High and Moderate Intensity Aerobic Training Effects on Galectin-3, Pentraxin-3, and Several Inflammatory Mediators Levels in Type 2 Diabetic Women, a Randomized Clinical Trial

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Abstract

Background: Galectin-3 and pentraxin-3 are recognized as cardiovascular risk factors, levels of which change in the pathological conditions, including type 2 diabetes. The aim of present research was to investigate the high and moderate intensity aerobic training effects on galectin-3, pentraxin-3, and some inflammatory mediators levels in type 2 diabetic women.

Methods: Our study was a randomized clinical trial, conducted on the 36 type 2 diabetic women with an average age of 46.95±3.49 years old, randomly assigned to three equal groups, including control, continuous training with moderate-intensity (MICT), and high intensity interval training (HIIT) groups. Both MICT and HIIT program performed three sessions per week over a 12-week period. Training intensity in HIIT and MICT group was 90 and 60-70 percent of maximum heart rate, respectively. Blood samples at the baseline and after the 12-week training intervention were collected and the variables levels were measured via ELISA method. Repeated measures ANOVA test and Tukey post-hoc test were employed for data analysis. The research is documented in the Iranian Registry of Clinical Trials (registration number: IRCT20200729048252N1).

Results: Galectin-3 levels significantly decreased in HIIT and MICT groups (P<0.001). However, no significant differences were observed for Pentraxin-3 levels between different group (P=0.306), yet paired t test indicated that Pentraxin-3 levels significantly decreased in HIIT group (P=0.003). In addition, serum levels of tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) significantly declined in HIIT and MICT groups (P<0.05).

Conclusion: HIIT and MICT intervention results in a significant decrease in inflammatory mediators and HIIT protocol was not superior to MICT protocol for observed changes in inflammatory mediators.

Keywords: Exercise, Type 2 diabetes mellitus, Inflammation, Galectin-3

1. Introduction

Obesity has become a major health problem worldwide, increasing the risk of various disorders significantly, including fatty liver disease, diabetes, hypertension, myocardial infarction, stroke, and different cancers types. Consequently, it leads to a decrease in the quality of life and life expectancy (1). Upregulation of glucose levels and insulin resistance is the main hallmark for type 2 diabetes, leading to high levels of glucose and free fatty acids (FFAa), increasing free radicals, impairing the islet beta cell function, oxidative stress, and inflammation reaction, and forming a vicious cycle (2). Type 2 diabetes is an inflammatory condition resulting from long-term immune imbalance, metabolic syndrome, and obesity-related excess energy (3). Inflammatory cytokines levels, such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), significantly increase in type 2 diabetic patients and inflammation plays an important role in pathogenesis of obesity and type 2 diabetes (4). IL-6 impairs the insulin function and increases insulin resistance through the inhibition of suppressor of cytokine signalling-3 (SOCS 3) (5) and affecting the JAK/STAT and mitogen-activated protein kinases (MAPKs) signaling pathway (6). TNF-α is another pro-inflammatory cytokine which disrupts the insulin signaling pathway, increases insulin resistance, and causes type 2 diabetes (7).

Galectin-3 is known as an important marker for various cardiovascular conditions (8). Galectin-3 belongs to the lectin family, which is mainly expressed through macrophages and affects the pathophysiology of immune/inflammatory diseases (metabolic diseases, cancer) (9). Elevated levels of galectin-3 have been
reported in obesity and type 2 diabetes and animal studies have shown that galectin-3 can affect the metabolic disorders with direct effects on adipose tissue, leading to increased insulin resistance (10). Upregulation of galectin-3 expression in different tissues (liver, white and brown adipose tissue) has been observed for obese subjects and FFAs and IL-6 stimulate the production of galectin-3 in adipocytes, which is responsible for higher levels of galectin-3 in obese individuals (11). Pentraxin-3 is an acute phase reactant characterized by a multiple cyclic structure produced by various tissues as an indicator of vascular endothelial function (12). Pentraxin-3 levels increase in various tissues in response to some stimulus, such as TNF-α and IL-1 (13). Elevated levels of pentraxin-3 lead to several inflammatory diseases, such as endothelial dysfunction, advanced vascular inflammation, chronic kidney disease, and nonalcoholic fatty liver disease (NAFLD) and an increased the expression of pentraxin-3 in visceral adipose tissue of high-fat diet induced-obese mice and diabetic rats was observed (14).

In addition to its role in preventing diabetes, exercise training also helps treat diabetes and improves insulin sensitivity and diabetes-related health disorders significantly with minimal unwanted side effects (15). Despite the positive effects on diabetes, different types of exercise training do not have the same effect on diabetes. HIIT is a well-known type of exercise training attracting a lot of attention for managing the type 2 diabetes (16). Several researchers observed a further decrease in HbA1c levels in type 2 diabetics patients following HIIT compared to MICT protocol and reported that HIIT is superior compared to MICT protocol (17, 18). In addition, it is suggested that HIIT result in cardiometabolic adaptations in prediabetes and type 2 diabetic patients similar to MICT protocol (19). In fact, the findings regarding that HIIT for type 2 diabetic patients are superior to MICT or not are contradictory and the effectiveness mechanism of both HIIT and MICT is remarkably unknown. Therefore, the objective of the present study was to investigate the high and moderate intensity aerobic training effects on galectin-3, pentraxin-3, and some inflammatory mediators levels in type 2 diabetic women.

2. Methods

2.1. Participants

The current work was a randomized clinical trial conducted on human subjects. The participants were type 2 diabetic women chosen in Tehran diabetes association, region 6 of Tehran hospital, Iran and from the volunteers in public places. Among the recruited patients, 36 women with type 2 diabetes with an age range of 35-50 years old were chosen for the present study. To calculate the sample size, we used the below previously reported formula (20) and 12 participants were considered for each group. Moreover, according to similar previously conducted studies, 8-12 subjects in each group can be suitable for conducting the research in the exercise physiology field (21, 22). Although further participants can increase the statistical analysis power, due to the COVID-19 pandemic, we were unable to carry out the present study protocol with further subjects.

$$n_A = k n_B \text{ and } n_B = \left(1 + \frac{1}{\kappa}\right) \left(\frac{\mu_A - \mu_B}{\sigma^2}ight)^2$$

$$1 - \beta = \Phi \left(\frac{z - z_{1-\alpha/2}}{\sigma}\right) + \Phi \left(-\frac{z - z_{1-\alpha/2}}{\sigma}\right), z = \frac{\mu_A - \mu_B}{\sigma \sqrt{\frac{1}{n_A} + \frac{1}{n_B}}}$$

2.2. Inclusion and Exclusion Criteria

Inclusion criteria: Confirmed diabetes (by physician, average glucose levels >125 mg/dl), lack of various malignancies (cancer) or cardiovascular disease, body mass index (BMI) 25-35 kg.m², being non-alcoholic, sedentary lifestyle in the year prior to study, no physical or medical limitation to take part in HIIT and MICT protocol, and signing the informed consent. Exclusion criteria: not taking part in HIIT and MICT sessions regularly, injuries during training period and subjects’ inability to complete the training program, subjects’ unwillingness to continue study protocol, forced consumption of drugs or supplements (except the glucose lowering drugs) during the 12-week intervention.

2.3. Ethics Statements

The present study intervention was confirmed and approved by the Ethics Committee of Islamic Azad University, Science and Research Branch, with the following documented ethical code: IR.IAU.SRB.REC.1399.011).

2.4. Study Design

This randomized clinical trial with pre- and post-test stages was conducted in the summer and autumn of 2020 in Tehran, Iran. This paper is documented in the Iranian Registry of Clinical Trials with the following registration number: IRCT20200729048252N1.
Herein, potential benefits or side effect of HIIT and MICT were explained to all the participants and among those accepting the explained conditions, 36 type 2 diabetic women were chosen randomly. For the random allocation of the subjects in the different groups (control, MICT, and HIIT groups), one number between 1-36 (1-12 for control group, 13-24 for MICT group, 25-36 for HIIT group) was considered for each subject by researchers at the baseline. Subsequently, the subjects selected a number from 1-36 randomly from a bag containing the numbers and each subject’s group was determined. All the participants took part in the present research voluntarily. Following baseline measurement, the subjects were assigned in three groups randomly (12 participants in each group), consisting of control, HIIT, and MICT groups. It seems that HIIT or MICT training protocol in the type 2 diabetic patients is applicable and safe (19). All the patients consumed the anti-diabetic and glucose-lowering drugs, including the metformin, sulfonylureas, and glibenclamide, and were asked not to change the type and dose of consuming drugs during the study intervention, except with their physician advice. The current study protocol stages are shown schematically in Figure 1.

2.5. Exercise Training Intervention (HIIT and MICT)

The HIIT and MICT exercise training protocol were conducted on treadmill in the indoor gym. HIIT protocol included the four intervals (4 min for each interval) with 85–95% of maximum heart rate (HRmax). Immediately after each high intensity interval, a 3-min active recovery (jogging with 50–60% of HRmax) was performed. The MICT protocol consisted of continuously walking for 47 min or running on treadmill with 60–70% of HRmax in each session (24, 25). Before and after each HIIT and MICT session, 10-min warm-up and 5-min cool-down were respectively performed. Training intensity during HIIT and MICT sessions were monitored with a polar belt. It should be noted that the control group was asked to continue their daily routine life. After starting both training protocols, subjects in the three groups (control, HIIT, MICT) were followed up for 12 weeks.

2.6. Blood Samples Collection

Herein, blood samples were collected in two stages: before and after 12 weeks HIIT and MICT intervention by a physician in similar conditions (after a 12-hour night fasting) from forearm vein. We collected the pre-test blood samples collected at the baseline and 3 days before starting the HIIT or MICT protocol. In addition, post-test blood sampling was carried out 72 hours after the last HIIT or MICT session. Blood samples were poured into falcon tubes and centrifuged for 10 minutes at 3000 rpm and obtained serum were stored at freezer for subsequent measurements.

2.7. Biochemical Analysis

The serum levels of pentraxin-3 (Raybiotech, catalog number: ELH-PTX3-1, sensitivity: 0.08 ng/ml), galectin-3 (Raybiotech, catalog number: ELH-Galectin3-1, sensitivity: 0.6 ng/ml), IL -6 (biovendor, catalog number: RD194015200R, sensitivity: 2.3 pg/ml), TNF-α (biovendor, catalog number: RAF128R, sensitivity: 0.65 pg/ml) were measured applying ELISA method. Moreover, insulin (Demeditec Elisa kit, catalog number: DE2935, sensitivity: 1.76 µIU/ml) and Glucose (Pars Azmoun kit, sensitivity: 5 mg/dL) levels were measured with special kits and insulin resistance calculates according to the formula below (26):

\[
\text{fasting insulin [µU/mL] } \times \text{ fasting glucose [mg/dL]} /405
\]

2.8. Statistical Analysis

We employed SPSS software version 24 for data analysis. The normality of data distribution was confirmed by the use of Shapiro-Vilk test (P>0.05). The difference between the groups were determined through repeated measures ANOVA test and Tukey post-hoc to compare the different groups together. In
addition, paired t-test was utilized for to analyze the group changes and significance levels was considered as \( P<0.05 \) for all the conducted tests.

3. Results

All the 36 subjects who were diabetic women completed the study protocol and were included in the final analysis of data. Demographic characteristics of the participants, including the age (years), height (cm), weight (kg), and BMI (kg.m\(^2\)) in the control, HIIT, and MICT groups are represented in Table 1.

The levels of pentraxin-3, galectin-3, TNF-α, and IL-6 as a Mean±SD for different groups before and after the 12-weeks intervention are reported in Table 2.

Repeated measures ANOVA test indicated a significant between-group difference in galectin-3 levels (\( P<0.001 \)). According to Tukey post-hoc test results, galectin-3 levels in both trained (HIIT and MICT) groups decreased significantly compared to the control group (\( P<0.001 \)). However, HIIT and MICT groups did not experience any significant differences between the observed changes in serum galectin-3 levels (\( P>0.99 \)). Pentraxin-3 levels did not change significantly in the groups (\( P=0.306 \)). Meanwhile, paired t-test revealed a significant decrease in pentraxin-3 levels in HIIT group (\( P=0.003 \)) but no significant changes for MICT (\( P=0.063 \)) and control (\( P=0.151 \)) groups were observed (Table 2).

After 12 weeks of intervention (HIIT or MICT), the between-group difference for IL-6 levels was significant (\( P=0.007 \)) and according to Tukey post-hoc test results, IL-6 decreased significantly in HIIT (\( P=0.010 \)) and MICT (\( P=0.029 \)) groups compared to the control group. The observed changes between HIIT and MICT group for IL-6 levels were of statistical significance (\( P=1.000 \)). Intragroup analysis reported that the decrease in IL-6 levels was significant in HIIT (\( P<0.001 \)) and MICT (\( P=0.017 \)) groups, but IL-6 levels did not change significantly in the control group (\( P=0.354 \)). According to repeated measures ANOVA test findings, the between-group difference for TNF-α levels was of significance (\( P=0.002 \)) and TNF-α in the HIIT (\( P=0.002 \)) and MICT (\( P=0.025 \)) groups decreased significantly compared to the control group. In addition, the observed changes for TNF-α levels between the HIIT with MICT groups was not statistically significant (\( P>0.99 \)). Paired t-test analysis noted a significant decrease in TNF-α for HIIT (\( P<0.001 \)) and MICT (\( P=0.003 \)) groups and no changes in the control group (\( P=0.218 \)) (Table 2).

Tukey post-hoc test indicated that insulin resistance (HOMA-IR), percent body fat and BMI decreased significantly in both HIIT and MICT groups in comparison with the control group (\( P<0.001 \)). However, the observed difference between HIIT and MICT groups for HOMA-IR (\( P=0.399 \)), percent body fat (\( P=0.368 \)), and BMI (\( p=1.000 \)) was not of statistical significance. Concerning paired t-test findings, HOMA-IR, percent

| Table 1: Characteristics of subjects (Mean±SD) |
|-----------------------------|-------------|-------------|-------------|---|
|                             | Control     | MICT        | HIIT        | P |
| Age (years)                 | 46.3±4.25   | 47.6±2.73   | 46.8±3.49   | 0.401 |
| Height (cm)                 | 157.6±4.12  | 158.4±4.79  | 157.6±4.12  | 0.372 |
| Weight (kg)                 | 80.1±6.21   | 79.6±6.52   | 78.7±6.60   | 0.173 |
| BMI (kg.m\(^2\))            | 32.2±1.87   | 31.3±2.57   | 31.4±2.72   | 0.471 |

| Table 2: The levels of inflammatory mediators (Mean±SD) |
|---------------------------------|-------------|-------------|-------------|---|
| Variables                       | Stage       | Control     | MICT        | HIIT        | Between groups P value |
| Galectin-3 (ng/ml)              | Pre test    | 6.8±1.16    | 7.2±1.34    | 7.6±1.58    | <0.001 |
|                                | Post test   | 6.9±1.27    | 6.4±1.08    | 6.8±1.34    |                       |
| Paired t test                   |             | 0.147       | <0.001      | <0.001      |                       |
| Pentraxin-3 (ng/ml)             | Pre test    | 0.65±0.11   | 0.64±0.09   | 0.71±0.13   | 0.306 |
|                                | Post test   | 0.64±0.11   | 0.62±0.08   | 0.67±0.10   |                       |
| Paired t test                   |             | 0.151       | 0.063       | 0.003       |                       |
| IL-6 (pg/ml)                    | Pre test    | 1.9±0.47    | 1.47±0.38   | 1.72±0.43   | 0.007 |
|                                | Post test   | 1.9±0.52    | 1.24±0.26   | 1.41±0.37   |                       |
| Paired t test                   |             | 0.354       | 0.017       | <0.001      |                       |
| TNF-α (pg/ml)                   | Pre test    | 3.7±0.74    | 3.3±0.96    | 4.1±1.17    | 0.002 |
|                                | Post test   | 3.6±0.82    | 3.0±0.87    | 3.6±1.04    |                       |
| Paired t test                   |             | 0.218       | 0.003       | <0.001      |                       |
body fat, and BMI decreased significantly after 12 weeks of intervention in HIIT and MICT groups (P<0.001). On the other hand, HOMA-IR (P=0.301), percent body fat (P=0.306), and BMI (P=0.228) did not change in the control group following the 12-week period. Moreover, repeated measures ANOVA test implied that VO_{2max} was affected significantly through the 12-week intervention (P<0.001) and VO_{2max} upregulation was of significance in HIIT and MICT groups compared to the control group (P<0.001) although no significant change for VO_{2max} values were observed between HIIT and MICT groups (P=0.074). Intragroup analysis suggested a significant increase in VO_{2max} following the 12-week intervention in HIIT and MICT groups (P<0.001), but VO_{2max} changes in the control group was not statistically significant (P=0.226) (Table 3).

4. Discussion

The main findings of our study were that the decrease in inflammatory mediators (including galectin3, IL-6, and TNF-α) following the 12-week HIIT and MICT was significant. However, the observed changes for galectin-3, IL-6, and TNF-α was not of statistical significance between HIIT with MICT groups and HIIT was not superior to MICT protocol for the decrease in inflammatory mediators. Galectin-3 levels upregulation led to an increase in the prevalence of diabetes, obesity, hypertension, hypercholesterolemia, and metabolic syndrome. Higher levels of galectin-3 have been observed in obese and type 2 diabetic patients (27). The increase in the levels of galectin-3 have been reported following acute exercise session (28). Meanwhile, it is suggested that long-term exercise training leads to decrease in the galectin-3 levels. Consistent with the present findings, Zaidi and colleagues reported that 12 months of combined training (endurance-resistance) in patients with type 2 diabetes contributes to a significant reduction in galectin-3 levels (29).

In another study, Moghadami and co-workers confirmed the findings of the present work and showed that 12 weeks of aerobic training (three sessions per week) in elderly women with metabolic syndrome reduced galectin-3 levels significantly. In accordance with our paper, it is reported that the downregulation of galectin-3 is associated with the decrease in the levels of other inflammatory mediators (including TNF-α, CRP) and a decrease in BMI and body weight. Researchers have suggested that reducing chronic inflammation (TNF-α and CRP) plays an effective role in lowering galectin-3 levels as a cardiovascular risk factor (30). Herein, a decrease in galectin-3 levels was observed along with a significant decrease in TNF-α levels in the trained groups. Contrary to the present findings, Khajeian and Moghadasi reported that eight weeks of endurance training in healthy young men had no significant effects on galectin-3 levels, but exercise training attenuated galectin-3 response to acute exercise significantly (31). The aforementioned contradictions can be attributed to the shorter duration of training period, different conducted exercise training program, and the different characteristics of the subjects. Additionally, it seems that the difference in subjects’ sexes can also affect the observed changes in galectin-3 levels (27).

Present findings demonstrated that the decreased levels of galectin-3 led to a decrease in insulin resistance. Consistent with these findings, it is reported that galectin-3 effects on reducing insulin sensitivity exerted through binding to the insulin receptor inhibiting

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the insulin receptor signaling pathway and glucose-transporter (GLUT-4) translocation, which finally increases the insulin resistance (32). Since the adipose tissue growth and expansion result in the upregulation of galectin-3 levels (33), it can be said that the observed decrease in our subjects’ adipose tissue is one of the possible mechanisms to reduce galectin-3 levels with HIIT and MICT protocols. However, identifying the exact mechanism for reducing galectin-3 levels with exercise training requires further investigation. In the present paper, the decrease in galectin-3 levels resulted in a significant decrease in inflammatory cytokines levels, including the IL-6 and TNF-α. On the other hand, the observed decrease in IL-6 and TNF-α levels between the HIIT and MICT groups was not statistically significant and both training programs were equally effective in reducing inflammation. In fact, both HIIT and MICT protocols can exert anti-inflammatory effects.

Farinha and colleagues in line with the present study, revealed that a 12-week aerobic training in women with metabolic syndrome contributes to a significant decrease in IL-6, IL-1β, and TNF-α levels, which is associated with a significant increase in IL-10 as an anti-inflammatory cytokine in inflammatory mediators. In addition, decreased body fat percentage, insulin resistance, oxidative stress, and the increase in VO$_{2\text{max}}$ and antioxidant capacity were also observed in the trained group (34). Unfortunately, in the present study, the changes in the antioxidant capacity and oxidative stress were not investigated. Exercise training anti-inflammatory effects exerted through various mechanisms, such as a decrease in visceral adipose tissue, secretion of anti-inflammatory myokines from working muscle, reducing TLR-like receptors (TLRs) expression on macrophages and monocytes, inhibiting the infiltration of monocytes and macrophages into adipose tissue, and changing the phenotype of adipose tissue macrophages from inflammatory to anti-inflammatory (35).

Another finding of present study was that 12 weeks of HIIT and MICT did not have a significant effect on pentraxin-3 levels compared to the control group although pentraxin-3 levels significantly decreased in HIIT group. Pentraxin-3 levels in the control, MICT, and HIIT groups decreased by 1.53%, 3.12%, and 10.52, respectively after the 12-weeks intervention. This indicates a greater effect of HIIT compared to MICT on pentraxin-3 levels although the observed changes in the HIIT group was not of statistical significance compared to other groups. Pentraxin-3 significantly increased in metabolic syndrome compared with healthy subjects, elevating the levels of pentraxin-3 in these subjects, attributed to low HDL-c levels (36). Wang and co-workers reported that the serum levels of pentraxin-3 in type 2 diabetic patients were higher than of those in people with normal glucose levels (37). Moreover, it has been reported that pentraxin-3 leads to an increased insulin resistance in gestational diabetes and is known as important risk factor for this disease (38).

In human subjects, the expression of pentraxin-3 in visceral adipose tissue has a positive correlation with BMI and increases in visceral adipose tissue of overweight and obese individuals (39). Although the reduction of pentraxin-3 levels in the HIIT group was not significant compared to other groups in this study, it was associated with a further reduction in body fat percentage in this group. Body fat percentage in MICT and HIIT groups decreased 5.82% and 7.85%, respectively. These findings emphasized the importance of changes in body fat mass for the observed changes in pentraxin-3 levels following exercise training intervention. Hovsepian and colleagues confirmed the present findings and suggested that 10 weeks of HIIT in obese women had no significant effect on pentraxin-3 levels compared to control group. However, contrary to the current findings, intragroup analysis implied that pentraxin-3 changes in the HIIT group was not of significance, which was associated with no change in insulin resistance (40). In another study, Madsen and co-workers confirmed the present study findings and reported that eight weeks of HIIT (pedaling) in type 2 diabetic patients had no significant effects on pentraxin-3 levels, but pentraxin-3 levels significantly increased in healthy individuals (41). These results emphasized that exercise training in subjects with different characteristics can exert different effects on pentraxin-3 levels. In support of these statements, Zempo-Miyaki and co-workers revealed that 12 weeks of caloric restriction alone or along with aerobic training in overweight and obese men resulted in a significant increase in circulating pentraxin-3. The researchers attributed the pentraxin-3 upregulation to the subject’s weight loss following intervention and increased pentraxin-3 particularly in the participants with a BMI of less than 25 (42). It seems that contradicting findings to the present study is related to different subjects’ characteristics (obese instead of type 2 diabetic). Increasing the levels of pentraxin-3 in the type 2 diabetics patients (37) may be a compensatory mechanism, such as increased insulin levels in these patients. However, due to limited findings regarding the effect of various exercises training on pentraxin-3
levels, including in type 2 diabetic patients, determining the effects of different exercise trainings on pentraxin-3 levels and its relationship with other inflammatory factors, require further studies. Consideration and attention to the present study limitations, including the small samples size in each group, not measuring the changes in the antioxidant capacity and oxidative stress markers, not measuring the different anti-inflammatory cytokines, inability to exact control of subject’s diet, and also inability to determine the leisure time activities of subjects for future similar studies have been suggested.

5. Conclusion

In conclusion, the findings of the present study suggested that HIIT and MICT intervention play an important role in reducing the inflammatory mediators levels and HIIT was not superior to MICT in diabetic patients. Moreover, 12 weeks of HIIT and MICT compared to that of the control group did not have a significant effect on pentraxin-3 levels although the HIIT was associated with a further decrease in pentraxin-3 levels.

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Ethical Approval

The present study was approved by the Ethics Committee of Islamic Azad University, Science and Research Branch (Ethical codes: IR.IAU.SRB.REC.1399.011). Also, the research is documented in the Iranian Registry of Clinical Trials (registration number: IRCT20200729048252N1).

Conflicts of interest: None declared.

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