the I-band width and the sarcomere length decreased according to myofilament O-GlcNAc level. Morphometry parameters measurement is one of the strongest parameters of the sarcomere structure; thus, changes of sarcomeric morphometry after O-GlcNAcylation modulations led to the conclusion that O-GlcNAcylation was a key modulator of the sarcomeric structure. Interestingly, protein-protein interactions within some protein complexes were modulated in parallel of the morphometry changes, including some key structural proteins of the sarcomere,  $\alpha$ -actinin,  $\alpha$ B-crystallin, desmin and filamin-C.<sup>28</sup> However, the direct link between the sarcomeric morphometry and the modulation of protein-protein interactions through O-GlcNAcylation was not clearly established and needs to be clarified.

Interestingly, it was demonstrated that the interaction between desmin and its molecular chaperone  $\alpha$ B-crystallin, was significantly changed according to global O-GlcNAcylation level.<sup>28</sup> Desmin is the major intermediate filament protein in skeletal muscle, crucial for structural integrity of sarcomere and the cell;<sup>86</sup> thus, a desmin knock-out in mice leads to sarcomeric structure impairments

with a misalignment of Z-lines.<sup>87</sup> Moreover, desmin is a hotspot for many post-translational modifications,<sup>88</sup> in particular O-GlcNAcylation, phosphorylation and ubiquitination. Interestingly, in addition to being O-GlcNAcylated, desmin has lectin-like properties<sup>89</sup> and a broad interactome in skeletal muscle cells.<sup>90</sup> While phosphorylation is known to modulate desmin polymerization,<sup>91,92</sup> O-GlcNAcylation could be also involved in the polymerization state of desmin since O-GlcNAcylation modulates cytokeratins filaments 8/18<sup>93</sup> and tubulin polymerization.<sup>94</sup> Recently, an O-GlcNAc site has been located on the Ser459 of desmin in murine skeletal muscle cells.<sup>29</sup> SW13 cells transfected with S459I mutant desmin show shorter desmin filaments and desmin aggregations in the cytoplasm.<sup>95</sup> Indeed, this amino-acid residue is located in the carboxy-terminal ‘tail’ domain of desmin, which controls lateral packing, thus the diameter of intermediate filaments,<sup>96,97</sup> as well as longitudinal head-to-tail tetramer assembly.<sup>96,98</sup> This domain also seemed to be involved in lateral interaction with other cytoskeletal proteins.<sup>98,99</sup> The second actor,  $\alpha$ B-crystallin, is known to be the molecular chaperone of desmin, playing a role in the localization and aggregation of desmin filaments<sup>100</sup> as well as in its assembly.<sup>101</sup> Activity of  $\alpha$ B-crystallin was first known to be regulated by phosphorylation,<sup>102,103</sup> while its O-GlcNAcylation on Thr170 seemed regulate its localization.<sup>104</sup> Recently, the Thr162 residue on  $\alpha$ B-crystallin was identified to be O-GlcNAcylated as well;<sup>29</sup> this residue is located on its C-terminal domain known to directly interact with desmin.<sup>105</sup> The deletion of amino acid residues from position 155 to 165 on  $\alpha$ B-crystallin leads to the increase of its cosedimentation with desmin and an improvement of filament-filament interactions.<sup>105</sup> Interestingly, repercussion of  $\alpha$ B-crystallin mutation, *i.e.* R120G, has been described in a pathological context showing desmin aggregation features.<sup>106,107</sup> However, the exact role of Thr162 residue on  $\alpha$ B-crystallin, as well as its O-GlcNAcylation have not been yet investigated. Altogether, this data suggests that the involvement of  $\alpha$ B-crystallin and desmin cannot be excluded to explain the modulation of the sarcomere structure by protein-protein interactions changes through O-GlcNAcylation.

Other skeletal muscle proteins should not be excluded as well, and many investigations about the role of O-GlcNAcylation in the modulation of protein-protein interactions must be pursued since several O-GlcNAc sites have been identified in interaction domains of several protein of interest. Indeed,

O-GlcNAc sites were found on MHC very close to its polymerization domain and its interaction domains with myomesin and titin.<sup>27</sup> It is worth noting that the O-GlcNAc site on MHC, which corresponds to the Ser1708, is adjacent to Leu1706 residue known to be mutated (L1706P) in Laing early onset distal myopathy.<sup>27</sup> Moreover, several O-GlcNAc sites were also identified on Kelch-12 domain in titin from mouse, corresponding to the Immunoglobulin-like domain 123 in human.<sup>29</sup> Titin is essential to sarcomere organization; a decrease of its expression was associated to changes of sarcomere structure, closely linked to a decrease of muscle performance in an hindlimb-unloading rat model.<sup>108</sup> Interestingly, the O-GlcNAc site is located onto a crucial region for myosin polymerization and sarcomere assembly since it interacts with MHC and MyBP-C.<sup>5</sup> Also, some O-GlcNAc sites have been identified into the PxxP domain of BAG3, and in the plakin domain repeat B5 of plectin known to interact with SH3-containing proteins and intermediate filament proteins respectively.<sup>29</sup>

Recently, it has been shown that O-GlcNAcylation could be involved in neuromuscular diseases.<sup>109</sup> In particular, it was demonstrated on human skeletal muscle biopsies that the O-GlcNAcylation signal seemed to be relocalized within fibers from patients suffering from muscular dystrophies, rhabdomyolysis, myositis, distal myopathies with rimmed vacuoles, sporadic inclusion body myositis and neurogenic muscular dystrophy. The O-GlcNAcylation involvement in the physiopathology of muscular disorders is also supported by the mapping of O-GlcNAc sites, as previously mentioned for Laing myopathy. In addition, an O-GlcNAcylation site was also mapped on Ser459 of desmin.<sup>29</sup> This site corresponds to Ser460 in human, known to be mutated in desminopathy.<sup>95</sup> Interestingly, SW13 cells transfected with desmin S460I mutant exhibited short desmin filaments, with irregular diameter and prominent aggregations randomly distributed in the cytoplasm, although C2C12 transfected cells did not seem to show abnormal desmin organization.<sup>95</sup> Moreover, patients with S460I desmin mutant displayed large autophagic vacuoles in muscle fibers.<sup>95</sup> Desminopathies are classified as myofibrillar myopathies and are characterized by a myofibrillar disorganization, especially from the Z-disk, an alteration of protein-protein interactions, many aggregations of myofibrillar products degradation and an ectopic expression of some proteins<sup>110</sup> leading to skeletal muscle and cardiac impairments. It is important to note that a modulation of the O-GlcNAc moiety could be involved in this



pathogenicity. In addition, other genes are known to cause myofibrillar myopathies, such as genes coding for  $\alpha$ B-crystallin, myotilin, ZASP, filamin C, BAG3, FHL1 (Four and a Half LIM domains protein 1) or plectin among others.<sup>110</sup> Although some of these proteins are known to be O-GlcNAc modified,<sup>29</sup> the role of some O-GlcNAc sites of interest have to be considered in the context of skeletal muscle diseases in the future.

## SUMMARY AND PERSPECTIVES

More and more studies strongly support the meaningful roles of O-GlcNAcylation in the skeletal muscle physiology, in particular the modulation of muscle contraction through calcium activation parameters, and the sarcomere structure among others cellular processes which were not considered in this review, such as cellular stress, muscle metabolism or myogenesis (for recent review<sup>62</sup>).

In this review, we focused on contractile and structural proteins of the sarcomere. However, from our recent study, many O-GlcNAcylated proteins of skeletal muscle cells (*i.e.* signaling proteins, transcription factors, epigenetic regulators) are also located in other compartments (*i.e.* nucleus, mitochondria), or are intended to go to these areas and might have a significant impact on the physiology of skeletal muscle.<sup>29</sup> For example, a conditional skeletal muscle OGT knock-out mouse model has been recently made and did not change the force production of the muscle but altered its tissue composition, glucose metabolism, and production of interleukin-15. The mice were less active and had higher whole-body expenditure. In skeletal muscle, O-GlcNAcylation of the histone-lysine-N-methyltransferase Enhancer of Zeste Homolog 2 (EZH2) represses the expression of *IL-15*.<sup>111</sup> Thus, it will be interesting to see the impact of conditional and specific knock-out of different OGT and OGA isoforms in the skeletal muscle physiology. Moreover, proteins known to have a primary role in the sarcomere and regulation of contraction might also be located to other compartments. For example, troponin T3 (full-length and fragmented forms from calpain 3 cleavage) can localize to the nuclei of skeletal muscle as well, closely associated with RNA polymerase activity and nucleolar regions regulating apoptosis and functioning as a transcription factors of genes involved in excitation-contraction coupling.<sup>112,113</sup> This

phenomenon has been shown to be associated with sarcopenia.<sup>112,113</sup> Interestingly, troponin T is known to be O-GlcNAcylated in skeletal muscle, but its function has not been yet investigated in this context.

From this review, O-GlcNAcylation seems to exert a key role in the sarcomeric cytoskeleton equilibrium; however, it is well established that the sarcomere is not only fundamental for the generation of force but is now considered as a nodal point for transduction involved in diseases and a target for pharmacological intervention. Therefore, it would be essential to better understand the role of O-GlcNAcylation in muscle function, including the organization and the reorganization of sarcomeric cytoskeleton, among other mechanisms, and to clarify its involvement in different pathologies. Indeed, the fine characterization of the impact of O-GlcNAcylation in skeletal muscle will allow the emergence of new therapeutics to limit or reverse the muscle dysfunction closely associated to muscular or neuromuscular diseases, but also resulting from other pathologies (such as diabetes, heart failure or cancer) or physiological changes (such as aging for example).

What we described in this review might only be the “tip of the iceberg” regarding the role of O-GlcNAcylation in the physio(patho)logy of skeletal muscle. Whether it’s from global studies interested in O-GlcNAc variations or recent targeted studies focused on identifying O-GlcNAc proteins and mapping O-GlcNAc sites utilizing inventive biochemistry and mass spectrometry techniques, there has been great progress in characterizing O-GlcNAcylation’s role in skeletal muscle physiology. In addition to computational prediction of O-GlcNAc sites (for available platforms<sup>114</sup>), precise O-GlcNAc sites identification studies need to be pursued in the near future. This would be one of the keys to understand O-GlcNAcylation role on a protein of interest and then within the whole cell, especially in the context of crosstalk with other post-translational modifications, such as phosphorylation or ubiquitination; most of O-GlcNAc residues already identified have not yet established a role in the O-GlcNAc-mediated skeletal physiology.

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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## REFERENCES

1. Clark KA, McElhinny AS, Beckerle MC, Gregorio CC. Striated muscle cytoarchitecture: an intricate web of form and function. *Annu Rev Cell Dev Biol.* 2002;18:637-706.
2. Gautel M. The sarcomeric cytoskeleton: Who picks up the strain? *Curr Opin Cell Biol.* 2011;23(1):39-46.
3. Gautel M, Djinovic-Carugo K. The sarcomeric cytoskeleton: from molecules to motion. *J Exp Biol.* 2016;219(2):135-145.
4. Gordon A, Homsher E, Regnier M. Regulation of Contraction in Striated Muscle. *Physiol Rev.* 2000;80(2):853-924.
5. Kontrogianni-konstantopoulos A, Ackermann MA, Bowman AL. Muscle Giants : Molecular Scaffolds in Sarcomerogenesis. 2009;89(4):1217-1267.
6. Boonyarom O, Inui K. Atrophy and hypertrophy of skeletal muscles: Structural and functional aspects. *Acta Physiol.* 2006;188(2):77-89.
7. Henderson CA, Gomez CG, Novak SM, Mi-Mi L, Gregorio CC. Overview of the Muscle Cytoskeleton. *Compr Physiol.* 2017;7(3):891-944.
8. Sanger JW, Wang J, Fan Y, White J, Sanger JM. Assembly and dynamics of myofibrils. *J Biomed Biotechnol.* 2010;2010:858606.
9. Sadikot T, Hammond CR, Ferrari MB. Distinct roles for telethonin N-versus C-terminus in sarcomere assembly and maintenance. *Dev Dyn.* 2010;239(4):1124-1135.
10. Obermann WM, Gautel M, Weber K, Fürst DO. Molecular structure of the sarcomeric M band: mapping of titin and myosin binding domains in myomesin and the identification of a potential regulatory phosphorylation site in myomesin. *EMBO J.* 1997;16(2):211-220.
11. Tskhovrebova L, Trinick J. Titin: properties and family relationships. *Nat Rev Mol Cell Biol.* 2003;4(9):679-689.

12. Huang X, Li J, Foster D, et al. Protein Kinase C-Mediated Desmin Phosphorylation is Related to Myofibril Disarray in Cardiomyopathic Hamster Heart 1. *Exp Biol Med.* 2002;227(11):1039-1046.
13. Ackermann MA, Kontogianni-Konstantopoulos A. Myosin Binding Protein-C Slow is a Novel Substrate for Protein Kinase A (PKA) and C (PKC) in Skeletal Muscle. *J Proteome Res.* 2011;10(10):4547-4555.
14. Stull JT, Kamm KE, Vandenboom R. Myosin light chain kinase and the role of myosin light chain phosphorylation in skeletal muscle. *Arch Biochem Biophys.* 2011;510(2):120-128.
15. Vandenboom R. Modulation of Skeletal Muscle Contraction by Myosin Phosphorylation. *Compr Physiol.* 2016;7(1):171-212.
16. Torres CR, Hart GW. Topography and polypeptide distribution of terminal N-acetylglucosamine residues on the surfaces of intact lymphocytes. Evidence for O-linked GlcNAc. *J Biol Chem.* 1984;259(5):3308-3317.
17. Bond MR, Hanover JA. A little sugar goes a long way: The cell biology of O-GlcNAc. *J Cell Biol.* 2015;208(7):869-880.
18. Banerjee PS, Lagerlöf O, Hart GW. Roles of O-GlcNAc in chronic diseases of aging. *Mol Aspects Med.* 2016;51:1-15.
19. Yang X, Qian K. Protein O-GlcNAcylation: emerging mechanisms and functions. *Nat Rev Mol Cell Biol.* 2017;18(7):452-465.
20. Haltiwanger RS, Holt GD, Hart GW. Enzymatic addition of O-GlcNAc to nuclear and cytoplasmic proteins. Identification of a uridine diphospho-N-acetylglucosamine:peptide beta-N-acetylglucosaminyltransferase. *J Biol Chem.* 1990;265(5):2563-2568.
21. Haltiwanger RS, Blomberg MA, Hart GW. Glycosylation of nuclear and cytoplasmic proteins. Purification and characterization of a uridine diphospho-N-acetylglucosamine:polypeptide beta-N-acetylglucosaminyltransferase. *J Biol Chem.* 1992;267(13):9005-9013.

22. Dong DL, Hart GW. Purification and characterization of an O-GlcNAc selective N-acetyl-beta-D-glucosaminidase from rat spleen cytosol. *J Biol Chem*. 1994;269(30):19321-19330.
23. Gao Y, Wells L, Comer FI, Parker GJ, Hart GW. Dynamic O -Glycosylation of Nuclear and Cytosolic Proteins. *J Biol Chem*. 2001;276(13):9838-9845.
24. Cieniewski-Bernard C, Bastide B, Lefebvre T, Lemoine J, Mounier Y, Michalski J-C. Identification of O-linked N-acetylglucosamine proteins in rat skeletal muscle using two-dimensional gel electrophoresis and mass spectrometry. *Mol Cell Proteomics*. 2004;3(6):577-585.
25. Cieniewski-Bernard C, Montel V, Berthoin S, Bastide B. Increasing O-GlcNAcylation level on organ culture of soleus modulates the calcium activation parameters of muscle fibers. *PLoS One*. 2012;7(10):e48218.
26. Hedou J, Cieniewski-Bernard C, Leroy Y, Michalski J-C, Mounier Y, Bastide B. O-linked N-acetylglucosaminylation is involved in the Ca<sup>2+</sup> activation properties of rat skeletal muscle. *J Biol Chem*. 2007;282(14):10360-10369.
27. Hédou J, Bastide B, Page A, Michalski J-C, Morelle W. Mapping of O-linked beta-N-acetylglucosamine modification sites in key contractile proteins of rat skeletal muscle. *Proteomics*. 2009;9(8):2139-2148.
28. Lambert M, Richard E, Duban-Deweere S, et al. O-GlcNAcylation is a key modulator of skeletal muscle sarcomeric morphometry associated to modulation of protein–protein interactions. *Biochim Biophys Acta - Gen Subj*. 2016;1860(9):2017-2030.
29. Deracinois B, Camoin L, Lambert M, et al. O-GlcNAcylation site mapping by (azide-alkyne) click chemistry and mass spectrometry following intensive fractionation of skeletal muscle cells proteins. *J Proteomics*. 2018;186:83-97.
30. Frontera WR, Ochala J. Skeletal muscle: a brief review of structure and function. *Calcif Tissue Int*. 2015;96(3):183-195.

31. Kooij V, Stienen GJM, van der Velden J. The role of protein kinase C-mediated phosphorylation of sarcomeric proteins in the heart—detrimental or beneficial? *Biophys Rev.* 2011;3(3):107-117.
32. Mounier R, Théret M, Lantier L, Foretz M, Viollet B. Expanding roles for AMPK in skeletal muscle plasticity. *Trends Endocrinol Metab.* 2015;26(6):275-286.
33. Reimann L, Wiese H, Leber Y, et al. Myofibrillar Z-discs Are a Protein Phosphorylation Hot Spot with Protein Kinase C (PKC $\alpha$ ) Modulating Protein Dynamics. *Mol Cell Proteomics.* 2017;16(3):346-367.
34. Kischel P, Bastide B, Potter JD, Mounier Y. The role of the Ca(2+) regulatory sites of skeletal troponin C in modulating muscle fibre reactivity to the Ca(2+) sensitizer bepridil. *Br J Pharmacol.* 2000;131(7):1496-1502.
35. Cieniewski-Bernard C, Montel V, Stevens L, Bastide B. O-GlcNAcylation, an original modulator of contractile activity in striated muscle. *J Muscle Res Cell Motil.* 2009;30(7-8):281-287.
36. Cieniewski-Bernard C, Lambert M, Dupont E, Montel V, Stevens L, Bastide B. O-GlcNAcylation, contractile protein modifications and calcium affinity in skeletal muscle. *Front Physiol.* 2014;5:1-7.
37. Huxley AF, Niedergerke R. Structural Changes in Muscle During Contraction: Interference Microscopy of Living Muscle Fibres. *Nature.* 1954;173(4412):971-973.
38. Huxley H, Hanson J. Changes in the Cross-Striations of Muscle during Contraction and Stretch and their Structural Interpretation. *Nature.* 1954;173(4412):973-976.
39. Herzberg O, Moulton J, James MN. Calcium binding to skeletal muscle troponin C and the regulation of muscle contraction. *Ciba Found Symp.* 1986;122:120-144.
40. Lehman W, Craig R, Vibert P. Ca<sup>2+</sup>-induced tropomyosin movement in Limulus thin filaments revealed by three-dimensional reconstruction. *Nature.* 1994;368(6466):65-67.

41. Blumenthal DK, Stull JT. Activation of skeletal muscle myosin light chain kinase by calcium(2+) and calmodulin. *Biochemistry*. 1980;19(24):5608-5614.
42. Lowey S, Trybus KM. Common structural motifs for the regulation of divergent class II myosins. *J Biol Chem*. 2010;285(22):16403-16407.
43. Greenberg MJ, Mealy TR, Watt JD, Jones M, Szczesna-Cordary D, Moore JR. The molecular effects of skeletal muscle myosin regulatory light chain phosphorylation. *Am J Physiol Regul Integr Comp Physiol*. 2009;297(2):R265-74.
44. Persechini A, Stull JT, Cooke R. The effect of myosin phosphorylation on the contractile properties of skinned rabbit skeletal muscle fibers. *J Biol Chem*. 1985;260(13):7951-7954.
45. Szczesna D, Zhao J, Jones M, Zhi G, Stull J, Potter JD. Phosphorylation of the regulatory light chains of myosin affects Ca<sup>2+</sup> sensitivity of skeletal muscle contraction. *J Appl Physiol*. 2002;92(4):1661-1670.
46. Sweeney HL, Stull JT. Alteration of cross-bridge kinetics by myosin light chain phosphorylation in rabbit skeletal muscle: implications for regulation of actin-myosin interaction. *Proc Natl Acad Sci U S A*. 1990;87(1):414-418.
47. Padrón R, Panté N, Sosa H, Kendrick-Jones J. X-ray diffraction study of the structural changes accompanying phosphorylation of tarantula muscle. *J Muscle Res Cell Motil*. 1991;12(3):235-241.
48. Levine RJ, Kensler RW, Yang Z, Stull JT, Sweeney HL. Myosin light chain phosphorylation affects the structure of rabbit skeletal muscle thick filaments. *Biophys J*. 1996;71(2):898-907.
49. Levine RJ, Yang Z, Epstein ND, Fananapazir L, Stull JT, Sweeney HL. Structural and functional responses of mammalian thick filaments to alterations in myosin regulatory light chains. *J Struct Biol*. 1998;122(1-2):149-161.
50. Ito M, Nakano T, Erdodi F, Hartshorne DJ. Myosin phosphatase: structure, regulation and function. *Mol Cell Biochem*. 2004;259(1-2):197-209.



51. Hartshorne DJ, Ito M, Erdödi F. Role of protein phosphatase type 1 in contractile functions: myosin phosphatase. *J Biol Chem.* 2004;279(36):37211-37214.
52. Ramirez-Correa GA, Jin W, Wang Z, et al. O-linked GlcNAc modification of cardiac myofilament proteins: A novel regulator of myocardial contractile function. *Circ Res.* 2008;103(12):1354-1358.
53. Cieniewski-Bernard C, Dupont E, Richard E, Bastide B. Phospho-GlcNAc modulation of slow MLC2 during soleus atrophy through a multienzymatic and sarcomeric complex. *Pflügers Arch - Eur J Physiol.* 2014;466(11):2139-2151.
54. Hart GW, Slawson C, Ramirez-Correa G, Lagerlof O. Cross talk between O-GlcNAcylation and phosphorylation: roles in signaling, transcription, and chronic disease. *Annu Rev Biochem.* 2011;80:825-858.
55. Hortemo KH, Aronsen JM, Lunde IG, Sjaastad I, Lunde PK, Sejersted OM. Exhausting treadmill running causes dephosphorylation of sMLC2 and reduced level of myofilament MLCK2 in slow twitch rat soleus muscle. *Physiol Rep.* 2015;3(2):e12285-e12285.
56. Hortemo KH, Lunde PK, Anonsen JH, et al. Exercise training increases protein O-GlcNAcylation in rat skeletal muscle. *Physiol Rep.* 2016;4(18):e12896.
57. Vandenboom R, Gittings W, Smith IC, Grange RW, Stull JT. Myosin phosphorylation and force potentiation in skeletal muscle: evidence from animal models. *J Muscle Res Cell Motil.* 2013;34(5-6):317-332.
58. Munkvik M, Lunde PK, Sejersted OM. Causes of fatigue in slow-twitch rat skeletal muscle during dynamic activity. *Am J Physiol Regul Integr Comp Physiol.* 2009;297(3):R900-10.
59. Hortemo KH, Munkvik M, Lunde PK, Sejersted OM. Multiple Causes of Fatigue during Shortening Contractions in Rat Slow Twitch Skeletal Muscle. Lionetti V, ed. *PLoS One.* 2013;8(8):e71700.
60. Peternelj TT, Marsh SA, Strobel NA, et al. Glutathione depletion and acute exercise increase

- O-GlcNAc protein modification in rat skeletal muscle. *Mol Cell Biochem.* 2015;400(1-2):265-275.
61. Cieniewski-Bernard C, Mounier Y, Michalski J-C, Bastide B. O-GlcNAc level variations are associated with the development of skeletal muscle atrophy. *J Appl Physiol.* 2006;100(5):1499-1505.
  62. Lambert M, Bastide B, Cieniewski-Bernard C. Involvement of O-GlcNAcylation in the Skeletal Muscle Physiology and Physiopathology: Focus on Muscle Metabolism. *Front Endocrinol (Lausanne).* 2018;9:578.
  63. Ramirez-Correa GA, MA J, Slawson C, et al. Removal of Abnormal Myofilament O-GlcNAcylation Restores Ca<sup>2+</sup> Sensitivity in Diabetic Cardiac Muscle. *Diabetes.* 2015;64(10):3573-3587.
  64. Stevens L, Bastide B, Hedou J, et al. Potential regulation of human muscle plasticity by MLC2 post-translational modifications during bed rest and countermeasures. *Arch Biochem Biophys.* 2013;540(1-2):125-132.
  65. Mounier Y, Tiffreau V, Montel V, Bastide B, Stevens L. Phenotypical transitions and Ca<sup>2+</sup> activation properties in human muscle fibers: effects of a 60-day bed rest and countermeasures. *J Appl Physiol.* 2009;106(4):1086-1099.
  66. Ricart-Firinga C, Stevens L, Canu MH, Nemirovskaya TL, Mounier Y. Effects of beta(2)-agonist clenbuterol on biochemical and contractile properties of unloaded soleus fibers of rat. *Am J Physiol Cell Physiol.* 2000;278(3):C582-8.
  67. Bozzo C, Stevens L, Toniolo L, Mounier Y, Reggiani C. Increased phosphorylation of myosin light chain associated with slow-to-fast transition in rat soleus. *Am J Physiol Physiol.* 2003;285(3):C575-C583.
  68. Gannon J, Doran P, Kirwan A, Ohlendieck K. Drastic increase of myosin light chain MLC-2 in senescent skeletal muscle indicates fast-to-slow fibre transition in sarcopenia of old age. *Eur J*

- Cell Biol.* 2009;88(11):685-700.
69. Fülöp N, Feng W, Xing D, et al. Aging leads to increased levels of protein O-linked N-acetylglucosamine in heart, aorta, brain and skeletal muscle in Brown-Norway rats. *Biogerontology*. 2008;9(3):139.
  70. Metzger JM, Westfall M V. Covalent and noncovalent modification of thin filament action: the essential role of troponin in cardiac muscle regulation. *Circ Res*. 2004;94(2):146-158.
  71. Bayliss CR, Jacques AM, Leung M-C, et al. Myofibrillar Ca<sup>2+</sup> sensitivity is uncoupled from troponin I phosphorylation in hypertrophic obstructive cardiomyopathy due to abnormal troponin T. *Cardiovasc Res*. 2013;97(3):500-508.
  72. Buscemi N, Foster DB, Neverova I, Van Eyk JE. p21-activated kinase increases the calcium sensitivity of rat triton-skinned cardiac muscle fiber bundles via a mechanism potentially involving novel phosphorylation of troponin I. *Circ Res*. 2002;91(6):509-516.
  73. Salhi HE, Hassel NC, Siddiqui JK, et al. Myofilament Calcium Sensitivity: Mechanistic Insight into TnI Ser-23/24 and Ser-150 Phosphorylation Integration. *Front Physiol*. 2016;7:567.
  74. Dubois-Deruy E, Belliard A, Mulder P, et al. Interplay between troponin T phosphorylation and O-N-acetylglucosaminylation in ischaemic heart failure. *Cardiovasc Res*. 2015;107(1):56-65.
  75. Kowlessur D, Tobacman LS. Low Temperature Dynamic Mapping Reveals Unexpected Order and Disorder in Troponin. *J Biol Chem*. 2010;285(50):38978-38986.
  76. Na I, Kong MJ, Straight S, Pinto JR, Uversky VN. Troponins, intrinsic disorder, and cardiomyopathy. *Biol Chem*. 2016;397(8).
  77. Darling AL, Uversky VN. Intrinsic Disorder and Posttranslational Modifications: The Darker Side of the Biological Dark Matter. *Front Genet*. 2018;9.
  78. Britto-Borges T, Barton GJ. A study of the structural properties of sites modified by the O-linked 6-N-acetylglucosamine transferase. Friedberg I, ed. *PLoS One*. 2017;12(9):e0184405.

79. Agarkova I, Perriard J-C. The M-band: an elastic web that crosslinks thick filaments in the center of the sarcomere. *Trends Cell Biol.* 2005;15(9):477-485.
80. Ehler E, Gautel M. The sarcomere and sarcomerogenesis. *Adv Exp Med Biol.* 2008;642:1-14.
81. Lange S, Ehler E, Gautel M. From A to Z and back? Multicompartment proteins in the sarcomere. *Trends Cell Biol.* 2006;16(1):11-18.
82. Frank D, Kuhn C, Katus HA, Frey N. The sarcomeric Z-disc: A nodal point in signalling and disease. *J Mol Med.* 2006;84(6):446-468.
83. Sanger JM, Sanger JW. The dynamic Z bands of striated muscle cells. *Sci Signal.* 2008;1(32):pe37.
84. Hu LYR, Ackermann MA, Kontogianni-Konstantopoulos A. The sarcomeric M-region: A molecular command center for diverse cellular processes. *Biomed Res Int.* 2015;2015.
85. von Nandelstadh P, Ismail M, Gardin C, et al. A Class III PDZ Binding Motif in the Myotilin and FATZ Families Binds Enigma Family Proteins: a Common Link for Z-Disc Myopathies. *Mol Cell Biol.* 2009;29(3):822-834.
86. Paulin D, Li Z. Desmin: a major intermediate filament protein essential for the structural integrity and function of muscle. *Exp Cell Res.* 2004;301(1):1-7.
87. Lovering RM, O'Neill A, Muriel JM, Prosser BL, Strong J, Bloch RJ. Physiology, structure, and susceptibility to injury of skeletal muscle in mice lacking keratin 19-based and desmin-based intermediate filaments. *Am J Physiol Cell Physiol.* 2011;300(4):C803-C813.
88. Winter DL, Paulin D, Mericskay M, Li Z. Posttranslational modifications of desmin and their implication in biological processes and pathologies. *Histochem Cell Biol.* 2014;141(1):1-16.
89. Ise H, Kobayashi S, Goto M, et al. Vimentin and desmin possess GlcNAc-binding lectin-like properties on cell surfaces. *Glycobiology.* 2010;20(7):843-864.
90. Hnia K, Ramspacher C, Vermot J, Laporte J. Desmin in muscle and associated diseases:

- beyond the structural function. *Cell Tissue Res.* 2014;360(3):591-608.
91. Farach AM, Galileo DS. O-GlcNAc modification of radial glial vimentin filaments in the developing chick brain. *Brain Cell Biol.* 2008;36(5-6):191-202.
  92. Sihag RK, Inagaki M, Yamaguchi T, Shea TB, Pant HC. Role of phosphorylation on the structural dynamics and function of types III and IV intermediate filaments. *Exp Cell Res.* 2007;313(10):2098-2109.
  93. Srikanth B, Vaidya MM, Kalraiya RD. O-GlcNAcylation determines the solubility, filament organization, and stability of keratins 8 and 18. *J Biol Chem.* 2010;285(44):34062-34071.
  94. Ji S, Kang JG, Park SY, Lee J, Oh YJ, Cho JW. O-GlcNAcylation of tubulin inhibits its polymerization. *Amino Acids.* 2011;40(3):809-818.
  95. Bar H, Goudeau B, Walde S, et al. Conspicuous involvement of desmin tail mutations in diverse cardiac and skeletal myopathies. *Hum Mutat.* 2007;28(4):374-386.
  96. Herrmann H, Haner M, Brettel M, et al. Structure and Assembly Properties of the Intermediate Filament Protein Vimentin: The Role of its Head, Rod and Tail Domains. *J Mol Biol.* 1996;264(5):933-953.
  97. Heimburg T, Schuenemann J, Weber K, Geisler N. Specific Recognition of Coiled Coils by Infrared Spectroscopy: Analysis of the Three Structural Domains of Type III Intermediate Filament Proteins. *Biochemistry.* 1996;35(5):1375-1382.
  98. Goldfarb LG, Olivé M, Vicart P, Goebel HH. Intermediate filament diseases: Desminopathy. *Adv Exp Med Biol.* 2008;642:131-164.
  99. Rogers KR, Eckelt A, Nimmrich V, et al. Truncation mutagenesis of the non-alpha-helical carboxyterminal tail domain of vimentin reveals contributions to cellular localization but not to filament assembly. *Eur J Cell Biol.* 1995;66(2):136-150.
  100. Elliott JL, Der Perng M, Prescott AR, Jansen KA, Koenderink GH, Quinlan RA. The specificity of the interaction between  $\alpha$ B-crystallin and desmin filaments and its impact on

- filament aggregation and cell viability. *Philos Trans R Soc Lond B Biol Sci*. 2013;368(1617):20120375.
101. Sharma S, Conover GM, Elliott JL, Der Perng M, Herrmann H, Quinlan RA.  $\alpha$ B-crystallin is a sensor for assembly intermediates and for the subunit topology of desmin intermediate filaments. *Cell Stress Chaperones*. 2017;22(4):613-626.
  102. Ecroyd H, Meehan S, Horwitz J, et al. Mimicking phosphorylation of  $\alpha$ B-crystallin affects its chaperone activity. *Biochem J*. 2007;401(1):129-141.
  103. Bakthisaran R, Akula KK, Tangirala R, Rao CM. Biochimica et Biophysica Acta Phosphorylation of  $\alpha$ B-crystallin : Role in stress , aging and patho-physiological conditions. *BBA - Gen Subj*. 2016;1860(1):167-182.
  104. Krishnamoorthy V, Donofrio AJ, Martin JL. O-GlcNAcylation of  $\alpha$ B-crystallin regulates its stress-induced translocation and cytoprotection. *Mol Cell Biochem*. 2013;379(1-2):59-68.
  105. Houck SA, Landsbury A, Clark JI, Quinlan RA. Multiple sites in  $\alpha$ B-crystallin modulate its interactions with desmin filaments assembled in vitro. *PLoS One*. 2011;6(11).
  106. Wang X, Klevitsky R, Huang W, Glasford J, Li F, Robbins J.  $\alpha$ B-Crystallin Modulates Protein Aggregation of Abnormal Desmin. *Circ Res*. 2003;93(10):998-1005.
  107. Andley UP, Hamilton PD, Ravi N, Weihl CC. A Knock-In Mouse Model for the R120G Mutation of  $\alpha$ B-Crystallin Recapitulates Human Hereditary Myopathy and Cataracts. Nikolaidis N, ed. *PLoS One*. 2011;6(3):e17671.
  108. Udaka J, Ohmori S, Terui T, et al. Disuse-induced preferential loss of the giant protein titin depresses muscle performance via abnormal sarcomeric organization. *J Gen Physiol*. 2008;131(1):33-41.
  109. Nakamura S, Nakano S, Nishii M, Kaneko S, Kusaka H. Localization of O-GlcNAc-modified proteins in neuromuscular diseases. *Med Mol Morphol*. 2012;45(2):86-90.
  110. Clemen CS, Herrmann H, Strelkov S V., Schröder R. Desminopathies: Pathology and

- mechanisms. *Acta Neuropathol.* 2013;125(1):47-75.
111. Shi H, Munk A, Nielsen TS, et al. Skeletal muscle O-GlcNAc transferase is important for muscle energy homeostasis and whole-body insulin sensitivity. *Mol Metab.* 2018;11:160-177.
  112. Johnston JR, Chase PB, Pinto JR. Troponin through the looking-glass: emerging roles beyond regulation of striated muscle contraction. *Oncotarget.* 2018;9(1).
  113. Pinto JR, Muller-Delp J, Chase PB. Will you still need me (Ca<sup>2+</sup>, TnT, and DHPR), will you still cleave me (calpain), when I'm 64? *Aging Cell.* 2017;16(2):202-204.
  114. Jia C, Zuo Y. Computational Prediction of Protein O-GlcNAc Modification. In: *Methods Mol Biol.* ; 2018:235-246.

## FIGURE LEGENDS

**Figure 1: Schematic cartoon of O-GlcNAc-modified proteins involved in the physiology of the skeletal muscle.** A non-exhaustive list of O-GlcNAc proteins identified in skeletal muscle cells is displayed with a blue (\*) symbol, whereas a non-exhaustive list of potential O-GlcNAc proteins is displayed with an orange (#) symbol, since they have been identified in other cell types.

**Figure 2: Impact of O-GlcNAcylation process on calcium activation properties of slow skeletal muscle fibers isolated from soleus.** Top, representative curve of the relation tension/pCa with  $pCa = -\log [Ca^{2+}]$ . Tension corresponds to the ratio  $P/P_0$  with P the tension developed by the fibers at a given pCa, and  $P_0$  the maximal tension developed by the fiber at a pCa4.2. The T/pCa curve following modification of O-GlcNAcylation process were indicated. Bottom, table indicates the variation of calcium activation parameters (threshold, Hill coefficient,  $pCa_{50}$  and  $P_0$ ) following the modification of the O-GlcNAcylation process.

**Figure 3: Representative scheme of the preferential localization of OGT and OGA within the sarcomere.** Both OGT (orange triangle) and OGA (blue circle) are preferentially localized to the Z-line, and in less extent, on I-band. According to physiological (exercise) or pathological (functional atrophy) signals, the localization of OGT and OGA, so as their interaction with MLCK, PP1 and MYPT2 within a multienzymatic complex, could change.

**Figure 4: Differential regulation and modulation of O-GlcNAc/Phospho-MLC2 in rat slow-twitch and fast-twitch muscles following exercises.** N/A means Non-Available data in the literature.



**Figure 5: Differential regulation and modulation of O-GlcNAc/Phospho-MLC2 in slow-twitch and fast-twitch muscles following disuse conditions in rat and human. N/A means Non-Available data in the literature.**

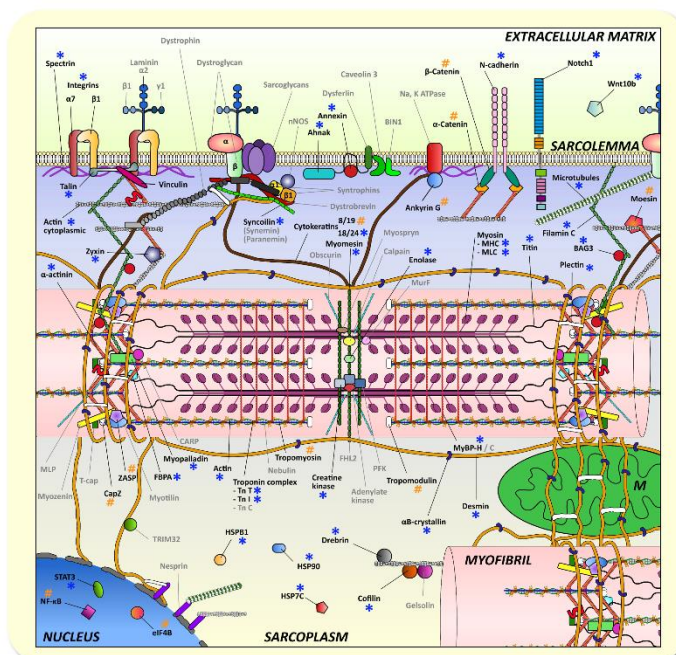


Figure 1

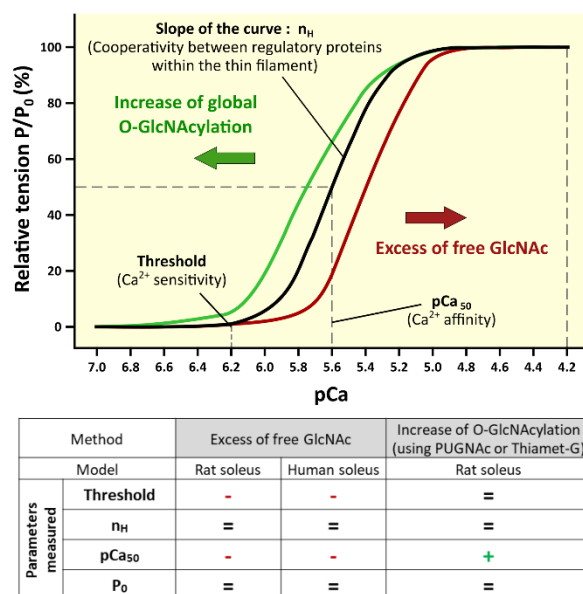


Figure 2

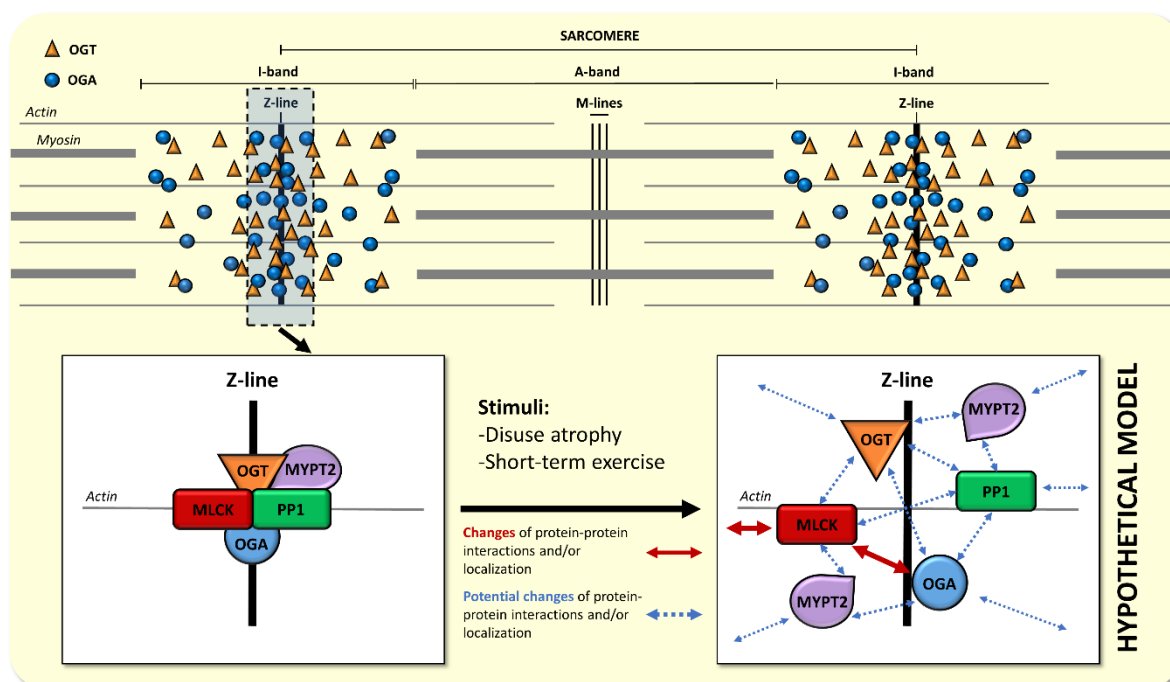


Figure 3

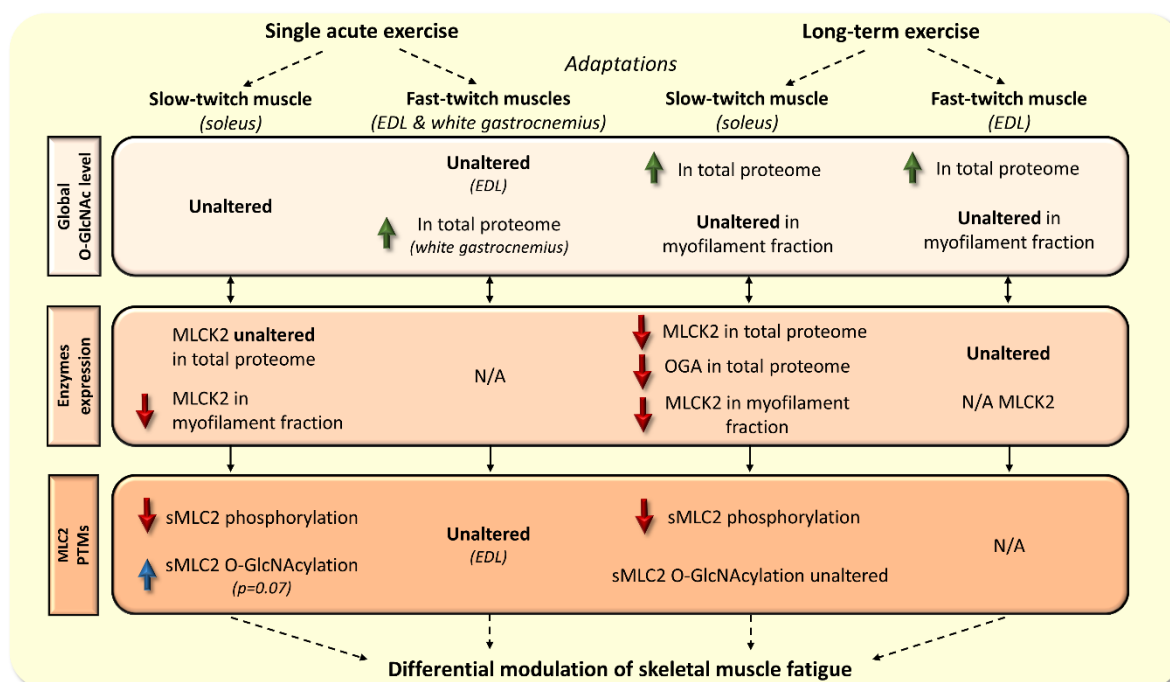


Figure 4

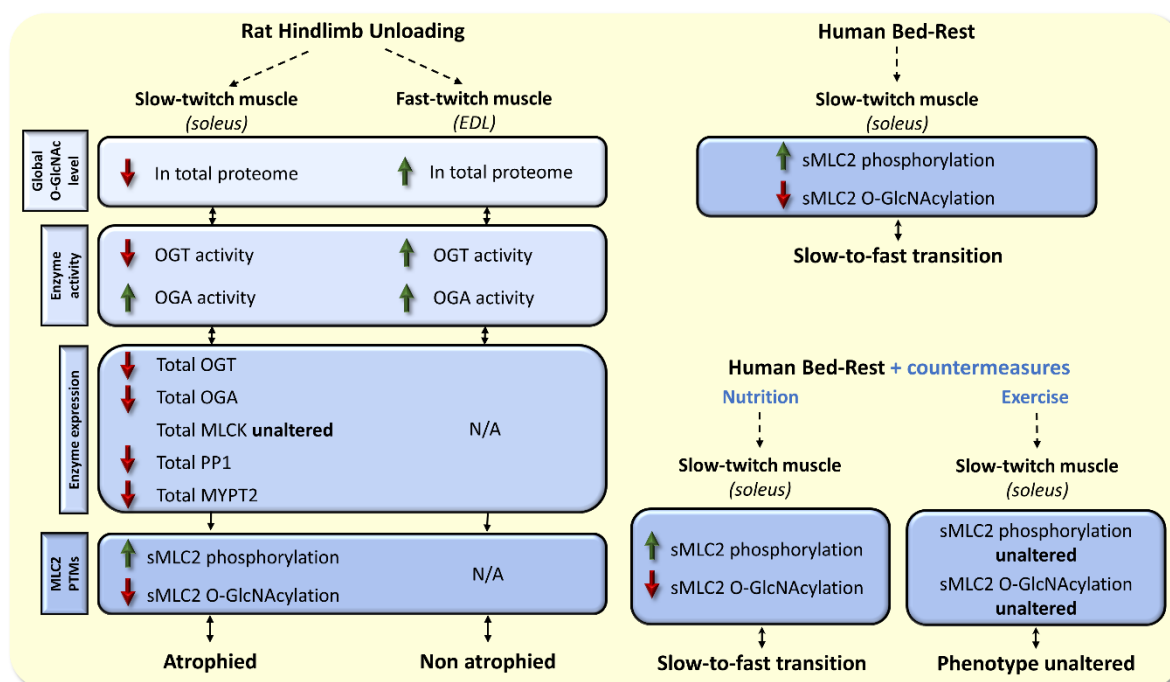


Figure 5