Effect of aging on response to exercise training in humans: skeletal muscle GLUT-4 and insulin sensitivity

JULIE H. COX, RONALD N. CORTRIGHT, G. LYNIS DOHM, and JOSEPH A. HOUMARD

1Human Performance Laboratory, Department of Exercise and Sport Science and 2Department of Biochemistry, School of Medicine, East Carolina University, Greenville, North Carolina 27858

Cox, Julie H., Ronald N. C ortright, G. Lynis Dohm, and Joseph A. H oumar d. Effect of aging on response to exercise training in humans: skeletal muscle GLUT-4 and insulin sensitivity. J. Appl. Physiol. 86(6): 2019–2025, 1999.—The purpose of this study was to compare the effects of short-term exercise training on insulin-responsive glucose transporter (GLUT-4) concentration and insulin sensitivity in young and older individuals. Young and older women (22.4 ± 0.8 (SE) yr, n = 9; and 60.9 ± 1.0 yr, n = 10) and men (20.9 ± 0.9, n = 9; 56.5 ± 1.9 yr, n = 8), respectively, were studied before and after 7 consecutive days of exercise training (1 h/day, ~75% maximal oxygen uptake). The older groups had more adipose tissue, increased central adiposity, and a lower maximal oxygen uptake. Despite these differences, increases in whole body insulin action (insulin sensitivity index, determined with an intravenous glucose tolerance test and minimal-model analysis) with training were similar regardless of age, in both the women and men (mean increase of 2.2 ± 0.3-fold). This was accompanied by similarrelative increases in muscle (vastus lateralis) GLUT-4 protein concentration, irrespective of age (mean increase of 3.1 ± 0.7-fold). Body mass did not change with training in any of the groups. These data suggest that older human skeletal muscle retains the ability to rapidly increase muscle GLUT-4 and improve insulin action with endurance training.

EXERCISE TRAINING can improve insulin action (6, 8, 20, 21, 23, 27, 28, 37). One of the cellular mechanisms that may contribute to this improvement is an increase in the concentration of the insulin-sensitive glucose transporter (GLUT-4) in skeletal muscle (1, 9, 18–21, 23). With insulin stimulation, the translocation of GLUT-4 to surface membranes is necessary for sugar transport into the cell; an increase in GLUT-4 may thus facilitate the transport of glucose into the muscle fiber (1). In support of this relationship, overexpression of GLUT-4 in the skeletal muscle of transgenic mice improved insulin action in healthy animals (31, 36) and restored insulin action in diabetic animals (31). Such data suggest that the increase in GLUT-4 in skeletal muscle with exercise training may be a functionally important mechanism associated with improved insulin action.

It is not evident, however, whether the exercise-induced increase in GLUT-4 is a universal phenomenon. For example, Kern et al. (29) reported that GLUT-4 concentration increased with exercise training in young and middle-aged, but not in oId Fischer 344 rats. They hypothesized that the old rodents did not exercise at a sufficient absolute workload to elicit an increase in the protein, although relative training intensities were similar. However, in another study (12), adult rats increased GLUT-4 to a greater extent than did young animals with exercise training. In contrast, 9–10 wk of one-legged cycle training increased GLUT-4 concentration to the same degree in young (mean age of 23 yr) and older men (mean age of 59 yr) (9). From these conflicting data, it is difficult to discern the impact of endurance-oriented training on muscle GLUT-4 concentration in older, compared with young, individuals.

The purpose of this study was, therefore, to compare the effect of exercise training on skeletal muscle GLUT-4 protein concentration in young (<30 yr) vs. middle- to older-aged subjects (50–70 yr). Insulin action can improve with 7 days of endurance training (6, 28, 37). Therefore, we compared the responses of GLUT-4 and insulin action in older and young subjects before and after a similar training regimen. Exercise intensity was held constant (70–75% maximal oxygen uptake (V\textsubscript{O\textsubscript{2max}})) to examine the impact of the same relative exercise stimulus on GLUT-4 regulation in young vs. older skeletal muscle.

METHODS

Study design. Four groups of subjects were examined: 1) young women (n = 9), 2) older women (n = 10), 3) young men (n = 9), and 4) older men (n = 8). Age criteria for the young groups were 18–30 yr and for the older groups 50–70 yr. Subjects were initially screened for body composition and cardiovascular fitness level. Pretraining insulin action was determined with an intravenous glucose tolerance test (IVGTT). A pretraining biopsy from the vastus lateralis was obtained for measurement of GLUT-4. Subjects then underwent 7 consecutive days of supervised exercise training. A posttraining IVGTT and muscle biopsy were performed 15–17 h after the final training bout.

Subjects. Subjects were volunteers who had not been active in an exercise program for at least the previous 2 yr. Subjects were also questioned concerning their normal daily activities, and only those with relatively sedentary lifestyles were included; this was verified by the V\textsubscript{O\textsubscript{2max}} and body composition data (see Tables 1–4). Other inclusion criteria were age-normative values for adiposity and V\textsubscript{O\textsubscript{2max}} (33, 35); no smoking; no medications that alter insulin action; and no evidence of coronary artery disease, hypertension, or orthopedic injuries that would inhibit exercise training. All of the older women were postmenopausal; five of the women were on estrogen-replacement therapy. There were no significant initial differences in insulin action or GLUT-4 with estrogen replacement vs. no medication; responses to exercise did not differ either. Thus the data from the older women were combined. The young women were tested in the follicular...
phase of the menstrual cycle, based on a recall of their previous menses.

Insulin action. Insulin action was determined with an IVGTT, as described by Bergman et al. (3) and as used previously in this laboratory (20–22). Subjects reported to the laboratory in the morning after a 12-h fast and had consumed at least 250 g of carbohydrate per day for the previous 3 days. The pretraining IVGTT was performed in the sedentary condition; the posttraining test was performed 15–17 h after the final exercise bout. Briefly, four baseline samples were obtained before the intravenous injection of glucose (1.7 mmol/kg) at time 0 and insulin (150 pmol/kg) 20 min later. Twenty-five samples were obtained between 0 and 180 min and subsequently analyzed by spectrophotometry for glucose (procedure HK 16-UV; Sigma Chemical, St. Louis, MO) and by microparticle enzyme immunoassay for insulin (IMx, Abbott, Abbott Park, IL). Insulin sensitivity [insulin-sensitivity index (ISI)] was calculated with the minimal model of insulin action (MINMOD, version 3.0). ISI measures the increase in glucose disappearance per increase in unit insulin (3). Insulin-independent glucose disappearance ($S_{glu}$) and insulin secretion [area under the insulin curve during the initial rise in glucose; acute insulin response (AIR$_{glu}$)] were also calculated.

GLUT-4 protein concentration. A muscle sample (~100 mg) was obtained with the percutaneous biopsy technique from the vastus lateralis of the same leg before and after training. Muscle was immediately frozen in liquid nitrogen. For the GLUT-4 assay, frozen muscle was pulverized by using a small ground-glass mortar and pestle and then homogenized with 400 µl of ice-cold buffer. Homogenization buffer contained 25 mmol/l HEPES, 4 µmol/l EDTA, 25 mmol/l benzamidine, 0.5 mmol/l phenylmethylsufonyl fluoride, 4.2 µmol/l leupeptin, 1.5 µmol/l pepstatin, and 0.6 µmol/l aprotinin (pH 7.4). Homogenates were pipetted into microfuge tubes and spun for 30 min at 4°C and 16,000 g. The pellet was resuspended in 150 µl of buffer containing 1% Triton X-100, kept on ice for 1.5 h, and vortexed periodically to ensure solubilization. The sample was spun again for 30 min at 16,000 g and 4°C, the supernatant was removed, and protein concentration was determined by using the bicinchoninic acid method (39). To determine GLUT-4 protein concentration, duplicate samples of supernatant containing 75 µg protein were solubilized in Laemmli buffer (30) containing 2.5% dithiothreitol and separated by electrophoresis on an 9% polyacrylamide resolving gel. The pre- and posttraining samples from a given subject were run on adjacent lanes of the same gel. Protein was then transferred (0.5 A, 2 h) to an Immobilon membrane (Millipore, Bedford, MA) and blocked overnight at 4°C with 5% nonfat dry milk in Tris-buffered saline (TBS-Blotto). This was followed by incubation at 4°C for 12 h in 4 µg/ml protein A-purified polyclonal antibody, specific for the COOH-terminal peptide of GLUT-4. Results with this antibody have been presented elsewhere (19, 21). The membrane was washed in TBS-Tween, followed by TBS, and incubated for 1 h at 25°C in 50 ml TBS-Blotto that contained horseradish peroxidase-conjugated donkey anti-rabbit IgG diluted 1:4,000 (Amer sham, Arlington Heights, IL). Antibody-antigen complexes were detected by enhanced chemiluminescence (ECL, Amer sham). Intensities of the bands were determined on a computer-controlled video densitometer (Hewlett-Packard Scan jet IIc/X/T hardware, Sunnyvale, CA) to quantify GLUT-4 by using ImageQuant software (Molecular Dynamics). Values are presented as arbitrary absorbance units (AAU). A rat heart standard was used as the molecular weight marker for GLUT-4.

Cardiovascular fitness and body composition. VO$_{2\text{max}}$ was measured during incremental exercise on an electrically braked cycle ergometer (Lode, Diversified, Brea, CA). Oxygen consumption was measured with open-circuit spirometry by using a metabolic cart (model 2900, Sensor Medics, Anaheim, CA). A 12-lead electrocardiogram (ECG) recorded heart rate and ECG tracings. The maximal exercise test was used to (1) screen for potential heart disease; 2) determine whether the subject was sedentary, as classified by VO$_{2\text{max}}$ (35); and 3) determine the heart rate and oxygen consumption needed to elicit the desired intensity (70–75% VO$_{2\text{max}}$) during the 7 days of training. A physician was present during the maximal testing of all older subjects and interpreted the exercise ECGs. Body composition was determined by body fat percentage with skinfolds (25), body mass index (BMI), and waist (level of umbilicus) and hip (maximum hip circumference) girths. Only subjects within normative values for their age for body fat percentage and BMI were studied (35).

Exercise training. Subjects exercised 1 h/day for 7 consecutive days on a cycle ergometer. Exercise intensity was adjusted to achieve 70–75% of VO$_{2\text{max}}$ as determined from Douglas bags collected at minute 5 and every subsequent 15 min of exercise. Heart rate was monitored with telemetry (Polar, Stamford, CT) to gauge exercise intensity. All subjects exercised continuously for 1 h during the 7 days of training.

Statistics. Data were compared with a two (age, young and older) by two (treatment, before and after exercise training) repeated-measures analysis of variance. Contrast comparisons were used to determine specific differences if a significant interaction or group effect (P < 0.05) was obtained. Descriptive data (age, body composition, exercise variables) were compared between the young and older groups within a gender with an independent t-test (P < 0.05).

RESULTS

Anthropometric data. Age and anthropometric data for the female and male subjects are presented in Tables 1 and 2, respectively. The older women and men had significantly (P < 0.05) more adipose tissue (body fat percentage, fat mass, BMI) than the younger groups, despite a similar fat-free mass. The older groups also exhibited higher waist and hip girths and an increased waist:hip ratio compared with the young subjects (P < 0.05). The older men were significantly (P < 0.05) heavier than the young men.

VO$_{2\text{max}}$ and exercise data. Maximal exercise and training data for the women and men are presented in Tables 3 and 4, respectively. VO$_{2\text{max}}$ was significantly (P < 0.05) lower in the older women and men, as was VO$_{2\text{max}}$.
maximal achieved workload and maximal heart rate. All groups exercised at ≈75% VO2max, however, heart rate and oxygen consumption at the workload that elicited this response were significantly (P < 0.05) lower in the older groups. Body mass did not change (P > 0.05) in any of the groups with the 7 days of training (mean for pooled data, 74.1 ± 2.6 vs. 74.2 ± 2.5 kg). VO2max was not measured after training, but it has been reported to not change with a virtually identical 7-day training regimen (see Ref. 37, unpublished observations).

Insulin action and secretion. There was a significant (P < 0.05) treatment (training) effect with no significant interaction for an improvement in insulin action (ISI) in the young (5.1 ± 0.9 vs. 7.1 ± 1.2 min⁻¹·µU⁻¹·ml⁻¹) and older (3.8 ± 0.7 vs. 8.3 ± 1.4 min⁻¹·µU⁻¹·ml⁻¹) women with training (Fig. 1). There were no significant (P > 0.05) differences in initial or posttraining ISI between these two groups (Fig. 1). In the men, ISI also improved significantly (P < 0.05) in the young (4.5 ± 0.6 vs. 6.4 ± 0.9 min⁻¹·µU⁻¹·ml⁻¹) and older (2.1 ± 0.5 vs. 3.8 ± 0.7 min⁻¹·µU⁻¹·ml⁻¹) groups with no interaction effect (Fig. 1). ISI was significantly (P < 0.05) lower in the older men before and after training (Fig. 1). In the women, there was no difference in glucose effectiveness (SGlu) with age (0.02 ± 0.01 min⁻¹). SGlu in the men was slightly but significantly (P < 0.05) higher in the young vs. older group (0.03 ± 0.01 vs. 0.02 ± 0.01 min⁻¹, respectively). SGlu did not change in the women or men with training.

Fasting blood glucose and insulin levels were within normal values and did not change with training in either the young (glucose, 4.6 ± 0.2 vs. 4.8 ± 0.2 mM; insulin, 36.6 ± 5.4 vs. 32.4 ± 4.8 pmol/l) or older women (glucose, 5.0 ± 0.4 vs. 5.1 ± 0.2 mM; insulin, 37.2 ± 4.8 vs. 34.2 ± 3.1 pmol/l, before vs. after training, respectively). Fasting glucose and insulin were not signifi-
cantly different between the young and older women. Fasting blood glucose and insulin did not change with training in either the older (glucose, 5.7 ± 0.2 vs. 5.6 ± 0.3 mM; insulin, 70.8 ± 10.2 vs. 66.0 ± 15.6 pM) or young (glucose, 5.4 ± 0.2 vs. 4.8 ± 0.2 mM; insulin, 30.6 ± 3.6 vs. 33.6 ± 7.8 pM) men (before vs. after training, respectively). Fasting glucose and insulin values were elevated in the older compared with the young men; this difference persisted after training.

The AIR$_{\text{glu}}$ obtained during the minimal model is indicative of the early phase of pancreatic insulin secretion (3). As presented in Fig. 2, there was a trend for insulin secretion to be reduced with exercise training in only the older subjects. This was particularly manifested in the men, as there was a significant ($P < 0.05$) interaction, with the older men decreasing AIR$_{\text{glu}}$ with training while the young group did not change. This interaction approached statistical significance ($P = 0.08$) in the women (Fig. 2).

Muscle GLUT-4 protein concentration. The GLUT-4 data are presented in Fig. 3. GLUT-4 protein concentration increased significantly ($P < 0.05$) in the young (92.4 ± 17.8 vs. 135.4 ± 24.6 AAU) and older women (107.1 ± 20.6 vs. 164.7 ± 25.9 AAU) with the 7 days of exercise training, with no significant ($P > 0.05$) interaction effect (Fig. 3). In the men, GLUT-4 concentration also increased significantly ($P < 0.05$) in the young (91.2 ± 16.9 vs. 169.5 ± 28.4 AAU) and older groups (82.9 ± 30.9 vs. 171.1 ± 36.5 AAU) with no interaction (Fig. 3). GLUT-4 protein concentration was not statistically different with age in the men and women before or after training.

Although there was evidence for a gender effect relative to aging and insulin action (Fig. 1), the insulin action and GLUT-4 data were pooled across genders to...
further compare responses to training in older vs. young subjects. There was no significant interaction effect but a significant treatment (training) effect (P < 0.05) for increasing ISI (young, 4.8 ± 0.5 vs. 6.8 ± 0.7 min⁻¹·µU⁻¹·ml⁻¹; older, 3.1 ± 0.5 vs. 6.3 ± 1.0 min⁻¹·µU⁻¹·ml⁻¹). A similar significant (P < 0.05) treatment effect with no interaction was evident for increasing GLUT-4 (young, 91.7 ± 17.4 vs. 152.5 ± 26.5; older, 93.8 ± 26.3 vs. 168.2 ± 31.7 AAU) with training (pre- vs. posttraining, respectively). The mean magnitude of increase (mean of postraining-to-petreining values for each subject) in ISI with training was 2.2 ± 0.3-fold, whereas the mean increase in GLUT-4 with training was 3.1 ± 0.7-fold.

DISCUSSION

The main finding of the present study was that exercise training at the same relative exercise intensity (=75% \( \text{V}_{\text{O2\text{max}}} \)) increased GLUT-4 protein concentration in human skeletal muscle to a similar extent in young and older men and women (Fig. 3). This increase occurred despite the significantly lower absolute workloads of the older subjects, as evidenced by oxygen consumption and heart rate responses during exercise (Tables 3 and 4). These findings suggest that the relative, rather than absolute, exercise stimulus elicited during training is an important factor controlling GLUT-4 regulation in human skeletal muscle. The present findings also indicate that skeletal muscle in older men and women retains the ability to rapidly increase muscle GLUT-4 concentration with endurance-oriented exercise training.

Such findings are relevant, as the presently available data concerning the ability of exercise to increase GLUT-4 in older skeletal muscle are not conclusive. Kern et al. (29) reported that GLUT-4 concentration increased approximately two- to threefold in the soleus and gastrocnemius of young (6–8 mo) and middle-aged (15–17 mo) Fischer 344 rats with 10–15 wk of training at 75% of maximal exercise capacity. However, there was no significant increase in GLUT-4 in these muscle groups, in older animals (27–29 mo), despite an identical relative exercise stimulus. The authors concluded that the lower absolute workload used by the older animals may not have been sufficient to trigger alterations in GLUT-4. Similar results were obtained by Gulve et al. (16) when they compared the effects of wheel-running training in adult and old rats. Exercise did not increase GLUT-4 in the older animals despite an increase in the young runners. The older runners, however, ran one-half of the distance of the younger rats. Ezaki et al. (12) obtained contradictory findings when they observed that adult (12-mo) Sprague-Dawley rats increased skeletal muscle GLUT-4 concentration to a greater degree than did young (1-mo) animals after 4-wk of training at the same absolute exercise intensity. These authors (12) concluded that exercise training increases glucose transporter concentration more efficiently in older vs. young muscle. Youngren and Barnard (40) also reported that 8 wk of training increased GLUT-4 concentration in the skeletal muscle of old (24 mo) Fischer 344 rats.

The present data suggest that older and young human skeletal muscle increases GLUT-4 to a similar extent when exercise training is performed at the same relative exercise intensity (Fig. 3). In agreement with these findings, Dela et al. (9) reported similar increases in GLUT-4 in older (59 yr, n = 8) and young (23 yr, n = 5) men after 9 wk of one-legged cycle training at \( \approx 70\% \text{V}_{\text{O2\text{max}}} \). Together, these results and the present findings suggest that GLUT-4 increases by approximately the same magnitude in young and older human skeletal muscle when an adequate (70–75% \( \text{V}_{\text{O2\text{max}}} \)) and equivalent relative exercise stimulus is presented. This increase in GLUT-4 can occur relatively rapidly, as evident from the present data, with such an exercise stimulus.

An increase in GLUT-4 with exercise may be functionally important in relation to improving insulin action. In transgenic animals, the overexpression of GLUT-4 results in a two- to fourfold increase of the protein in skeletal muscle (31, 36). An increase of this degree was sufficient to improve insulin action in healthy animals. An interesting finding in the present (Fig. 3, RESULTS) and other studies (19–21, 23, 34) is that GLUT-4 concentration increases \( \approx 1.5 \)- to 3.0-fold in human skeletal muscle with 7 days to 14 wk of endurance-oriented training. In light of the transgenic animal data (31, 36), it appears that an increase of this magnitude may contribute, at least in part, to the improvement in insulin action with training.

Insulin action was determined 15–17 h after the last exercise bout; the residual effects from the last training bout may thus account for the enhanced ISI evident in the present study. In support of this relationship, insulin action is enhanced for up to 12 h after only a single bout of exercise (10, 11). This enhancement of insulin action is primarily driven by the need for glycogen repletion following an exercise bout (4, 24). The acute effects of exercise may not, however, fully account for the enhanced insulin action observed in the aged subjects studied in the present study. We (18) and others (6, 37) have observed that a single bout of physical activity (40–60 min at 70–75% \( \text{V}_{\text{O2\text{max}}} \)) in older individuals (>50 yr) did not improve insulin action 15–18 h after the exercise bout. Such data suggest that the enhanced insulin action evident in the present study was a combination of both the acute effects of a single exercise bout and cumulative effects from the 7 days of training. This hypothesis is in agreement with conclusions derived from other 7-day training studies (6, 37).

Aging is associated with a progressive decrement in insulin action, which can be initiated as early as the third decade of life (7, 26). This decline has been attributed to chronological age itself and/or to a variety of secondary factors associated with the aging process, such as an increase in body fat, increased central adiposity, and a reduction in spontaneous physical activity (13, 17, 26, 38). Our data were consistent in demonstrating that older men and women display a
reduction in cardiorespiratory fitness, increased overall adiposity, and an increase in central adiposity, compared with younger individuals (Tables 1, 2). Such differences in body composition (Table 2) may explain why the older men displayed lower ISI and $S_{glu}$ values than did their younger counterparts both before and after training (Fig. 1 and RESULTS). In contrast, despite differences in body composition (Table 1), insulin action and $S_{glu}$ did not differ in the young and older women (Fig. 1). A gender difference has been reported in relation to the insulin resistance of aging (13, 14), with women being more resistant to this phenomenon (14). This observation (14) is in agreement with the present data (Fig. 1). However, another large-scale study reported similar decrements in glucose tolerance in men and women with progressing age (38). Such findings suggest that a variety of factors are involved with the insulin resistance of aging and that the role of gender is not definitive.

Other 7-day exercise training studies have reported improvements in insulin action in older (6), obese (28), and non-insulin-dependent diabetes mellitus (37) subjects, despite no changes in body mass. However, in these studies (6, 28, 37), there has been no determination of the relative improvement in insulin action, i.e., comparison with a healthy control group. The present data provide the additional information that insulin action improves to a similar extent in young and older individuals with a relatively acute exercise stimulus that does not change body mass.

Insulin secretion is often reduced in older and young subjects with exercise training, reciprocal to enhanced peripheral insulin sensitivity (17, 27). The present data suggest that this adaptation can occur relatively rapidly and may be more pronounced in older subjects (Fig. 2). Thus, even though islet function may be compromised in older individuals (17), it appears as though the $\beta$-cell maintains it ability to adapt to an improvement in insulin action with training.

In a previous study, we reported a negative relationship between age and GLUT-4 protein concentration in the vastus lateralis of both men ($r = -0.28$, $P < 0.05$) and women ($r = -0.51$, $P < 0.01$) (22). In the present study, we observed no decline in GLUT-4 in this muscle group with aging (Fig. 3). An explanation for these differences may lie in the populations examined. A tendency for GLUT-4 to decline with aging was evident when we studied individuals over a relatively wide age range (18–80 yr) (22). In the present work, the age range of the subjects was smaller, and we did not examine a large number of individuals >65 yr old. The reduction in GLUT-4 with aging may only become more pronounced when an elderly group is included. Another contributing factor may be the inherent variability evident in insulin action among older human populations (7, 13, 14, 17, 22, 26). We observed such variability in skeletal muscle GLUT-4 concentration with age (22). In support of this relationship, Dela et al. (9) did not observe a reduction in GLUT-4 when comparing similar, small groups of young (23 yr, $n = 5$) and older (59 yr, $n = 8$) men. In rodents, several studies have shown decreases in the concentration of GLUT-4 in some skeletal muscle groups in rapidly growing young (1–4 mo) vs. adult (>10 mo) animals (2, 5, 12, 15, 32). In studies comparing adult (7–13 mo) vs. older rats (>25 mo), either no change (5, 15, 16) or a decrease (29) in GLUT-4 in skeletal muscle has been reported.

In conclusion, 7-days of endurance-oriented training (1 h/day) at the same relative exercise intensity (=75% $V_{O_{2max}}$) increased GLUT-4 protein concentration to a similar extent in the skeletal muscle of young and older men and women. This increase was evident despite the markedly lower absolute workloads during training and the increased adiposity and reduced cardiovascular fitness of the older groups. Whole body insulin action also increased by a similar magnitude in young vs. older men and women. These data suggest that older human skeletal muscle retains the ability to increase muscle GLUT-4 and improve insulin action with endurance training.

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Address for reprint requests and other correspondence: J. A. Houmard, Human Performance Laboratory, Ward Sports Medicine Bldg., East Carolina Univ., Greenville, NC 27858 (E-mail: HOUMARDJ@MAIL.ECU.EDU).

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