

Skeletal muscle glycogen content and particle size of distinct subcellular localizations in the recovery period after a high-level soccer match

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Abstract Whole muscle glycogen levels remain low for a prolonged period following a soccer match. The present study was conducted to investigate how this relates to glycogen content and particle size in distinct subcellular localizations. Seven high-level male soccer players had a vastus lateralis muscle biopsy collected immediately after and 24, 48, 72 and 120 h after a competitive soccer match. Transmission electron microscopy was used to estimate the subcellular distribution of glycogen and individual particle size. During the first day of recovery, glycogen content

increased by ~60% in all subcellular localizations, but during the subsequent second day of recovery glycogen content located within the myofibrils (Intramyofibrillar glycogen, a minor deposition constituting 10–15% of total glycogen) did not increase further compared with an increase in subsarcolemmal glycogen (−7 vs. +25%, respectively, $P = 0.047$). Conversely, from the second to the fifth day of recovery, glycogen content increased (53%) within the myofibrils compared to no change in subsarcolemmal or intermyofibrillar glycogen ($P < 0.005$). Independent of location, increment in particle size preceded increment in number of particles. Intriguingly, average particle size decreased; however, in the period from 3 to 5 days after the match. These findings suggest that glycogen storage in skeletal muscle is influenced by subcellular localization-specific mechanisms, which account for an increase in number of glycogen particles located within the myofibrils in the period from 2 to 5 days after the soccer match.

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Introduction

Biochemical measures of cell glycogen concentrations have demonstrated an inability to continue prolonged high-intensity exercise when glycogen reserves are depleted (Hermansen et al. 1967). Additionally, there is a strong correlation of endurance capacity with pre-exercise glycogen level (Bergström et al. 1967). Recently, however, the role of glycogen metabolic compartmentalization and individual glycogen particles in muscle function have

gained increased attention (Graham et al. 2010; Marchand et al. 2002; Prats et al. 2011), implying that the traditional biochemical measure of whole cell glycogen concentrations may overlook important aspects of glycogen metabolism. Indeed, detailed transmission electron microscopy (TEM) analysis of the subcellular distribution of glycogen has shown that utilization of glycogen during exercise is dependent on localization characterized by a greater utilization of glycogen located within the myofibrils (intra-myofibrillar (Intra) glycogen) compared with depositions located between the myofibrils (intermyofibrillar (IMF) glycogen) and in the subsarcolemmal region (SS glycogen) (Nielsen et al. 2011). Intriguingly, we have previously shown that low amount of Intra glycogen decreases the fatigue resistance capacity of mechanically skinned rat fibres (Nielsen et al. 2009) and impairs the Ca^{2+} release rate of isolated human sarcoplasmic reticulum vesicles (Ørtenblad et al. 2011) suggesting an explanatory link between compartmentalized glycogen depletion and muscle fatigue. Thus, the specific subcellular localization of glycogen is important for key events in excitation–contraction coupling and to fully understand the role and regulation of glycogen metabolism it is important to take into account the subcellular organization.

In the recovery period after glycogen-depleting exercise, the resynthesis of glycogen has been suggested to be dependent on localization. Marchand et al. (2007) showed that Intra glycogen was preferentially resynthesised during the initial 4 of 48 h of recovery from exercise and it was suggested that the initial increase in glycogen content was due to an increase in particle number and later by an increase in particle size. Interestingly, this difference in resynthesis rate between localizations of glycogen is supported by the translocation of glycogen synthase to glycogen particles located within the myofibrils in response to exercise (Prats et al. 2009). Conversely, another study did not show any difference between subcellular localizations in the initial 4 h of glycogen resynthesis after exercise. (Nielsen et al. 2011). Thus, knowledge of how and why the resynthesis of glycogen after exercise is compartmentalized remains largely elusive.

Interestingly, in some conditions the resynthesis of glycogen is impaired as seen after severe eccentric exercise (Doyle et al. 1993; Widrick et al. 1992) or a soccer match (Bangsbo et al. 2006; Jacobs et al. 1982). Soccer is an intermittent sport consisting of energy demanding activities involving eccentric contractions as repeated high-intensity running, sprinting, short accelerations, tackling and jumping (Bangsbo et al. 2006). Muscle glycogen content of elite soccer players was only 50% of pre-match level 2 days after a match (Jacobs et al. 1982) and carbohydrate enriched diet could not fulfil this gap (Bangsbo et al. 2006). Therefore, the aim of the present study was to estimate the

glycogen content and size of individual glycogen particles with respect to subcellular localization immediately and 24, 48, 72 and 120 h after a soccer match. The hypothesis was that the increase in glycogen content during recovery would be subcellular localization-dependent and that the increase in glycogen content during recovery is explained by an initial increase in particle number and subsequently an increase in particle size.

Materials and methods

Subjects

Seven first and second division Danish soccer players participated in the study, which was a part of a larger study (Krustrup et al. 2011). Their mean (\pm SD) age, height, weight, fat percentage and maximum oxygen uptake ($\dot{V}\text{O}_{2\text{max}}$) were 27 ± 3 years, 180 ± 7 cm, 80 ± 6 kg, $14 \pm 5\%$, and 57 ± 7 ml $\text{kg}^{-1} \text{min}^{-1}$, respectively. The project was approved by the Regional Ethical Review Board in Copenhagen, Denmark. Before giving their written informed consent to participate, the subjects were fully informed about the project, the risks involved, discomfort associated with the experiment, and reminded that they could withdraw from the project at any time.

Experimental protocol

All the players (2 defenders, 2 central midfielders, 2 lateral midfielders and 1 striker) completed a competitive soccer match (2 \times 45 min of duration) in the last part of the competitive season. The match started early in the afternoon (2–4 p.m.). Prior to the match the players refrained from strenuous exercise and intake of alcohol for 48 h, as well as from tobacco and caffeine for 12 h. Throughout the initial 72 h of the recovery period the players consumed a controlled diet (see dietary intake) and were instructed to abstain from any prolonged or strenuous activities during the entire 5-day recovery period. Biopsies were taken from vastus lateralis of the right leg immediately after as well as 24, 48, 72 and 120 h after the match. In the recovery period after the match, all the biopsies were taken within 2–3 cm from the position of the biopsy obtained immediately after the match. Six of the seven players had the right leg as the dominant leg. The muscle specimen was dried on a filter paper placed on a glass plate cooled on ice. After the removal of visible connective tissue and fat, a portion of the muscle specimen was prepared for transmission electron microscopy (see below). Due to multiple purposes of the project, 4 out of 35 muscle biopsy samples did not have adequate size to prioritize transmission electron microscopy analysis (1 at 24 and 48 h, and 2 at 120 h).

Dietary intake

The players recorded their diet 3 days prior to the match. During this period the intake of carbohydrates, proteins and fats were 5.0, 1.6 and 1.1 g kg⁻¹ body mass day⁻¹, respectively, corresponding to 55, 18 and 26% of energy intake (En%), respectively. After the match, the subjects received their diet for the following 3 days, consisting of 9.5, 2.7 and 0.8 g kg⁻¹ body mass day⁻¹ of carbohydrates, proteins and fats, respectively, corresponding to 67, 20 and 13 En%, respectively. The diet was supplemented with 0.20, 0.25, and 0.20 g creatine (Cr) per kg⁻¹ body mass on the 1st, 2nd and 3rd day after the match, respectively.

Maximal voluntary contraction force

Maximal voluntary contraction (MVC) force was evaluated as previously reported (Krustrup et al. 2011). Briefly, players were placed in a custom-built chair, adjusted for a knee angle of 90°. Trunk and hips were fixed to the chair and the experimental leg was fixed in a metal-cuff connected to a strain-gauge. After 10 min of warm-up (80 W on a cycle ergometer), each player performed three knee extensions at maximal voluntary effort separated by 45 s. Each MVC was sustained for more than 3 s and strong verbal encouragement was given during every contraction to promote maximal voluntary effort.

Transmission electron microscopy (TEM)

Muscle biopsy specimens were prepared for enhanced glycogen visualization by transmission electron microscopy as described previously (Nielsen et al. 2010a, b). In order to obtain as many fibres as possible, the ultra-thin sections were cut using a Leica Ultracut UCT ultramicrotome (ROWAKO AB, Vendelsö, Sweden) in three depths separated by 150 µm. The sections were contrasted with uranyl acetate and lead citrate, and examined and photographed in a pre-calibrated Philips EM 208 electron microscope and a Megaview III FW camera (FEI Company, Eindhoven, Netherlands). An average of nine (range 7–13) longitudinal oriented fibres from each biopsy were photographed at 40,000× magnifications in a randomized systematic order, including 12 images from the subsarcolemmal region and 12 images from the myofibrillar region, as previously described (Nielsen et al. 2010b).

Subcellular glycogen distribution

The estimation of glycogen content in distinct subcellular localizations was performed as described in detail elsewhere (Nielsen et al. 2010b, 2011). In brief, three localizations were defined (Fig. 2): (1) the intermyofibrillar

(IMF) space; (2) the intramyofibrillar (Intra) space (this is the space within the myofibrils along with the contractile filaments); and (3) the subsarcolemmal (SS) space (Fridén et al. 1985, 1989; Sjöström et al. 1982b). The glycogen volume fraction of the IMF, Intra and SS localizations are expressed relative to the myofibrillar space, intramyofibrillar space and fibre surface area, respectively. The glycogen volume fraction was estimated by point counting (Weibel 1980) and the average glycogen profile diameter was directly measured using iTEM (FEI Company, The Netherlands).

The TEM derived total glycogen volume percentage, and the relative distribution of glycogen was estimated as previously described (Nielsen et al. 2011). This TEM derived total glycogen volume density correlated with the biochemical determined glycogen concentration ($r = 0.66$, $P < 0.001$) and there was significant concordance ($r_c = 0.52$, $P < 0.001$) between the two estimates as evaluated by Lin's concordance correlation coefficient (r_c) (Lin 1989). The images from the different time points and fibre types were distributed equally between two investigators and all of the analyses were blinded to the investigators. The variation in the parameters between images was used to estimate a coefficient of error (estCE) as proposed for stereological ratio-estimates by Howard and Reed (2005). The estCE were 0.15, 0.19 and 0.22 in IMF, Intra and SS glycogen, respectively.

Fibre typing

All the fibres were classified as type I or II, based on a combination of IMF mitochondrial volume and Z-line width as described elsewhere (Nielsen et al. 2011; Sjöström et al. 1982a). The procedure generated two distinct groups of fibres, where the IMF mitochondria volume fraction and Z line width were (mean ± SD) 0.10 ± 0.03 and 78 ± 8 nm for type I fibres and 0.05 ± 0.02 and 68 ± 5 nm for type II fibres, respectively. Thus, when fibres are referred to as type I or II throughout the present study it refers to clear distinctions in mitochondria content and Z line width, the combination of which have previously been shown to relate to myofibrillar ATPase properties (Sjöström et al. 1982a).

Statistics

Statistical analyses were performed using STATA 10.1 (StataCorp. 2007. Stata Statistical Software: Release 10. College Station, TX: StataCorp LP). All interactions or main effects were tested using a linear mixed-effects model with subjects and fibre as random effects and with time, fibre type and location as fixed effects. Model assumptions about normal distribution of residuals and homogeneity of

variance were satisfied by transformation of data. Values are presented as geometric means and 95% confidence intervals, unless stated otherwise. Significance level was set at $\alpha = 0.05$.

Results

Total glycogen volume percentage

Total glycogen volume percentage was 1.7 (1.5–2.0) immediately after the match, increased by 59% to 2.7 (2.4–3.0) after 24 h of recovery ($P < 0.001$), and further by 19% to 3.2 (3.0–3.5) after 48 h of recovery ($P = 0.02$ vs. 24 h). The values obtained after 72 and 120 h of recovery were not different from 48 h [3.2 (3.0–3.5) and 3.3

(2.8–3.8), respectively]. This pattern of TEM estimated total muscle glycogen content in the recovery period after the soccer match is in accordance with the biochemical measure of muscle glycogen concentration presented in a companion paper (Krustrup et al. 2011).

Subcellular glycogen localization

The transmission electron microscopy analysis of the subcellular distribution of glycogen in the recovery period after the soccer match revealed that the measure of total glycogen content was covering distinct subfractions, which had differential temporal associations with duration of recovery (Fig. 1a–c): The initial increase in glycogen content of 54% in the IMF location, 69% in the Intra location, and 59% in the SS location during the 1st day was

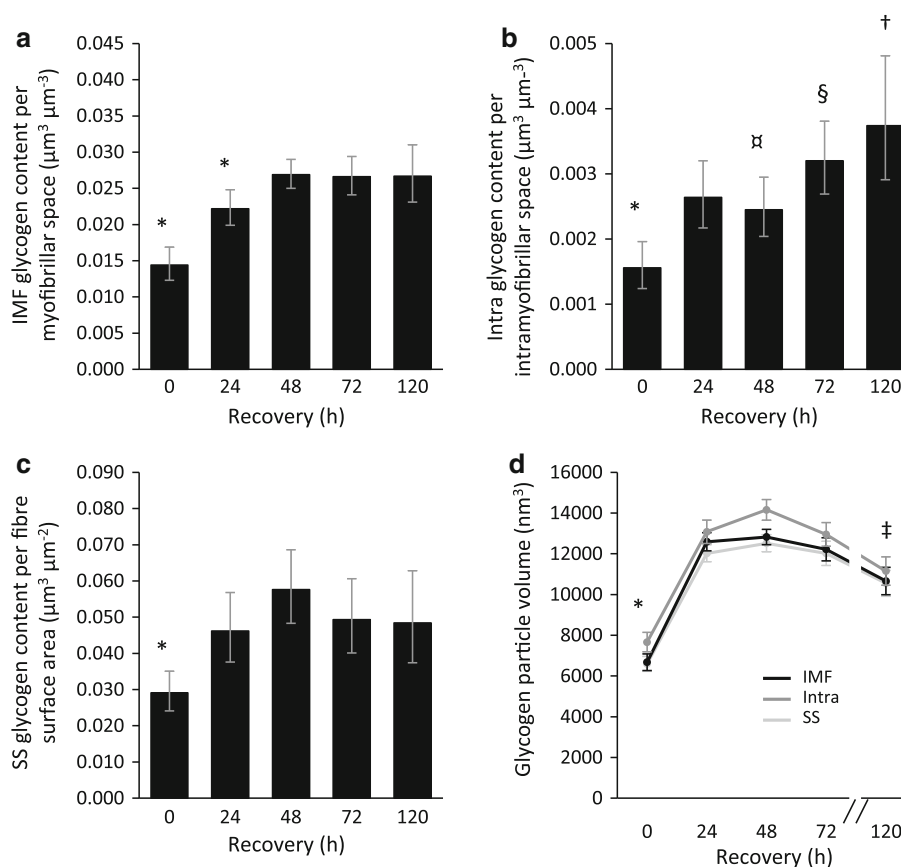


Fig. 1 Muscle glycogen content and particle size of three subcellular localizations in the recovery period after a high-level soccer match. In **a**, **b**, **c**, TEM analysis was performed to estimate the content of three spatial distinct subfractions of glycogen: Intermyoibrillar (IMF) glycogen (**a**); intramyofibrillar (Intra) glycogen (**b**); and subsarcolemmal (SS) glycogen (**c**). Values represent geometric means and horizontal bars represent 95% confidence interval. Asterisk denotes different from all other time points ($P < 0.05$); currency sign denotes that Intra glycogen is different from SS glycogen compared with their respective 24 h values (two-way interaction, $P = 0.047$); section

symbol denotes that Intra glycogen is different from SS glycogen compared with their respective 48 h values (two-way interaction, $P = 0.004$); dagger symbol denotes that Intra glycogen is different from SS and IMF glycogen compared with their respective 48 h values (two-way interaction, $P < 0.005$). In **d**, the size of glycogen particles was estimated in three subcellular localizations. Values are means \pm SE. Asterisk denotes different from all other time points ($P < 0.001$); double dagger denotes different from 48 h ($P = 0.008$). IMF intermyofibrillar, Intra intramyofibrillar, SS subsarcolemmal

Table 1 The relative distribution of glycogen in three subcellular localizations in the recovery period after a high-level soccer match

	Hours of recovery				
	0	24	48	72	120
IMF	85 (82–87)	83 (79–87)	84 (79–88)	83 (79–88)	83 (77–88)
Intra	7 (5–10)	7 (5–10)	6 (4–8)*	8 (5–10)†	10 (5–13)†
SS	8 (6–11)	9 (6–11)	9 (7–12)	7 (5–13)	8 (5–11)

Values are percentages of total glycogen and expressed as medians with interquartile range in parentheses

IMF Intermyofibrillar, Intra Intramyofibrillar, SS subsarcolemmal

* Denotes different from 24 h ($P = 0.039$)

† Denotes different from 48 h ($P < 0.02$)

not different between subfractions ($P > 0.48$; Fig. 1a–c), but during the subsequent 2nd day of recovery Intra glycogen remained unchanged, while SS glycogen continued to increase (25%, $P = 0.047$) and IMF glycogen tended to increase (21%, $P = 0.067$). This difference between subcellular localizations mediated a decrease from day 2 to 3 in the relative contribution of Intra glycogen to total glycogen from 7 to 6% (Table 1).

On the 3rd day of recovery this pattern turned around, so that an increase of 31% in Intra glycogen was observed compared with a small decrease in SS glycogen ($P = 0.004$ vs. Intra glycogen) and unchanged IMF glycogen ($P = 0.056$ vs. Intra glycogen).

At day 5 after the soccer match, the difference between subfractions became more pronounced (although not different from day 3) showing that Intra glycogen had increased more than SS glycogen compared to immediately after the soccer match (140 vs. 66%, $P = 0.016$) and after 1 day (42 vs. 5%, $P = 0.049$) and 2 days of recovery (53 vs. –16%, $P < 0.001$), and more than IMF glycogen compared with day 2 (53 vs. –1%, $P = 0.005$) (Fig. 1a–c). The players' individual values at the different time points can be found in electronic supplementary material Fig. 2. Representative transmission electron micrographs of Intra glycogen are shown in Fig. 3. The difference between subfractions at day 5 after the soccer match, combined with the lack of increase in Intra glycogen during day 2, increased the relative contribution of Intra glycogen to total glycogen from 6% at day 2–8 and 10% at day 3–5, respectively (Table 1).

Discriminating between fibre types revealed that type I fibres had lower glycogen content (30–39%) immediately after the match than found in type II fibres; however, this was not related to the subcellular localization ($P = 0.76$) and the two fibre types displayed no difference in localization-dependent resynthesis of glycogen ($P = 0.76$).

Glycogen particle size

The average glycogen particle volume increased by 80% during the first 24 h of recovery and did not change any

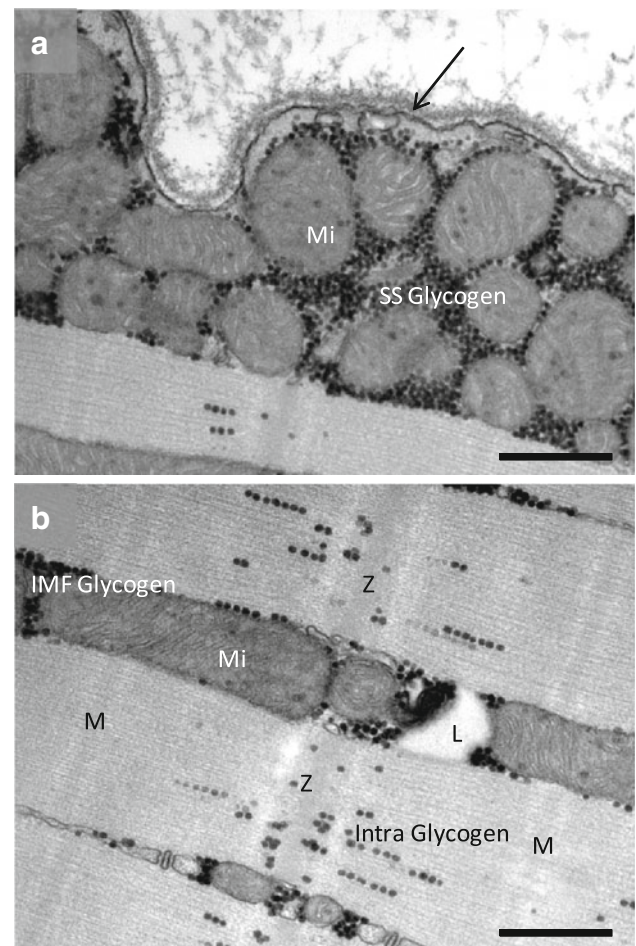


Fig. 2 TEM images illustrating three distinct subcellular localizations of muscle Glycogen. In **a** TEM image of the subsarcolemmal space illustrating the localization of SS glycogen (black dots) in conjunction with mitochondria (Mi) deposited just beneath the sarcolemma (arrow) ($\times 40,000$ original magnification, scale bar: 500 nm). In **b** TEM image of the myofibrillar space illustrating the localization of IMF and Intra glycogen (black dots). Mi mitochondria, M M-band, Z Z-line, ($\times 40,000$ original magnification, scale bar: 500 nm). Both images are from a biopsy collected 24 h after the high-level soccer match

further during the subsequent 2 days (Fig. 1d). Conversely, the particle volume was 18% smaller on the 5th day compared with the second day after the soccer match

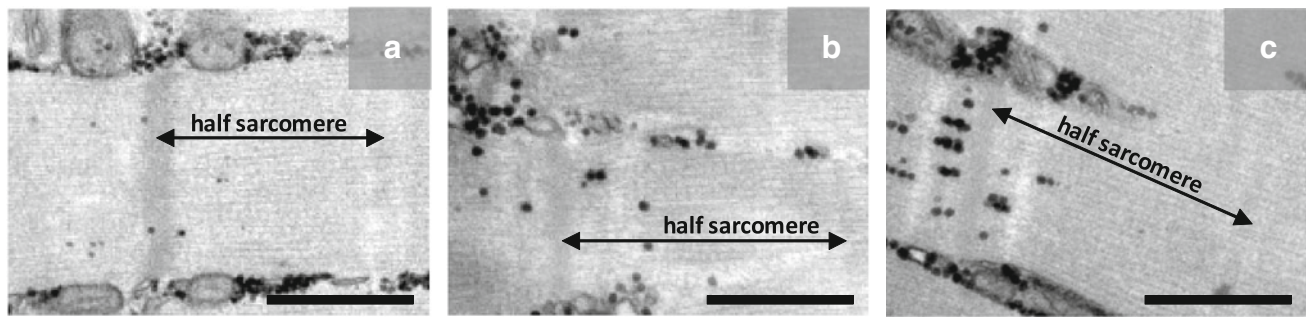


Fig. 3 Representative TEM images showing glycogen particles located within the myofibrils (Intra glycogen) immediately, 24 h, and 120 h after soccer match. Immediately after the match, the glycogen content located within the myofibrils was comprised of relatively few and small particles (*black dots*) (a). After 24 h of

($P = 0.008$, Fig. 1d). Thus, the continued increase in glycogen content (Fig. 1a, b) after the 1st day of recovery is characterized by an increase in number of particles. The time-dependent change of particle size was not different between subfractions ($P = 0.31$) or fibre types ($P = 0.58$). However, the particles were in general 11% smaller in type I fibres than in type II fibres ($P < 0.001$) and subtle differences were observed between subfractions in the order: Intra >IMF >SS, where Intra and IMF particles have 10% ($P < 0.001$) and 2% ($P = 0.05$) greater volumes than SS particles, respectively.

Maximal voluntary contraction force after the match

The MVC force can also be found in a companion paper (Krustrup et al. 2011). The soccer players showed a 10% ($P = 0.004$) and 8% ($P = 0.02$) higher MVC peak force 5 days after the match compared with immediately after and 1 day after the match, respectively, which are in accordance with previous studies (Thorlund et al. 2009; Rampinini et al. 2011). Furthermore, the MVC peak force tended to be higher 5 days after the match compared with 2 days after the match (6%, $P = 0.06$). The relative increase in MVC peak force throughout the recovery period did not correlate with the relative increase in any of the distinct subcellular localizations of glycogen.

Discussion

In the present study, transmission electron microscopy was used to monitor the glycogen content and individual particle size of distinct subcellular localizations of skeletal muscle in the recovery period after a high-level soccer match. While all subcellular depositions of glycogen increased by $\sim 60\%$ during the initial 24 h of recovery, the increase in Intra glycogen content was impaired compared to that of SS glycogen during the 2nd day of recovery.

recovery, the increase in glycogen content was mainly ascribed an increase in the size of particles (b), whereas continued increase of Intra glycogen content 120 h after the match was accompanied by an increase in number of particles (c) ($\times 40,000$ original magnification, scale bar: 500 nm). All images originate from the same subject

Conversely, from the 2nd day to 5 days after the match, the increase in glycogen content exclusively occurred in the Intra location compared to the SS and IMF locations. In all subcellular locations, the initial increase in glycogen content could be attributed to an increased size of existing glycogen particles, whereas continued increase in glycogen content later in recovery was accompanied by an increase in the number of visible particles.

Subcellular localization

Recently, several studies have investigated the role and regulation of compartmentalized glycogen metabolism in skeletal muscle. It has been shown to be important that glycogen and its associated proteins can be found both in between the myofibrils (intermyofibrillar (IMF) space) and within the myofibrils (intramyofibrillar (Intra) space) (Nielsen et al. 2001; Prats et al. 2011, Wanson and Drochmans 1968). Moreover, glycogen synthase translocates to Intra glycogen particles in response to exercise (Prats et al. 2005, 2009) and Intra glycogen is preferentially resynthesised after glycogen-depleting cycling exercise (Marchand et al. 2007). However, in the present study, the IMF, Intra and subsarcolemmal (SS) glycogen showed similar increments ($\sim 60\%$) during the initial 24 h of recovery after a soccer match, which is in line with a previous study on cross country skiers showing a relative equal resynthesis of IMF, Intra and SS glycogen during the initial 4 h of recovery from 1 h exhaustive exercise (Nielsen et al. 2011). A lower post-exercise glycogen concentration ($36 \text{ mmol kg dw}^{-1}$) in the study of Marchand et al. (2007) compared with the present study ($193 \text{ mmol kg dw}^{-1}$, see Krustrup et al. 2011) and the cross country skiers ($167 \text{ mmol kg dw}^{-1}$) could indicate that preferential resynthesis of Intra glycogen following exercise is a response to high degree of glycogen depletion.

Interestingly, while all defined subcellular localizations showed equal initial (24 h) increase in glycogen content,

there were marked differences between localizations in the remaining recovery period. During the 2nd day of recovery IMF and SS glycogen continued to increase, whereas Intra glycogen did not increase further. On contrary, Intra glycogen levels increased by additional 50% from the 3rd to the 5th day of recovery and IMF and SS glycogen remained unchanged. These unexpected findings may be explained by a localization-specific regulatory mechanism initiated by muscle damage during the soccer match. Indeed, myoglobin and creatine kinase were elevated after the match as described in a companion paper (Krustrup et al. 2011) indicating muscle damage, which is known to impair resynthesis of glycogen at the 2nd and 3rd day of recovery (Widrick et al. 1992). Furthermore, we have previously shown that resynthesis of glycogen in the absence of CHO-intake preferentially impairs resynthesis of Intra glycogen (Nielsen et al. 2011) corroborating the present finding that Intra glycogen could be affected by muscle damaging-induced impaired glucose uptake. Interestingly, we have recently reported a decrease in Intra glycogen of 50% following 2 weeks of muscle disuse (Nielsen et al. 2010b), which suggests that muscle disuse and eccentric contraction-induced muscle damage influences glycogen storage by common but yet unknown mechanisms.

During the period from the 3rd to the 5th day of recovery resynthesis of glycogen exclusively occurred in the Intra deposition. In conjunction with the constant total glycogen content during this period, the question is whether glycogen has been resynthesized or translocated from IMF and SS stores. Interestingly, it has been shown in hepatocytes that glycogen particles can move from the periphery towards the centre of the cell (Fernandez-Novell et al. 2002). Thus, translocation of glycogen particles between distinct localizations may occur in response to or in combination with cells' adaptations to environmental alterations. Furthermore, attention should be given whether movement of glycogen particles takes place within the specific localizations, which could serve as a mechanism to optimize glycogen availability for local processes related to mitochondrial function, sarcoplasmic reticulum and surface membrane ion-transport, or the contractile apparatus. In order to facilitate resynthesis of glycogen following the soccer match, the players of the present study consumed an optimized diet rich in carbohydrates and creatine, the combination of which have previously been shown to enhance glycogen resynthesis following exercise (Robinson et al. 1999). The present study was not designed to elucidate the role of creatine supplementation on localization-dependent glycogen resynthesis, but creatine supplementation may increase $\text{Na}^+\text{-K}^+\text{-ATPase}$ content (see Wyss and Kaddurah-Daouk, 2000) and, in turn, have an effect on the subcellular distribution of glycogen. Based on

our previous findings that Intra glycogen may be the primary glycogen source for energy provision to T-tubuli $\text{Na}^+\text{-K}^+\text{-ATPases}$ (Nielsen et al. 2009), the results from the present study indicate that creatine supplementation could up-regulate $\text{Na}^+\text{-K}^+\text{-ATPase}$ content and Intra glycogen, concomitantly, explaining the exclusive increase in Intra glycogen from the 2nd to 5th day of recovery.

Inspection of players' individual values at the different time points revealed that one player had a marked decrease (40%) in Intra glycogen content from the 2nd to 5th day of recovery while all the other players showed increments (range 25–150%). Importantly, the player's content of IMF and SS glycogen decreased also indicating a general depletion of glycogen. In addition, a high variation in SS glycogen content between players was found at the 5th day of recovery indicating that some factors yet unknown may influence SS glycogen more than the other depots.

Glycogen particle size

Further support for a redistribution theory is that the glycogen particles decreased in size from the 3rd to 5th day of recovery, indicating that the increase in Intra glycogen during this period could be ascribed to an increased number of particles. However, it is known that skeletal muscle prefer smaller particles as shown by a glycogenin-over-expression study (Skurat et al. 1997) and by the notion that the particle, in theory, could grow much larger than observed (Graham et al. 2010). The present study is to the best of our knowledge, the first to report quantitative data on glycogen particle size beyond 48 h of recovery from exercise showing that a mechanism related to the interplay between or optimization of particle size and number is active between the 3rd and 5th day of recovery.

The finding of a very high increase in particle volume during the 1st day of recovery, followed by no further increase in the remaining recovery period in spite of a continued increase in SS and IMF glycogen during the 2nd day of recovery, suggests that an increase in particle size is the main mechanism of initial glycogen resynthesis and that an increase in number of particles is a subsequent mechanism to further increase glycogen content. This finding conflicts with a previous study showing that glycogen resynthesis during the first 4 h after glycogen-depleting exercise was mediated through an increase in number of particles and continued increase in glycogen content the subsequent 20 h was mediated through an increase in particle size (Marchand et al. 2007). The post-exercise level of glycogen was much lower ($36 \text{ mmol kg dw}^{-1}$) in the study by Marchand and colleagues compared to the present study ($193 \text{ mmol kg dw}^{-1}$, Krustrup et al. 2011), suggesting that the interplay between increase in number of particles and growth of existing particles depends on the glycogen

content. However, as noted by Marchand and colleagues, the initial increase in number of particles could solely be due to an increase in the size of particles, which immediately after exercise were too small to be detected. Additionally, during the subsequent 2nd day of recovery in the study of Marchand et al (2007), glycogen content increased by an additional 30%, which could only be ascribed, although statistically insignificant, to an increased number of particles. Thus, there seems to be equivocal in existing data that the initial replenishment of glycogen is due to an increase in the size of existing particles and the continued resynthesis of glycogen later in recovery is mediated through an increase in number of visible particles.

Muscle performance and subcellular glycogen localization

Although the present study was not designed to investigate the role of glycogen on muscle performance, the results on subcellular glycogen localization after the soccer match have interesting perspectives in relation to muscle function: Recently, we have shown that exercise-induced very low levels of Intra glycogen (half the level of the present study) is associated with an impaired Ca^{2+} release rate from sarcoplasmic reticulum (Ørtenblad et al. 2011). Further, Intra glycogen may be the exclusive glycogen source for energy delivery to sodium potassium pumps of the t-tubuli as indicated by electric field stimulation-evoked repeated contractions of mechanically skinned rat muscle fibres (Nielsen et al. 2009). Thus, the increase of ~50% in Intra glycogen content from the second to the 5th day of recovery in the present study could be important for optimal muscle function at the end of a soccer match.

Conclusion

In conclusion, the increase of glycogen content after a soccer match has a biphasic dependency on its subcellular localization: while Intra glycogen content does not increase during the 2nd day of recovery; it increases exclusively during the subsequent 3 days. Furthermore, single particle size analysis revealed that the increase in glycogen during the initial 24 h of recovery could be ascribed an increase in the size of particles, while further increments in glycogen is due to increased number of visible glycogen particles. In perspective, these results provide important information regarding differential regulatory mechanisms of spatial distinct depositions of glycogen and underscore the necessity of consideration of the subcellular arrangement when investigating glycogen metabolism in skeletal muscle.

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Conflict of interest The authors declare that they have no conflict of interest.

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