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Subcellular localization- and fibre type-dependent utilization of muscle glycogen during heavy resistance exercise in elite power and Olympic weightlifters

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Abstract

Aim. Glycogen particles are found in different subcellular localizations, which are utilized heterogeneously in different fibre types during endurance exercise. Although resistance exercise typically involves only a moderate use of mixed muscle glycogen, the hypothesis of the present study was that high-volume heavy-load resistance exercise would mediate a pattern of substantial glycogen depletion in specific subcellular localizations and fibre types.

Methods. 10 male elite weightlifters performed resistance exercise consisting of 4 sets of 5 (4x5) repetitions at 75% of 1RM back squats, 4x5 at 75% of 1RM deadlifts and 4x12 at 65% of 1RM rear foot elevated split squats. Muscle biopsies (vastus lateralis) were obtained before and after the exercise session. The volumetric content of intermyofibrillar (between myofibrils), intramyofibrillar (within myofibrils) and subsarcolemmal glycogen was assessed by transmission electron microscopy.

Results. After exercise, biochemically determined muscle glycogen decreased by 38 (31:45)%. Location-specific glycogen analyses revealed in type 1 fibres a large decrement in intermyofibrillar glycogen, but no or only minor changes in intramyofibrillar or subsarcolemmal glycogen. In type 2 fibres, large decrements in glycogen were observed in all subcellular localizations. Notably, a substantial fraction of the type 2 fibres demonstrated near-depleted levels of intramyofibrillar glycogen after the exercise session.

Conclusion. Heavy resistance exercise mediates a substantial utilization of glycogen from all three subcellular localization in type 2 fibres, while mostly taxing intermyofibrillar glycogen stores in type 1 fibres. Thus, a better understanding of the impact of resistance training on myocellular metabolism and performance requires a focus on compartmentalized glycogen utilization.

Keywords. Resistance exercise, glycogen, transmission electron microscopy, skeletal muscle fibres, fibre types

Introduction

High-volume resistance training is primarily performed to increase muscle mass, strength and power,[1] which is crucial for the athletic performance as well as for patient groups suffering from muscle fibre atrophy or in old adults with severe sarcopenia.[2] During acute resistance exercise, the progressive decline in maximal muscle force and power production represents a limitation to exercise intensity (load and shortening velocity per set) and exercise duration (repetitions per set), and therefore also training volume

(load x repetitions). A key energy fuel during resistance exercise is the endogenous stores of muscle glycogen, where most studies have found that 100-250 mmol/kg are used by the active muscle mass during typical high-volume exercise sessions. This includes studies on trained male strength athletes,[3,4,5] male body builders[6,7,8] and untrained men[9] and women.[10] In contrast to endurance exercise, the total work performed during resistance exercise seem to determine the net utilization of glycogen more than exercise intensity per se,[3] which provide an explanation for the low variation in glycogen use between studies. Even when study participants (untrained) performed repeated resistance exercise sets until fatigue (contraction failure), muscle glycogen stores were only depleted by 160 mmol/kg corresponding to a decrease of 30% of the total glycogen stores.[9] Since, skeletal muscle tissue may easily store 400-600 mmol/kg it has been argued that the glycogen stores are far from depleted to critical low values (i.e. < 200 mmol/kg) and therefore do not pose a limitation to the performed training volume.[11] However, while the biochemical determination of muscle glycogen from homogenized muscle segments does not consider fibre type specific glycogen depletion, phenotypical differences in activation pattern and metabolic characteristics between type 1 and 2 fibres may affect the utilization of glycogen. Indeed, previous studies have reported a higher glycogen utilization by type 2 fibres than by type 1 fibres during acute resistance exercise[12-14]. Since these studies used a semi-quantitative method (brightfield microscopy on periodic acid-Schiff stainings) to estimate intracellular glycogen content, it remains unknown whether the type 2 fibres were depleted to reach critical low levels.

Moreover, using quantitative transmission electron microscopy it was shown by Marchand et al.[15] as well as in our laboratory[16-18] that the depletion of glycogen during exercise may be compartmentalized as characterized by a higher utilization from a subcellular store located within the myofibrils (intramyofibrillar glycogen) than from any other localizations (intermyofibrillar and subsarcolemmal glycogen deposits). Furthermore, applying different experimental models we have previously shown that the specific store of intramyofibrillar glycogen correlates positively with fatigue resistance capacity during endurance exercise in humans[18], in mechanically skinned single fibres of the rat,[19] with tetanic Ca^{2+} in stimulated intact single fibres from mice,[20] and with Ca^{2+} release rate in isolated sarcoplasmic reticulum (SR) vesicles obtained from human skeletal muscle biopsies.[21,22] Therefore, if glycogen is depleted from the intramyofibrillar space during acute exercise this would likely mediate a reduction in SR Ca^{2+} release rate and in turn compromise maximal force and power production. Together, the available data suggest that there may exist a glycogen-depletion component in the progressive decline in maximal muscle force and power production during acute resistance exercise sessions. However, no studies so far have estimated the localization and fibre type-specific glycogen utilization during this type of high-intensity muscle work. Therefore, the present study aimed to investigate the effect of acute high-volume resistance exercise on the utilization of

intramuscular glycogen stores from distinct subcellular localizations in male competitive powerlifters and Olympic weightlifters.

Results

Myosin heavy chain (MHC) distribution

The mean (SD) MHC distribution was 50% (6), 48% (4), and 2% (3) MHCI, MHCIIa and MHCIIx, respectively (Fig. 1).

Muscle glycogen and lactate

Muscle glycogen concentration (mmol kg dw^{-1}) decreased by 150 (123:176) from 398 (361:435) before the exercise session to 249 (202:296) after the exercise session corresponding to a decrease of 38 (31:45) % ($P < 0.0001$). Muscle lactate concentration (mmol kg dw^{-1}) increased by 340 % from 7.3 (5.2:9.5) before the exercise session to 32.1 (18.9-45.3) after the exercise session ($P < 0.0001$).

Spatially distinct subfractions of glycogen

The volumetric content of three subfractions of glycogen (intermyofibrillar, intramyofibrillar and subsarcolemmal) was estimated by quantitative transmission electron microscopy. MHC-weighted whole muscle volume fraction of glycogen (see methods) showed substantial concordance with biochemically determined glycogen concentration both before and after the exercise session (Fig. 2D).

The acute exercise session resulted in a fibre type-specific utilization of intermyofibrillar, intramyofibrillar and subsarcolemmal glycogen (exercise x fibre type interaction: $P = 0.019$, $P = 0.0005$ and $P = 0.001$, respectively) as shown in Fig. 2E-G and described below.

Type 1 fibres demonstrated a decrement in intermyofibrillar glycogen (-33% (-42:-22), $P < 0.001$, Fig. 2E), but no or only small changes in intramyofibrillar glycogen (-20% (-47:+21), $P = 0.30$, Fig. 2F) or subsarcolemmal glycogen (-8% (-29:+18), $P = 0.51$, Fig. 2G). In type 2 fibres, decrements in glycogen were observed in all subcellular localizations (intermyofibrillar: -48% (-56:-40), $P < 0.001$, Fig. 2E; intramyofibrillar: -54% (-70:-30), Fig. 2F, $P < 0.001$; subsarcolemmal: -47% (-59:-31), $P < 0.001$, Fig. 2G). Interestingly, after the exercise session a substantial fraction (48%) of the type 2 fibres demonstrated very low levels of intramyofibrillar glycogen (< 2 $\mu m^3 \mu m^{-3}$, Fig. 2F). In contrast, a single type 1 fibre exhibited extreme amounts of intramyofibrillar glycogen (> 9 $\mu m^3 \mu m^{-3}$, Fig. 2F) after the exercise session.

In both fibre types, intermyofibrillar glycogen was the major subfraction contributing with around 80% of the whole muscle glycogen content, and with intramyofibrillar and subsarcolemmal glycogen contributing with around 10% each (Table 2). However, due to the above described heterogenous utilization of these subfractions, the contribution of intermyofibrillar glycogen fell to 76% in type 1 fibres after the acute exercise session (Table 2).

Size and number of glycogen particles

In all three subfractions and for both fibre types, glycogen particles were smaller after the acute exercise session than prior to the exercise (Fig. 3). By assuming a spherical structure of the glycogen particles, the decrease in glycogen particle diameter corresponded to a decrease in particle volume which matched the decrease in the volumetric content of glycogen for all three subfraction in type 2 fibre and for intermyofibrillar and intramyofibrillar glycogen, but not for subsarcolemmal glycogen in type 1 fibres. An estimation of the average number of particles (volumetric content divided by particle volume) showed a tendency for an exercise mediated increase in the number of particles in the subsarcolemmal region of type 1 fibres (4896 (3653:6892) μm^{-2} vs 6816 (4870:8770) μm^{-2}, $P = 0.052$).

Existence of crystal-like structures in the most depleted fibres

Interestingly, a striking distribution was noted for the remaining glycogen particles in the most depleted fibres after the exercise session. Scattered throughout the fibres, glycogen particles appeared to group into small crystal-like structures located in both the intermyofibrillar and subsarcolemmal spaces (Fig. 4). They were observed in 7 type 2 fibres and one type 1 fibre (originating from a total of 4 participants) and were strongly associated with very low (near-depleted) levels of both intermyofibrillar and intramyofibrillar glycogen (Fig. 2). The morphometry of the crystal-like structures could best be described as small elongated (elliptical) structures (aspect ratio of 1.7 and sphericity of 0.4). There were no large differences between intermyofibrillar and subsarcolemmal crystal-like structures (Table 3 and Fig 4).

Discussion

Using quantitative transmission electron microscopy, the present study demonstrates that an acute session of high-volume resistance exercise performed in elite power and weightlifters is associated with a marked depletion of local glycogen stores within type 2 skeletal muscle fibres. This contrasts with only a modest decrease in biochemically measured glycogen concentrations reflecting a mixture of all subcellular stores.

In addition, crystal-like structures containing small glycogen particles were observed in the most glycogen-depleted fibres.

The modest reduction (38%) in the biochemically measured mixed glycogen concentration by 150 mmol/kg are in line with previous reports demonstrating reductions between 100 and 250 mmol/kg using various resistance exercise protocols.[3-10] Interestingly, total training volume seems to be a stronger determinant of the overall glycogen depletion during acute exercise sessions than exercise intensity per se (% of 1RM),[3,12] probably reflecting that a high energy turnover exits for all loading intensities. In the present study, a discrimination between fibre types revealed a slightly higher glycogen use by type 2 fibres than by type 1 fibres in all localizations (47-54% vs 8-33%, respectively, Fig. 2). This is in line with previous reports demonstrating a higher glycogen use in type 2 compared to type 1 fibres.[12-14] In the present high-intensity resistance exercise session we expected all type 1 fibres and most of the type 2 fibres in the vastus lateralis to be recruited[23] and, therefore, the higher glycogen utilization observed in type 2 fibres is likely to be explained by their more glycolytic, less oxidative phenotype that would require usage of more glycogen for a given amount of work produced.[24] The notion of such a predominantly anaerobic utilization of glycogen was further supported by the large increase in muscle lactate concentration observed acutely post-exercise in the present study. Further, during fast contraction velocities the reduced work per crossbridge cycle of type 2 fibres (compared to type 1 fibres) also make type 2 fibres less metabolically efficient.[25]

The main aim of the present study was to examine the subcellular localization of glycogen. Prior to exercise we found in both fibre types that intermyofibrillar glycogen was the major subfraction comprising around 80% of the total glycogen content and with corresponding values of intramyofibrillar and subsarcolemmal of around 10 % for each (cf. Table 2). The 10% share of intramyofibrillar glycogen was higher than previously observed in elite endurance trained athletes[16,17]especially when comparing the type 2 fibres, where endurance athletes appear to store only 5-7% of their glycogen in this compartment. This athlete specific difference could be explained by a lower absolute amount of intermyofibrillar and subsarcolemmal glycogen (and hence reduced mixed muscle glycogen content) in the weightlifters examined in the present study, demonstrating that despite having less glycogen overall, these athletes still have a very high content of intramyofibrillar glycogen. Of notion, high levels of intramyofibrillar glycogen content have also been observed in untrained or recreational active subjects[15,26-28] and may be elevated in response to just a single exercise bout.[15,29,30]

We found that the volumetric content of glycogen decreased in type 2 fibres for all three pre-defined localizations, to reach low and comparable levels post exercise (Fig. 2). The comparable degree of utilization of glycogen stores from all three subcellular localizations is in accordance with quantitative data

observed for from type 1 fibres after continuous prolonged or short-term aerobic exercise.[16-18] However, a closer analysis of individual fibres revealed that in the most depleted fibres (presumably most recruited fibres) intramyofibrillar glycogen decreased to lower levels than intermyofibrillar and subsarcolemmal glycogen stores, as exemplified by the lower quartile of intramyofibrillar glycogen decreasing by 72% compared with 60% and 62% in intermyofibrillar and subsarcolemmal glycogen, respectively (Fig. 2). We have previously observed similar large-magnitude decrements in intramyofibrillar glycogen to be associated with impaired excitation-contraction coupling and reduced contractile force production.[19-21] Therefore, it is likely that a large proportion of the type 2 fibres were fatigued during the acute resistance exercise session as a result of pronounced depletion of local glycogen stores. Since half of the fibres of the whole muscle in the present study was comprised of MHC isoforms IIa (48%) and IIx (2%) (Fig. 1), this would most likely impair whole muscle performance.

In type 1 fibres we found a striking pattern, with a clear preferential utilization of intermyofibrillar glycogen, which contrasts with the depletion pattern presently observed in type 2 fibres. One explanation for this fibre type specificity could be the lower glycogen utilization by type 1 fibres, suggesting that intermyofibrillar glycogen is used before the other two localizations during the time-course of glycogen depletion. We have previously found that a high glycogen utilization by type 1 fibres after 1 hr exhaustive cross-country skiing (endurance exercise) was associated with a preferential utilization of intramyofibrillar glycogen, whereas a low utilization of glycogen by the type 2 fibres was associated with a preferential utilization of intermyofibrillar glycogen, altogether supporting the concept that intermyofibrillar glycogen is preferentially used during exercise with low levels of glycogen utilization.[16] On the other hand, 4-min maximal ski sprinting resulted in only 22-24% reduced glycogen stores and if anything, intramyofibrillar and not intermyofibrillar glycogen deposits were preferentially used[17] and during endurance exercise a recent time-course study found no preferential depletion of intermyofibrillar glycogen (or intramyofibrillar and subsarcolemmal glycogen) after 60 min of exercise compared with the depletion pattern of the different subcellular stores observed at exhaustion after around 112 min.[18] It remains to be understood how the continuous time-course of glycogen depletion is characterised at the subcellular level, and how this is affected by the intensity and volume of exercise as well as the training state of the subject.

As a methodological limitation to the present study, only net utilization of glycogen was estimated, which did not consider resynthesis of glycogen during the exercise session. All participants were served a carbohydrate-rich meal 90 min before the exercise to mimic the conditions of real-life exercise practice and to prevent impaired performance due to progressive decrements in blood glucose levels.[11] As a consequence of the meal, there may have been a high circulatory delivery of glucose to the active muscles

during the exercise and given the repeated intermittent work-rest periods typical of resistance exercise, it is likely that some amount of glycogen resynthesis may have occurred during the pause periods between exercise sets. Thus, the preferential utilization of intermyofibrillar glycogen by type 1 fibres could theoretically result from a preferential resynthesis of intramyofibrillar and subsarcolemmal glycogen during such rest periods. The concept of compartmentalized glycogen metabolism is corroborated by the distribution of glycogen synthase, where exercise is found to mediate a translocation of a de-phosphorylated, and hence more active form of glycogen synthase from the intermyofibrillar to the intramyofibrillar region, with no clear data on the subsarcolemmal region.[31]

Except for the subsarcolemmal space in type 1 fibres (discussed below), the decrease in the volumetric content of glycogen was matched by a corresponding decrease in particle volume (cf. Figs. 2 and 3). Within the range of the particle diameters observed in the present study, the theoretically number of glycosyl units per particle[32] is comparable to the calculated volume of the particle (assuming a spherical particle shape). Therefore, the results of the present volumetric content analysis of glycogen in each subcellular location is likely to resemble the theoretically number of glycosyl units. Interestingly, we found that while the volumetric content of subsarcolemmal glycogen in type 1 fibres did not change, the estimated number of glycogen particles increased by 39% during the exercise session. This may support the notion that some glycogen resynthesis occurred during rest periods, and that, at least at the particle level, resynthesis was dissociated from the degradation of glycogen particles. I.e. some glycogen particles may have been degraded to smaller sized particles while during rest periods, yet other particles may have been formed. This concept is in line with cell culture experiments, showing a concurrent degradation and synthesis of glycogen particles, where some particles appeared more severely affected than others.[33] This notion was corroborated in the present study by the pre-to-post changes in size distribution for subsarcolemmal glycogen particles in type 1 fibres, showing a Gaussian distribution curve before exercise that approached a bipolar distribution after exercise (Fig. 3F). *De novo* synthesis of new glycogen particles is generally considered to be initiated by the self-glucosylating protein glycogenin.[34] Although glycogenin has been recognized as the protein-back bone of the glycogen particle and therefore is considered a limiting factor for the numerical density of glycogen particles, recent studies have observed synthesis of glycogen particles in the absence of glycogenin.[35,36] Thus, it can be speculated if the acute (and fast) increase in the numerical density of glycogen particles can occur without the biosynthesis of glycogenin and therefore through an alternative and more dynamic mechanism.

While the average decrease in intramyofibrillar glycogen of type 1 fibres could be explained by a comparable relative decrease in average particle volume, a few fibres unexpectedly demonstrated an

extremely high content of intramyofibrillar glycogen post exercise (Fig. 2F). The glycogen particle size of these fibres was between 22.0 and 24.7 nm, which is considerably smaller than particle size of most of the fibres obtained prior to exercise (Fig. 3D). This observation suggests that the fibres had been active during the exercise, but during the exercise may have experienced a pattern of concurrent continuous resynthesis and degradation of glycogen (discussed in detail above) or alternatively may have been de-recruited during the last sets of exercise resulting in an earlier onset of glycogen resynthesis compared to other fibres.

The current consensus is that glycogen-depletion during resistance exercise is unlikely to play a main role in the concerted action of potential intracellular fatigue mechanisms. A recent study, however, found a correlation (although weak) between the rate of glycogen utilization and the decrease in peak force during resistance exercise, when sets were performed to task failure.[12] Here, we confirm and extend these results by analysing subcellular localization specific glycogen utilization in different fibre types. Our results demonstrate very low levels (near-full depletion) of intramyofibrillar glycogen in half of the investigated type 2 fibres, suggesting that glycogen-depletion could at least in part contribute to the time course of fatigue development during on-going intense muscle exercise. This notion is based on previous findings from our laboratory demonstrating a lower Ca^{2+} release rate from the sarcoplasmic reticulum in conditions of very low intramyofibrillar glycogen content.[19-21] Thus, it is plausible that marked glycogen depletion leads to impaired maximal force and power production at the single fibre level, and consequently results in a reduced total training volume at the muscle level.

Compartmentalization is well known to exist at the subcellular level, where glycogenolytic-glycolytic derived ATP is preferentially consumed by Na,K-ATPases[37-39], SR Ca^{2+} ATPases[40] and myosin ATPases.[41] It has been speculated if the distinct pools of glycogen play a differential role in supporting these different energy consuming processes[42,43], however experimental data addressing this aspect are lacking. In initial support of this notion, we have previously shown that a high content of intermyofibrillar glycogen correlates with a fast relaxation of tetanic contractions in vitro, which suggests that this specific pool of glycogen may provide energy for Ca^{2+} to be pumped back into the sarcoplasmic reticulum by the SR Ca^{2+} ATPases.[19]

Interestingly, crystal-like glycogen structures were found to be distributed throughout the cytoplasm in muscle fibres most markedly depleted of glycogen (Fig. 4). While reported previously,[16,31,44-47] Prats and colleagues[31,47] found these structures to contain β-actin, α-actinin and tropomyosin in addition to glycogen synthase and glycogen phosphorylase and suggest that these crystal-like structures bind metabolic enzymes creating a local availability of metabolites to enhance the initiation of glycogen resynthesis.[31] Prats et al.[48] have proposed a model describing that critical low glycogen levels initiates the formation of the

crystal-like structures, which could enhance glycogen resynthesis by glycogenin dimerization and interaction with glycogen synthase. Then, when the glycogen particles grow the crystal-like structures would dissolve and the glycogen particle become free unbound particles. Since there seems to be clear propensity that they dominantly are present in glycogen-depleted type 2 fibres, while largely absent in glycogen-depleted type 1 fibres[16,18] as well as in isolated cardiac myocytes exposed to prolonged ischemia mediating a very high glycogen utilization[49], we suggest that it may not be the critical low glycogen level *per se*, which initiates the formation of such crystal-like structures, but it may also be influenced by a factor related to the specific fibre types. Whether these crystal-like structures are formed because of glycogen depletion combined with the typical high-frequency innervation pattern of type 2 fibres currently remains unknown.

In perspective, future studies should be undertaken to investigate glycogen-dependent fatigue mechanisms during resistance exercise and to identify strategies (i.e. nutritional supplementation and tapering) to ensure high levels of skeletal muscle glycogen located in the intramyofibrillar space during preparations for training and competition. If the depletion of intramyofibrillar glycogen accelerates fatigue development, the accomplished training volume, and in turn, the degree of resulting hypertrophy may be attenuated. This may have important implications for the implementation of nutrition strategies and the design of resistance training programs for athletes and patient groups suffering from muscle fibre atrophy or in old adults with severe sarcopenia.[2]

In conclusion, by quantitative investigation of the content and subcellular distribution of glycogen in type 1 and 2 fibres before and after an acute bout of resistance exercise in elite power and Olympic weight lifters, we show that a large portion of type 2 fibres drop very low in volumetric content of glycogen located within the myofibrils, reflecting near-depleted amounts of intramyofibrillar glycogen. Since low intramyofibrillar glycogen has been associated with myocellular fatigue mechanisms, we suggest that preservation of this glycogen pool may be important for achieving optimal performance during the execution of high-intensity resistance exercise, while potentially also contributing to ensure optimal hypertrophic adaptations with more long-term training.

Materials and Method

Participants

Ten male competitive powerlifters and Olympic weightlifters were included in the study. Their mean (SD) age, height, body mass, body fat percentage and fat free mass were 24 (4) years, 182 (4) cm, 95 (8) kg, 18

(5) % and 76 (6) kg, respectively. They had an average of 7 (4) years of strength training experience. Their 1 repetition maximum (1RM) for back squat, bench press and deadlift were 210 (25), 150 (18) and 243 (29) kg, respectively. The participants were fully informed of potential risks associated with the experimental conditions before obtaining their verbal and written consent. The project was approved by the local Ethics Committee in the Region of Southern Denmark (project ID S-20160116). The experiments conformed to the standards set by the *Declaration of Helsinki*. The authors declare that the material conform with good publishing practice in physiology.[50]

Experimental Overview

The participants visited the laboratory on two separate days at least one week apart. During their first visit, preliminary measurements including height, body mass and fat mass were obtained. The experimental part of the study was performed on the second visit and consisted of a single high intensity resistance exercise session, designed to assemble a typical exercise session during an off-season training cycle. The participants were requested to refrain from any resistance or endurance exercise the 48 h preceding the experimental trial. Upon arrival, after an overnight fast, the participants were served a standardized pre-exercise meal consisting of bread, chicken, nuts and greens. The total meal contained ~560 kcal with a macronutrient energy distribution of 45% CHO, 26% P and 29% F. The meal was served 1-1½ hour before starting the exercise session. The participants did not intake any nutrition during the exercise, while no limitations were put on the participants' water intake during the experiment. Participants then performed a preliminary free individual warm-up, mostly involving dynamic stretching. The exercise session consisted of 3 lower body exercises and lasted for approximately 1.5 h. Muscle biopsies were obtained from the ~~m.~~ vastus lateralis muscle before (pre) and immediately after (post) the exercise session. The pre-biopsy was obtained 55-85 min after the meal, and 5-10 minutes before initiation of the exercise session. The post-biopsy was obtained immediately (within 2-5 min) after the participant finished the exercise session. All procedures were conducted in laboratories at the Institute of Sports Science and Clinical and Biomechanics, University of Southern Denmark, Odense.

Preliminary Measurements

Body composition was assessed after overnight fasting, using dual energy X-ray absorptiometry (DEXA, Lunar Prodigy Advance; GE Healthcare, Little Chalfont, United Kingdom). Participants were instructed to maintain dietary habits and to refrain from physical activity 24 h prior to the test day. All measurements were carried out by the same trained technician, and the equipment was calibrated daily according to the

manufacturer's specifications.

Resistance Exercise Protocol

The exercise protocol was designed to closely mimic a typical resistance exercise session performed during the strength athletes' normal off-season training. The session lasted 70-90 min and consisted of three lower body exercises (barbell back squats, barbell deadlifts from a deficit, and dumbbell rear foot elevated split squats), all chosen to best incite a high activation of m. vastus lateralis. The exercises were performed with progressive training loads of 40-75% of the subject's self-reported one-repetition maximum (1RM) (Table 1). Subjects performed three warm up sets in the barbell back squat consisting of 10 repetitions at 40%, 8 repetitions at 50% and 6 repetitions at 60% of 1RM, followed by four working sets of 5 repetitions at a prescribed intensity between 70-75%. One warm up set of 5 repetitions at 60% was then completed in the barbell deadlift from deficit, before 4 working sets of 5 repetitions at 70-75 was carried out. Lastly 4 sets of 10-12 repetitions of the dumbbell rear foot elevated split squats was performed on each leg, alternating the working leg each set and aiming for a rating of perceived exertion (RPE) of 8-9 based on the RPE/RIR scale[51]. Rest intervals between sets were maintained at 3-6 minutes in the barbell back squat and the barbell deadlift from deficit and 1-2 minutes in the dumbbell rear foot elevated split squats. All participants completed all of the exercises at the required intensities. In the working sets the mean (SD) intensities were 74% (5) of 1RM in the barbell back squat and 71% (6) of 1RM in the barbell deadlift from deficit.

No restrictions in squat technique were made in relation to the participants' preferred bar placement. Regarding squat depth, participants were instructed to follow the The International Powerlifting Federation (IPF) standards for a qualified squat.[52] The deficit deadlift was performed with the participants standing on 10-cm elevated platform. The rear foot elevated split squat was performed with two dumbbells; one in each hand, and the foot of the rear leg was placed on a training bench, while the front leg was actively working. All support equipment currently allowed at IPF sanctioned competitions were permitted during training. All sessions were supervised by one of the investigators. None of the participants reported any discomfort from the pre-biopsy during the exercise session.

Analytical Procedures

Muscle biopsy sampling

Muscle biopsies were obtained from the quadriceps muscle (m. vastus lateralis) just before (pre-biopsy) and immediately after (post-biopsy) the exercise session. As described in detail elsewhere[53] biopsies were obtained from the middle portion of vastus lateralis muscle utilizing the percutaneous needle biopsy technique of Bergström.[54] The biopsies were taken in a random order of the left and right leg. During the pre-biopsy in one leg, an incision was made in the other leg as well, to ensure a fast post-biopsy and to avoid the potential influence of muscle damage from repeated biopsies.[55] All biopsies were taken by the same trained medical doctor to ensure standardization of the location on the muscle and muscle depth. The muscle sample was placed on a filter paper, on an ice-cooled ~5°C petri dish and divided for transmission electron microscopy imaging and the remaining part was frozen directly in liquid N_2 and stored for later use for biochemical determination of muscle glycogen and lactate.

Myosin Heavy Chain (MHC) composition

Myosin heavy chain (MHC) composition was determined from homogenate using gel electrophoresis as previously described.[56] Briefly, muscle homogenate (80 µL) was mixed with 200 µL of sample-buffer (10% glycerol, 5% 2-mercaptoethanol and 2.3% SDS, 62.5 mM Tris and 0.2% bromophenolblue at pH 6.8.), boiled in water at 100ºC for 3 min and loaded (10-40µL) on a SDS-PAGE gel (6% polyacrylmide (100:1 acrylmid : bis-acrylmid), 30% glycerol, 67.5 mM tris-base, 0.4% SDS, and 0.1 M glycine). Gels were run at 80V for at least 42 h at 4°C and MHC bands made visible by staining with Coomassie. The gels were scanned (Lino-scan 1400 scanner, Heidelberg, Germany) and MHC bands quantified densitometrically (Phoretix 1D, nonlinear, Newcastle, UK) as an average of three separate lanes loaded with 0.03 to 0.05 mg protein, respectively, for each of the two biopsies (Fig. 1). The MHC composition of each subject was determined as an average of the two biopsies. MHC II was identified with Western blot using monoclonal antibody (Sigma M 4276) with the protocol Xcell IITM (Invitrogen, Carlsbad, CA, USA). Data are averages of the pre and post exercise biopsies.

Biochemical determination of muscle glycogen and lactate

Total muscle glycogen content was determined by spectrophotometry (Beckman DU 650) as described in detail elsewhere.[22] Briefly, freeze dried muscle tissue (1.5 mg) was boiled in 0.5 ml 1 M HCL for 150 min. before it was rapidly cooled, whirl-mixed and centrifuged at 3500g for 10 min. at 4° C. 40 µL of boiled muscle sample and 1 ml of reagent solution containing Tris-buffer (1M), distilled water, ATP (100mM), $MgCl_2$ (1M), $NADP^+$(100mM) and G-6-PDH were mixed before the process was initiated by adding 10µL of diluted hexokinase. Absorbance was recorded for 60 min. before the glycogen content was calculated. Lactate was determined from specimen, which was freeze-dried, dissected free of non-muscle tissue,

powdered and extracted with $HClO_4$ as previously described.[57] Muscle glycogen and lactate was expressed as mmol·kg^{-1} dw.

Transmission electron microscopy (TEM) analyses

Subcellular glycogen localization was estimated by TEM as previously described.[16] In brief, muscle specimens were fixed with a 2.5 % glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.3) for 24 h at 4°C and subsequently rinsed four times in 0.1 M sodium cacodylate buffer. Following rinsing, fibres were post-fixed with 1 % osmium tetroxide (OsO_4) and 1.5 % potassium ferrocyanide ($K_4Fe(CN)_6$) in 0.1 M sodium cacodylate buffer for 90 min at 4°C. After post-fixation, the fibres were rinsed twice in 0.1 M sodium cacodylate buffer at 4°C, dehydrated through a graded series of alcohol at 4–20°C, infiltrated with graded mixtures of propylene oxide and Epon at 20°C, and embedded in 100 % Epon at 30°C. Ultra-thin (60 nm) sections were cut (using a Leica Ultracut UCT ultramicrotome) in three depths (separated by 150 µm) and contrasted with uranyl acetate and lead citrate. Sections were examined and photographed in a pre-calibrated transmission electron microscope (JEM-1400Plus, JEOL Ltd, Tokyo, Japan and a Quemesa camera). All longitudinally oriented fibres (n = 8-10) were photographed at x10,000 magnification in a randomized systematic order, including 12 images from the subsarcolemmal region and 12 images from the myofibrillar region.

Fibre typing

Based on intermyofibrillar mitochondrial volume and Z-line width, muscle fibres were classified as either type 1 or type 2 based on their content of myosin ATPase isoforms[58], which other studies have shown to correlate also with myosin heavy chain isoform composition.[59] Intermyofibrillar mitochondrial volume was plotted against Z-line width from all the photographed fibres from each biopsy. The 2-3 fibres demonstrating the highest mitochondria volume fraction and thickest Z-line width were classified as type 1 fibres, and vice versa for type 2 fibres. Only distinct type 1 and type 2 fibres were included, whereas intermediate fibres were discarded. A total of 106 fibres were included in the TEM analyses of intracellular glycogen localization (2-3 fibres of each type per biopsy). Thus, each muscle biopsy was represented by a low number of fibres for this analysis, which limits potential inter-subject analyses. This is emphasised by the large variation in total glycogen[60] and phosphocreatine concentrations[61] as well as protein phosphorylation[62] between fibres from the same muscle. Nonetheless, the total amount of analysed fibres was 25-28 fibres of each fibre type per time-point ensured the estimates at the group level to have CE

values of 5-14% in the present study.

Subcellular glycogen localization

Within each muscle fibre classified as either type 1 or 2, the volumetric content of glycogen was estimated in three distinct localizations: 1) the intermyofibrillar space; 2) the intramyofibrillar space; and 3) the subsarcolemmal space. Based on stereological counting of glycogen in each space, and by taking section thickness into account, glycogen volume fraction (V_V) was calculated as proposed by Weibel: $V_V = A_A - t\{(1/\pi)\cdot B_A - N_A\cdot[(t\cdot H)/(t+H)]\}$, where A_A is glycogen area fraction, t is the section thickness (60 nm), B_A is the glycogen boundary length density, N_A is the number of particles per area (A_A / ($\pi \cdot \frac{1}{2}H^2$), and H is the average glycogen particle diameter.[63] Glycogen particles were assumed to be spherical.[32] A_A was estimated by point counting using different grid sizes for the different locations in order to achieve satisfactorily precision of the estimates (see below). B_A was calculated as $\pi / 4 \cdot S_V + t \cdot N_V \cdot \pi \cdot H$, where S_V is $N_V \cdot \pi \cdot H^2$ and N_V is $N_A / (t + H)$. The average glycogen particle diameter for each subcellular location was calculated by directly measuring at least 60 particles per location per fibre (IQR: 85-102) using iTEM (iTEM software, version 5.0, Olympus, Germany). Based on this formula, the calculation of the volumetric fraction based on the area fraction attained from the projected images was corrected for potentially an overestimating the volumetric fraction due to stochastic cutting of some glycogen particles at the upper and lower slice surface.[63] Intermyofibrillar glycogen was expressed relative to the myofibrillar space and estimated using grid sizes of 180 nm and 300 nm, respectively. The myofibrillar space consists of myofibrils (intramyofibrillar space), mitochondria, SR, t-system and lipids. The amount of intramyofibrillar glycogen was expressed relative to the intramyofibrillar space and estimated using grid sizes of 60nm and 300nm, respectively. The subsarcolemmal glycogen was expressed relative to the muscle fibre surface area and estimated using a grid size of 180 nm. The fibre surface area was estimated by measuring directly the length of the fibre accompanying with the area of the subsarcolemmal region, which is perpendicular to the outer most myofibril (Fig. 2B) and then multiplied by the section thickness (60 nm). The variation in the parameters between images was used to estimate a coefficient of error ($_{est}$CE) as proposed for stereological ratio-estimates by Howard & Reed.[64] The $_{est}$CE were 0.13, 0.14 and 0.19 in intermyofibrillar, intramyofibrillar and subsarcolemmal glycogen, respectively. All fibres were analysed in a blinded and randomized order by three independent and trained investigators. There was only a small bias (<9%) and a low coefficient of variation (<5%) between the three investigators as verified by Bland-Altman plotting.[65]

In some of the most depleted fibres, we observed a grouping of small glycogen particles compressed in crystal-like structures. The morphology of these crystal-like structures was examined using RADIUS (EMSIS

GmbH, Muenster, Germany). After a manual outline of each structure, the following characteristics were estimated: area, perimeter (the length of the boundary), feret diameter (the distance of parallel tangents at opposing boundaries), convexity (the area relative to the area of the convex hull), aspect ratio (the maximum ratio between the length and width of a bounding box), shape factor (the area relative to the area of a circle with an equal perimeter), and sphericity (the squared quotient of width relative to length).

Statistical analysis

Statistical analyses were performed using Stata, version 15 (StataCorp LP, College Station, TX, USA). All interactions or main effects were tested using a linear mixed-effects model, with time and group as fixed effects. Subject was included as random effect to take into account the nested design with 2-3 fibres obtained per fibre type per subject. The TEM derived data on the three subfractions of glycogen were log-transformed before analyses and assumptions on heteroscedasticity and normal distribution were evaluated by inspecting the distribution of residuals and a standardized normal probability plot, respectively. Concordance was evaluated by the concordance correlation coefficient (r_c) as proposed by Lin[66] and the r_c value was interpreted using the following scheme: r_c of 0.2-0.4 was considered fair; 0.4-0.6 moderate; 0.6-0.8 substantial; and 0.8-1.0 almost perfect. Values are presented as medians and interquartile range, unless otherwise stated. Level of statistical significance was set at $\alpha = 0.05$. The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Conflict of Interests

The authors have no conflict of interests.

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Tables

Table 1. Acute resistance exercise protocol

Exercise	Sets	Repetitions	% 1RM
Back squat	1	10	40
	1	8	50
	1	6	60
	4	5	70-75
Deficit Deadlift	1	5	60
	4	5	70-75
Rear foot elevated split squat	4	12	60-70

1RM, 1 repetition maximum.

Table 2. The relative distribution (%) of glycogen at three subcellular locations in human skeletal muscle before and after resistance exercise

Fibre type	Pre-exercise			Post-exercise		
	IMF	Intra	SS	IMF	Intra	SS
Type 1	81 (78-83)	11 (8-14)	8 (7-10)	76 (72-80)*	12 (9-16)	10 (8-15)
Type 2	82 (77-87)	10 (7-14)	7 (5-9)	83 (78-85)	9 (7-14)	8 (6-12)

IMF, intermyofibrillar glycogen; Intra, intramyofibrillar glycogen; SS, subsarcolemmal glycogen. Values are medians and IQR (n = 24-30 fibres). *, $P < 0.05$ vs. Pre-exercise

Table 3. Morphology of crystal-like structures

Location	Area (nm^2 10^{-3})	Perimeter (nm 10^{-3})	Feret diameter (nm)	Convexity	Aspect ratio	Shape factor	Sphericity
Intermyofibrillar	54 (17)	1.1 (0.2)	206 (37)	0.87 (0.04)	1.8 (0.2)	0.51 (0.07)	0.34 (0.08)
Subsarcolemmal	44 (23)	1.0 (0.3)	188 (46)	0.88 (0.03)	1.7 (0.2)	0.53 (0.06))	0.40 (0.10)

Values are means (SD) (n = 8 fibres).

Figure legends

Fig. 1. MHC distribution

Representative gel analysis of the MHC isoform composition (a). Bands with MHC I, IIa, and IIx are identified by arrows. The whole muscle homogenate MHC bands made visible by staining with Coomassie and the relative distribution of the MHC isoform bands was estimated by densitometrically quantification and given as an average of three separate lanes (loaded with 0.03, 0.04 and 0.05 mg protein, respectively) for each biopsy (A1-3 and B1-3). The MHC composition of each subject was determined as an average of the two biopsies (A and B). Three MHC isoforms (MHC I, IIA, and IIX) are detectable in a mixed sample of human vastus lateralis muscle (lane A1-A3). In (b) bars and vertical lines represent mean and SD. N = 10 subjects.

Fig. 2. TEM-estimated glycogen localization

Representative TEM images show the distribution of images (a), the myofibrillar space, where glycogen particles are located within the myofibrils (intramyofibrillar) and between myofibrils close to the sarcoplasmic reticulum and mitochondria (intermyofibrillar space) (b), and the subsarcolemmal space with glycogen particles located in between mitochondria (c). In (c) the horizontal dotted line shows how the length of the fibre was measured. TEM estimated total glycogen volume fraction plotted against the biochemically determined muscle glycogen concentration in biopsies obtained before (filled circles) and after (open circles) the acute exercise session (d). Dotted line indicates line of identity. Analyses of concordance showed fair to substantial concordance prior to exercise (r_c = 0.68 (95% CI: 0.34-1.02); P < 0.001) and substantial to almost perfect concordance post exercise (r_c = 0.86 (95% CI: 0.67-1.04); P < 0.001). Volumetric content of intermyofibrillar (e), intramyofibrillar (f) and subsarcolemmal (g) glycogen in type 1 and 2 fibres before and after acute resistance exercise. Dots represent single fibres (Type 1: n = 26 (pre) and 28 (post) fibres; Type 2: n = 25 (pre) and 27 (post) fibres). Boxes depict medians with interquartile

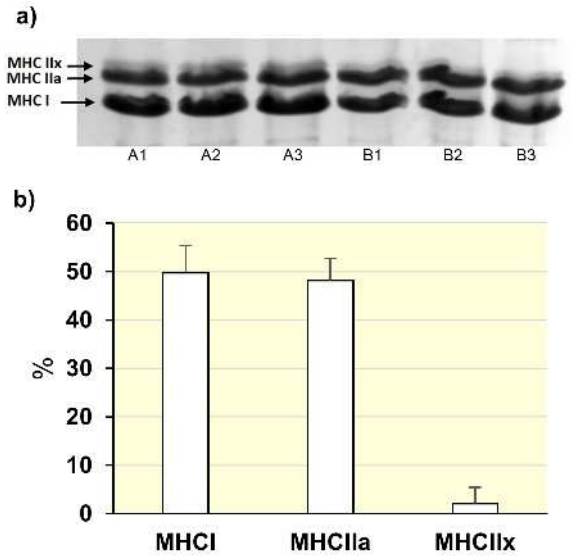
range. * indicate different from pre ($P < 0.001$). Dark grey dots represent fibres with glycogen particles arranged in crystal-like structures (cf. Figure 3).

Fig. 3. Histograms of glycogen particle diameter in different subcellular localizations and fibre types

The diameter of glycogen particles was measured on TEM images (a). In each subfigure (b-g) the indicated values are mean (SD). Pre, before the acute resistance exercise. Post, after the acute resistance exercise. For each histogram the diameter was measured of 2153-2779 glycogen particles originating from 26 (type 1 pre), 28 (type 1 post), 25 (type 2 pre) and 27 (type 2 post) muscle fibres (corresponding to 85-102 (IQR) particles of each localization per fibre) from 10 participants (2-3 fibres of each type per participant). The diameter of each particle was measured directly with steps of approximately 0.4 nm. The histogram bin size is 2 nm.

Fig. 4. Glycogen in crystal-like structures

Representative TEM images of a highly glycogen depleted fibre with glycogen in crystal-like structures located in the intermyofibrillar space (a) and in the subsarcolemmal space (b). Glycogen particles are the black dots. White arrows point at the crystal-like structures. Z, z-disc; M, mitochondria; SR, sarcoplasmatic reticulum, T-sys, T-system. Histogram of the area of the crystal-like structures (c) and aspect ratio (d) located in both the intermyofibrillar space (black bars) and subsarcolemmal space (grey bars).



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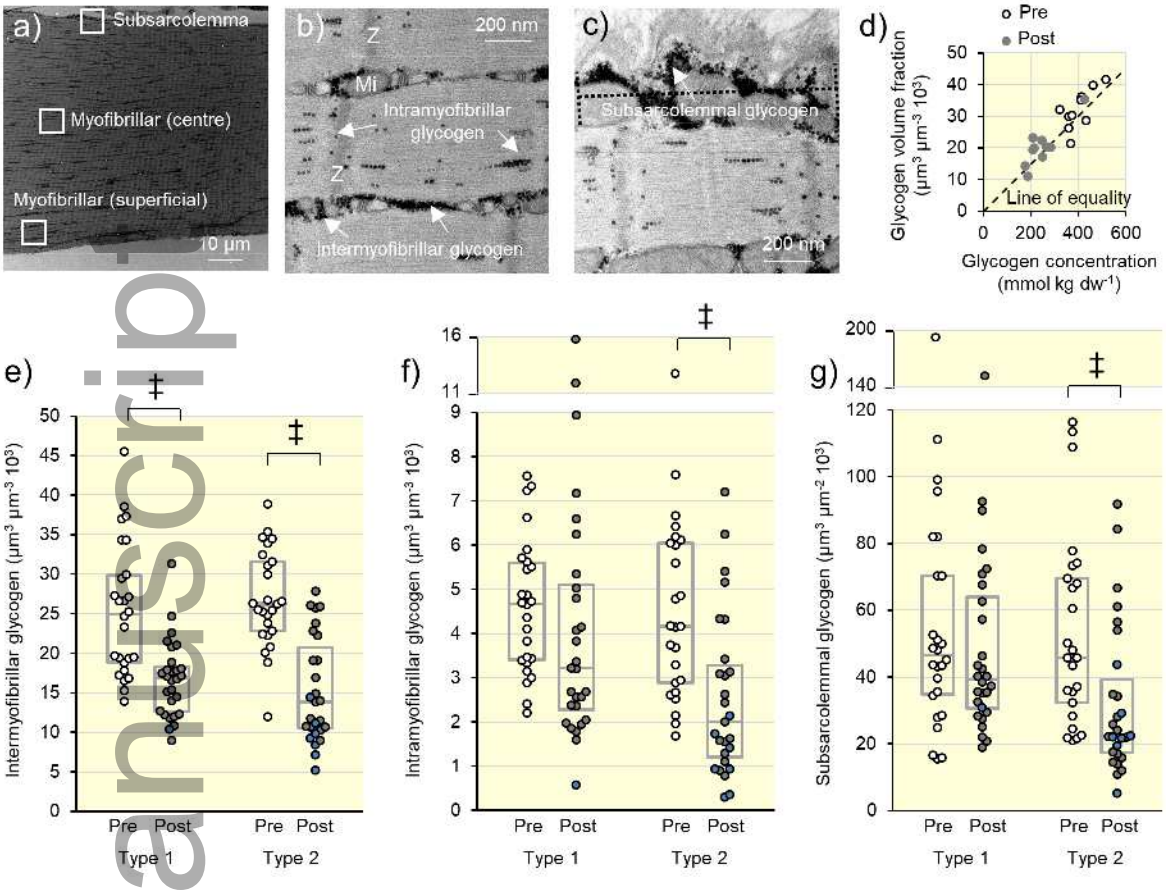

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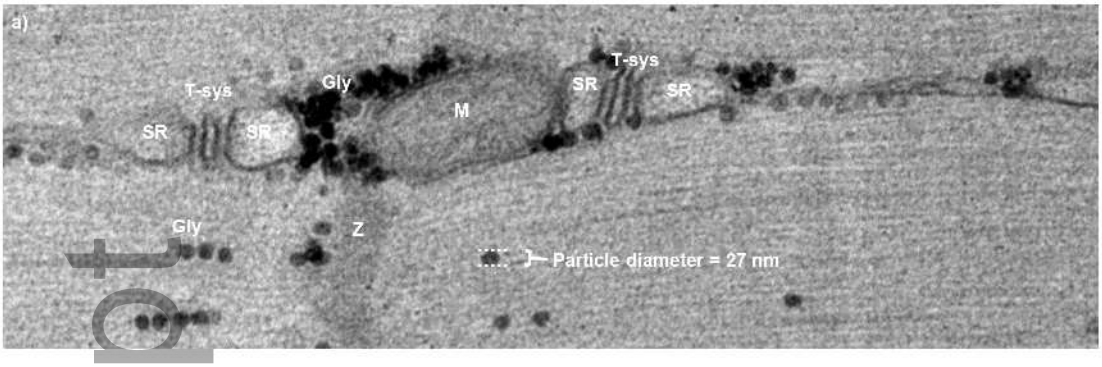

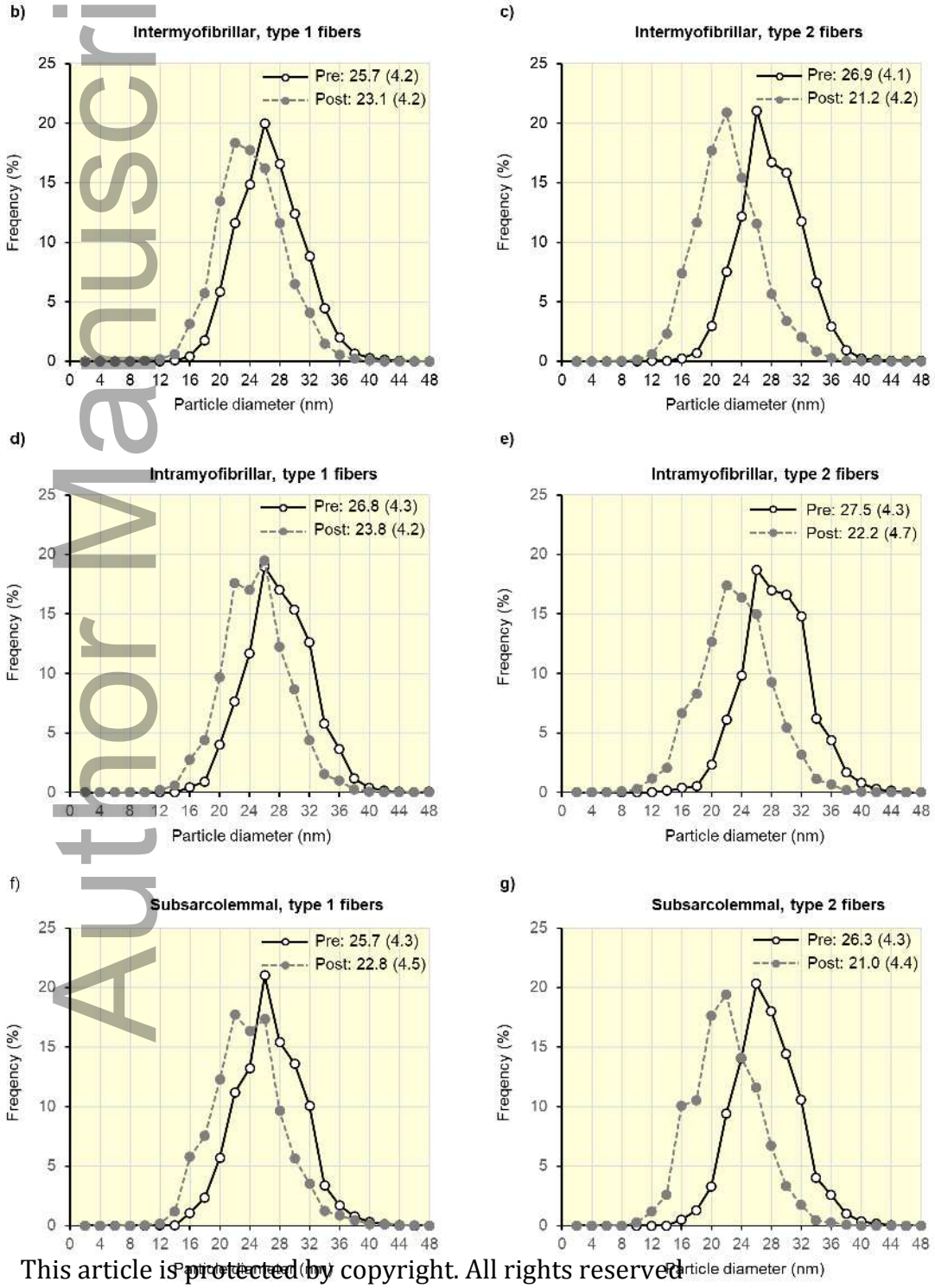

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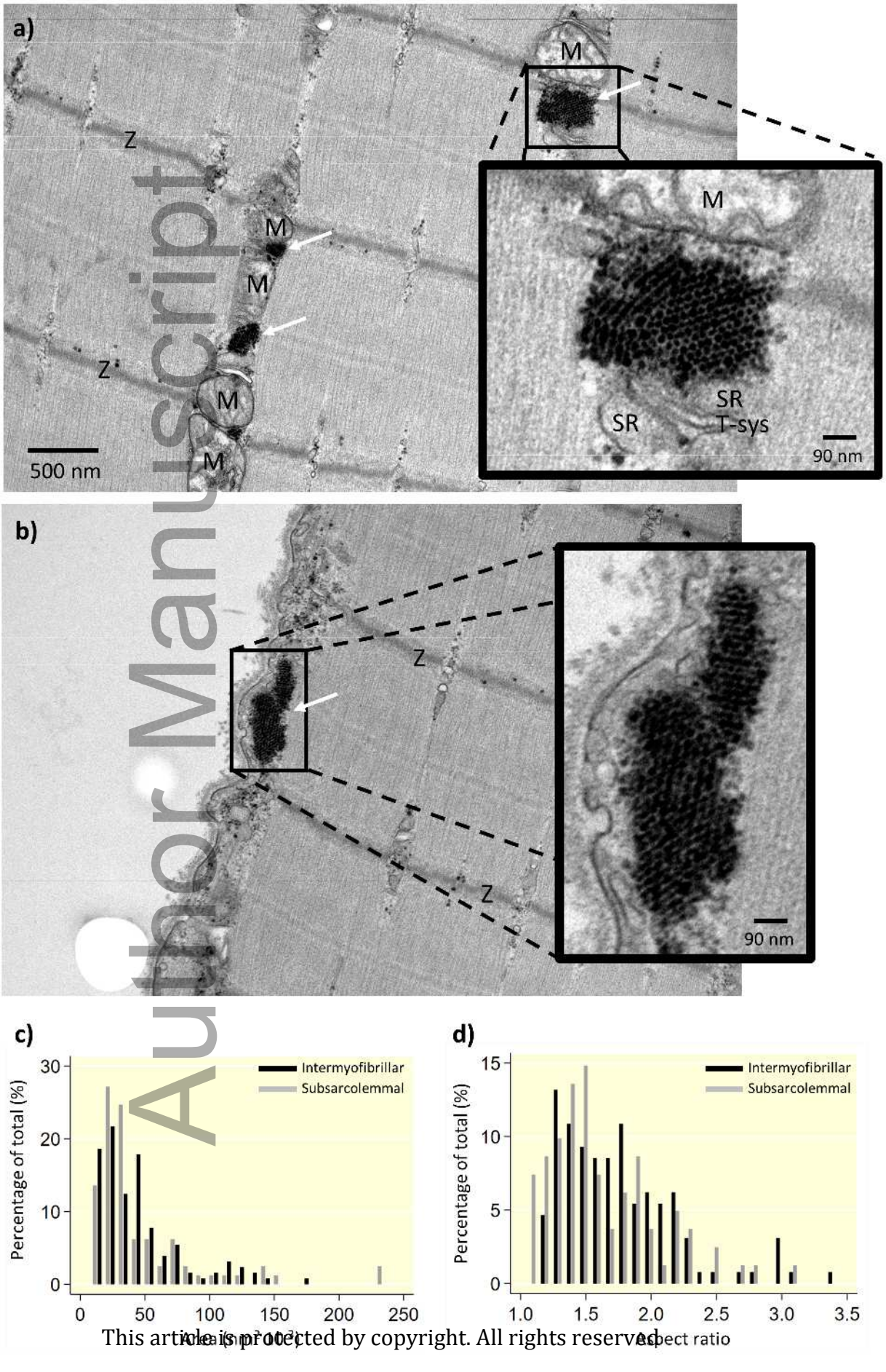

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