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COMMENTARY

Excitation–Contraction Coupling

The spatial distribution of glycogen and glycogen consumption in muscle cells

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Introduction

Muscles are impressive machines that can produce force or shortening over a wide range of intensities in a precisely timed sequence. However, they cannot do so indefinitely, and the decline in performance with time is generally known as fatigue. When intense activity is continued for about 1 h, a type of fatigue, often called exhaustion, occurs, and it has been known for at least 50 yr that this occurs at around the time when muscle glycogen falls to low levels (Hermansen et al., 1967) and that muscles that commence exercise with a high level of glycogen can exercise for longer before fatigue occurs (Bergström et al., 1967). These discoveries have led to continuing interest in muscle glycogen and whether glycogen depletion causes fatigue and the possible mechanism(s) involved. The study by Nielsen et al. (2022) in this issue adds a further step in this understanding by showing that the three main ATPases in muscle consume glycogen in a spatially distinct manner.

Glycogen granules and their distribution

Glycogen is a branched glucose polymer and a major source of energy in muscle cells. The glucose chains are organized in concentric layers known as the tiered model (for review see Prats et al., 2018). Up to 12 tiers are possible, giving a maximum size of 42 nm. In addition to the glucose chains, each glycogen granule contains multiple proteins, which regulate the synthesis and breakdown of glycogen and other proteins that have a scaffolding role. When muscles contract, breakdown of glycogen is rapidly triggered by activation of glycogen phosphorylase producing glucose-1-phosphate, which enters the glycolytic pathway and provides a rapidly available supply of ATP (for review see Katz, 2022). Each glycogen particle is thought to act as an independently regulated source of metabolic fuel, raising the possibility that there is local regulation and supply of metabolites.

Glycogen granules are visible in transmission electron microscopy and are recognized to have a distinctive distribution in muscles that depends on species, fiber type, and the history of activity (for review see Ørtenblad et al, 2013). In Nielsen et al. (2022), rat soleus muscles were studied and glycogen particles were measured at three different sites using standard stereological techniques. The sites chosen were (1) intramyofibrillar, i.e., within the myofibrils where the granules are concentrated near the Z line; (2) intermyofibrillar, i.e., between the myofibrils, a space that includes SR, transverse tubules (T-tubules), the triads (a diffusion-restricted junctional space where SR and T-tubule membranes come in close contact and SR Ca^{2+} release occurs), and mitochondria; and (3) subsarcolemmal, i.e., a relatively narrow space adjacent to the sarcolemma. These sites and the distribution of glycogen granules are shown in Figs. 1 and 2 of Nielsen et al. (2022). Around 29% of the glycogen granules were intramyofibrillar, 65% intermyofibrillar, and 6% subsarcolemmal.

The causes of muscle fatigue

The causes of muscle fatigue are intimately bound up with the changes in intracellular metabolites, which occur when muscles are stimulated intensively (for review see Allen et al., 2008). Three main categories are recognized: (1) decline in maximum Ca^{2+}-activated force produced by the myofibrillar proteins, (2) decline in Ca^{2+}-sensitivity of the myofibrillar proteins, and (3) decline in Ca^{2+} release from the SR. Effects of lactic acid accumulation and inorganic phosphate on the contractile proteins were the earliest established causes of fatigue. Glycogen breakdown contributes to the lactate and H^+ accumulation during intense activity when the aerobic capacity is exceeded. Nevertheless, the main focus of the role of glycogen depletion and its association with fatigue (or exhaustion) during prolonged exercise is on its possible role in regulating SR Ca^{2+}

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release. This emphasis on SR Ca^{2+} release arises from a number of experimental findings.

Muscle glycogen and fatigue

Initial evidence of involvement of muscle glycogen with failure of SR Ca^{2+} release comes from a study where single mouse fibers were stimulated to fatigue and a fall in tetanic $[Ca^{2+}]_i$ was noted (Chin and Allen, 1997). Then muscles were allowed to recover for 1 h either in the presence of 5 mM glucose or without glucose and the fatigue protocol repeated. With glucose present, force and tetanic $[Ca^{2+}]_i$ were similar to the first fatigue run, whereas without glucose, tetanic force and $[Ca^{2+}]_i$ did not fully recover and the second fatigue run was greatly accelerated. Parallel glycogen measurements showed that glycogen fell to 27% in the first fatigue, recovered to 150% during the 1-h recovery with glucose, but did not recover at all without glucose. These authors suggested that when glycogen fell to some critical level, ATP supply to the triads might become inadequate and ATP requiring processes within the triads might fail.

In an interesting development of the above experiments, Stephenson et al. (1999) studied the relationship between glycogen and contractions of skinned muscle induced by depolarizing solutions. Depletion of glycogen occurred with repeated contractures, and the degree of depletion correlated with a reduction in the contracture force. Intriguingly, although these results are qualitatively similar to the intact fibers described above, in the skinned fiber experiments both ATP (8 mM) and PCr (10 mM) were present, so ATP concentrations would be expected to remain close to normal. Several possible explanations have been proposed to explain this apparent paradox. One is that glycogen depletion is exerting a non-metabolic role; for instance, one of the proteins of the glycogen complex could modulate the ryanodine receptor. An example of this is that the active form of glycogen phosphorylase has been shown to inhibit Ca^{2+} release from SR vesicles (Hirata et al., 2003). Another possibility is that a pool of ATP essential for SR Ca^{2+} release depends on the presence of glycogen, and declines despite the presence of ATP and PCr in the bulk solution. There is some support for this idea from the work of Han et al. (1992). They showed that isolated triads contained many of the glycolytic enzymes, and that if triads were incubated with labeled glycolytic precursors (glyceraldhyde-3-phosphate or fructose-1,6-biphosphate), then labeled ATP was produced slowly, suggesting that ATP produced within the triads diffused slowly into the bulk solution. Furthermore, if a phosphatase inhibitor, fluoride, was added, ATP production was enhanced, suggesting phosphorylation of endogenous proteins was normally occurring. Thus, glycogenolysis in or near the triads can produce ATP, which might be essential for SR Ca^{2+} release.

Spatially localized glycogen consumption

This interest in glycogen located near the triads has led to a number of experiments correlating various aspects of muscle function with glycogen distribution. Nielsen et al. (2009) were interested in whether the effects of glycogen depletion were metabolic or non-metabolic. They therefore used the skinned fiber preparation with constant high levels of ATP and PCr and manipulated the muscle glycogen levels by fasting some animals. Muscles were examined by electron microscopy and the volumes of glycogen in the three sites defined above were measured in unfatigued fibers. The rate of early relaxation after a tetanus in unfatigued fiber, thought be to an indicator of the rate of Ca^{2+} uptake by the SR Ca^{2+} pump, showed a correlation with the intermyofibrillar glycogen. The authors suggested that, because of the know association of glycogen and glycolytic enzymes with SR membrane (Xu et al., 1995), SR Ca^{2+} pumps preferential consumed ATP produced glycolytically rather than ATP present in the surrounding solution. A second interesting observation was that the time to fatigue correlated with the amount of glycogen present prior to fatigue in the intramyofibrillar space. They considered two explanations for this observation: action potential propagation in the T-tubule depend on glycolytically produced ATP that powers the Na^+K^+ pump or SR Ca^{2+} release requires glycolytically produced ATP within the triads. Either way, the result is somewhat surprising, as both the T-tubules and the triads lie in the intermyofibrillar rather than the intramyofibrillar space. Nevertheless, the study by Nielsen et al. (2009) is important for demonstrating that various aspects of muscle function seem to associate with different pools of muscle glycogen.

In a further attempt to understand the role of glycogen distribution, Ørtenblad et al. (2011) turned to humans engaged in cross-country skiing. SR Ca^{2+} release rate was determined in SR vesicles obtained from biopsy samples from the arm taken before, immediately after, or 4 h or 24 h after the event. Glycogen was manipulated by giving some subjects glucose by mouth in first 4 h whilst others were denied glucose in this period, whereas all subject received glucose from 4 to 24 h. Glycogen fell to 31% after the exercise and recovered to 59% in the subjects who received glucose but remained at 29% in those who were denied glucose. The SR Ca^{2+} release rate fell to 85% of control after the exercise and recovered to normal in the glucose subjects; conversely there was no recovery of SR Ca^{2+} release in the subjects who had no glucose. Glycogen and SR Ca^{2+} release rate returned to normal in all subjects at 24 h. The distribution of glycogen showed the best correlation between SR Ca^{2+} release rate and intramyofibrillar glycogen. Bearing in mind that the vesicles were in standardized solutions containing ATP, this result again suggests that some relation exists between glycolytically produced ATP in the intramyofibrillar space and SR Ca^{2+} release, even though these sites are not directly opposed (see Fig. 6 B of Ørtenblad et al, 2011).

Subcellular glycogen depletion has also been studied in mouse single fibers in which SR Ca^{2+} release was measured more directly as tetanic $[Ca^{2+}]_i$ (Nielsen et al., 2014). Several different fatiguing protocols were used so that a wide range of final force and $[Ca^{2+}]_i$ values were obtained. In this study, there were correlations between both intramyofibrillar and intermyofibrillar glycogen levels and tetanic $[Ca^{2+}]_i$, but the correlation was somewhat stronger for intramyofibrillar glycogen. In a subgroup of fibers there were substantial reductions in tetanic $[Ca^{2+}]_i$ release and glycogen but without other manifestations of fatigue, i.e., slowing of relaxation. This again suggests that

glycogen depletion is affecting some spatially limited site in the absence of generalized metabolic depletion.

Glycogen consumption by various muscle ATPases

The study by Nielsen et al. (2022) represents a further attempt to understand how the spatial consumption of glycogen is affected by various ATPases in muscle. It is generally thought that in working muscles myosin ATPase consumes around 50–60% of ATP, SR Ca^{2+} ATPase around 40–50%, and Na^+K^+ ATPase around 5–10%. Repeated tetani in isolated rat soleus muscles caused substantial reductions of both intermyofibrillar and intramyofibrillar glycogen. When myosin ATPase was blocked by N-benzene-p-toluene sulphonamide and blebistatin, glycogen consumption in the intramyofibrillar pool was eliminated, whereas consumption of glycogen in the intermyofibrillar space was reduced by about one half. The authors argue that both inter- and intramyofibrillar glycogen pools contribute to myosin ATPase activity whilst the consumption of glycogen when myosin ATPase is blocked is largely due to SR Ca^{2+} ATPase, leading to reduction of only the intermyofibrillar glycogen. One surprising aspect of these results is that intramyofibrillar glycogen was completely depleted when myosin ATPase was active, but most intramyofibrillar glycogen was near the Z-line whereas most myosin ATPase activity would be in the overlap region of the thick and thin filaments (see their Fig. 2 J). This suggests that the stimulus to glycogen breakdown and the provision of ATP must be capable of considerable mobility within the cell.

Nielsen et al. (2022) studied the third ATPase within muscle, the Na^+K^+ ATPase, in resting muscles by increasing the Na^+K^+ ATPase activity with salbutamol and then blocking it with ouabain. The only ouabain-sensitive pool was the intramyofibrillar glycogen and consumption was reduced by about one half. Na^+K^+ pumps are present in the surface membrane and the T-tubules, so this result is surprising given that the intramyofibrillar pool does not co-locate with T-tubules or sarcolemma. The authors point out the similarity of this result to the correlation between SR Ca^{2+} release and intramyofibrillar glycogen (Ørtenblad et al., 2011; Nielsen et al., 2014). They note that glycolytic enzymes are present in the T-tubular membranes, bound to myofilaments and bound to glycogen particles and suggest that breakdown of glycogen provides glycolytic intermediates, which are effectively channeled through the sequential steps of glycolysis (Ovádi and Srere, 1992) toward the ATPases where the ATP is consumed. Intriguing evidence for this possibility is a study of flight muscles in *Drosophila*, which found that failure of the glycolytic enzymes to co-localize led to muscle failure even though the full complement of active glycolytic enzymes was present (Wojtas et al., 1997).

It seems clear that localized depletion of glycogen affects Na^+K^+ pumping/SR Ca^{2+} release/SR Ca^{2+} uptake independent of the generalized metabolic state. In the case of the SR Ca^{2+} pumping, there is co-localized glycogen, co-localized glycolytic enzymes, and blocking either glycogenolysis or glycolysis inhibits pump function (Xu et al., 1995). Thus, is seems that glycolytically produced ATP has preferential access to the pump over the myoplasmic ATP, perhaps due to steric hindrance. For the Na^+K^+ pump located in the T-tubules and SR Ca^{2+} release located in the triads it is less clear how distant glycogen particles provide ATP preferentially over myoplasmic ATP. One possibility is channeling by co-located glycolytic enzymes; another is the diffusional barrier provided by the narrow triadic space, which may restrict diffusion of myoplasmic ATP whilst allowing the glycolytic production of ATP.

How can these issues be further investigated? One problem is that measurement of glycogen in the intramyoplasmic pool is not close to the T-tubules or triadic space and measurement in a space located around the triad seems more appropriate (Stephenson, 2011). Nielsen et al. (2022) accept this criticism and propose that some form of automated pattern recognition could be used. In most hypotheses localized depletion or production of ATP occurs, and imaging of ATP distribution might be helpful. Novel methods to study skinned fibers allow introduction of indicators to the sealed T-tubules, the myoplasm, and the SR (Meizoso-Huesca et al., 2022) so that SR Ca^{2+} release can be determined. Because the triadic space is accessible, it might be possible to introduce glycogenolytic or glycolytic inhibitors into the junctional space to see whether these inhibited Ca^{2+} release, and in a glycogen-depleted preparation it might be possible to restore release with glycolytic intermediates.

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