Effects of different resistance training protocols over the morphofunctional, hormonal and immunological parameters

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ABSTRACT

The purpose of this study was to assess the influence of two different resistance training protocols on the anthropometric (weight, BMI, fat mass), functional parameters (1-MR test, and maximal repetition test) and the parameters related to the endocrine system (testosterone and cortisol concentrations), as well as to the immunological system (glutamine and IgC concentrations). The study was composed by twelve trained men (27.4 ± 4.8 years), who were randomly divided in two groups that later were submitted to two different training protocols: the Multiple Series (MS), and Tri-set (TS). Blood samplings were collected before and after an resistance training session in the beginning and the end of the 8 weeks training period. It was observed no alterations in the morphofunctional parameters (except as to the maximal repetition test for the squat). As to the endocrine parameters, it was observed that the TS caused a significant increase in the cortisol immediately after the training session both in the beginning and in the end of the eight weeks ($p < 0.05$) period. Upon the observation of the testosterone vs. cortisol ratio (T:C) behavior, it can be observed a noticeable increase in the group submitted to the MS protocol after the 8 weeks training period ($p < 0.05$). As to the immunological parameters, it was observed no alterations in the concentration of the immunoglobulin G. The concentration of the glutamine suffered a decrease after 8 weeks in both groups. That decrease had a higher accentuation in the TS group ($p < 0.05$). Results attained suggest that the TS method imposed a higher stress to the body. Furthermore, these data also indicate that the MS protocol promotes a more propitious environment to the anabolism after the 8 weeks training period. However, both methods did not succeed in promoting significant changes in the morphofunctional parameters.

Keywords: Resistance training. 1-MR. Testosterone. Cortisol. Glutamine. IgC.

INTRODUCTION

The muscular hypertrophy is one of the most desirable goals among the endurance training practitioners. Today, there are different training methods or systems aiming to attain such goal, generating a big number of discussions on the superiority of a method compared to other[1]. The difference between both methods lies in the way the acute training variables (intensity, amount, resting period between series and exercises and order of the exercises) are disposed[1].

Among the most popular endurance training methods, it can be pointed out: the Multiple Series (MS), and the Tri-set (TS) methods. The MS method is most spread among the endurance training practitioners, and it can be used from sedentary individuals up to athletes with the highest training level to achieve any goal, since it an adjustment in the amount of series and repetitions is made. That method is characterized by the accomplishment of more than one series of exercises[1]. The TS method consists in the utilization of three different exercises in the same portion of the body with the least or without any interval between series, and usually with three series of each exercise[1]. The TS is generally used by well-trained individuals who has as main target the muscular hypertrophy, since small intervals between series induce a significant metabolic overload[1].

The distinction between positive and negative points related to different training methods is more complicated in trained individuals, since in these individuals it is imperative to use increasing overloads, in order to promote the desired adaptations. Nevertheless, such increase in the level of the stress applied (overload) must not induce unhealthy effects on the practitioner's organism. Although quite spread, this issue has been less explored.

Aiming to answer to the energetic demand imposed by the exercise, the cellular, neural and hormonal mechanisms are activated, in order to regulate-control the methabolism[2]. While performing the exercise, the alterations in the hormone concentration increases the availability of the energetic substrates with prevalence of the catabolism. In the recovering phase, such balance between the anabolic and catabolic hormones with prevalence of the anabolic ones, will be responsible by the increase in the adaptable protean synthesis[3].

Apparently, these hormonal changes constitute an appropriate parameter to verify the acute and chronic effects of the physical treatment[4]. as the hormonal response to the endurance training (for instance, an increase in the testosterone concentration compared to the cortisol) is correlated to the hypertrophy and the muscular power[5].

Added to the endocrine system, the analysis of the physical training effects on other systems such as the nervous and the immunological systems may supply more encompassing information on how such stimulus affects the physiology of the body[2,6,7], once these systems are also determinant factors to the stress adaptation[3]. The change in the hormone concentration, mainly the increasing levels of the cortisol is also able to change immunological parameters. It is recognized that the cortisol exerts an unhealthy effect on the functionality of the immunological system[6-9].

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Considering that the neural, hormonal, and immunological responses responsible by the adaptations to the physical training depend on their characteristics, it is expected that different training systems also cause different organic responses. Thus, the purpose of this paper was to assess the influence of two resistance training protocols known as Multiple Series (MS), and Tri-set (TS) on the parameters related to the endocrine and the immunological systems.

The body composition, the maximal voluntary contraction, and the located muscular resistance were also assessed, in order to determine the effect of the hormonal and immunological changes on the morphofunctional parameters.

METHODS

Individuals: Twelve resistance training practitioners were selected, all of them with more than 12 months experience (70.2 ± 4.5 kg; 172 ± 5 cm; 27.4 ± 4.8 years old). The individuals were randomly divided, and later, they were submitted to two different training protocols: Multiple Series (MS) (n = 6), and Tri-Set (TS) (n = 6). The data collection was performed in the beginning and after the eight training weeks. According to a specific resolution set by the National Health Council (no. 196/96), the participants received detailed information on the procedures used, and they agreed in voluntarily participate in the study signing an informed and privacy protection consent term. The trial was approved by the Human Research Ethics Committee of the Institute of Biomedical Science of the São Paulo University (Statement no. 72/00). All individuals were submitted to the analysis of the urine (in the beginning, 4 weeks, and after 8 weeks) in order to verify the presence of anabolic steroids. Such analysis was performed at the facilities of the Laboratory for toxicological analysis of the USP School of Pharmaceutical Sciences, and the result was negative for every individual.

Tests to determine the MR and the maximal repetition test values: After a short stretching and warm up time, the maximal muscular power was determined through the maximal repetition test (1-MR) using four tries of one sole repetition, with a 3 minute interval of the following exercises: supine, squat and arm curl[1] using the natural amplitude of the movement, except for the squat exercise that was performed in the eccentric phase up to the moment the individual's thigh was parallel to the ground. Seven days after that exercise, another test was performed: the muscular endurance, maximal repetitions, in which the weight used was 50% of the 1-MR value (50%-1MR) to perform up to achieving the exhaustion point, or an incapacity to keep the standard movement[1], and the velocity of the execution of these exercises respected the following condition: approximately 2 seconds both in the concentric and in the eccentric phase of the movement.

Description of the resistance training protocol: For 8 weeks, all individuals trained 4 times a week (Mondays, Tuesdays, Thursdays, and Fridays), and on Mondays and Thursdays it was performed the A training, when the chest, back and shoulders were worked out, and on Tuesdays and Fridays the B training was performed, consisting in exercises for thighs and arms. Each day, the intensity was differentiated: Mondays and Tuesdays, 100% weight in 10 maximal repetitions (10MR), and 90% weight of 10 maximal repetitions (90% of 10MR) on Thursdays and Fridays, and the maximal number of repetitions per each exercise was determined along the training, and every two weeks this was adjusted, in order to provide a real work out with the forecasted load. The endurance training was restricted to 20 minutes maximum, twice a week, separated for at least 8 hours from each strength sessions. The Multiple Series (MS) method consisted in two exercises for each group of muscle, except for the muscles of the thigh amounting three exercises in four 10 repetitions series of each exercise, with a 90 seconds interval between series. The exercises utilized were: A training: supine, inclined dumbbell supine, low rowing, lat pull down, lateral raise, development with dumbbells; B training: leg press, extensor bench, flexor bench, bar-arm curl, alternate curl, high-pulley triceps, French triceps press. The Tri-Set method (TS) consisted in the accomplishment of three different exercises for the same muscular group with no interval, three 10 repetitions series. After finalizing the third exercise, there was a 90 second interval to reinitiate the whole sequence.

The exercises used in the A training were the following: supine, inclined dumbbell supine, crucifix, low rowing, lat pull down, high rowing, lateral raise, development with dumbbells, frontal raise. B training: leg press, extensor bench, flexor bench, bar-arm curl, alternate curl, simultaneous curl with inclined bench, high-pulley triceps, French triceps press, supine with closed catch. Both protocols were previously equalized as to their total training volume (tonnage) by means of a pilot-test, that is, the total amount of raised weight in both groups was quite similar. For this, it was used the following formula: total volume = series x repetitions x weight (kg)[1].

Assessment of the body composition: The body composition was assessed by means of a Lange sector for the cutaneous fold, which was the protocol used by Jackson and Pollock[10] for men.

Plasmatic determinations: After a 5 hour fasting, the blood was collected before (7 p.m.) and after the end of the training session (around 8 p.m.), those blood collections were performed in the beginning of the training and after the 8 week period of the trial. In order to assess the plasmatic concentration of the testosterone and cortisol, DPC© COAT-A-COUNT radio-immune-assay kits were used. All participants were instructed to follow a standard menu in a fixed 24 hours schedule prior to the blood collection[11]. Aiming to decrease the inter-assay variability, every sampling was assessed at a time at the end of the trial (the analytic variation was lower than 10.5%, and 9%, respectively to the testosterone and cortisol, according to the proposal set by Tremblay and Chu[12]. The glutamine concentration was determined according to the method described by Windmueller and Spaeth[13] using asparaginase enzymes and glutamate dehydrogenase (GDh). The immunoglobulin G (IgG) was dosed through the ELISA assay (double antibody, enzyme-linked immunosorbent assay) in a DPC© 96-wheel plaque assay.

Statistical analysis: In order to make a comparison before and after the training sessions, and in the pre- and post-training, a paired t-Student test was used. To make a comparison between methods, it was used the non-paired t-Student test. The minimum significance level of $p < 0.05$ was set.

RESULTS

Related to the body composition (weight, BMI, and fat mass), it was observed no significant differences in both training interventions (MS and TS) related to the initial amount (table 1).

TABLE 1
Body composition of individuals submitted to the Tri-set (TS) and Multiple series (MS) training methods

MS		TS	
Body weight (Kg)			
Initial	8 weeks	Initial	8 weeks
72.6 ± 6.5	73.0 ± 6.9	67.0 ± 6.6	67.4 ± 6.6
Body mass index			
Initial	8 weeks	Initial	8 weeks
23.2 ± 0.6	23.4 ± 0.5	22.8 ± 1.1	22.9 ± 1.1
Fat mass (%)			
Initial	8 weeks	Initial	8 weeks
12.6 ± 3.8	11.1 ± 3.1	12.8 ± 2.4	12.2 ± 3.0

Values expressed in mean ± standard deviation. The measurement was performed in the beginning and end of the 8 week training.

The value of the maximal voluntary contraction recorded in the 1-MR test in the supine, squat and arm curl exercises did not present a significant difference as well compared to the beginning of the training in both protocols. Related to the initial value, the ability to perform the maximal repetition has increased only in the squat exercise. Such increase was observed in both protocols (MS ~56%, and TS ~66%; $p < 0.05$ (tables 2 and 3).

TABLE 2
Determination of the 1-MR value in individuals submitted to the Tri-set (TS) and Multiple series (MS) training methods

1-RM (kg)			
MS		**TS**	
Supine			
Initial	8 weeks	Initial	8 weeks
70.1 ± 11.1	84.8 ± 9.1	67.1 ± 9.2	76.0 ± 8.4
Squat			
Initial	8 weeks	Initial	8 weeks
100.9 ± 11.6	119.6 ± 5.8	90.4 ± 9.9	104.9 ± 12.9
Arm curl			
Initial	8 weeks	Initial	8 weeks
44.2 ± 7.1	49.1 ± 8.3	37.4 ± 2.1	40.2 ± 4.1

Values expressed in mean ± standard deviation. The tests were performed in the beginning and end of the 8 week training.

TABLE 3
Determination of the maximal repetitions performed at 50% 1-MR in individuals submitted to the Tri-set (TS) and Multiple series (MS) training methods

Maximal repetitions at 50% 1-MR			
MS		**TS**	
Supine			
Initial	8 weeks	Initial	8 weeks
31.2 ± 4.4	26.6 ± 4.4	30.5 ± 3.3	27.7 ± 5.6
Squat			
Initial	8 weeks	Initial	8 weeks
27.2 ± 10.2	42.2 ± 11.3[a]	21.5 ± 5.2	35.7 ± 7.6[a]
Arm curl			
Initial	8 weeks	Initial	8 weeks
21.6 ± 3.3	23.40 ± 3.7	25.2 ± 4.9	27.5 ± 4.3

Values expressed in mean ± standard deviation. The tests were performed in the beginning and end of the 8 training weeks. a – statistical difference related to pre-exercise.

As to the plasmatic parameters, it was observed no change in the plasmatic concentration of the testosterone in none of the protocols, whether before or after the training session in the beginning or end of the 8 weeks period. In the beginning of the treatment, it was observed an increase in the concentration of the cortisol in the post-training in both protocols (MS ~38%, and TS ~250%; $p < 0.05$). Nevertheless, the magnitude of this increase was more evident in the Ts compared to the MS ($p < 0.05$). After the 8 weeks training, only the group submitted to the TS method presented an increase (~38%; $p < 0.05$) in the cortisol secretion after the training session. Still, related to the concentration of the cortisol after the 8 weeks training, it was observed immediately after the training session that the TS group presented a raise in the concentration of the cortisol compared to the MS group (~230%; $p < 0.05$) (table 4).

TABLE 4
Plasmatic concentration of the total testosterone, cortisol and glutamine in individuals submitted to the Tri-set (TS) and Multiple series (MS) training methods

	MS		TS	
	Testosterone (ηmol.L^{-1})			
	Before	After	Before	After
Initial	16.0 ± 3.5	17.3 ± 6.2	21.5 ± 6.8	20.2 ± 6.9
8 weeks	16.7 ± 3.6	18.0 ± 2.8	17.1 ± 2.4	18.9 ± 4.6
	Cortisol (ηmol.L-1)			
	Before	After	Before	After
Initial	304.7 ± 39.1	421.8 ± 101.5[a]	285.8 ± 70.2	715.4 ± 105.7[ac]
8 weeks	207.4 ± 94.6	209.6 ± 114.5[b]	349.5 ± 54.2	485.2 ± 69.8[abc]
	T:C Ratio			
	Before	After	Before	After
Initial	0.05 ± 0.01	0.04 ± 0.01	0.06 ± 0.01	0.03 ± 0.005[a]
8 weeks	0.09 ± 0.01[b]	0.11 ± 0.01[b]	0.04 ± 0.01[c]	0.04 ± 0.01[c]

Values expressed in mean ± standard deviation. The collections were performed before (pre-training) and after (post-training) each training session in the beginning and end of the 8 training weeks. a – statistical difference related to the pre-exercise; b – statistical difference related to the initial condition; c – statistical difference related to the MS method.

In the beginning of the training period (initial condition), the T:C ratio was reduced (~50%; $p < 0.05$) after performing the TS protocol. Related to the MS method, the T:C ratio was evident in the resting period (pre-training) and after the exercise (post-training) in the end of the 8 week period related to the beginning of the trial (initial condition). Upon the comparison of the groups (TS x MS) in the end of the 8 training weeks, in the post-training, it was also verified a remarkable increase in the T:C ratio in the MS group compared to the TS ($p < 0.05$) group (table 4).

Related to the immunological parameters in both protocols, MS and TS, the plasmatic glutamine concentration presented a decrease after the 8 week training period both before (pre-training) and after (post-training) the strength exercise related to the initial value. Particularly in the TS group, it was detected a remarkable reduction after the resistance training related to the resting situation (pre-training) after the 8 weeks training period. Comparing the TS and MS groups, it is possible to note that the concentration of the glutamine presented a reduction in the TS method after the training session in the end of the 8 week period (~40%; $p < 0.05$) (table 5).

TABLE 5
Plasmatic concentration of glutamine and immunoglobulin G (IgG) in individuals submitted to the Tri-set (TS) and Multiple series (MS) training methods

	MS		TS	
	Glutamine (mmol.L-1)			
	Before	After	Before	After
Initial	1,110.6 ± 105.3	928.6 ± 77.1	1,318.3 ± 320.3	1,156.9 ± 288.2
8 weeks	663.2 ± 214.2[b]	540.2 ± 198.7[b]	836.7 ± 301.2[b]	340.0 ± 151.6[ab]
	IgG (mg.L^{-1})			
	Before	After	Before	After
Initial	15.3 ± 2.7	14.2 ± 5.3	15.4 ± 3.7	13.7 ± 5.6
8 weeks	13.9 ± 4.1	14.0 ± 4.9	16.6 ± 3.0	14.3 ± 3.9

Values expressed in mean ± standard deviation. The collections were performed before (pre-training) and after (post-training) each training session and in the beginning and end of the 8 training weeks. a – statistical difference related to the pre-exercise; b – statistical difference related to the initial condition.

DISCUSSION

The alterations in the hormonal and immunological parameters can be used in order to assess the effect overload on the body, whether acute or chronically[3,14].

In the present study, it was performed the acute analysis (before and after a training session), but with a significant interval between them (8 weeks between the beginning and the end of the training period). Thus, the results presented in this study not only represent the acute effect of two different strength training protocols, but also the chronic response to both methods along 8 training weeks.

Endocrine alterations

In terms of the acute response, it is believed that the hormonal changes can modify the exercise performance by means of several mechanisms, as for instance metabolic alterations and/or in the nervous and immunological system's functionality. As an example, Bosco *et al.*[2] have shown an association between the concentration of the testosterone and a reduction in the neural activity along a high intensity strength training session. Due to this, they have concluded that the testosterone (in an adequate concentration) may compensate the quick contraction-fibers' fatigue (present according to the advancement of the training), thus assuring a higher neuromuscular efficiency. That conclusion is confirmed by the Tamaki *et al.*'s data[15], who demonstrated an increase in the work capacity and a higher resistance to the fatigue through the training using anabolic steroids.

Individuals from the TS group worked the same muscular groups out compared to the MS group, but they performed three exercises per group (rather than two exercises in the majority of the training sessions of the MS group, except for the thigh), with no interval between the three exercises (with the MS group performing a 90 second pause between exercises). In such sense, if the T:C ratio would be used as a physiological overload indicator[16,17], possibly the individuals from the TS group were submitted to a higher level of stress in the beginning of the trial. In agreement with this supposition, Smilos *et al.*[18] proposed that the response of the cortisol to the strength training depends on the metabolic needs and the total stress[18]. In the present study, the fall in the T:C ratio occurred in function of the significant increase of the cortisol concentration at the end of the TS training, especially at the beginning of the exercise (a 250% increase).

Passelergue and Lac[19] observed the same increase. Coincidently, those authors have shown that the concentration of the salivary cortisol increased 2.5 times along a wrestling competition. Elloumi *et al.*[20] have also shown that the cortisol is associated to the stress level in a rugby game, and it was also observed a similar increase in the concentration of that hormone.

As to the chronic responses after 8 training weeks, both groups (MS and TS) presented a decrease in the plasmatic concentration of the cortisol in the end of the training session, thus indicating attenuation in the stress imposed by the training session. Nevertheless, even so, the TS group presented a higher concentration of cortisol compared to the MS group in the end of the 8 training weeks. Considering that the secretion of the cortisol is a physiological mechanism in response to the stress, this would reinforce the hypothesis of the TS method is more stressing even after a chronic period of 8 weeks.

Considering the meaning of the falling in the T:C ratio, the apparent nonsense of the fact the TS protocol is more stressing can be understood, but it does not compromise the morphofunctional parameters assessed along the 8 weeks period. Initially, Adlercreutz *et al.*[4] recommended to use the free testosterone vs. cortisol ratio as an overtraining indicator whenever it presents a higher than 30% reduction.

Next, it was preconized that the T:C ratio could also be a physiological indicator for the training overload.

Several authors point out that the T:C ratio is not necessarily associated to the Overtraining syndrome[3,14,16,17,21]. Nevertheless, despite the reduction (50%) in the T:C ratio observed after the 8 week TS training at the beginning of the training session (resting condition), it is not possible to assert that such method induced the overtraining.

It is already set that the functionality of the endocrine system is deeply changed by the overtraining syndrome. But the interpretation of such alterations is restrained through four factors: type of the exercise (endurance vs. strength), circadian variation of the hormones, difference between the acute (along the exercises) and chronic (noted during resting periods) responses, and especially on the strength exercise, the number of the sets and repetitions, pause intervals, and order of the exercises[21]. Sill related to the overtraining, it is important to point out the existence of two ways: the first one is marked by the predominance of the parasympathetic nervous system. The second one is marked by the higher activity of the sympathetic nervous system[14,21]. This last overtraining form is most frequently observed in strength athletes and in those performing anaerobic activities[21]. The hormonal adaptation of the overtraining (sympathetic) are: increasing, decreasing or unchanged cortisol levels, decreasing testosterone, GH, and prolactin. As to the adrenalin and noradrenalin, their behavior is directly related to the stress level (duration and intensity)[21].

Facing such complexity of the endocrine responses verified in the overtraining, is quite difficult to make an interpretation on the T:C ratio as an indicator of that syndrome. Presently, it is believed that the T:C ratio is related to the metabolic picture (anabolism vs. catabolism) that cannot be directly associated to the overtraining[21].

So, the behavior of the T:C ratio suggests that individuals submitted to the TS method were submitted to a high stress level. It can be asserted that in the end of such session training (TS) there was a prevalence of the catabolic stimulus. It is important to point out that in the present study, the behavior of this relationship was punctually assessed in the end of the training. It were not performed determinations of the resting period following the training session.

Alterations related to the immunological system

The plasmatic concentration of glutamine has been associated to the functionality of the immunological system in individuals submitted to intense physical training[8,9]. In the beginning of the trial, it was observed no alteration in the plasmatic glutamine before and after the training session in both protocols. Despite the reduction in the T:C ratio (promoted by the increasing cortisol level) in the beginning of the trial (acute effect) after the TS protocol training, such fall was not followed by a reduction in the plasmatic glutamine and by immunosuppressant signals.

After 8 weeks, it was observed that the glutaminemia presented a fall as to the prior and next moments of the training session in both groups. Nevertheless, the relevance of the maintenance of the plasmatic glutamine has been discussed[22]. Recently, Hiscock and Pedersen[22] asserted that is unlike that a fall in the plasmatic concentration of glutamine might exert some kind of influence on the immunodepression induced by the exercise (normally studies assessing the endurance activities). In the present study, the glutamine fall did not affect the IgG production in both groups.

Besides, it is common that the immunological parameters present a falling pattern in the immediately post-training periods. Bush *et al.*[23], have shown that two strength training protocols (same total amount of work performed) were able to promote a reduction in the concentrations of the interferon-gamma and interleukin 2. This response would indicate a reduction in the immunity, but it was a temporary fall.

Simonson[24] still points out in his review found in the literature on the immune-response to the strength exercises that conditioned individuals do not present changes in their immunological resting parameters along a several years period.

The other immunological parameter assessed, the salivary concentration of IgG, did not present any difference related to the type of the protocol. These data are confirmed by McDowell *et al.*[25] and Calabrese *et al.*[26] in their studies.

Morphofunctional alterations

Thus, despite the hormonal and immunological alterations observed in the TS group, the morphofunctional parameters assessed (body composition, 1-MR and maximal repetition tests) were not different from the MS group after the 8 weeks period. Nevertheless, it is relevant to mention that although it has not been observed any statistical difference, the 1-MR value in the supine and squat exercises increased in both protocols (such difference was higher than 10 kg). It is possible that the statistical differences have not been observed due to the reduced number of individuals in each group. Although a 10 kg increase (in absolute terms) cannot be despised mainly because it contemplated trained individuals presenting a lower adaptation reserve.

Main limitations

An important limitation of this paper is the fact that the analysis of the T:C ratio was performed immediately after the end of the training session. Other researches[19,20,27] also observed an increasing cortisol along a competition/training and the maintenance of the testosterone concentration, similar to what was found in the present study. Nevertheless, these researches still have shown that in hours and days after the stress, there is a high plasmatic concentration of the testosterone, while the cortisol concentration suffers a reduction. Possibly, the punctual analysis did not allow observe such response pattern in this study. Other factors that limit our conclusions are: the reduced amount of participants, and the short period of the intervention time.

FINAL CONSIDERATIONS

Concluding, the acute and chronic alterations in the T:C ratio suggest that the TS training method was more stressing. As to the MS protocol, it can be asserted that it has promoted a more anabolic environment. Especially as to the MS protocol, a prior study performed at our facilities showed an alteration pattern similar to the T:C ratio in women in the resting condition after 8 week training[28].

However, the induction of that more favorable environment to the anabolism through the MS protocol did not result in a higher morphofunctional adaptation level compared to the changes induced by the TS protocol along the 8 weeks period. Thus, these data confirm the belief that the use of two different strength training methods results in different hormonal and immunological responses in trained individuals within a short period of time (2 months). In the present study, it was impossible to set a relationship between the endocrine/immunological alterations, and the alterations in the body composition and muscular power.

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