

SYMPOSIUM REVIEW

Muscle glycogen stores and fatigue

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Abstract Studies performed at the beginning of the last century revealed the importance of carbohydrate as a fuel during exercise, and the importance of muscle glycogen on performance has subsequently been confirmed in numerous studies. However, the link between glycogen depletion and impaired muscle function during fatigue is not well understood and a direct cause-and-effect relationship between glycogen and muscle function remains to be established. The use of electron microscopy has revealed that glycogen is not homogeneously distributed in skeletal muscle fibres, but rather localized in distinct pools. Furthermore, each glycogen granule has its own metabolic machinery with glycolytic enzymes and regulating proteins. One pool of such glycogenolytic complexes is localized within the myofibrils in close contact with key proteins involved in the excitation–contraction coupling and Ca^{2+} release from the sarcoplasmic reticulum (SR). We and others have provided experimental evidence in favour of a direct role of decreased glycogen, localized within the myofibrils, for the reduction in SR Ca^{2+} release during fatigue. This is consistent with compartmentalized energy turnover and distinctly localized glycogen pools being of key importance for SR Ca^{2+} release and thereby affecting muscle contractility and fatigability.

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Corresponding author N. Ørtenblad: Institute of Sports Science and Clinical Biomechanics, University of Southern Denmark, Odense, DK-5230 Odense M, Denmark. Email: nortenblad@health.sdu.dk**Abbreviations** CHO, carbohydrate; E-C coupling, excitation–contraction coupling; SR, sarcoplasmic reticulum; t-system, transverse tubular system.

Niels Ørtenblad is Associate Professor based at the University of Southern Denmark. His research is focused on basic mechanisms linking cell energy production with energy utilization, with special reference to mechanisms of muscle fatigue. In his research he has used various techniques from the whole body level to mechanically skinned muscle fibres, and organelles. **Håkan Westerblad** is professor in cellular muscle physiology at the Karolinska Institutet in Stockholm, Sweden. He and his colleagues developed techniques to study contractile function and Ca^{2+} handling in isolated, intact fibres from mammalian skeletal muscle. A major focus of his research has been on cellular mechanisms of skeletal muscle fatigue. **Joachim Nielsen** is post doc at the Institute of Sports Science and Clinical Biomechanics, University of Southern Denmark. His major research focus has been to elucidate the role and regulation of local storage of glycogen molecules in specific micro-domains within muscle fibres.



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Introduction

Glycogen is a complex glucose polymer found in most species in the animal kingdom. It serves as a storage form for glucose found in a variety of tissues, although quantitatively mainly in skeletal muscles and the liver. The wide occurrence of glycogen in skeletal muscles indicates that it is essential in providing a mechanism by which ATP rapidly can be produced in muscle cells, which display a high and rapidly fluctuating energy turnover. The responses of muscle glycogen to such perturbations as exercise and diet are very well documented in studies of humans as well as various animal models (Bergström *et al.* 1967; Jensen & Richter, 2012). In addition, it has been understood for more than half a century that the ability of muscle to exercise is seriously compromised when the glycogen store is reduced to low levels, even when there is an abundance of other fuel sources (Bergström *et al.* 1967). Thus, Hultman and co-workers demonstrated a strong correlation between muscle glycogen content and endurance capacity during prolonged cycling exercise (Bergström *et al.* 1967) and an inability to continue such exercise when the glycogen stores were exhausted (Hermansen *et al.* 1967). These observations have subsequently been confirmed in numerous studies and it is now well established that there is a close relationship between muscle glycogen content and fatigue resistance, both during prolonged (more than 1 h) and during high-intensity intermittent exercise (Pernow & Saltin, 1971; Gollnick *et al.* 1972; Bangsbo *et al.* 1992; Hargreaves *et al.* 1995). However, the precise mechanism linking glycogen to muscle function remains elusive. The fundamental aspects of glycogen regulation are also very clearly documented in any biochemistry context, with most single enzymatic and regulatory steps well described. Thus, it is noteworthy that we cannot explain precisely why muscle function is impaired when muscle glycogen is low and how the glycogen stores affect basic cell function. The relationship between low muscle glycogen stores and fatigue is generally considered to be explained by a compromised rate of ATP regeneration and this aspect has been covered in other reviews (Sahlin *et al.* 1998; Allen *et al.* 2008; Jensen & Richter, 2012). In this review we put forward evidence that in skeletal muscle, glycogen should not only be considered as a form of global carbohydrate storage but also a dynamic molecule regulating distinct and spatially restricted cellular functions.

Role of glycogen in muscle fatigue

The most recognized theory for the association between low muscle glycogen levels and impaired contractile function is that glycogen is an essential substrate, the depletion of which results in a reduction in the rate of ATP regeneration. As a consequence, the muscle is unable to maintain an adequate global energy supply

to one or more of the processes involved in excitation and contraction, leading to an inability to translate the motor drive into an expected force, i.e. fatigue develops. This is supported by observations of phosphocreatine (PCr) decreases along with an increase in free ADP and IMP (inositol monophosphate) following prolonged glycogen-depleting exercise (Norman *et al.* 1988; Sahlin *et al.* 1997). However, the energy deficiency theory in skeletal muscle fatigue is challenged by both *in vitro* and *in vivo* studies demonstrating a strong association between low glycogen and decreased muscle function even after recovery periods, where ATP levels would be normal (Bangsbo *et al.* 1992; Chin & Allen, 1997). However, observations on whole body experiments or intact muscle preparations will not provide direct evidence for a specific role of glycogen on muscle function, because changes in total muscle glycogen content bring about other metabolic changes, which may affect the excitation–contraction coupling (E-C coupling; Stephenson *et al.* 1999; Allen *et al.* 2008). Thus, our knowledge of the mechanism responsible for glycogen modulation of muscle function mainly originates from studies at the single fibre or organelle level.

Studies on both rodent single fibres and human studies have pointed to a modulating role of glycogen availability on sarcoplasmic reticulum (SR) Ca^{2+} handling (Chin *et al.* 1997; Duhamel *et al.* 2006b; Nielsen *et al.* 2009; Ørtenblad *et al.* 2011). Experiments with simultaneous measurements of force and intracellular free $[\text{Ca}^{2+}]$ ($[\text{Ca}^{2+}]_i$) have been used to identify the cellular mechanisms linking low glycogen to fatigue. By using both rat and toad fibre bundles it has been demonstrated that when recovery after glycogen-reducing contractions occurs in the absence of glucose, glycogen does not recover and fibre bundles fatigue more rapidly and show reduced tetanic $[\text{Ca}^{2+}]_i$ transients in a subsequent fatigue run (Chin *et al.* 1997; Kabbara *et al.* 2000; Helander *et al.* 2002). Thus, a reduced level of glycogen is associated with a faster decrease of tetanic $[\text{Ca}^{2+}]_i$ during repeated contractions (Fig. 1). Studies on SR vesicles from human muscle support a link between reduced muscle glycogen and decreased tetanic $[\text{Ca}^{2+}]_i$ (Duhamel *et al.* 2006a,b; Ørtenblad *et al.* 2011). Duhamel *et al.* (2006b) examined the relationship between muscle glycogen content and SR vesicle Ca^{2+} release rate during a prolonged fatiguing cycling session at 70% $\dot{V}_{\text{O}_{2\text{peak}}}$. To manipulate muscle glycogen concentrations, exercise was preceded by a glycogen-depleting exercise session followed by 4 days of either low or high carbohydrate (CHO) diet. Muscle glycogen content was markedly reduced at the initiation of exercise with the low CHO diet and deteriorations in SR Ca^{2+} release occurred earlier during exercise in this condition compared to the high CHO diet (Duhamel *et al.* 2006b). We have recently demonstrated an association between muscle glycogen and SR vesicle Ca^{2+}

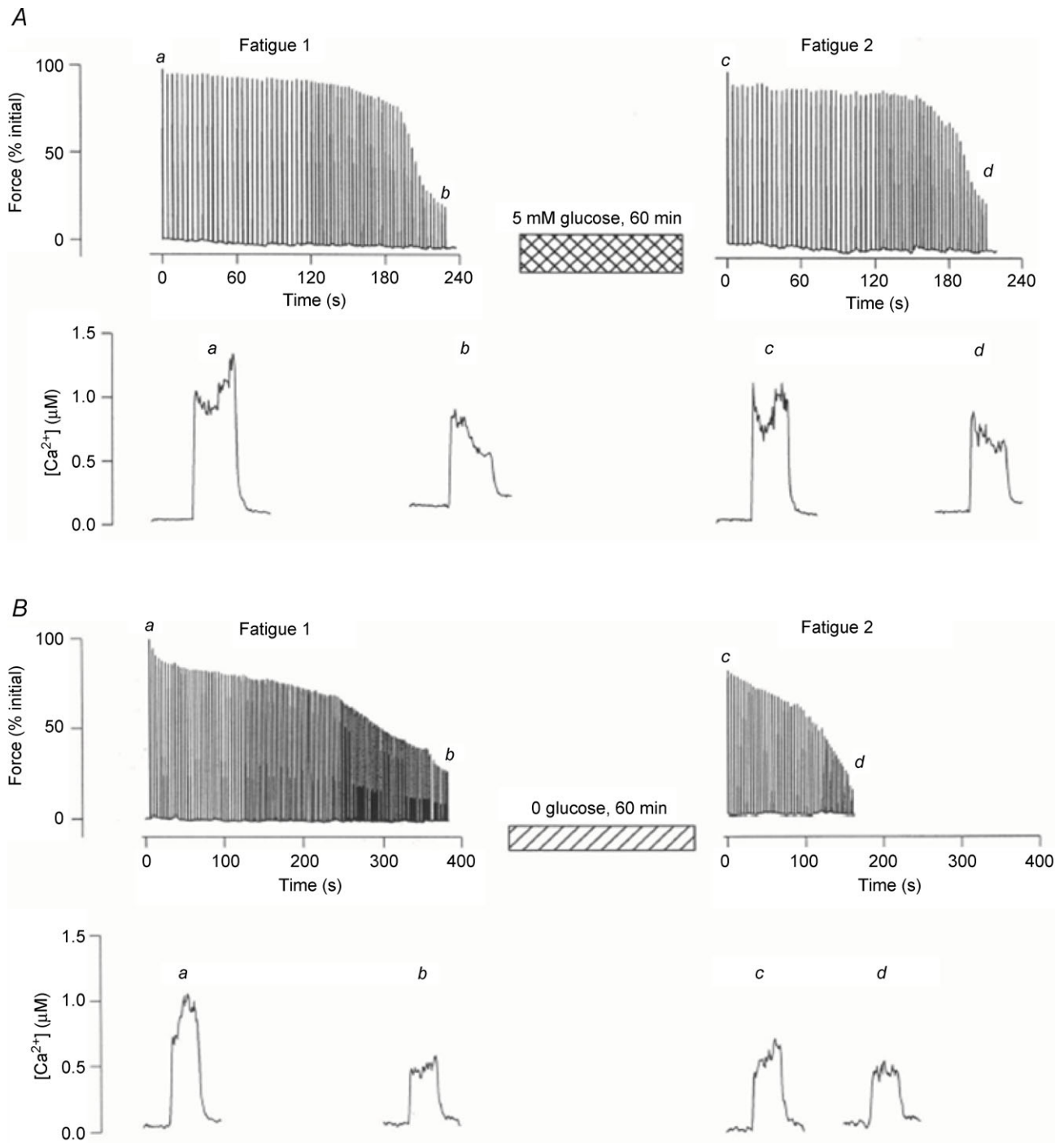


Figure 1. Fatigue occurs more rapidly after recovery without glucose

Original data traces of force (upper panels) and $[Ca^{2+}]_i$ (lower panels) obtained in single fast-twitch mouse muscle fibres during fatigue induced by repeated tetanic contractions (100 Hz, 350 ms). This type of fatiguing stimulation resulted in marked decrease in glycogen to ~30% of the control value (Chin & Allen, 1997). Force and $[Ca^{2+}]_i$ were well maintained during a second fatigue run after 60 min of recovery in 5.5 mM glucose (A), which restored glycogen to pre-fatigue levels. Conversely, force and $[Ca^{2+}]_i$ were not fully restored and fatigue occurred more rapidly after 60 min of recovery without glucose (B), where glycogen stores remained depleted. Adapted from Figs 5 and 6 of Chin & Allen (1997).

release rate by manipulating muscle glycogen levels in the recovery period following fatiguing cross country skiing exercise (Ørtenblad *et al.* 2011). Skiers were provided with either a CHO-enriched diet or water during the initial 4 h following exercise. After 4 h of recovery with CHO, SR vesicle Ca^{2+} release rate was normalized and muscle glycogen content was markedly increased compared to post exercise, whereas both SR release and glycogen remained depressed in the group which did not receive CHO. Together, data from SR vesicle experiments indicate a mechanistic role of glycogen on SR Ca^{2+} release. A reduced SR Ca^{2+} release rate will per se cause a decrease in tetanic $[\text{Ca}^{2+}]_i$, which is very much in line with the studies on isolated fibres demonstrating a faster decrease of tetanic $[\text{Ca}^{2+}]_i$ in fibres with low levels of glycogen.

Furthermore, in mechanically skinned muscle fibres, where global ATP can be kept high and constant, low glycogen content is associated with an irreversible force depression during repeated tetanic contractions (Stephenson *et al.* 1999; Barnes *et al.* 2001; Nielsen *et al.* 2009). In this preparation the extensive transverse tubular system (t-system), which represents the greater part of the plasma membrane, reseals and becomes normally polarized when placed in a medium mimicking the cytosolic environment of the intact cell (Lamb *et al.* 1995; Stephenson, 2006). With this preparation it is possible to measure fibre excitability and force production while at the same time having direct access to the intracellular environment. This makes it possible to estimate the effect of muscle fibre glycogen content per se without changes in other metabolites, i.e. keeping PCr and ATP high and constant. In mechanically skinned toad muscle fibres the ability to respond to t-tubular depolarizations by ion substitution correlated with muscle glycogen content (Stephenson *et al.* 1999). This was later both confirmed (Barnes *et al.* 2001) and disproved (Goodman *et al.* 2005) using rat muscle fibres. In a later experiment, mechanically skinned rat muscle fibres were activated by electric field stimulation and glycogen content was estimated by electron microscopy, also providing an estimate of the subcellular localization of glycogen (Nielsen *et al.* 2009). Here, the fatigability (number of tetanic contractions until 50% force reduction) correlated only with the distinct deposition of glycogen located within the myofibrils (intramyofibrillar glycogen, see next section; Nielsen *et al.* 2009). These results demonstrate that the muscle glycogen content affects muscle function in contracting single muscle fibres under conditions where the global myoplasmic ATP level is kept high and constant. Thus, glycogen affects the E-C coupling despite global ATP being held constant, which argues against a direct metabolic effect of low glycogen levels at the whole cell level. However, this does not exclude a role of glycogen on compartmentalized energy transfer in the cell. To sum up, depletion of glycogen during prolonged, exhausting

exercise may contribute to fatigue by causing decreased SR Ca^{2+} release and the underlying mechanism seems to be independent of the overall energy level of muscle fibres.

The glycogen granule and glycogen localization

Glycogen is often interpreted as if it is uniformly distributed in the cell, providing an average concentration of the cell. Interestingly, electron microscopy has revealed that glycogen is found as discrete glycogen particles located in distinct pools within the fibres (Wanson & Drochman, 1968; Friden *et al.* 1989; Marchand *et al.* 2002, 2007; Nielsen *et al.* 2010, 2011). Furthermore, each glycogen granule has its own metabolic machinery with glycolytic enzymes and regulating proteins (Wanson & Drochman, 1972; Graham *et al.* 2010). Based on two-dimensional electron microscopy images, three distinct intracellular pools of glycogen have been identified: (1) subsarcolemmal glycogen, just beneath the sarcolemma; (2) intermyofibrillar glycogen, located between the myofibrils, mainly at the level of the I-band close to mitochondria and SR; and (3) intramyofibrillar glycogen, in the myofibril, mainly near the z-line (Fig. 2). Estimates of the relative distribution of glycogen in the distinct pools in humans have revealed that the major glycogen pool is the intermyofibrillar one, constituting roughly 75% of the total glycogen store, whereas intramyofibrillar and subsarcolemmal glycogen accounts for 5–15% each. The relative distribution is, however, to a large degree dependent on fibre type, training status, immobilization, exercise and species (rat, human; see Nielsen & Ørtenblad, 2013).

Role of glycogen localization during exercise

Early studies of the subcellular localization-dependent utilization of glycogen during exercise were performed in the 1980s by Ekblom and colleagues (Sjöström *et al.* 1982; Fridén *et al.* 1985, 1989). More recently, quantitative methods following unbiased stereological rules have demonstrated a larger relative utilization of intramyofibrillar glycogen during exercise compared to intermyofibrillar and subsarcolemmal glycogen (Marchand *et al.* 2007; Nielsen *et al.* 2011). In the study by Marchand *et al.* (2007), recreationally active subjects completed an exhaustive glycogen-depletion cycling exercise at 70% of $\dot{V}\text{O}_{2\text{max}}$ before biopsies were obtained during a 48 h recovery period. Immediately after the cycling exercise, glycogen levels were very low [$36 \text{ mmol (kg dw)}^{-1}$] and the relative contribution of intramyofibrillar glycogen was only 3% compared to 12–14% in resting controls (Marchand *et al.* 2002) or after 48 h recovery (Marchand *et al.* 2007). In the study by Nielsen *et al.* (2011), muscle biopsies were obtained both before and after exercise consisting of completion of a approximately 1 h cross country skiing time trial performed by elite skiers. In type

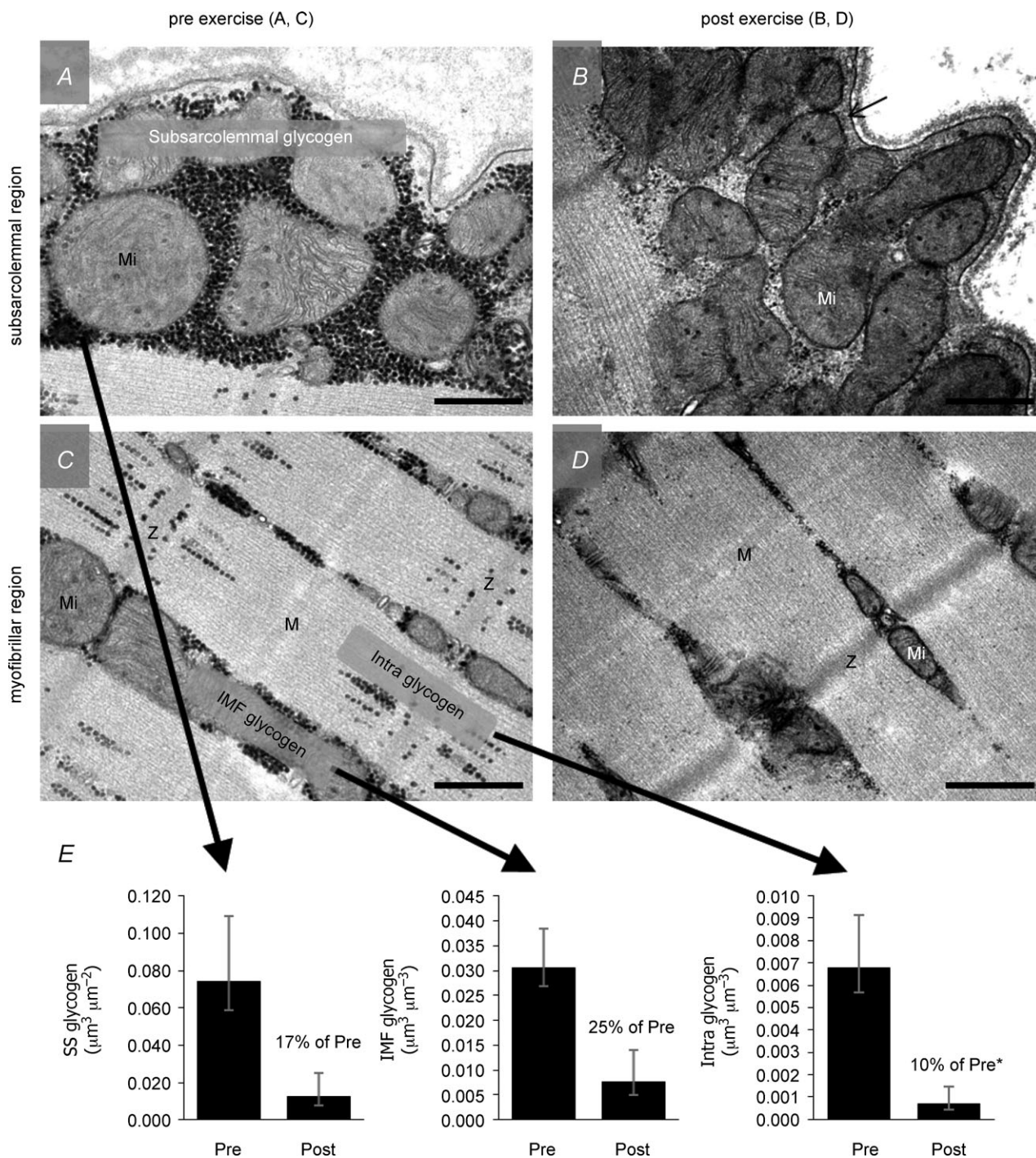


Figure 2. Glycogen content in three subcellular localizations in muscle before and after approximately 1 h of exhaustive exercise

A–D, overview showing the typical localization pattern of glycogen particles in the subsarcolemmal region (A, B) and the myofibrillar region (C, D) of a muscle fibre, pre (A, C) and post (B, D) approximately 1 h of exhaustive exercise. Representative images originate from an arm (m. triceps brachii) type I fibre from trained subjects. Glycogen particles are visualized as black dots, with the intermyofibrillar (IMF) glycogen located between the myofibrils and the intramyofibrillar (Intra) glycogen within the myofibrils, mainly located in the I-band. Subsarcolemmal (SS) glycogen is located between the sarcolemma and the outermost myofibril. E, geometric mean glycogen content in the three localizations, with a significantly higher relative utilization of the Intra glycogen (5–15% of total glycogen) during the exercise compared to SS and IMF glycogen (5–15 and 75% of total glycogen, respectively). Mi, mitochondria; Z, Z-line; M, M-band. Scale bars = 0.5 μm. Original ×40,000 magnification. Adapted from Fig. 4 of Nielsen *et al.* (2011).

I fibres before exercise, glycogen was distributed with 77% in the intermyofibrillar space, 12% in the intramyofibrillar space and 11% in the subsarcolemmal space. After exercise, intramyofibrillar glycogen was reduced by 90% compared to reductions by only 75% in intermyofibrillar glycogen and 83% in subsarcolemmal glycogen (Fig. 2); thus, the relative content of intramyofibrillar glycogen was decreased from 11% before exercise to only 6% after exercise. Interestingly, after exercise there was a correlation between the decreased rate of Ca^{2+} release in SR vesicles and the reduction in the intramyofibrillar glycogen content, corroborating the results from rat single fibres (Nielsen *et al.* 2009). In the latter study (Nielsen *et al.* 2009), a fast partial recovery of force might indicate that the force reduction was due to some loss of excitability, hence suggesting that decreased intramyofibrillar glycogen may also be related to reduced Na,K-ATPase activity. An interesting perspective is that intramyofibrillar glycogen may be related to both SR Ca^{2+} release and t-system Na,K-ATPase activity, both processes located in the restricted space of the triadic junction, which ensures conversion of the action potential to SR Ca^{2+} release and subsequently muscle contraction. Together the t-system and the SR form a triadic junction, around 12 nm wide, with a diffusional restricted space for more than 90% of the t-system length, and with a high metabolic activity (Dulhunty *et al.* 1984; Han *et al.* 1992). In line

with this, it has been shown that Na,K-ATPase preferential uses glycolytically derived ATP (Lynch & Paul, 1987; James *et al.* 1999; Dutka & Lamb, 2007).

In support for a role of an essential use of glycogen for Na,K-ATPase, it has been demonstrated that, in astrocytes, glycogen is a critical energy source for neurons with significant implications in brain function, i.e. synaptic activity and memory formation (Obel *et al.* 2012). Taken together, the distinct pool of intramyofibrillar glycogen may be important for energy status in the triad junction and low amount of glycogen in this restricted area between t-tubules and the SR may decrease SR Ca^{2+} release rate and Na,K-ATPase activity.

Mechanisms of glycogen modulating E-C coupling

What is the basis of the mechanism responsible for glycogen modulation of Ca^{2+} release from the SR? A consequence of microenvironments with high ATPase activity and the limited diffusability in the muscle cell is the need to compartmentalize metabolic pathways. In other words, the main ATPases depend on a structural organization of substrate and metabolic enzymes in the direct vicinity and cannot rely on simple diffusion (Ovadi & Saks, 2004). Thus, distinct channelling of the intermediates from the substrate through enzymatic pathways and transfer of ATP to the metabolically active sites will

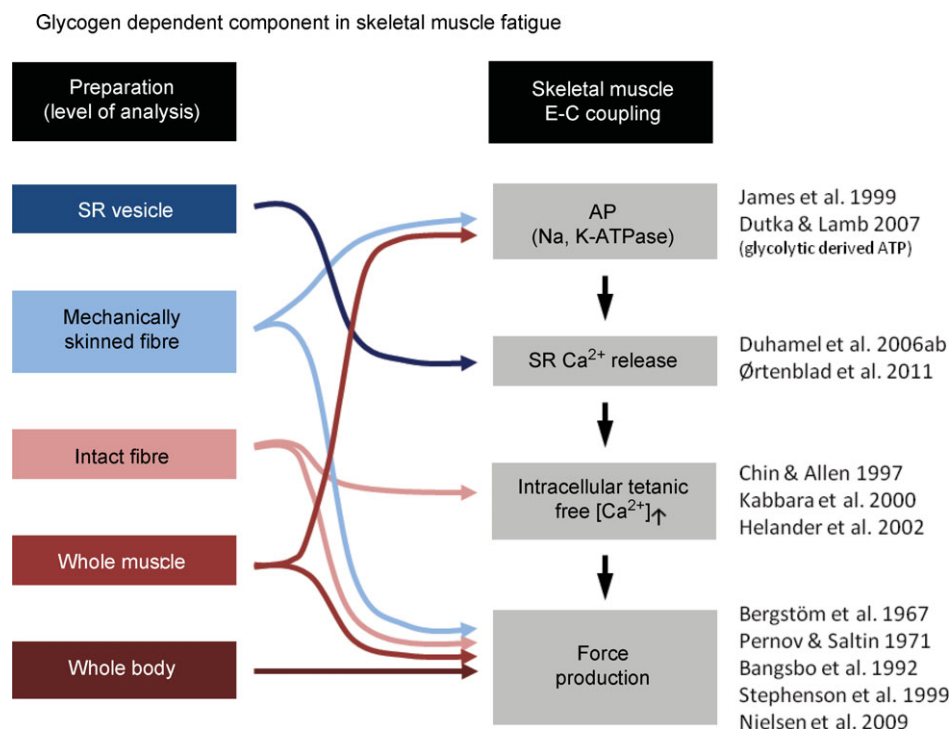


Figure 3. Tentative glycogen-dependent components in muscle fatigue

Measurements at the level from organelles (SR vesicles) to whole body experiments indicate a glycogen-dependent role in the E-C coupling failure leading to muscle fatigue. Arrows indicate the level in the series of events in the E-C coupling, where glycogen has been demonstrated to play a significant role.

ensure a sufficient metabolic power without the need of free diffusion. In line with this, glycogen particles are found in distinct locations within this organization including just beneath the plasma membrane, close to SR and mitochondria, and within the myofibrils between the contractile filaments. The CK does channel ATP from sites of production to buffer free ADP and Pi (free phosphate) concentrations. CK (creatine kinase) is localized at the mitochondrial membrane, on the M- and I-band, and on the SR membrane (Rossi *et al.* 1990; Wegmann *et al.* 1992; Wallimann *et al.* 2011). CK may be present in the triad, but to our knowledge this has not been demonstrated. From a functional aspect, there is a clear association of muscle glycogen contents and muscle function in experiments with the mechanically skinned fibre (Stephenson *et al.* 1999; Nielsen *et al.* 2009). This association has been established under conditions where ATP and PCr are kept high and constant. Furthermore, the t-tubular Na,K-ATPase function is improved by addition of phospho(enol)pyruvate (supported by endogenous pyruvate kinase bound within the triad) even with 40 mM PCr present (Dutka & Lamb, 2007). Together, these results show that the triad junction is a highly restricted micro-environment, which may not be adequately buffered by CK channelling.

The observed effect of low glycogen on mechanically skinned fibres, where global ATP can be kept high and constant, might be taken as an argument for a non-metabolic role of glycogen (Stephenson *et al.* 1999; Nielsen *et al.* 2009). However, as discussed above, an alternative explanation is that restricted areas in the muscle cell (e.g. the triadic junction) with a limited access and high metabolic activity may still be metabolically affected by low glycogen levels. The association between the specific pool of intramyofibrillar glycogen and SR Ca²⁺ release rate combined with the preferential depletion of this glycogen pool during most forms of exercise argues for a specific role of this pool to secure a sufficient metabolic milieu in the triads. This idea supports the energy-deficient theory, stating that low glycogen levels may compromise ATP synthesis and importantly this may be exacerbated in the triads, which are out of diffusion equilibrium with global ATP (Han *et al.* 1992; Ovadi & Saks, 2004). However, at this stage a non-metabolic (e.g. structural) role of glycogen cannot be discarded. Furthermore, it is possible that the connection between glycogen and SR Ca²⁺ release is not related to glycogen as an energy source, but to the action of enzymes associated with the glycogen particles, which themselves might modulate the function of the SR Ca²⁺ release channels in their proximity, e.g. by phosphorylation or de-phosphorylation of proteins involved in E-C coupling (Hamilton & Serysheva, 2009; Sharma *et al.* 2012).

Conclusion

In this review we present evidence that the subcellular localization of glycogen has to be considered to fully understand the role of glycogen metabolism for skeletal muscle function in general and especially in relation to fatigue. Studies performed from the whole-body to organelle level suggest that the pool of intramyofibrillar glycogen has a key role during repeated contractions by counteracting contractile impairments caused by defective SR Ca²⁺ release (Fig. 3).

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Additional information

Competing interests

None.