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ORIGINAL ARTICLE

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Increased muscle glycogen content is associated with increased capacity to respond to T-system depolarisation in mechanically skinned skeletal muscle fibres from the rat

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Abstract The ability of mechanically skinned muscle fibres from the rat to respond to T-system depolarisation was studied in relation to muscle glycogen content. Muscle glycogen was altered by incubating extensor digitorum longus (EDL) muscles in Krebs solution without glucose or in Krebs solution with glucose (10 mM) and insulin (20 $U \cdot l^{-1}$). The glycogen content of muscles stored without glucose was rather stable between 30 and 480 min (11.27±0.39 $\mu mol \cdot g^{-1}$), while the muscles stored with glucose and insulin maintained an elevated and stable level of glycogen (23.48±1.67 $\mu mol \cdot g^{-1}$) between 100 and 360 min. Single mechanically skinned fibres from paired muscles, incubated in either glucose-free Krebs or in Krebs with glucose and insulin, were subjected to cycles of T-system depolarisation–repolarisation in a controlled environment (8 mM ATP, 10 mM creatine phosphate, 1 mM Mg^{2+}, pH 7.10) and the force response was monitored until the force had declined to 50% of the maximum response (50% rundown). Fibres from muscles with a higher glycogen content reached 50% rundown after a larger number of depolarisations and displayed consistently larger average response capacity values, calculated as the sum of the force responses to 50% rundown divided by the maximum Ca^{2+}-activated force response in that fibre. Thus skinned fibres originating from muscles with a higher glycogen content have an increased ability to respond to T-system depolarisation when the effect of metabolite accumulation is minimised and the function of glycogen acting as an energy source is by-passed. This provides direct support to the hypothesis that glycogen has a protective role in maintaining fibre excitability.

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Introduction

Muscle glycogen stores are depleted at fatigue induced by medium- to high-intensity exercise in humans [1, 8] and there is now a general acceptance in the field that glycogen depletion corresponds with fatigue at medium- to high-intensity exercise [5]. Chin and Allen [3] have demonstrated in mouse skeletal muscle that a reduced muscle glycogen concentration is closely associated with reductions in sarcoplasmic reticulum (SR) Ca^{2+} release and force production following fatiguing stimulation. The mechanism by which glycogen depletion is linked to reduced Ca^{2+} release is not known. It has long been assumed that fatigue associated with glycogen depletion is due to the loss of an energy source, although attempts to demonstrate near total ATP depletion at fatigue have failed [2, 7]. In their study of fatigue in humans, Vøllestad et al. [20] report minimal depletion of ATP but substantial depletion of glycogen and creatine phosphate. This suggests that one cannot simply link fatigue to the function of glycogen as an energy store because the ATP pool was not greatly reduced when fatigue had occurred. One possible reason why fatigue is correlated with glycogen breakdown is that when glycogen is low, creatine phosphate is also low and various metabolites such as inorganic phosphate (Pi) are high, and it is well established that Pi has a powerful inhibitory effect on both Ca^{2+} available for release from the sarcoplasmic reticulum [6, 11] and the contractile apparatus [5]. The alternative possibility is that glycogen per se exerts a protective action on excitation–contraction coupling. Evidence for this hypothesis was obtained in a study of muscle fibres from cane toads using the skinned fibre technique [19]. However, there is no direct evidence for this hypothesis in mammalian muscle, for which most data on glycogen function in muscle have been obtained.

Table 1 Composition of solutions (mM except for [Ca^{2+}] which is expressed in pCa=–log[Ca^{2+}]) used in skinned fibre experiments. All solutions also contained 8 mM total ATP, 10 mM creatine phosphate and 90 mM HEPES buffer (pH 7.10±0.01)

	K^+	Na^+	Mg_{TOTAL}	Mg^{2+}	HDTA	$EGTA_{TOTAL}$	pCa
Repriming	127	37	8.5	1	50	0.05	7
T-system depolarising	0	164	8.5	1	50	0.05	7
Low Mg^{2+}	139	37	1.08	0.02	50	0.05	7
Maximally Ca^{2+} activating	126	37	8.12	1	–	50	4.5
Relaxing	127	37	10.3	1	–	50	>9.0

The mechanically skinned fibre technique can bypass the role of glycogen as an energy source and is also capable of eliminating the effects of metabolites; therefore, it can be used to distinguish between these possibilities. In the mechanically skinned fibre the T-system reseals [13] and becomes normally polarised when the preparation is placed in a solution mimicking the internal environment. The sealed T-system can be then depolarised, initiating contractile activation by the usual sequence of events. The effects of glycogen content on fibre excitability can be studied without interference from possible changes in ATP supply and accumulation of metabolites, because the fibre can be bathed in solutions containing high and constant concentrations of ATP (8 mM) and creatine phosphate (10 mM). The aim of this study was to use the mechanically skinned fibre preparation and investigate whether the glycogen level in fast-twitch mammalian muscle fibres is related to the ability to respond to T-system depolarisation in an environment of high energy (ATP) and where any metabolites produced can be washed away from the fibre's internal environment.

Materials and methods

Muscle preparation

Male rats (Long-Evans Hooded), 4–6 months old, were anaesthetised by halothane inhalation and killed by asphyxiation as approved by the Animal Experimentation and Ethics Committee at La Trobe University. Both extensor digitorum longus (EDL) muscles were removed and pinned out under Krebs solution (mM: NaCl 118; KCl 4.75; KH_2PO_4 1.18; $MgSO_4$ 1.18; $NaHCO_3$ 24.8; $CaCl_2$ 2.5; pH 7.4, 23.5±1.5°C) with or without glucose (10 mM) and insulin (20 $U \cdot l^{-1}$) as appropriate. Krebs solutions were constantly bubbled with 95% O_2, 5% CO_2.

Glycogen levels

Whole muscles or muscle strips exposed to Krebs solution with or without glucose (10 mM) and insulin (20 $U \cdot l^{-1}$) were rapidly frozen in capped polypropylene test tubes in 2-methylbutane at –76°C for glycogen analysis.

Glycogen content was determined using the phenol–sulfuric acid method modified from Lo et al. [16]. After freezing, portions of muscles (15–50 mg) were separated from visible connective tissue and weighed into polypropylene test tubes. A 0.5-ml aliquot of a solution of 30% potassium hydroxide saturated with sodium sulfate was added to the sample. Tubes were placed in a boiling water bath for 60 min and then allowed to cool before adding 2 ml of 95% ethanol, vortexing, and leaving on ice for 12 h. Samples were then centrifuged at 840 *g* (Jouan CR412) for 30 min. The supernatants were removed and the remaining precipitate was dissolved in 2 ml water. Aliquots of the glycogen solutions, including standards, were made up to 1.0 ml with water and produced in triplicate. One millilitre of a 5% phenol solution and 5 ml of a sulfuric acid solution were added rapidly in succession to each tube. The tubes were allowed to stand for approximately 20 min before their content was thoroughly mixed and absorbance was read at 490 nm using a Varian Carey spectrophotometer. Controls have been run where known amounts of glycogen were added to a test tube instead of portions of muscle and then extracted by the same procedure as for the muscle samples. These results show that a 90±5.5% yield is achieved using this method over the range of glycogen concentrations measured in all samples. No correction has been applied to our results.

Single fibre preparation

Following a suitable incubation (between 2 and 6 h) muscles were removed from the Krebs solutions, blotted thoroughly on filter paper and placed under paraffin oil.

Single fibres were dissected and mechanically skinned under oil from each muscle using fine forceps as previously described [15]. While under oil, the skinned fibres were attached to a force transducer (AME 875, Horten, Norway) using a braided silk loop (Deknatel 10.0) and clamped at the other end in the jaws of a fine pair of forceps. Fibres were then stretched by 20% of their slack length, which is optimal for recording force production within any section of the preparation. In mechanically skinned fibres the T-system reseals [13] and becomes normally polarised when the preparation is placed in a K^+-based solution (see repriming solution in Table 1) mimicking the internal environment in a muscle fibre at rest [14, 15]. By transferring the fibre into a similar solution in which all K^+ is replaced by Na^+ (see T-system depolarising solution in Table 1), the T-system can be rapidly depolarised resulting in a force response [14, 15].

The preparation was allowed to equilibrate for 2 min in the K^+-based repriming solution before being subjected to successive cycles of T-system depolarisation–repolarisation. The fibre was allowed 1 min in the repriming solution between depolarisations. The cycle was terminated when the force response declined to 50% of its highest value, i.e. at 50% rundown. At the end of the experiment the fibres were transferred to a low-Mg^{2+} solution (see Table 1) to release Ca^{2+} from the sarcoplasmic reticulum (SR), to ensure that the decline in the T-system depolarisation-induced force response was not due to Ca^{2+} depletion in the SR. All experiments were performed at 23.5±1.5°C.

All results are presented as mean ±SEM (*n*).

Results

Muscle glycogen

Muscle glycogen can be manipulated in isolated EDL muscles of the rat by simply incubating the muscles in Krebs solutions with or without glucose and insulin. The glycogen content did not change in whole EDL

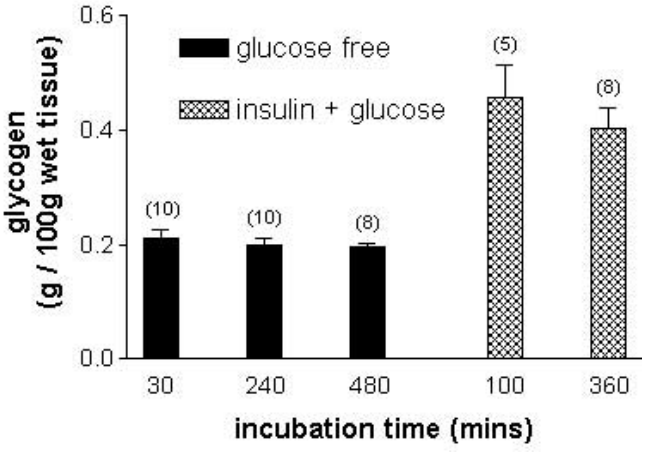

Fig. 1 Glycogen levels in muscles frozen following various lengths of time in bubbled (95% O_2; 5% CO_2) glucose-free Krebs solution and in Krebs solution containing insulin (20 U·l^{-1}) and glucose (10 mM). The *numbers* above the *bars* indicate the number of muscles used

muscles placed, at rest, in glucose-free Krebs solution for a period of between 30 min and 8 h after dissection (Fig. 1). After 30 min the glycogen content was 11.71± 0.72 µmol·g^{-1} (*n*=10) and there was no significant change in glycogen content after 8 h (10.88±0.33 µmol·g^{-1}, *n*=8). When whole muscles were left idle in Krebs solution containing insulin (20 U·l^{-1}) and glucose (10 mM) for 100 min, the glycogen content was considerably higher (25.31±2.78 µmol·g^{-1}, *n*=5) and was not significantly different after 6 h (22.31± 2.00 µmol·g^{-1}, *n*=8). Since it is known that glycogenesis is stimulated by activity [5], we also tried to further increase the glycogen content by placing muscle strips in Krebs solution containing glucose and insulin with stimulation. The stimulation protocols consisted of 30 min stimulation at 0.1 Hz, 1 ms pulse duration or 30 min stimulation at 0.1 Hz, 1 ms pulse duration followed by 60 min of stimulation at 30 Hz with a 1:20 duty cycle. The level of glycogen attained after 30 min of stimulation at 0.1 Hz was 16.93±1.61 µmol·g^{-1} (*n*=6) and after 30 min stimulation at 0.1 Hz followed by 60 min of stimulation at 30 Hz with a 1:20 duty cycle, the glycogen level was 27.37±1.94 µmol·g^{-1} (*n*=12). The latter protocol resulted in a higher level of glycogen, but it was not significantly different from that of muscles kept idle for the same length of time in the Krebs solution containing insulin and glucose. Since there was no further increase in glycogen content with stimulation, which would have also introduced complicating factors due to fatigue, it was decided to use the simple incubations in either glucose-free Krebs solution or Krebs solution containing insulin and glucose which gave stable and different glycogen levels over the period of interest. Thus incubation in Krebs solution containing insulin and glucose for 6 h resulted in a 2.13±0.18 (*n*=8) times increase in the muscle glycogen content when compared to that of paired muscles from the same animals incubated in glucose-free Krebs solution. Pairs of EDL muscles from the same rat, where one muscle was treated by incubation in Krebs solution containing insulin and glucose and the other in glucose-free Krebs solution, were used to provide muscle fibres from two distinctly different populations with respect to glycogen content.

Rundown and fibre response capacity

As indicated in Materials and methods, the mechanically skinned fibre preparation can be used to study the entire sequence of events in excitation–contraction coupling, by first polarising and then depolarising the sealed T-system [14, 15]. Typical responses to T-system depolarisation in fibres from the two EDL muscles of the same animal are shown in Fig. 2. The trace in Fig. 2A is from a fibre dissected from the muscle incubated for 120 min in glucose-free Krebs solution and the trace in Fig. 2B is from a fibre dissected from a muscle incubated in Krebs solution containing insulin and glucose for 165 min. As can be seen in Fig. 2, the depolarisation-induced forces decreased to 50% (50% rundown) after 17 depolarisations in Fig. 2A and after 25 depolarisations in Fig. 2B, while the maximum level of activation of these two fibres was not different.

The decrease in the depolarisation-induced force response at "rundown" is not due to depletion of Ca^{2+} in the SR, because the SR contained sufficient Ca^{2+} to produce near-maximal force responses when Ca^{2+} was released directly from the SR by lowering the Mg^{2+} concentration in solution, as shown in the second last responses on each trace (Fig. 2). Also the decrease in the force response at rundown is not due to impairment of the regulatory system and contractile apparatus as evidenced by the last response on each trace in Fig. 2, which is due to direct activation of the contractile apparatus by placing the fibre in a strongly buffered Ca^{2+} solution (pCa 4.5) and indicates the maximum Ca^{2+}-activated response that can be elicited in the fibre at the end of the experiment. Therefore, events preceding the opening of the Ca^{2+} channels must be causing the decrease in the T-system depolarisation-induced responses.

In this study we used the fibre response capacity as an indication of overall fibre excitability. The fibre response capacity is calculated as the sum of the amplitudes of the force responses to rundown divided by the maximum Ca^{2+}-activated force response measured at the end of the experiment. This measurement indicates the capacity of a fibre to repeatedly develop maximum Ca^{2+}-activated force when the T-system is depolarised and is a more encompassing indicator than time to 50% rundown or peak force. The average fibre response capacity for fibres from all muscles incubated in the absence of glucose and insulin was 9.7±2.3 (*n*=18) and the fibre response capacity of the low-glycogen fibre in Fig. 2A was 12. The fibre from the high-glycogen muscle in Fig. 2B had a fibre response capacity of 21 and the average fibre response capacity for fibres from all muscles incubated in insulin and glucose was 10.6±1.3 (*n*=23).

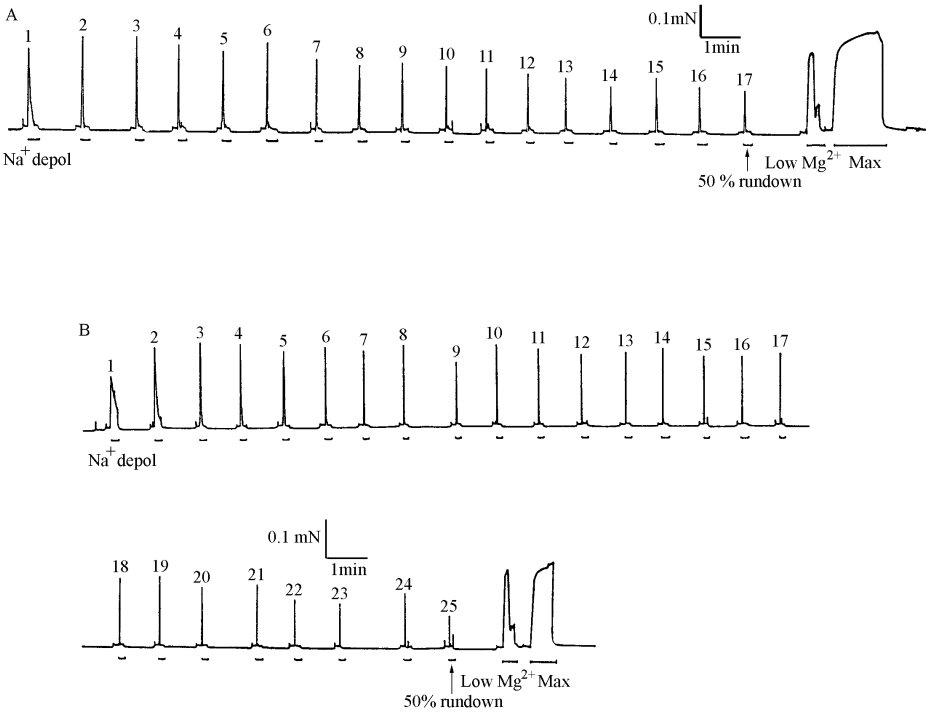

Fig. 2A, B Force responses induced by depolarisations of the sealed T-system in mechanically skinned muscle fibres from the rat. **A** Fibre from a muscle treated with glucose-free Krebs solution. The fibre response capacity (see text for definition) of this fibre is 12. **B** Fibre from a muscle treated with Krebs solution containing insulin and glucose. The fibre response capacity of this fibre is 21. The force responses obtained in low-Mg^{2+} solution at the end of the experiments indicate that the sarcoplasmic reticulum was not depleted of Ca^{2+} when the depolarisation-induced responses decreased to less than 50% of their respective maximum response. The last force response in **A** and **B** represents the maximum Ca^{2+}-activated force in that particular fibre and was used to estimate the fibre response capacity. Fibre dimensions (diameter, slack length): in **A** 17.5 µm, 2 mm; in **B** 20 µm; 2 mm

There was a large variation between average values for fibre response capacity from muscles of different rats following identical incubations in either glucose-free Krebs solution or Krebs solution containing insulin and glucose. In order to reduce the possibility that factors other than glycogen affect the results, the average fibre response capacity for muscles incubated in glucose-free Krebs solution was compared with that of the contralateral muscle incubated in the presence of glucose and insulin for each individual rat.

Results for individual rats were recorded as "+" or "–" depending on whether the muscles treated with insulin and glucose yielded fibres with a higher response capacity than fibres from the muscles incubated in glucose-free Krebs solution. Out of nine experiments, only once did a fibre from a low-glycogen muscle result in a higher fibre response capacity than the average response of fibres from the high-glycogen muscle for the same animal. The proportion of animals demonstrating a positive effect of higher muscle glycogen on fibre response capacity was significantly greater than that expected by chance (Sign Test, $P<0.03$). On average, the increase in fibre response capacity in muscles incubated in the presence of glucose and insulin compared with that in paired muscles from the same rat which were not incubated in glucose and insulin was by a factor of 1.88±0.43 ($n=9$, $P<0.05$). We also considered the time or number of contractions to 50% rundown and the maximal level of depolarisation-induced activation expressed as a percentage of maximum Ca^{2+}-activated force in fibres from contralateral muscles incubated in the absence and in the presence of glucose and insulin. The number of contractions to 50% rundown were significantly greater for fibres from muscles with a higher glycogen content compared to fibres from contralateral control muscles (Sign Test, $P<0.03$). However, there was no significant difference between the maximal level of depolarisation-induced activation and glycogen content (Sign Test, $P>0.25$).

Discussion

This study demonstrates that the muscle glycogen content can be successfully manipulated by incubating whole muscles in Krebs solution containing or not containing glucose (10 mM) and insulin (20 $U \cdot l^{-1}$), achieving stable levels of muscle glycogen content which differ by a factor of 2.13±0.18 ($n=8$) for up to 6 h. This study also demonstrates that fibres from muscles with a higher glycogen content have a higher fibre response capacity to T-system depolarisation-induced responses. The concentration of glycogen in EDL muscles measured in this study is at the low end of results reported by others (e.g. [9, 10, 18]). This may be because of differences between strains of rats and feeding. Importantly, when the muscle was incubated in the presence of glucose and insulin the glycogen concentration was considerably higher and remained stable for the duration of the experiments used in this study.

Fibres from muscles with the higher glycogen content were better able to respond to successive T-system depolarisations, producing more force before running down, as indicated by the significantly greater fibre response capacity and number of contractions to 50% rundown. Importantly the increase in fibre response capacity in muscles incubated in the presence of glucose and in-

sulin (high glycogen) compared with that in paired muscles from the same rat incubated in the absence of glucose and insulin (by a factor of 1.88±0.43, n=9) was similar to the increase in glycogen seen in paired muscles from rest to 6 h of incubation with glucose and insulin (2.13±0.18, n=8). A similar observation was also made with skinned muscle fibres from the toad [19]. This shows that a higher glycogen content is somehow related to the better preservation of the integrity of events preceding Ca^{2+} release from the SR.

One should bear in mind that the range of fibre glycogen content in a muscle is rather broad [19] and, therefore, it is possible that particular individual fibres from the muscle incubated in glucose and insulin may have a lower glycogen content than individual fibres from the contralateral muscle incubated in the absence of glucose and insulin. This explains why on one out of nine occasions the average response capacity of fibres from the muscle with a lower glycogen content was greater than that of fibres from the muscle with a higher glycogen content.

Although ATP has not always been demonstrated to be depleted at fatigue [2, 7], it has been suggested that microenvironments may exist within the fibre, where ATP could become limiting in the region of the SR while remaining high in the rest of the fibre [21] or metabolites may accumulate which may impair function [17]. In this study, the skinned fibre experiments were performed in the presence of an essentially infinite pool of high ATP and creatine phosphate, by-passing the function of glycogen as an energy source and ensuring that local ATP was not limiting at the time when the T-system was depolarised. Therefore, we can conclude that the fibre's enhanced capacity to respond to T-system depolarisation associated with a higher glycogen content is not related to the function of glycogen as an energy source in muscle.

A non-energy-related mechanism by which glycogen can be involved has also been proposed, whereby glycogen acts as a Pi sink [19], preventing Pi accumulation with the breakdown of glycogen and the formation of glucose-1-phosphate. If a cell contains 20 mM glycogen it has the potential to remove 20 mM Pi without any ATP resynthesis. However, this mechanism cannot explain these results, because the sheer volume of the solutions used compared to the volume of the skinned fibre (ratio $\cong 10^6$) would have ensured that all metabolites, including phosphate, would have been washed away from the site of generation between responses.

Mechanisms that could explain all our results involve glycogen particles associated with the SR. These particles are known to contain a number of potentially active molecules such as phosphatases and kinases [4] which can be liberated when the glycogen particles become smaller than a certain size. These agents may directly interfere with the excitation–contraction coupling. Alternatively, the glycogen particles may anchor molecules that are locally important for excitation–contraction coupling as suggested for the anuran muscle fibres [19]. Therefore, when the particle size is reduced, these molecules would be redistributed within the cell and become unavailable at specific sites. Such mechanisms would explain the longer time to 50% rundown because it would take longer for the glycogen particles to reach the minimum critical size if the initial glycogen concentration in the fibre was larger. It would also explain why the maximum depolarisation-induced response is not significantly different when the initial glycogen is lower, because it is only when the glycogen is below threshold that an impairment would be expected. This is consistent with human research, where running speed was not altered by the initial level of muscle glycogen [12]. This study provides direct support for the idea that glycogen has a non-energy-dependent protective role in maintaining fibre excitability in mammalian muscle.

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References

1. Ahlborg B, Bergström J, Ekelund L-G, Hultman E (1967) Muscle glycogen and muscle electrolytes during prolonged exercise. Acta Physiol Scand 70:129–142
2. Byrd SK, McCutcheon LJ, Hodgson DR, Gollnick PD (1989) Altered sarcoplasmic reticulum function after high-intensity exercise. J Appl Physiol 67:2072–2077
3. Chin ER, Allen DG (1997) Effects of reduced muscle glycogen concentration on force, Ca^{2+} release and contractile protein function in intact mouse skeletal muscle. J Physiol (Lond) 498:17–29
4. Entman ML, Keslensky SS, Chu A, Van Winkle WB (1980) The sarcoplasmic reticulum–glycogenolytic complex in mammalian fast-twitch skeletal muscle. Proposed in vitro counterpart of the contraction-activated glycogenolytic pool. J Biol Chem 255:6245–6252
5. Fitts RH (1994) Cellular mechanisms of muscle fatigue. Physiol Rev 74:49–94
6. Fryer MW, Owen VJ, Lamb GD, Stephenson GD (1995) Effects of creatine phosphate and P(i) on Ca^{2+} movements and tension development in rat skinned skeletal muscle fibres. J Physiol (Lond) 482:23–40
7. Green HJ (1991) How important is endogenous muscle glycogen to fatigue in prolonged exercise? Can J Physiol Pharmacol 69:290–297
8. Hermansen L, Hultman E, Saltin B (1967) Muscle glycogen during prolonged severe exercise. Acta Physiol Scand 71:129–139
9. Holness MJ, Howard RM, Sugden MC (1992) Glucose utilization and disposition by skeletal muscle during unrestricted feeding. Biochem J 286:395–398
10. Hutber CA, Bonen A (1989) Glycogenesis in muscle and liver during exercise. J Appl Physiol 66:2811–2817
11. Kabbara AA, Allen DG (1999) The role of calcium stores in fatigue of isolated single muscle fibres from the cane toad. J Physiol (Lond) 519:169–176
12. Karlsson J, Saltin B (1971) Diet, muscle glycogen, and endurance performance. J Appl Physiol 31:203–206
13. Lamb GD, Junankar PR, Stephenson DG (1995) Raised intracellular $[Ca^{2+}]$ abolishes excitation–contraction coupling in skeletal muscle fibres of rat and toad. J Physiol (Lond) 489: 349–362

14. Lamb GD, Stephenson DG (1990) Control of calcium release and the effect of ryanodine in skinned muscle fibres of the toad. J Physiol (Lond) 423:519–542
15. Lamb GD, Stephenson DG (1994) Effects of intracellular pH and [Mg^{2+}] on excitation–contraction coupling in skeletal muscle fibres of the rat. J Physiol (Lond) 478:331–339
16. Lo S, Russell JC, Taylor AW (1970) Determination of glycogen in small tissue samples. J Appl Physiol 28:234–236
17. Nagesser AS, Van Der Laarse WJ, Elzinga G (1993) ATP formation and ATP hydrolysis during fatiguing, intermittent stimulation of different types of single muscle fibres from *Xenopus laevis*. J Muscle Res Cell Motil 14:608–618
18. Soefrankova A (1975) Glycogen metabolism in white and red muscle of normal and diabetic rats. The glycogen concentration and synthesis from glucose. Phys Bohemo 24:509–514
19. Stephenson DG, Nguyen LT, Stephenson GMM (1999) Glycogen content and excitation–contraction coupling in mechanically skinned muscle fibres of the cane toad. J Physiol (Lond) 519:177–187
20. Vøllestad NK, Sejersted OM, Bahr R, Woods JJ, Bigland-Ritchie B (1988) Motor drive and metabolic responses during repeated submaximal contractions in humans. J Appl Physiol 64:1421–1427
21. Westerblad H, Allen DG, Bruton JD, Andrade FH, Lannergren J (1998) Mechanisms underlying the reduction of isometric force in skeletal muscle fatigue. Acta Physiol Scand 162:253–260