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Original Article

Simultaneous Effects of Aerobic Training and Berberine Chloride on Plasma Glucose, IL-6 and TNF-α in Type 1 Diabetic Male Wistar Rats

Javad Ramezani[1], Mohammad Ali Azarbayjani[*2], Maghsoud Peeri[2]

1- Ph.D. Student, Department of Exercise Physiology, Central Tehran Branch, Islamic Azad University, Tehran, Iran
2- Professor, Department of Exercise Physiology, Central Tehran Branch, Islamic Azad University, Tehran, Iran

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ABSTRACT

Background and Objectives: Aerobic training and berberine chloride include antioxidant and anti-inflammatory characteristics. In the current study, simultaneous effects of aerobic training and berberine chloride on plasma glucose, IL-6 and TNF-α were investigated in type 1 diabetic male Wistar rats.

Materials and Methods: In this experimental study, 64 male Wistar rats were randomly divided into eight groups (n=8), including healthy control, diabetic control, diabetic-berberine (15 and 30 mg/kg), diabetic-training, diabetic-training-berberine (15 and 30 mg/kg) and health-trained. Diabetes was induced using a single intraperitoneal injection (IP) of streptozotocin (STZ) of 60 mg/kg body weight (BW). The training schedule included running on a treadmill for six weeks, five sessions a week and daily intakes of berberine using gavage. The IL-6 and TNF-α values were assessed using enzyme-linked immunosorbent assay (ELISA) method.

Results: Results showed that the glucose concentration in diabetic groups with aerobic training and intake of berberine simultaneously was significantly lower than that in the diabetic control group (P<0.05). Plasma IL-6 in the diabetic control group significantly increased, compared to that in the normal control group (P<0.05). In diabetic groups that received the two treatments simultaneously, IL-6 significantly increased, compared to that in the diabetic control group (P<0.05). The quantity of TNF-α in the diabetic control group was significantly higher than that in the normal control group (P<0.05). The plasma level of TNF-α significantly decreased in all treatment groups, compared to that in the diabetic control group (P<0.05).

Conclusions: Combined effects of berberine chloride use and aerobic training on blood glucose, IL-6 and TNF- α are more evident than their individual effects. Furthermore, a dose of 30 mg/kg of berberine chloride included a greater effect on the highlighted blood parameters, compared to that a dose of 15 mg/kg of berberine chloride did.

Keywords: Type 1 Diabetes, Aerobic training, Berberine chloride, IL-6, TNF-α

Introduction

Diabetes mellitus is a chronic and progressive metabolic disorder that is currently a public health problem and one of the most important causes of mortality in the world (1). Type 1 diabetes (T1D), which includes approximately 5–10% of the diabetic cases, is an autoimmune disorder caused by abnormalities in the function or degradation of pancreatic beta-cells (2). This is dependent on cellular infiltration and inflammatory responses in Langerhans Island cells within the pancreas (3). Cellular components of this infiltration include monocytes, macrophages, and CD^{4+} and CD^{8+} T cells (4). Balances between T-helper 1 (Th1) and Th2 cells are essential in the pathogenesis of this disease (5). Cytokines play important roles in the development and activation of the immune cells as they act as cell-signaling molecules, especially in autoimmune diseases such as type 1 diabetes (3). Plasma levels of pro-inflammatory and Th1 cytokines such as interleukin 1 beta (IL-1β), IL-2, IL-6, IL-12, tumor necrosis factor alfa (TNF-α) and interferon gamma (IFN-γ) may be upregulated in patients with T1D (3).

***Address for correspondence:** Mohammad Ali Azarbayjani, Professor Department of Exercise Physiology, Central Tehran Branch, Islamic Azad University, Tehran, Iran. E-mail address: Ali.azarbayjani@gmail.com

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The IL-6 is most often classified as a pro-inflammatory cytokine. However, IL-6 includes anti-inflammatory properties as well (6). Evidence shows that IL-6 is regulated by muscle contractions (7–9). The IL-6 anti-inflammatory effects are imposed by inhibiting TNF-α and IL-1β and activating IL-1ra and IL-10 (10). Furthermore, TNF-α is believed to play an important role as an inflammatory cytokine in autoimmune type 1 diabetes. *In Vitro* studies on isolated Islets of Langerhans have shown that TNF-α is directly involved in degradation of beta-cells (11). Moreover, *in vivo* studies have shown that this cytokine includes deep inflammatory effects, including a direct effect, on antigen-presenting cells (APCs) and T-cell lymphocytes (12).

In recent decades, herbs and their active components have been widely used in the treatment of diabetes, particularly in T2D (13). Of these active components in herbal plants, berberine is an isoquinoline alkaloid that exhibits promising potential for its potent anti-inflammatory (14) and hypoglycemic effects (15). However, exact berberine effects on serum glucose levels in T1D patients remain unknown. Use of oral hypoglycemic agents for the treatment of T1D is still in its early stages. Berberine has been shown to include hypoglycemic and insulin-sensitizing effects in high-fat diet and streptozocin-induced diabetic rats (16). Berberine is found in many herbal plants, such as *Hydrastis Canadensis* (Goldenseal), *Rhizoma coptidis* (Huang Lian), *Berberis vulgaris* (barberry) and *B. aristata* (tree turmeric) widely used in Chinese traditional medicine (17,18). Iran is the largest producer of barberries in the world. Barberries can be used as one of the most important medications in controlling and treating diabetes. Previous studies have shown that berberine chloride includes negative regulation of pro-inflammatory cytokines such as IFN-γ, TNF-α, and IL-6 in animal models (19,20). Recently, berberine chloride at doses of 50 mg/kg for 45 days have been shown to significantly decrease TNF-α (21). Other studies have revealed that the lowest dose of berberine chloride (as an antioxidant and anti-inflammatory agent) is 50 mg/kg (21,22). In the current study, berberine chloride doses of 15 and 30 mg/kg were used to compare the interactive effects of the chemical and aerobic training.

Physical activity is another way of decreasing blood glucose in diabetics. Evidence suggests that physical activity reduces the progression of impaired glucose tolerance (23). the treadmill training program has been demonstrated to decrease plasma glucose concentrations 5 min after the glucose challenge (24). Research has shown that circulating IL-6 levels increase in an exponential mode (up to 100-fold) in response to exercises and decrease post exercises (25). The magnitude by which plasma IL-6 increases is associated to the exercise duration and intensity, the muscle mass involved in mechanical works and the endurance capacity (25). Duration of exercise is the most importantly unique factor that determines the magnitude of the systemic IL-6 response. The longer the duration of exercises, the further systemic IL-6 responses. Following intense exercises such as running at 75% of VO_2^{max}, the basal plasma IL-6 may increase 5-folds after 30 min. However, plasma IL-6 concentration may increase up to 100-fold after a marathon (26). The exercise-induced an increase in plasma IL-6 is followed by increased circulating levels of well-known anti-inflammatory cytokines such as IL-1ra and IL-10 (27). Furthermore, infusion of IL-6 to healthy donors mimics the exercise response of IL-1ra and IL-10 and enhances systemic levels of cortisol (28). The IL-1ra inhibits IL-1b signal transduction (29) and IL-10 is able to inhibit synthesis of pro-inflammatory cytokines such as TNF-α (26). However, other studies have shown no decreases in TNF-α (30). Therefore, the aim of the current study was to investigate interactive effects of aerobic training and low-doses of berberine chloride on plasma glucose, IL-6, and TNF- α in streptozocin (STZ) induced diabetic male Wistar rats.

Materials and Methods

Animals: In this experimental study, 64 male Wistar rats (240–280 gr) were purchased from the Pasteur Institute of Iran, Tehran, Iran, and transferred to the Animal Room of the International Campus, Shahid Sadoughi University of Medical Sciences, Yazd, Iran. All standard parameters, including temperature condition (24 ±1°C), relative humidity (55 ±3%), free access to water, standard special diet (Behparvar, Iran) and a dark/light cycle (12/12 h) were included. Animals were housed in special polycarbonate cages for adaptation to the new environment for two weeks. To acquaint with the treadmill in the adaptation period, animals walked on the treadmill five days a week, each time for 5–10

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min at a speed of 4–5 m/min. Then, rats were randomly assigned to eight groups (n = 8 per group). Groups were matched based on the BW. The experimental type 1 diabetes was induced using intraperitoneal (IP) injections of 60 mg/kg per BW of fresh streptozocin solution (pH 4.5) (Sigma, USA) dissolved in 0.1 M of citrate buffer (31). An equivalent volume of normal saline solution was injected into two non-diabetic groups. To avoid severe drops in blood glucose after STZ injection, rats were provided with 5% glucose instead of water (21). Induced diabetes was verified after three days by measuring glucose in blood samples collected from tail veins using the glucocard-01 device (ARKRAY, Japan). Rats with a blood glucose level of 300 mg/dl or greater were included in the study as diabetic animals. The day of blood glucose test was set as zero. The experimental groups in this study included: 1) normal control group, 2) diabetic control group, 3) diabetic and berberine chloride group (15) [D-Br (15 mg)], 4) diabetic and berberine chloride group (30) [D-Br (30 mg)], 5) diabetic and aerobic training group [D-AT], 6) diabetic, aerobic training and berberine chloride group (15) [D-AT-Br (15 mg)], 7) diabetic, aerobic training and berberine chloride group (30 mg) [D-AT-Br (30 mg)], and 8) healthy and aerobic training group (H-AT).

Aerobic training protocol: In the present study, a moderate training intensity (55–50% maximum consumed oxygen) and physiological efficacy were used (Table 1) (32). Training groups trained for six weeks with a 3-day exercise on a treadmill and a 1-day rest. In each training session, 5 min of warming up and 5 min of cooling down were used at a speed of 4–5 m/min; added to the main training time. Speed and duration of training gradually increased according to Table 1. No electrical shocks were used during the exercise. If necessary, hand touches or acoustic stimuli were used to force the animals to continue training.

Table 1. The aerobic training program

Practice variable	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Speed (m/min)	10	10	14–15	14–15	17–18	17–18
Duration (min)	10	20	20	30	30	40

Berberine chloride: Berberine chloride supplement was administrated every day at a specific time using gavage. In this study, doses of 15 and 30 mg/kg were used based on EC50 (50% effective concentration) of the complementary. In groups that did aerobic training, the drug was administrated through gavage one hour before exercises. Drug administration was continued in days that the animals did not do physical activities.

Sacrifice and sampling: Briefly, 48 h after the last exercise session and after 12 h of fasting, samples were collected from control and treatment groups. To collect the samples, rats were initially anesthetized using intraperitoneal (IP) injection of a combination of ketamine (30–50 mg/kg) and xylazine (3–5 mg/kg). Then, the chest of the animals was split and blood was collected directly from the heart. Blood was immediately poured into tubes containing ethylene diamine tetra acetic acid (EDTA). Samples were centrifuged at 3000 rpm for 15 min and plasma was stored at -80 °C until use.

Biochemical assays: A commercial kit (Cat No. ZB-10135S-R9648; Zellbio, Germany) with a sensitivity of 0.05 ng/l was used for testing plasma IL-6 and another commercial kit (Cat. No: 865,000.096; Diaclone, France) with a sensitivity of less than 15 pg/ml for TNF-α. Blood Glucose was assessed using the glucocard-01 device (ARKRAY, Japan). Furthermore, blood glucose, BW and body mass index (BMI) was measured at the beginning of Week 1 and end of Week 6.

Statistical analysis: Data were analyzed using SPSS software v.25 (IBM Analytics, USA). The Kolmogorov-Smirnov test was used to show the normal distribution of the samples. For the comparison of the groups, two-way ANOVA and Tukey's post hoc test were used. Results were presented as mean ±SD (standard deviation) and significant differences were reported when $P < 0.05$.

Results

Changes in BW and BMI have been shown in Table 2. Blood glucose in the diabetic control group was significantly higher than that in the normal control group (P<0.05), which indicated the successful induction of diabetes with STZ. Based on the results from two-way ANOVA, neither aerobic training alone nor berberine chloride alone included significant effects on blood glucose in diabetic rats

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(P > 0.05). However, with the intervention of these two variables (aerobic training and berberine chloride with doses of 15 and 30 mg/kg), blood glucose significantly decreased (P<0.05) (table 3). A dose of 30 mg/kg of berberine chloride included greater effects on decreasing blood glucose, compared to a dose of 15 mg/kg of berberine chloride (Fig. 1).

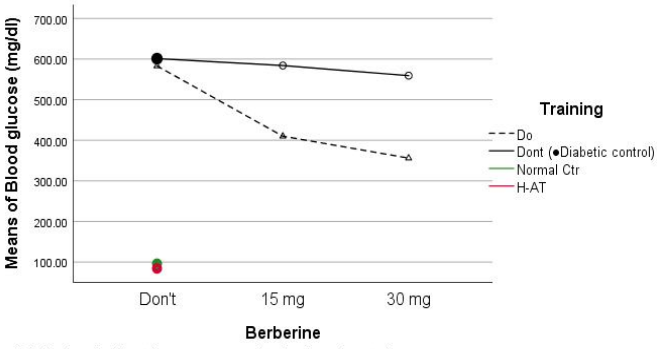

Figure 1. Blood glucose changes in the study groups

The IL-6 in the diabetic control group was significantly higher than that in the normal control group (P<0.05). Consumption of 15 mg/kg of berberine chloride [D-Br (15 mg)] included no significant effects on the increase or decrease in IL-6, compared to the diabetic control group. Consumption of 30 mg/kg of berberine chloride [D-Br (30 mg)] significantly decreased IL-6, compared to the diabetic control group (Fig. 2). The IL-6 slightly increased in diabetic groups that simultaneously received berberine chloride and aerobic training, compared to that in the diabetic control group. However, this increase was not statistically significant (P>0.05)(table 3). Plasma IL-6 was significantly higher in the health-trained group (H-AT) than that in other groups.

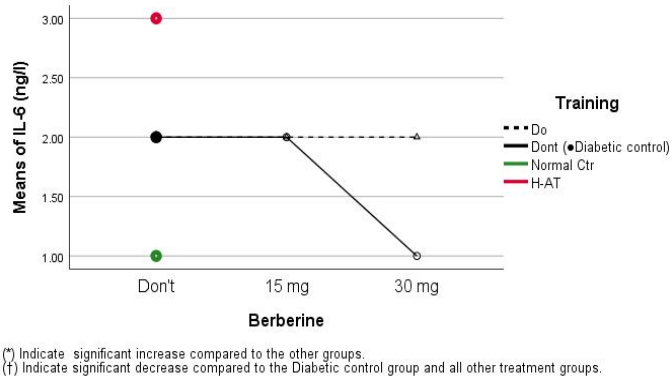

Figure 2. Changes in IL-6 of plasma in the study groups

Plasma TNF-α increased significantly in the diabetic control group, compared to that in the normal control group (P<0.05). Furthermore, TNF-α decreased significantly in all treatment groups compared to that in the diabetic control group (P<0.05). However, the interactive effect of the two variables (aerobic training and berberine chloride) was greater resulting in further the decreases in plasma TNF-α. Moreover, a dose of 30 mg/kg of berberine chloride was more effective than a dose of 15 mg/kg of the chemical (Fig. 3).

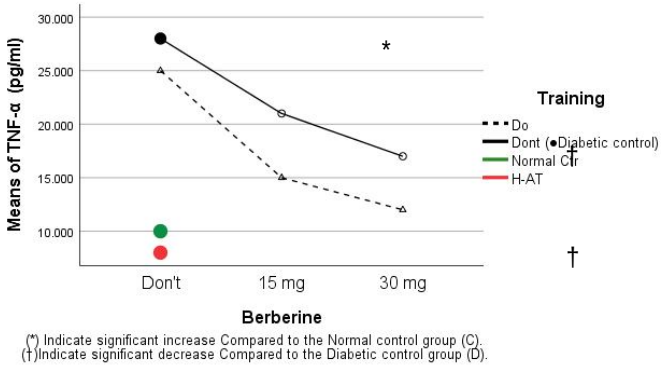

Figure 3. Changes in TNF-α of plasma in the study groups

Table 2. Initial and final body weights and body mass indices in study groups receiving various doses of berberine chloride

variable	week	Group							
		C	D	D-Br (15 mg)	D-Br (30 mg)	D-AT	D-AT-Br (15 mg)	D-AT-Br (30 mg)	H-AT
BW (gr)	1	283.63 ±18.20	285.12 ±13.37	283.81 ±21.84	281.57 ±14.04	281.78 ±3.01	283.63 ±5.79	282.78 ±17.05	282.80 ±7.26
	6	343.87 ±21.48	198.57 ±4.47	228.81 ±10.65	283.88 ±10.05*	205.36 ±11.59	289.63 ±7.95*	323.85 ±18.07*	354.56 ±16.63
BMI (g/cm^2)	1	0.63 ±0.02	0.61 ±0.02	0.60 ±0.01	0.62 ±0.03	0.61 ±0.01	0.60 ±0.01	0.60 ±0.02	0.61 ±0.01
	6	0.64 ±0.0	0.46 ±0.03	0.45 ±0.03	0.50 ±0.03	0.48 ±0.06	0.61 ±0.01£	0.62 ±0.02£	0.65 ±0.02

Values include means ±SD assayed by two-way ANOVA and Tukey's post hoc test; significant differences were seen between the experimental groups; *indicates a significant increase, compared to diabetic control group; £indicates a significant increase, compared to diabetic control group; BW, body weight; BMI, body mass index

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Table 3: two-way analysis of variance

Dependent variable	Independent variable	F	Sig	Eta
Glucose	Aerobic training	91.761	0.076	0.286
	Berberine chloride	88.555	0.081	0.214
	Aerobic training* Berberine chloride	110.244	0.001	0.797
IL-6	Aerobic training	33.753	0.070	0.277
	Berberine chloride	3.072	0.001	0.842
	Aerobic training* Berberine chloride	9.394	0.062	0.441
TNF-α	Aerobic training	165.237	0.001	0.090
	Berberine chloride	84.806	0.001	0.478
	Aerobic training* Berberine chloride	180.555	0.001	0.990

Table 4. Pearson correlation coefficient between the variables

Variable	Variable	Pearson correlation	Significance
BW	Blood glucose	-.838**	0.001
BW	TNF-α	-.911**	0.001
BW	BMI	.858**	0.001
BMI	TNF-α	-.819**	0.001
BMI	Blood glucose	-.848**	0.001
Blood glucose	TNF-α	.876**	0. 001

**Correlation is significant at 0.001 levels; BW, body weight; BMI, body mass index

Discussion

At the end of 6 weeks, blood plasma glucose level significantly decreased in the groups that received Berberine chloride and Aerobic Training Simultaneously compared to the diabetic control group, but in other treatment groups, this reduction was not significant. The Results suggested that berberine administration at indicated doses (15 and 30 mg/kg/d) and aerobic training alone did not significantly affect the mean blood glucose of the experimental rats, but the simultaneous intervention of these two variables has a significant effect on decreased blood glucose in diabetic mice. Normally, blood glucose in type 1 diabetic patients decreases during the aerobic exercises but increases again (33), which is due to the insulin deficiency in the body. Reaven and Chang concluded that the exercise exerts a decrease in blood glucose (34). Exercise training enhances insulin sensitivity in normal rats (24,35) and improves carbohydrate and fat metabolism abnormalities in type 1 diabetic subjects (34). Effects of the exercise training increase the efficiency of insulin-stimulated glucose disposal by the muscles (34). Based on the results from the current study and other studies, aerobic training alone possibly cannot lower blood glucose for a long time in type 1 diabetes. However, berberine was shown to be beneficial for the functional recovery of the Langerhans Islets in diabetes or prediabetes (36). Results from studies showed that berberine supplementation significantly increased the number of Langerhans Islets (37). Results from another study indicated that berberine decreased serum blood glucose in a dose-dependent manner after 14 weeks of berberine oral supplementation. However, blood glucose was not significantly changed after seven weeks (7). Considering the effects of berberine on Langerhans Islets the cells and those of aerobic training on insulin sensitivity, it can be concluded from the present study that the interactive effect of these two variables results in a significant decrease in the blood glucose.

In the present study, plasma IL-6 in the diabetic control group increased significantly, compared to that in the normal control group. Cytokines, as one of the intracellular signaling molecules, cause the development and activation of immune cells, especially in autoimmune diseases, such as type 1 diabetes. Moreover, cytokines may serve as additional biomarkers of type 1 diabetes. Cytokines can provide valuable information on the pathways involved in the regulation of type 1 diabetes processes (3). The IL-6, a multifunctional cytokine, is secreted by T cells and macrophages and stimulates immune responses in inflammations and infections (38). In the current study, plasma IL-6 significantly decreased in the group that received 30 mg/kg of berberine chloride alone [D-Br (30 mg/kg)], compared to that in other treatment groups. Previous studies have shown that berberine chloride negatively regulates pro-inflammatory cytokines such as IFN-γ, TNF-α, and

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IL-6 in animal models (19, 20).In the present study, a dose of 30 mg/kg of berberine, reduced the level of plasma IL-6 to the level of the normal control group. However, a dose of 15 mg/kg could not decrease blood IL-6. Furthermore, in health trained group (H-AT), IL-6 significantly increased, compared to that in all other groups. The IL-6 increased in all trained groups, compared to that in untrained groups. A study has shown that circulating IL-6 increases in an exponential mode (up to 100-fold) in response to exercises (25), which may occur due to the release of IL-6 from contractile muscles (39). Hiscock et al. provided definitive evidence that myocytes *per se* are major sources of contraction induced IL-6 (39). In 2000, Steensberg et al. published the first article demonstrating that most of IL-6 seen in blood circulation are likely derived from contracting limbs (40). Plasma concentrations of IL-6 have been known to increase considerably during physical activities. Studies have demonstrated that IL-6 is first produced by contracting skeletal muscles and then released into the blood circulation (8). An accompanying editorial by Gleeson suggests that muscle-derived IL-6 includes metabolic roles (41). Moreover, contraction-induced increases in IL-6 may result in activation of anti-inflammatory pathways and increase endogenous glucose production and clearance. These data provide the first evidence that skeletal muscles can release substances capable of modulating metabolic processes (8). The IL-6 increases insulin sensitivity, glucose transporter type-4 translocation, and glucose uptake in human muscle fibers (42) and enhances fat oxidation in skeletal muscles via the activation of the AMPK pathway (43). In fact, myokines exert their anti-inflammatory effects by inhibiting effects of TNF-α and IL-1β as well as activating IL-1ra and IL-10 and thereby improve conditions of diabetes (46, 47).

Another cytokine, TNF-α, is closely linked to metabolic disorders and diabetes. This cytokine was originally identified as an endogenous factor that influenced energy balance and was associated with weight loss, hypermetabolism and increased resting energy expenditure in infectious or malignant diseases (48, 49). In type 1 diabetes, TNF-α was found to involve in autoimmune processes leading to beta-cell damages (10, 42). Furthermore, TNF-α has been shown to increase the binding of molecules and activate macrophages, resulting in the development of type 1 diabetes (48). In the current study, plasma TNF-α was significantly higher in the diabetic control group than that in the normal control group. This possibly occurred due to induced diabetes with STZ. Furthermore, plasma TNF-α decreased in all treatment groups, compared to that in the diabetic control group. Berberine alone with the doses of 15 and 30 mg/kg significantly decreased TNF-α in diabetic mice while aerobic training alone could not significantly decrease plasma TNF-α. Plasma TNF-α decreased significantly in groups that received berberine chloride and aerobic training simultaneously, compared to that in other treatment groups (Figure 4). Based on the literature reviews, blood IL-6 increases in response to exercises. The released IL-6 from contractile muscles acts as an anti-inflammatory cytokine (myokine). Moreover, aerobic training inhibits TNF-α production and hence decreases inflammation (49).

Conclusion

Based on the results from the current study, aerobic training and berberine synergistically affect blood glucose, IL-6, and TNF-α in type-1 diabetic male Wistar rats. Therefore, if berberine chloride is used at the same time that aerobic training is carried out, better results will be achieved. Furthermore, a dose of 30 mg/kg of berberine was more effective than a dose of 15 mg/kg. Higher doses of berberine chloride or longer times of aerobic training possibly include better results. Further studies are necessary to prove this hypothesis.

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