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Short report

OGT and OGA expression in postmenopausal skeletal muscle associates with hormone replacement therapy and muscle cross-sectional area

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ABSTRACT

Protein glycosylation via O-linked N-acetylglucosaminylation (O-GlcNAcylation) is an important post-translational regulatory mechanism mediated by O-GlcNAc transferase (OGT) and responsive to nutrients and stress. OGT attaches an O-GlcNAc moiety to proteins, while O-GlcNAcase (OGA) catalyzes O-GlcNAc removal. In skeletal muscle of experimental animals, prolonged increase in O-GlcNAcylation associates with age and muscle atrophy. Here we examined the effects of hormone replacement therapy (HRT) and power training (PT) on muscle OGT and OGA gene expression in postmenopausal women generally prone to age-related muscle weakness. In addition, the associations of OGT and OGA gene expressions with muscle phenotype were analyzed. Twenty-seven 50–57-year-old women participated in a yearlong randomized placebo-controlled trial: HRT ($n = 10$), PT ($n = 8$) and control ($n = 9$). OGT and OGA mRNA levels were measured from muscle samples obtained at baseline and after one year. Knee extensor muscle cross-sectional area (CSA), knee extension force, running speed and vertical jumping height were measured. During the yearlong intervention, HRT suppressed the aging-associated upregulation of OGT mRNA that occurred in the controls. The effects of PT were similar but weaker. HRT also tended to increase the OGA mRNA level compared to the controls. The change in the ratio of OGT to OGA gene expressions correlated negatively with the change in muscle CSA. Our results suggest that OGT and OGA gene expressions are associated with muscle size during the critical postmenopausal period. HRT and PT influence muscle OGT and OGA gene expression, which may be one of the mechanisms by which HRT and PT prevent aging-related loss of muscle mass.

1. Introduction

Protein glycosylation via O-linked N-acetylglucosaminylation (O-GlcNAcylation) is an important post-translational regulatory mechanism, mediated by O-GlcNAc transferase (OGT) (Ozcan et al., 2010). OGT catalyzes the addition of a single N-acetylglucosamine (GlcNAc) moiety to serine and threonine amino acid residues in nuclear, cytoplasmic and mitochondrial proteins, providing reversible modification comparable to protein phosphorylation. Unlike phosphorylation, O-GlcNAcylation is controlled by only two enzymes, OGT and O-GlcNAcase (OGA), the latter being an enzyme that releases O-GlcNAc moiety from proteins. O-GlcNAcylation has an important role in insulin signaling since O-GlcNAcylation of insulin signal pathway proteins by O-GlcNAc attenuates the insulin signal transduction (Yang et al., 2008).

Studies on rats have revealed that several muscle proteins are O-GlcNAcylated, including myosin and actin (Cieniewski-Bernard et al., 2009). Prolonged increase in O-GlcNAcylation of muscle proteins causes insulin resistance in rat skeletal muscle (Arias et al., 2004), and it has been hypothesized that sustainably increased protein O-GlcNAcylation might be involved in the muscular pathology (i.e. muscle atrophy, fiber type changes and metabolic disturbance) associated with diabetes (Cieniewski-Bernard et al., 2009). In addition, inactive splice variant of OGA has been shown to induce muscle atrophy, concomitantly with an increase in proapoptotic proteins (Huang et al., 2011). Furthermore, studies with mice showed that in cardiac muscle, exercise-induced hypertrophy is associated with an improvement in contractile function and decrease in protein O-GlcNAcylation, independent of blood glucose and fatty acid levels (Belke, 2011; Bennett et al., 2012). However, a recent study in type 2 diabetic mice showed that exercise increases O-GlcNAcylation level (Cox and Marsh, 2013), suggesting that an increase in O-GlcNAcylation is a beneficial effect of exercise in cardiac muscle. In skeletal muscle, bed rest induced decrease in

Abbreviations: CO, control; CSA, cross-sectional area; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; HRT, hormone replacement therapy; OGA, O-GlcNAcase; O-GlcNAc, O-linked N-acetylglucosamine; OGT, O-GlcNAc transferase; PI3K, phosphatidylinositol-3-kinase; PT, power training.

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O-GlcNAcylation is prevented by exercise in humans (Mounier et al., 2009), which also suggests that O-GlcNAcylation has a protective effect on muscle. In rats, ageing increases O-GlcNAcylation levels in many tissues including skeletal muscle (Fulop et al., 2008). This may have an important role in age-related impairments like muscle weakness and loss of muscle mass. However, knowledge of the effects of ageing on human muscle protein O-GlcNAcylation is currently lacking.

The decline in muscle mass and strength with advancing age is well established and has several health concerns. Women are especially prone to this phenomenon after menopause when estrogen levels decrease dramatically (Maltais et al., 2009). We used a yearlong randomized controlled trial, the Exercise and Hormone Replacement Therapy-study (Ex/HRT), to investigate the effects of plyometric power training (PT) and HRT on muscle mass and performance in postmenopausal women (Sipilä et al., 2001; Taaffe et al., 2005). We showed that HRT induced on average a 6% increase in knee extensor muscle cross-sectional area while the change in PT and control groups was minimal. Moreover, muscle power production increased by 6% with PT and 7% with HRT, while in those not undergoing either treatment it was reduced by 5%. In addition, running speed increased by 4% both after HRT and PT and was reduced by 2% without treatments.

The transcriptome-wide study conducted with muscle samples of the Ex/HRT participants revealed that the expression of many genes related to energy metabolism and its regulation are affected by HRT and PT (Pöllänen et al., 2010). OGT was one of the genes upregulated in the muscles of postmenopausal women in the control group in comparison to those receiving HRT or PT. In the present study we investigated the associations of OGT and OGA gene expression with muscle phenotype and improvements obtained due to HRT and PT.

2. Material and methods

2.1. Study design

The original yearlong randomized placebo-controlled Ex/HRT-trial (ISRCTN49902272) has been previously described in detail by Sipilä et al. (2001). Briefly, 80 early postmenopausal women (50–57-years) were randomly assigned to one of the four groups: PT ($n = 20$), HRT ($n = 20$), PT + HRT ($n = 20$) and control (CO, $n = 20$). Study participants in the HRT group received combined oestradiol (2 mg) and noretisterone acetate (1 mg) product (Kliogest®; Novo Nordic) continuously, one tablet per day, for 1 year. Women not receiving HRT took placebo one tablet per day. PT participants performed progressive plyometric power training under supervision twice a week and a series of exercises at home four days per week. The training was performed in a circuit fashion. During the first two circuit training periods (8–10 weeks each interspersed with 2 weeks of high-impact aerobic dance), three rotations were performed of skipping (30 s), bounding over soft hurdles (13–16 cm), drop jumping (10–15 cm), and hopping (on one leg 10 times, added during the second training period). The following three periods (8–10 weeks each) comprised four rotations of bounding (19–25 cm), drop jumping (20–25 cm), hopping (10 times per leg), and leaping (10 times). In addition, all circuit training sessions included three or four resistance exercises for the upper body. The home exercise program was also designed as a circuit-training routine comprising three rotations of skipping (30 s), hopping (10 times per leg), and drop jumping (15 cm). In addition, exercises to strengthen the abdominal and lower back region were included. Women who were not in the exercise groups were advised to maintain their daily routines and physical activity level. In the present study we used muscle phenotype and microarray data from participants, from whom baseline and 12 month muscle samples were available. These included eight PT, ten HRT and nine CO women. The PT + HRT group was excluded from gene expression analysis due to the small number of muscle samples available. The study was carried out in accordance with the Declaration of Helsinki (1989) of the World Medical Association and was approved by the ethics committee of the Central Finland Health Care District. Written informed consent was obtained from all subjects.

2.2. Microarray analyses for muscle gene expression

Muscle biopsy sampling has been previously described in detail (Pöllänen et al., 2007). Briefly, needle muscle biopsies were obtained from the mid-part of *vastus lateralis* (midpoint between the greater trochanter and the lateral joint line of the knee) on the side of dominant hand. All the visible fat and connective tissue were removed and samples were snap-frozen in liquid nitrogen. Samples were stored at −80 °C until analyses. Total RNA was isolated according to manufacturer's instructions with Trizol-reagent (Invitrogen, Carlsbad, CA, USA). The whole genome gene expressions arrays (HumanRef-8 v1.0 or HumanWG-6 v1.0 BeadChips; Illumina Inc., San Diego, CA) were used to measure gene expression in baseline and follow-up muscle samples as described by Pöllänen et al. (2010), with the expression of OGT and OGA genes determined from the arrays.

2.3. Data analysis

Data analyses were carried out with PASW Statistics 18 (SPSS, Inc., Somers, NY, USA). Normality of the distributions was tested using the Shapiro–Wilk test. Since the gene expression data did not fulfill the criteria for parametric testing, the differences in percent changes between groups were analyzed by the Kruskal–Wallis test followed by the post-hoc Mann–Whitney U test. Percent change was calculated as (12 month value − baseline value)/baseline value × 100. The Pearson correlation coefficient was used to determine the relationship between OGT and OGA gene expression, muscle CSA, knee extension force, running speed and vertical jumping height. Although the gene expression data were not normally distributed, it was considered acceptable based on skewness and kurtosis, and therefore parametric tests were employed for all correlations. An α level of 0.05 was required for significance in all statistical analyses.

3. Results

3.1. Gene expressions of OGT and OGA enzymes

The increase in OGT gene expression was significantly greater in the CO group (22.1%) than in the HRT group (6.2%, $p = 0.01$) (Fig. 1a). The increase tended to be smaller in the PT group (14.3%), but it did not differ significantly from the increase in the CO and HRT groups (Fig. 1a).

Change in OGA gene expression did not differ significantly between the groups, although in the HRT group the OGA gene expression tended to increase (13.8%, −0.6% and 2.5% in HRT, PT and CO groups, respectively) (Fig. 1b).

The ratio of OGT to OGA gene expression decreased significantly in the HRT group (−3.9%) compared to the CO group (20.0%, $p = 0.004$) (Fig. 1c). There were no significant differences in the OGT/OGA ratio change between PT (15.2%) and CO and HRT groups.

3.2. Associations of OGT and OGA gene expressions with muscle mass and performance

There was a trend for the change in OGT gene expression to be negatively associated with change in knee extensor muscle CSA, while the change in the OGA gene expression tended to correlate positively with the change in muscle CSA (Table 1). Consequently, the change in OGT/OGA ratio, was correlated negatively with the change in muscle CSA ($r = -0.61$, $p = 0.001$, Fig. 2), but no significant correlation was found with the change in knee extension force, running speed or vertical jumping height (Table 1).