Immediate effects of a single exercise bout on protein O-GlcNAcylation and chromatin regulation of cardiac hypertrophy

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Medford HM, Porter K, Marsh SA. Immediate effects of a single exercise bout on protein O-GlcNAcylation and chromatin regulation of cardiac hypertrophy. Am J Physiol Heart Circ Physiol 305: H114–H123, 2013.—Cardiac hypertrophy induced by pathological stimuli is regulated by a complex formed by the repressor element 1-silencing transcription factor (REST) and its corepressor mSin3A. We previously reported that hypertrophic signaling is blunted by O-linked attachment of β-N-acetylglucosamine (O-GlcNAc) to proteins. Regular exercise induces a physiological hypertrophic phenotype in the heart that is associated with decreased O-GlcNAc levels, but a link between O-GlcNAc, the REST complex, and initiation of exercise-induced cardiac hypertrophy is not known. Therefore, mice underwent a single 15- or 30-min bout of moderate- to high-intensity treadmill running, and hearts were harvested immediately and compared with sedentary controls. Cytosolic O-GlcNAc was lower (P < 0.05) following 15 min exercise with no differences in nuclear levels (P > 0.05). There were no differences in cytosolic or nuclear O-GlcNAc levels in hearts after 30 min exercise (P > 0.05). Cellular compartment levels of O-GlcNAc transferase (OGT, the enzyme that removes the O-GlcNAc moiety from proteins), REST, mSin3A, and histone deacetylases (HDACs) 1, 2, 3, 4, and 5 were not changed with exercise. Immunoprecipitation revealed O-GlcNAcylation of OGT and HDACs 1, 2, 4, and 5 that was not changed with acute exercise; however, exercised hearts did exhibit lower interactions between OGT and REST (P < 0.05) but not between OGT and mSin3A. These data suggest that hypertrophic signaling in the heart may be initiated by as little as 15 min of exercise via intracellular changes in protein O-GlcNAcylation distribution and reduced interactions between OGT and the REST chromatin repressor.

Exercise; cardiac hypertrophy; chromatin; β-N-acetylglucosamine; histone deacetylase

CELLULAR REGULATION OF CARDIAC hypertrophy has primarily been examined in the context of pathological remodeling resulting from pressure overload and hypertension (4). While the focus has traditionally been on activation of the fetal gene program by pathological stimuli and subsequent downstream mediators of hypertrophic remodeling, it is now evident that these processes are regulated by chromatin remodeling induced by reduced activation of the repressor element 1-silencing transcription factor (REST) complex. REST is a key transcription repressor found in numerous tissues, including the heart, and interacts with corepressors such as mSin3A to form a complex that recruits histone deacetylases (HDACs), thus altering chromatin structure, inducing epigenetic alterations, and subsequently resulting in gene silencing of hypertrophic mediators (15). Class I HDACs (HDAC 1, 2, 3) promote transcription of hypertrophic genes while class IIa HDACs (HDAC 4, 5) repress hypertrophy (14, 20). While it has long been accepted that exercise training induces a healthy, “physiological” hypertrophic phenotype, the mechanisms underlying this process are not well described. Consistent with its role in pathological hypertrophy, cardiac HDAC 2 activity is increased following 3 days of forced swimming (13), and, in skeletal muscle, HDACs 4 and 5 are exported from the nucleus in response to exercise (19). The role of the REST complex in exercise-induced cardiac hypertrophy is not known.

We recently demonstrated that the O-linked attachment of a β-N-acetylglucosamine (O-GlcNAc) sugar on serine and threonine residues of nuclear and cytoplasmic proteins blunted the hypertrophic response in diabetic hearts (18). O-GlcNAcylation is a dynamic, transient, and reversible regulatory mechanism that is analogous to protein phosphorylation (12, 28–30) and can be upregulated by either increases in substrate availability or cellular stressors such as glucose deprivation (27) or heat shock (33). In contrast to phosphorylation, only two enzymes control O-GlcNAcylation with the attachment of O-GlcNAc mediated by O-GlcNAc transferase (OGT) and the removal catalyzed by β-N-acetyhexosaminidase (O-GlcNAcase; OGA). Recent studies by others reported increased cardiac hypertrophy and decreased cardiac O-GlcNAc and OGT protein in both diabetic and nondiabetic adult mouse hearts after 6 wk of swimming (2, 3). Interestingly, OGT forms a corepressor complex with mSin3A, which regulates gene transcription (5, 31), and both OGT and O-GlcNAc are reported as part of the “histone code” in chromatin (11). However, the role of O-GlcNAc and OGT in regulation of the REST-mSin3A complex control of cardiac hypertrophy is not known.

Exercise-induced cardiac hypertrophy becomes evident after 3 wk of training and is the result of repeated bouts of acute exercise and subsequent recovery (22). Therefore, the goals of this study were to examine the effect of a single bout of exercise on protein O-GlcNAcylation and the REST corepressor complex and to explore potential interactions between these two processes. We hypothesized that acute exercise would upregulate O-GlcNAcylation of components of the REST-mSin3A complex and that interactions between OGT, REST, and mSin3A would be enhanced in exercised hearts.

METHODS

Ethical approval. This protocol was approved by the Washington State University Institutional Animal Care and Use Committee and conformed to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH publication no. 85–23, 1996).
Experimental design. Ten-week-old, male CD-1 mice (Harlan Laboratories, Kent, WA) were housed up to four per cage, maintained on a 12:12-h light-dark cycle, and provided with food and tap water ad libitum. Mice were randomly assigned to sedentary or exercise groups. Irrespective of activity group, all animals were accommodated to a small animal treadmill before acute exercise for 1 h/day for three consecutive days; the first 30 min were spent with the treadmill turned off after which the treadmill was turned on, and the belt was set to move at a very low speed (5 m/min) for the second 30 min. Following accommodation, animals were rested for 48 h, and those that were randomized to the exercise group then underwent either 15 or 30 min (n = 18/group) of treadmill running at 17 m/min for a single bout.

Table 1. Animal characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>15 Min Sedentary</th>
<th>15 Min Exercise</th>
<th>30 Min Sedentary</th>
<th>30 Min Exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt, g</td>
<td>39.3 ± 1.2</td>
<td>40.3 ± 1.1</td>
<td>39.9 ± 1.2</td>
<td>39.1 ± 0.7</td>
</tr>
<tr>
<td>Heart wt, mg</td>
<td>193 ± 8</td>
<td>205 ± 11</td>
<td>211 ± 8</td>
<td>193 ± 8</td>
</tr>
<tr>
<td>HW/BW, mg/g</td>
<td>4.9 ± 0.2</td>
<td>5.1 ± 0.2</td>
<td>5.3 ± 0.2</td>
<td>4.9 ± 0.2</td>
</tr>
<tr>
<td>Blood glucose, mmol/l</td>
<td>11.1 ± 0.6</td>
<td>11.6 ± 0.6</td>
<td>12.7 ± 1.0</td>
<td>11.3 ± 0.4</td>
</tr>
</tbody>
</table>

Values are means ± SE. HW, heart weight; BW, body weight.

Fig. 1. Effect of 15 min of exercise (EX) on β-N-acetylglucosamine (O-GlcNAc) levels and O-GlcNAc transferase (OGT) protein in cytosolic and nuclear compartments of mouse hearts compared with sedentary (SED) controls. Representative Western blots (A) and densitometric analyses of protein levels of O-GlcNAc using analysis of the entire lane (B) or high-, mid-, and low-molecular weights in the cytosolic (C) or nuclear (D) fractions are shown in addition to cytosolic (E) and nuclear (F) OGT. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and TATA-binding protein (BP) antibodies are shown as purity and loading controls for the cytoplasmic and nuclear fractions, respectively; n = 8/group. *P < 0.05 vs. sedentary.
Sedentary, control animals (n = 18/group) were placed on the stationary belt for the same duration as their respective exercise-trained cohort. To examine the acute effect of exercise, mice were quickly anesthetized immediately after cessation of exercise using 2–4% isoflurane in 100% oxygen and killed by decapitation. Hearts were rapidly removed, cleaned in ice-cold phosphate-buffered saline (PBS), snap-frozen (n = 16/group) or placed in 4% paraformaldehyde (n = 2/group), and stored for later use. Nonfasting blood glucose levels were measured in whole trunk blood using the Accu-Chek Advantage analyzer (Roche Diagnostics, Basel, Switzerland).

Western blot analysis. Frozen heart tissue was ground into a fine powder under liquid nitrogen and fractionated into nuclear and cytosolic components using a commercially available kit (Thermo Fisher Scientific, Rockford, IL) in the presence of O-(2-acetamido-2-deoxy-D-glucopyranosylidene)amino-N-phenylcarbamate (PUGNAc; Sigma-Aldrich, St. Louis, MO) to inhibit OGA and prevent the removal of O-GlcNAc as previously described (16) and a protease inhibitor cocktail (Sigma-Aldrich). Protein concentrations were determined using the Bio-Rad Protein Assay Kit (Bio-Rad, Hercules, CA). Solubilized nuclear and cytosolic proteins were suspended in Laemmli buffer (Bio-Rad), and 25 μg protein/sample were separated using SDS-PAGE and transferred to either nitrocellulose (for O-GlcNAc only) or polyvinylidene difluoride membranes at a constant voltage of 100 volts for 75 min. Equal protein loading was initially confirmed by

Fig. 2. Effect of 30 min of exercise on O-GlcNAc levels and OGT protein in cytosolic and nuclear compartments of mouse hearts compared with sedentary controls. Representative Western blots (A) and densitometric analyses of protein levels of O-GlcNAc using analysis of the entire lane (B) or high-, mid-, and low-molecular weights in the cytosolic (C) or nuclear (D) fractions are shown in addition to cytosolic (E) and nuclear (F) OGT. GAPDH and TATA-binding protein antibodies are shown as purity and loading controls for the cytoplasmic and nuclear fractions, respectively; n = 8/group. *P < 0.05 vs. sedentary.
Ponceau-S staining, and immunoblots were then probed for O-GlcNAc (CTD110.6, 1:5,000; Epitope Recognition and Immunodetection Facility, University of Alabama at Birmingham, Birmingham, AL; or RL-2, 1:2,000; Abcam, ab2739, Cambridge, MA), OGT (1:1,500; Sigma-Aldrich, O6139), actin (1:2,000; Abcam, ab28052), atrial natriuretic peptide (1:500; Abcam, ab91250), HDACs 1–5 [1:1,000; Cell Signaling stock nos. 5356, 5113, 3949, 5392, and 2082 (HDAC1–5 respectively), Danvers, MA], mSin3A (1:2,000; Abcam, ab3479), or REST (1:1,000; Millipore, 07–579, Billerica, MA). Blots were then stripped using 1 M NaOH and reprobed with glyceraldehyde-3-phosphate dehydrogenase (1:1,500; Abcam, ab9484) and TATA-binding protein antibodies (1:1,000; Abcam, ab51841) as purity and loading controls for the cytoplasmic and nuclear fractions, respectively, or calsequestrin (1:4,000; Abcam, ab3516) as the protein-loading control for whole cell lysates. After incubation with appropriate secondary antibodies, blots were washed with SuperSignal West Pico or Femto Chemiluminescent Substrate (Thermo Fisher Scientific) and either exposed to film or visualized using the Chemidoc XRS imager (Bio-Rad). Densitometric analysis was performed using ImageJ software (National Institutes of Health, Bethesda, MD).

Coimmunoprecipitation. Heart tissue was homogenized in 10× wt/vol T-PER buffer (Thermo Fisher Scientific) containing 1% protease inhibitor cocktail (Sigma-Aldrich), 1% each phosphatase inhibitor cocktail 2 and 3 (Sigma-Aldrich), and 0.1% PUGNAc (20 mM stock) and incubated 45 min on ice. Tissue debris was pelleted at 12,000 g for 10 min, and the supernatants were stored at −80°C. Lysates were made to 3 mg/ml with Dulbecco’s PBS and precleared over protein A/G resin beads (Thermo Fisher Scientific) for 4 h at 4°C. Beads were pelleted at 10,000 g, and the supernatants were collected. Fresh beads were incubated with antibody (anti-OGT, Abcam, ab50271) or anti-O-GlcNAc (RL-2, Abcam, ab2739) for 4 h at 4°C. Precleared lysate was directly immunoprecipitated onto the antibody-conjugated beads overnight with slow rotation at 4°C. Beads were then pelleted by centrifugation, washed with wash buffer, eluted by boiling for 5 min in Laemmli buffer, and subjected to Western blotting as described above. Heart tissue lysate diluted to the immunoprecipi-

Fig. 3. Cytosolic distribution of O-GlcNAc (green) in sedentary and exercised mouse hearts compared with z-lines indicated by desmin (red). Dashed lines indicate the direction of the corresponding line scans that are shown in the panels on the right. Scale bars, 10 μm.
tation (IP) concentration was used for input; precleared lysates immunoprecipitated without antibody were used as IgG controls. Analysis for co-IP was performed by normalizing the densitometry of the communoprecipitated protein to that of the IP target protein.

**Immunohistochemistry.** To visualize the effect of acute exercise on intracellular distribution of O-GlcNAc, horizontal short-axis sections through the mid-left ventricle were fixed in 4% paraformaldehyde, transferred to 70% ethanol until being paraffin embedded, sectioned at 5 µm, and mounted on slides. Slides were then deparaffinized in xylene, rehydrated in ethanol, and blocked with 5% goat serum in 1% bovine serum for 1 h at room temperature. Sections were incubated with primary antibodies against O-GlcNAc (CTD110.6, 1:50; Epitope Recognition and Immunodetection Facility, University of Alabama at Birmingham) and desmin (1:400; Abcam, ab15200) diluted in 5% goat serum in 1% bovine serum overnight at 4°C; appropriate secondary antibodies conjugated to either Alexa Fluor 488 (green) or 594 (red) (Invitrogen, Carlsbad, CA) were used to visualize the specific proteins with 4',6-diamidino-2-phenylindole (DAPI; blue) to identify nuclei. Image acquisition was performed on a Zeiss Axioplan 2 epifluorescence microscope with an AxioCam MRm cooled CCD camera and AxioVision software (Carl Zeiss Microimaging, Thornwood, NY). Line scans to detect intracellular patterns of O-GlcNAc distribution were generated using ImageJ software.

**Statistical analysis.** Data were analyzed using a two-tailed unpaired Student’s t-test or by two-way ANOVA or ANOVA on ranks, where appropriate. Interactions for ANOVA were investigated using Bonferroni post hoc analysis. Values are presented as means ± SE, and significance between groups was established at \( P < 0.05 \).

**RESULTS**

Acute exercise differentially alters cytosolic and nuclear O-GlcNAc and OGT protein. Physical characteristics for sedentary and exercised animals are presented in Table 1. Body weight and heart weight were not different between groups. It is important to note that postexercise blood glucose levels were not
different compared with sedentary controls, irrespective of the duration of exercise ($P > 0.05$), since protein O-GlcNAcylation can be upregulated by increased production of UDP-GlcNAc, the substrate for O-GlcNAc. A lack of difference between sedentary and exercise blood glucose values indicates that any differences observed in O-GlcNAc and OGT protein are not due to changes in glucose-mediated substrate availability.

The effects of a single exercise bout on nuclear and cytosolic levels of O-GlcNAcylated and OGT protein were initially analyzed using Western blotting. Densitometry was first performed on whole lanes to determine the effect of exercise on overall O-GlcNAc protein levels and revealed that cytosolic O-GlcNAc was significantly lower following 15 min of exercise ($P < 0.05$) with no difference in nuclear levels compared with sedentary hearts (Fig. 1, A and B). There were no differences in cytosolic or nuclear O-GlcNAc levels in hearts from animals that exercised for 30 min (Fig. 2, A and B). Because densitometry of the entire sample lane is dominated by the intense immunoreactive bands at 40 and 90 kDa, the analysis was also performed over the high-, mid-, and low-molecular-weight ranges as indicated in Figs. 1A and 2A. This analysis revealed that the decrease in overall cytosolic O-GlcNAc following 15 min exercise was largely the result of lower O-GlcNAc levels in the mid- and low-molecular-weight protein ranges (Fig. 1C). Despite no effect of exercise on overall nuclear O-GlcNAc levels, lower-molecular-weight proteins exhibited significantly higher O-GlcNAcylated following both 15 and 30 min exercise (Figs. 1D and 2D). Acute changes in O-GlcNAc levels in response to cellular stress are likely due to changes in enzyme activity or substrate availability rather than protein levels of the regulatory enzymes (27, 33). Therefore, it was not surprising that a single bout of exercise did not alter OGT protein in either cytosolic (Figs. 1E and 2E) or nuclear (Figs. 1F and 2F) fractions. OGT reportedly exists in three isoforms: 110- to 116-kDa nucleocytoplasmic (ncOGT), 103-kDa mitochondrial (mOGT), and 78-kDa short cytosolic (17); the immunoblots shown in Figs. 1A and 2A are consistent with this, since all three isoforms were present in cytosolic fractions, but we were only able to detect the 110-kDa band in nuclear fractions.

**Exercise does not alter intracellular O-GlcNAc distribution.** The effects of exercise on the distribution of cytosolic and nuclear O-GlcNAcylated and OGT can be qualitatively compared via the line scans shown in Figs. 3 and 4, respectively. Cytosolic O-GlcNAcylated exhibited a striated pattern in cardiomyocytes that appears to be closely associated with the z-lines indicated by desmin staining (Fig. 3); to our knowledge, this is the first study to demonstrate this pattern in the mouse heart and is consistent with our previous report showing a similar distribution in the rat heart (16). Interestingly, neither 15 nor 30 min of exercise alters the localization of O-GlcNAcylated proteins to the z-lines.

Punctate O-GlcNAcylated and DAPI staining was present in the nuclei of both sedentary and exercise-trained hearts (Fig. 4). The punctate DAPI staining is characteristic of mouse nuclei, since mouse chromatin contains increased AT-rich regions of DNA to which DAPI preferentially binds (10). The line scans shown in Fig. 4 clearly demonstrate that the pattern of O-

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**Fig. 5.** ImmunobLOTS (A) and densitometric analysis of cytosolic (B) and nuclear (C) distribution of histone deacetylase (HDAC) 1, HDAC2, HDAC3, HDAC4, HDAC5, repressor element 1-silencing transcription factor (REST), and mSin3A in sedentary and exercised mouse hearts compared with GAPDH or TATA-binding protein; $n = 6$/group. ND, not detected.

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GlcNAc immunofluorescence is markedly different from that seen with DAPI, particularly in the AT-rich DAPI-positive regions where O-GlcNAc staining is very low. Moreover, the nuclear distribution of protein O-GlcNAcylation does not appear to be altered by either 15 or 30 min of exercise compared with sedentary controls.

**Chromatin proteins are not altered by acute exercise.** Figure 5 demonstrates the presence of class I HDACs 2 and 3, class II HDACs 4 and 5, and mSin3A in both cellular and nuclear compartments, whereas HDAC1 and REST appear to reside exclusively in the nucleus. To our knowledge, this is the first demonstration of compartment localization of HDACs, REST, and mSin3A in the adult heart. Regardless of duration, an acute bout of intense exercise does not alter compartmentalized protein levels of class I or II HDACs, REST, or mSin3A.

**O-GlcNAcylation of OGT is decreased with acute exercise.** Because protein O-GlcNAcylation was increased in nuclear extracts following 15 min of exercise (Fig. 1), we subsequently examined O-GlcNAylation of proteins that modulate chromatin regulation of hypertrophic signaling through IP. As shown in Fig. 6A, HDACs 1, 2, 4, and 5 and OGT are O-GlcNAcylated in the mouse heart, but this is not altered by acute exercise. Furthermore, as shown in Fig. 6A, the 110-kDa nOGT isoform appears to be more O-GlcNAcylated than are the 103-kDa mOGT and 78-kDa OGT isoforms; however, this may be a result of antibody-antigen affinity. We were unable to identify O-GlcNAcylation of HDAC3 through IP because of its molecular weight corresponding to the signal of the IgG band. We show here, for the first time, that both REST and mSin3A interact with OGT in the mouse heart (Fig. 6, C and D). Interestingly, IP of OGT primarily captured the 110-kDa nucleocytosolic isoform that was associated with decreased levels of total protein O-GlcNAcylation (P < 0.05), and a trend toward lower protein O-GlcNAcylation of the 110-kDa band (P = 0.072) in response to exercise. Acute exercise was associated with a lower interaction between REST and OGT (P < 0.05), with no differences in

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Fig. 6. Coimmunoprecipitation (IP) of O-GlcNAc (A and B) and OGT (C and D) and subsequent densitometric analysis with immunoblots (IB) for O-GlcNAc, OGT, HDAC1, HDAC2, HDAC4, and HDAC5. Note that the input and IgG lanes were present on the same membranes as the sedentary and exercise samples; the position from which additional lanes were removed is indicated by the lined box and white spaces; n = 6/group. *P < 0.05, main effect of exercise.
OGT-mSin3A and no effect of exercise duration when analysis was performed relative to the OGT 110-kDa band.

DISCUSSION

The mechanisms regulating cardiac hypertrophy have primarily focused on pathological remodeling, particularly downstream mediators of this process, whereas the processes that mediate exercise-induced physiological hypertrophy are not well understood. The REST corepressor complex alters chromatin architecture via interactions with HDACs and subsequent acetylation and deacetylation of histones, thus regulating access of transcription factors to DNA and gene transcription of hypertrophic mediators (9, 21, 23, 32). We have previously demonstrated that protein O-GlcNAcylation blunts the hypertrophic response in cardiomyocytes (18). When taken together with other reports that mSin3A, a component of the REST complex, interacts with OGT and is subsequently O-GlcNAcylated (31), and that cardiac O-GlcNAc levels are decreased following endurance exercise training (2, 3), we postulated that exercise-induced hypertrophy may be mediated by interactions between the REST complex and OGT. In the current study, we have demonstrated 1) a differential effect of acute exercise on nuclear and cytosolic O-GlcNAc levels, 2) O-GlcNAcylation of HDACs and components of the REST complex that are not altered with exercise, and 3) an established interaction between OGT and REST that decreases in the heart with exercise. It must be emphasized that these changes are independent of metabolic increases in UDP-GlcNAc, the substrate for O-GlcNAc. We actually found that acute exercise differentially alters cytosolic and nuclear protein O-GlcNAcyl ation in the mouse heart. Indeed, a single 15-min bout of exercise induced a decrease in cytosolic O-GlcNAc in the mouse heart that was not evident after 30 min of exercise. We also found that 15 min of exercise induced a significant elevation in O-GlcNAcyl ation of low-molecular-weight proteins in the nucleus that remained elevated after 30 min of exercise. Because the previous studies in this area focused solely on analysis of whole tissue lysates, it is not known whether the decreases reported were evident in both the cytosolic and nuclear compartments. The differential response between subcellular compartments in response to 15 min of exercise, particularly in the lower-molecular-weight range, is intriguing and may reflect translocation of O-GlcNAcyl ated proteins to the nucleus, a decrease or increase in cytosolic and nuclear protein O-GlcNAcyl ation, respectively, or a combination of these processes. Another possibility is the utilization of cellular glucose as fuel for exercise within 15 min, which results in decreased O-GlcNAc. Protein O-GlcNAcyl ation is then increased through a cellular stress response in exercise lasting 30 min, which may occur independent of substrate availability (27, 33). Because increased levels of O-GlcNAc are cardioprotective in ischemia-reperfusion (16), and we observed decreased O-GlcNAcyl ation of OGT and lower association of REST with OGT in exercised hearts, we postulate that this may be an early initiator of physiological, or exercise-induced, cardiac hypertrophy. More specifically, we propose that decreased O-GlcNAcyl ation of OGT allows initiation of exercise-induced cardiac hypertrophy via reduced recruitment of the chromatin repressor REST. In human skeletal muscle, it was previously established that exercising for 60 min induces ejection of class II HDACs from the nucleus to cytoplasm, a mechanism known to initiate hypertrophic signaling (19). However, to our knowledge, our study is the first to report a link between REST and OGT in regulation of cardiac hypertrophy. As such, we propose the mechanism illustrated in Fig. 7 with inhibitors diagram.
bition of REST/mSin3A signaling and cardiac hypertrophy when OGT is present in this complex (Fig. 7A). O-GlcNAcylation of OGT during acute exercise causes dissociation of OGT and the anti-hypertrophic class II HDACs from the REST/mSin3A complex, thus triggering physiological hypertrophy signaling (Fig. 7B). The O-GlcNAc moiety is a very small, uncharged molecule, and its attachment does not prevent proteins crossing membranes, for example, O-GlcNAc modification of NeuroD1 and cyclic adenosine monophosphate response element-binding protein 2 induces translocation of the modified protein to the nucleus (1, 7). The fact that lower-molecular-weight molecular proteins were lower in the cytosol and higher in the nucleus following 15 min of exercise suggests that translocation of O-GlcNAcylated proteins to the nucleus may have been induced by exercise; however, the lack of discernible exercise-induced changes in patterns of cytosolic and nuclear distributions of O-GlcNAc visualized using immunohistochemistry do not fully support this notion. Changes in metabolic demand may contribute to the differential responses between intracellular compartments, since it was recently reported that nicotinamide adenine dinucleotide (NAD\(^+\)) decreases O-GlcNAc in cardiomyocytes in a time- and dose-dependent manner that is independent of both OGT and OGA (8); however, because increased cardiac work induced by intense exercise is driven by a transient reduction in cytosolic and mitochondrial NAD\(^+\) (34), it appears unlikely that this mechanism can explain the decreased cytosolic O-GlcNAc following 15 min of exercise.

A limitation of this study is the use of forced treadmill exercise, since this elicits a clear psychological and physiological stress response that may confound any changes observed in the heart (6). Ideally, a voluntary exercise protocol would be used to limit the stress response; however, this is not practical for the purposes of examining the response to a single bout of exercise because of the inability to control the duration or intensity of exercise in the rodent model. Thus, to limit the stress associated with the treadmill and/or forced exercise, all animals were accommodated to the treadmill in the days preceding the experiment, and mice in the sedentary group were placed on the treadmill for the same duration as the exercise-trained animals before death. Finally, because our observations suggest transient modifications in the state of protein O-GlcNAcylaton, caution should be used if extrapolating these data to any longer-duration or chronic exercise applications. However, training-induced adaptations occur through repeated exposures to acute stress; therefore, we suggest that such adaptations occur because of cumulative effects of the transient findings observed in our study.

To our knowledge, this study is the first to demonstrate an effect of a single bout of exercise on cardiac O-GlcNAcylation in the heart and an effect of exercise on interactions between O-GlcNAc, OGT, and chromatin corepressors of hypertrophic signaling. Specifically, O-GlcNAc differentially modifies nuclear and cytosolic cardiac proteins in response to a single bout of exercise, and this occurs independently of changes in compartment levels of OGT and OGA. Although others have clearly demonstrated a role of O-GlcNAc and OGT in regulation of gene transcription (24–26), our study is the first to demonstrate O-GlcNAcylation of HDACs 1, 2, 4, and 5, mSin3A, and REST in cardiac tissue. Finally, the finding that acute exercise decreases the interaction between OGT and REST suggests that this could be the mechanism by which the O-GlcNAc pathway regulates cardiac hypertrophy and may be the initial step in stimulation of exercise-induced physiological hypertrophy. This finding is important, since HDACs are involved in both pathological and physiological hypertrophic signaling, and changes in O-GlcNAc have been demonstrated in both processes in the heart. We believe this is the first step toward increasing understanding of the paradoxical nature of protein O-GlcNAcylation and may provide a new and novel target for therapies specifically designed to mimic the beneficial effect of exercise on the heart.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


REFERENCES

EXERCISE, O-GlcNAc, CHROMATIN, AND CARDIAC HYPERTROPHY


