Changes in myosin structure and function in response to glycation

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ABSTRACT Nonenzymatic glycosylation (glycation) is recognized as an important post-translational modification underlying alterations of structure and function of extracellular proteins. The effect of glycation on intracellular proteins is, on the other hand, less well known despite the vital importance of intracellular proteins for cell, tissue, and organ function. The aim of this study was to explore the effects of glycation on the structure and function of skeletal muscle myosin. Myosin was incubated for up to 30 min with glucose and subsequently tested for structural and functional modifications by matrix-assisted laser desorption/ionization (MALDI) mass spectrometry and a single-fiber in vitro motility assay, respectively. MALDI spectra revealed glycation-related structural alterations as evidenced by the disappearance of specific Lys-C proteolysis products and the appearance of higher mass peaks that are attributed to cross-linking by glucose. This change was paralleled by a significant reduction in the in vitro motility speed, suggesting a structure-related decline in myosin mechanics in response to glucose exposure. Further evidence that early glycation products form in the regulatory regions of the myosin molecule is derived from the fact that there is complete reversal of motility speed after reaction with the Schiff base-cleaving agent hydroxylamine hydrochloride. Thus, glycation of skeletal muscle myosin has a significant effect on both the structural and functional properties of the protein, a finding that is important in understanding the mechanisms underlying the impairment in muscle function associated with aging and diabetes.—Ramamurthy, B., Höök, P., Jones, A. D., Larsson, L. Changes in myosin structure and function in response to glycation. FASEB J. 15, 2415–2422 (2001)

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Skeletal muscle myosin generally accounts for 15–25% of the total body protein, and the coding sequence for the adult myosin isoforms is among the most heavily amplified in mammalian species (1). Myosin is considered to be the molecular motor that converts free energy derived from the hydrolysis of ATP into the mechanical work that drives muscle contraction. Studies using single muscle fibers have shown that the expression of myosin isoforms is the major, though not the only, determinant for the wide range of shortening velocities under which muscle cells operate. There is a close relationship between the maximum velocity of unloaded shortening, the actin-activated ATPase activity of myosin, and the myosin isoform expression of single muscle fibers in different species (2–7).

Skeletal muscle myosin has been reported to have a half-life as long as ~30 days (8). An aging-related decrease in the myosin turnover rate has also been observed in human muscle (9). The slow turnover rate makes myosin a potential target for post-translational modifications. For example, changes due to nonenzymatic glycosylation (glycation) have been suggested to play a major role in the functional and morphological changes associated with diabetes and aging (10). Glycation of proteins occurs by a chemical reaction of reducing sugars with primary amino groups, i.e., aldehyde groups of free unbound sugars react preferentially with free amino groups of proteins to reversibly form Schiff base adducts. These structures may undergo further Amadori rearrangements and free radical-mediated oxidation to finally generate irreversible advanced glycation end products (11, 12).

Many have studied the effects of glycation on protein structure and function have focused on extracellular proteins, such as collagens, lens crystallin, hemoglobin, myelin, and albumin. Considerably less scientific attention has focused on the effects of glycation on intracellular protein structure and function, even though intracellular glucose metabolites such as glucose-6-phosphate and glyceraldehyde-3-phosphate have been reported to be more potent glycation agents than glucose (11, 13). Intracellular proteins such as myosin accordingly may be susceptible to glycation. The two most lysine-rich regions of myosin and potential targets for glycation are the actin binding site and the ATPase pocket, two functionally important parts of the myosin molecule. However, the effects of glycation on myosin
structure and function remain to be established; there is a compelling need for a detailed understanding of the effects of glycation-induced post-translational modifications of myosin on regulation of muscle contraction. It is hypothesized that 1) glycation primarily affects myosin function via an effect on the nucleotide binding site, and 2) glycation of myosin has a significant effect on myosin structure and function independent of myosin isoform. To test these hypotheses, we used 1) matrix-assisted laser desorption/ionization (MALDI) mass spectrometry to analyze glycation-induced structural modifications of myosin and 2) an in vitro motility assay that allows detailed studies of actomyosin function after myosin and myosin-associated proteins have been extracted from a short, single muscle fiber segment (14, 15, 16). These methods are advantageous because minute quantities of myosin can be used to study post-translational modifications in specific myosin isoforms. Preliminary results from this study have been presented in short form elsewhere (17, 18).

MATERIALS AND METHODS

Animal care and muscle dissection

The study was carried out on young (2–4 months) male Wistar (n=5) and Sprague Dawley (n=1) rats. The animals were kept in conventional facilities at 20–22°C, with constant humidity and a 12 h light/12 h dark cycle, and were fed standard laboratory chow and tap water ad libitum. The rodents were anesthetized by intramuscular injections of fentanyl-fluanisone (0.2–0.3 ml/kg), followed by pentobarbital (30 mg/kg) administered intraperitoneally. The skin over the right and left hind limb was removed, and the soleus and extensor digitorum longus (EDL) muscles were dissected from surrounding tissue. The use of animal material in this study was approved by the ethical committees at both the Karolinska Hospital, Stockholm, Sweden, and Pennsylvania State University.

Mass spectrometry

Myosin was extracted from soleus muscle according to the method of Svensson et al., (19). That is, muscle samples were homogenized in Guba-Straub solution and extracted on ice for 20 min. The samples were then centrifuged and the supernatant was diluted in 12 volumes of low-salt buffer. After 30 min of incubation on ice, the residue pelleted by centrifugation was resuspended in 12 volumes of low-salt ATP buffer. After a final incubation of 30 min on ice and centrifugation, the pellet (which contained myosin) was dissolved in buffer, stored on ice, and used within 2 days. Protein concentration was estimated using the modified method of Lowry et al. (20). That is, aliquots (0.5 μl) of digests were applied to a thin film of α-cyano-4-hydroxycinnamic acid matrix on the MALDI sample target and allowed to dry under ambient conditions. On-plate desalting was achieved by adding 5 μl of 0.01% trifluoroacetic acid, which was removed after 10 s. Ions formed by a pulsed UV laser beam from a nitrogen laser (337 nm) were accelerated at 20 KeV. Mass spectra were obtained as averages of 256 laser shots, and three independent MALDI mass measurements were made from each sample to evaluate reproducibility (21).

The reported amino acid sequence for the β/slow myosin isoform from Wistar rat was obtained from the SwissProt sequence database (accession number P02564). The SwissProt entry identifies the ATP binding site at amino acid residues 178–185 and actin binding regions corresponding to residues 655–677 and 757–771. Average isotopic masses of Lys-C digestion products were calculated from the SwissProt sequence using PAWS software v. 8.1.1 for MacOS (ProteoMetrics, Inc., New York, NY).

Muscle fiber preparation

Small bundles of ~25–50 fibers were disected free from the muscle and tied to a glass microcapillary tube. The bundles were then placed in skinning solution at 3°C for 24 h and treated with a cryoprotectant (sucrose) for long-term storage at ~80°C (5, 22, 23). Before use in the in vitro motility assay, a sucrose-treated bundle was transferred to a 2.0 M sucrose solution for 30 min and incubated in solutions of decreasing sucrose concentrations (1.5–0.5 M). The bundle was then stored in skinning solution at ~20°C and used within 2 wk.

Figure 1. Schematic depiction of the chemistry of stabilization of Schiff base between glucose and myosin and subsequent digestion of the protein by endoproteinase Lys-C for measurement by MALDI MS.
In vitro motility assay

Actin was purified from rabbit skeletal muscle as described previously (24) and fluorescent-labeled with rhodamine-phal-loidin (Rh-PH; Molecular Probes Inc., Eugene, OR). A muscle fiber segment 2–4 mm in length was placed on a glass slide between two strips of grease and a coverslip precoated with 0.1% nitrocellulose in amyl acetate was placed on top, creating a flow cell of ∼5 μl volume. Myosin was extracted from the fiber segment through addition of high-salt buffer (0.5 M KCl, 25 mM HEPES pH 7.6, 4 mM MgCl₂, 4 mM EGTA, 2 mM ATP, 1% β-mercaptoethanol). After 30 min incubation on ice, a low-salt buffer (25 mM KCl, 25 mM HEPES pH 7.6, 4 mM MgCl₂, 1 mM EGTA, 1% β-mercaptoethanol) was applied, followed by BSA (1 mg/ml) in low-salt buffer. To block nonfunctional myosin molecules, unlabeled F-actin filaments in low-salt buffer (15 μM) were sonicated for 1 min and infused into the flow cell. To remove F-actin from the functional myosin heads, low-salt buffer containing 2 mM ATP was applied, followed by low-salt buffer. Rh-PH-labeled actin filaments in low-salt buffer (20 nM) and low-salt buffer were added; to initiate filament movement, motility buffer (2 mM ATP, 0.1 mg/ml glucose oxidase, 23 μg/ml catalase, 2.5 mg/ml glucose in low-salt buffer) was infused.

The flow cell was placed on the stage of an inverted epifluorescence microscope (Olympus IX 70, Olympus America, Melville, NY) and the fluorescent-labeled actin filaments were visualized through an ×60 objective (NA 0.7) by illumination from a 200 watt mercury lamp. The temperature of the flow cell was thermostatically controlled (Bionomic Controller, BC-100, 20/20 Technology) by a thermometer probe (HH21, Omega Engineering, Stamford, CT) placed in contact with the surface of the glass slide next to the flow cell. Actin movement was filmed with an image intensified SIT camera (SIT 66, DAGE-MIT) and recorded on VCR tape (14).

Motility data analysis

From each single-fiber preparation, 10 actin filaments moving with constant speed in an oriented motion were selected for speed analysis. Except for the preparations incubated with glucose, in which a larger fraction of the filaments moved randomly, recordings and analyses were performed from preparations in which >90% of the filaments moved bidirectionally. Using an image analysis package (OPTIMAS 6.0, Optimas Corp., Del Mar, CA), a filament was tracked from the center of mass and the speed was calculated from 20 frames at an acquisition rate of 5 or 1 frame(s)/s, depending on the fiber myosin heavy-chain (MyHC) composition. The average speed and standard deviation of the 10 filaments were calculated. Since the standard deviation in this group of filaments was small (between 10–15% of the mean), the average speed was taken as representative of the muscle fiber (14–16).

Incubations and motility speed analyses

Preincubation data were obtained from motility speed measurements on myosin extracted from a single fiber as described above. To acquire postincubation values, the extracted myosin was incubated with low-salt buffer (with or without β-mercaptoethanol), glucose (with or without β-mercaptoethanol), sucrose (without β-mercaptoethanol), or hydroxylamine hydrochloride (post-glucose). Motility speed was analyzed before and after the respective incubations. Two methods of obtaining postincubation data were tested and compared.

1. Myosin from one fiber segment was used to obtain pre- and postincubation values. After preincubation measurements, the labeled actin was removed from the flow cell by increasing the ionic strength of the buffer. After proper reconditioning and incubation of the exposed myosin, labeled actin filaments were reintroduced into the experimental chamber and postincubation motility speed measurements were carried out.

2. Two segments of a fiber were used to obtain the pre- and postincubation values, i.e., myosin isolated from the first fiber segment was assayed to obtain preincubation data and myosin from the second segment was incubated with selected solutions to obtain postincubation data.

Pilot experiments using the first method described showed there were no differences in the motility speeds between pre- and postincubation values with low-salt buffer. However, on occasion, removal of the labeled actin from the preincubation preparation proved difficult and interfered with postincubation measurements. Therefore, the second method—using two segments of the same fiber—was chosen to study the effects of glucose exposure on actomyosin performance, since this method eliminated the ambiguity due to the interference created by the presence of actin filaments remaining from the preincubation preparation. Furthermore, the accuracy of using separate preparations from the same muscle fiber gains support from a previous study in our lab, which showed that no significant difference exists between in vitro motility speeds of two halves of a single fiber assayed under identical conditions (15).

The extracted myosin from the second halves of slow- and fast-twitch fibers was exposed to 6 mM glucose for 15, 20, and 30 min and 10, 20, and 30 min, respectively. After selective blockage of inactive myosin with fragmented F-actin, fluorescent-labeled actin was added and motility was recorded. A relatively high concentration of glucose representative of extracellular levels of 6 mM was used to compensate for the duration of time that myosin could be kept functional in the in vitro preparation. Control incubations with sucrose (a nonreducing sugar used as an osmotic control) and low-salt buffer were carried out for 30 min on both slow and fast myosin. Hydroxylamine hydrochloride, an agent that cleaves Schiff base linkage between reducing sugars and proteins, was used to test the reversal of early glycation of myosin. After 30 min of glucose exposure and motility speed measurements, the myosin was incubated for an additional 20 min in a buffer containing hydroxylamine hydrochloride, and motility speeds before and after incubation with hydroxylamine hydrochloride were compared. Changes in postincubation motility speed are expressed either as percent changes from preincubation values or as individual motility speed values.

Statistics

Means and SD of data collected from both Wistar and Sprague Dawley rats were calculated from individual values by standard procedures. A two-tailed t test was used for comparisons of two groups. Differences were considered significant at P < 0.05.

RESULTS

Effects of glucose exposure on myosin structure

MALDI mass spectra of Lys-C digests of myosin showed numerous peaks attributable to digestion products. The myosin heavy chain, with a mass of 223 kDa, is expected to give ~202 proteolysis products of < 6 kDa.
after reduction of disulfides assuming 100% cleavage efficiency (25). Myosin contains 201 lysine residues and offers numerous potential sites for glycation.

MALDI spectra of digests of control and glucose-incubated myosin show great similarity (Fig. 2). Several of the peaks observed in spectra of control myosin digests either were not present or were less abundant in spectra of digests of glucose-treated myosin. One prominent peak at m/z 4196 disappeared upon glucose treatment (Fig. 2). Examination of the amino acid sequence suggests this proteolysis product corresponded to amino acid residues 147–184, which is the consensus ATP binding domain. Treatment with glucose leads to complete disappearance of this proteolysis product.

Changes in actin motility pattern in response to glucose incubation of myosin

The criterion for acceptance of motility recordings from the single muscle fiber in vitro motility assay has been determined to be a bi-directional movement by at least 90% of the total number of moving actin filaments. All preincubation and control postincubation recordings fulfilled this criterion. In glucose postincubation of muscle myosin, single 2–4 mm muscle fiber segments were dissected from the fast-twitch EDL and the slow-twitch soleus muscles from 2–4 month male Wistar and Sprague-Dawley rats. Muscle fibers were divided into two groups based on the MyHC isoform expression: a fast-twitch group expressing type IIB MyHC isoform or a combination of IIX and IIB MyHC isoforms, and a slow-twitch group expressing the β/slow (type I) MyHC isoform.

Myosin heavy-chain isoform expression had a strong effect on actin filament speed and no overlap in speed was observed between the slow and fast isoforms (Fig. 3). The average motility speeds for types I, IIXB, and IIB MyHC isoforms were 1.12 ± 0.43 μm/s (n = 11), 4.20; 5.57 μm/s (n = 2), and 5.11 ± 1.29 μm/s (n = 7), respectively. A significant (P < 0.001) strain-related difference in actin motility speed propelled by type I myosin was observed between Wistar (1.42 ± 0.33 μm/s) and Sprague Dawley (0.81 ± 0.17 μm/s) rats. The reason for this strain difference is not known. This observation deserves further scientific attention, but is beyond the scope of the present study.
bation preparations, on the other hand, an increased deviation from the bi-directional linear movement of actin filaments was observed with increasing duration of glucose exposure (Fig. 4). Owing to the random-like nature of their motility, the speed of these filaments was not included in the analysis. However, a fraction of the bi-directionally moving filaments expressed a uniform but wavy motion, and a comparative analysis of the motility speed of these did not reveal a significant difference from the speed of linear bi-directionally moving filaments. Hence, these filaments were included in the analyses of glucose postincubation recordings.

Effects of glucose, sucrose, and low-salt buffer incubations on actin motility speed

Incubations for 30 min with low-salt buffer, with or without β-mercaptoethanol, had no significant effect on motility speed. These values were therefore pooled and are presented as control postincubation data. In the slow myosin, motility speed after 30 min of incubation with low-salt buffer or sucrose (0.79±0.19 μm/s, n=3; 1.25 μm/s, n=1) did not differ significantly from preincubation motility speed (0.82±0.33 μm/s, n=3; 1.12 μm/s, n=1; Fig. 5A). Similar to the slow myosin, in vitro motility speed on fast myosin after 30 min of exposure to the low-salt buffer (6.42±0.32 μm/s, n=3) showed no difference when compared with the preincubation values (5.78±0.25 μm/s, n=3; Fig. 5B). In the slow myosin in vitro motility preparations, actin filament speed decreased to 87 ± 16, 82 ± 14, and 48 ± 22% (P<0.05) of preincubation values after 15, 20, and 30 min of glucose exposure, respectively. A similar result was observed in the fast myosin preparations, in which motility speed after 10, 20, and 30 min exposure to glucose decreased to 89 ± 3, 65 ± 26, and 21 ± 33% (P<0.05) of preincubation values. Incubation of type I myosin with glucose in low-salt buffer containing β-mercaptoethanol did not affect postincubation motility speed (1.31 μm/s, n=1) when compared with the preincubation value (0.90 μm/s, n=1; Fig. 5A).

Effects of hydroxylamine hydrochloride on glucose incubated myosin

Exposure of myosin to 10 mM hydroxylamine hydrochloride for 20 min restored the slowing of motility speed observed after 30 min of incubation with glucose, i.e., the preincubation in vitro motility speed of filaments (0.98±0.25 μm/s, n=16) decreased after exposure to glucose (0.21±0.23 μm/s, n=17) and was restored to preincubation levels (1.04±0.26 μm/s, n=17) after exposure to hydroxylamine hydrochloride (Fig. 6). Incubation with hydroxylamine hydrochloride reduced the relative proportion of the randomly to bi-directionally moving actin filaments (Fig. 7). Post-glucose incubation for 20 min with low-salt buffer did not change motility from glucose incubation levels at 30 min.

DISCUSSION

The major findings from this study are as follows: 1) structural modifications of myosin are documented by
the selective disappearance of specific proteolytic fragments upon incubation of myosin with glucose, reflecting a relationship in myosin structure and function in response to exposure to a reducing sugar; 2) the dramatic effect of both fast and slow myosin function in response to glucose exposure; and 3) reversal of the glycation-induced changes in myosin function after incubation with the glucose-cleaving agent hydroxylamine hydrochloride.

Reactive protein side chains, particularly those involving lysine residues, undergo a reversible reaction with glucose to form Schiff base adducts. Schiff base adducts may undergo spontaneous reactions during sample handling, including Amadori rearrangements or cleavage of the Schiff base by nucleophiles such as thiols. To explore the sites on the protein that are modified by reaction with glucose, the glycated protein is treated with sodium cyanoborohydride, which forms a reduced adduct that is not susceptible to displacement. After reduction of disulfide bridges, the myosin undergoes proteolysis by endoproteinase Lys-C, which hydrolyzes the protein on the carboxyl-terminal side of lysine residues. In the case of myosin, proteolysis is expected to form more than 200 peptide fragments. However, lysine side chains that have been modified by glycation are not cleaved by the endoproteinase. As a result, the enzyme will not cleave at sites where it would have hydrolyzed myosin that had not undergone glycation. Analysis of the products of proteolytic digestion is accomplished using MALDI mass spectrometry, which indicates the molecular masses of the digestion products. Extensive glycation of a specific lysine side chain should result in the near-complete disappearance of the corresponding proteolysis product, with the appearance of at least one new digestion product from glucose-treated protein.

MALDI analyses show that glucose treatment resulted in 100% loss of the proteolysis product corresponding to amino acid residues 147–184 in the consensus ATP binding region of the protein. The reduced abundance of the proteolysis product corresponding to amino acids 1263–1279 and the appearance of a glycated Schiff base product point to glycation at Arg-1268 or Arg-1275. The appearance of several peaks > 4 kDa for the glucose-treated myosin, but not in controls treated with hydroxylamine, suggests two possibilities: 1) glycation blocked proteolysis at sites where lysine modification occurred, leading to higher mass products of glycation, and/or 2) glycation led to formation of cross-links between proteolysis products, which would also result in formation of higher mass products. These high mass products were not observed in significant abundance in digests of glucose-treated myosin that were subsequently treated with hydroxylamine, as would be expected from early-stage glycation products. Studies are under way to isolate and characterize these high mass products and identify other myosin sites that are reactive to glycation.

A significant reduction in actin filament speed was observed for both slow and fast myosin after the incubation with glucose, demonstrating an isoform-independent decline in the mechanical performance of the motor protein. The gradual decrease in motility speed correlated with the duration of glucose exposure, although a marked drop in speed was observed after ~20 min. A glycation-induced alteration in the myosin structure is proposed, since a complete reversal of actin sliding speed was observed after incubation with hydroxylamine hydrochloride. Hydroxylamine hydrochloride is a nucleophilic reagent that is more reactive than lysine side chains in binding aldehyde and ketone groups. As a result, various derivatives of hydroxylamine hydrochloride are useful for displacing carbonyl adducts from lysine residues in proteins (26, 27). Restoration of motility by the use of hydroxylamine hydrochloride implies a reversal of the Schiff base formation. Since Schiff base formation occurs in the early reaction of glycation, we surmise that the modification of myosin by glucose in our experiments is in the initial glycation phase. Additional evidence provides strong support for the argument that the changes observed in myosin function are caused by an early formation of glycation...
products between the reducing sugar glucose and lysine residues in the myosin heavy chain domain. This is evidenced by the lack of change in motility speed in response to 30 min of incubation with the nonreducing sugar sucrose and the inhibition of the effect of the reducing sugar glucose by β-mercaptoethanol.

Oxidative damage by free radicals has been implicated as an important factor in the impairment of protein function (28). However, it is unlikely that oxidative damage of myosin contributes to the observed slowing in the in vitro motility speed after 30 min of glucose incubation, since motility was retained after 50 min of incubation (30 min with glucose and subsequently 20 min with hydroxylamine hydrochloride) without the use of the reducing agent β-mercaptoethanol. It is unlikely that the effect of hydroxylamine hydrochloride incubation was nonspecific, since a similar 20 min incubation with low-salt buffer did not restore motility to preincubation levels.

An interesting observation was the decreasing linearity in actin filament movement with increasing incubation time, i.e., filaments displayed a gradual change from a linear to a more random movement with longer exposure to glucose. This transformation in F-actin motion pattern was not observed in control incubations, and might be explained by a glycation-related decrease in the number of functionally working myosin molecules. In a previous study, we showed that actin filament directionality is dependent on the density of interacting myosin. In the center of the flow cell where the concentration of myosin was high, the filaments moved bi-directionally, whereas in the periphery of the myosin streak where the myosin density was lower, actin filaments demonstrated a more random motion (15). This observation is supported by a study in which a microlithographic material was used to pack myosin with high density and on which actin filaments gilded in a smooth and linear movement. Conversely, on a myosin-poor surface, the filaments moved unevenly and with a wider distribution in motility speed (29). However, it has been demonstrated that the speed of actin filament movement is largely independent of the density of myosin immobilized to the surface provided myosin density exceeds a lower limit (30, 31). It is further suggested that the targeting of reducing sugars to the lysine-rich ATPase catalytic site and the actin binding region of the myosin molecule is the most likely mechanism for causing the reduction in the in vitro motility speed. This is supported by biochemical experiments on glucose-exposed myosin in solution that demonstrated a decrease in actin-activated ATPase activity (11, 32) and by cell physiological experiments using the skinned fiber preparation. These experiments showed reduced ATPase activity in parallel with a decrease in the specific tension after incubation with physiological levels of glucose-6-phosphate (33). Kinetic studies of actomyosin preparations, however, have shown that the rate-limiting factor in the cross-bridge cycle is the release rate of ADP from myosin, and not the ATPase activity (34). The correlation between ATPase activity, ADP release, and actomyosin mechanics has been further investigated using various chimeras of two loop structures, one located close to the ATPase catalytic site (loop 1) and another in the actin binding domain (loop 2). Structural changes in loop 1 demonstrated a significant effect on the rate of ADP release and actin filament speed (35), whereas alteration in the loop 2 sequence had a strong influence on the actin-activated ATPase activity but no effect on the in vitro motility speed (36). Sweeney and co-workers (35) showed that either removal or reversal of the positive charge from three adjacent lysine residues within the loop 1 sequence resulted in a slowing of the motility speed. Based on these results, it is reasonable to conclude that the decrease in actin gliding speed in the present study is the result of glycation-induced structural alterations in or close to the ATPase hydrolytic cleft. These are probably parallel to a similar change in the actin binding site, although structural modifications in this region are of less functional importance.

Nonenzymatic glycosylation has been known to be an important post-translational modification underlying aging-related alterations of protein structure, function, and digestibility (37). To our knowledge, this study is the first to show that glycation of skeletal muscle myosin has a significant effect on both protein structure and function. Given the reported increase in glycated myosin from old rodent muscle and in muscle of diabetic subjects (38–40), the results of this study may prove useful in understanding the mechanisms underlying the aging-related decrease in motility speed propelled by type I myosin observed at the cellular and molecular levels in rodents and humans (14, 15, 41–45) and in the pathophysiological changes of muscle function associated with diabetes (38).

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