**INTRODUCTION**

The increase of body weight and excessive accumulation of lipids in adipose cells in the body, due to the ingestion of large amounts of fat and a low energy expenditure, thereby increasing the concentration of body fat, can be a factor to obesity development. Excessive consumption of a hyperlipidic diet can contribute for this process, by the high caloric values and leads fat mass increase, due to the metabolic conversion of the lipids contained in food into triglycerides, which are then stored in the adipose tissue (Assaad et al., 2014), which can be conducive to the development of obesity.

The Epididymal Adipose Tissue (EAT) has the function of storing triglycerides in its adipocytes and the gain of adipose fat can contribute to the development of the complications of obesity. Non-alcoholic Fatty Liver Disease (NAFLD) is characterized by an increase in the concentration of intrahepatic triglycerides (IHTG), which can lead to excessive tissue injury, evolving into chronic inflammation. This chronic inflammation caused by the accumulation of IHTG may result in hepatic steatosis or cirrhosis (Song et al., 2014).

Among the non-pharmacological therapeutic approaches for treating obesity and its deleterious effects on body composition and hepatic tissue, physical exercise appears as one of the most indicated to reduce body mass and the concentrations of IHTG (Spassiani & Kuk, 2008). Intermittent training proves to be effective in reducing ab-
dominal fat and intrahepatic lipids in obese people, decreasing the concentration of adiponectin and of the adiponectin/leptin ratio, subcutaneous fat, LDL, total cholesterol and in the amount of alanine aminotransferases (Lee et al., 2012).

However, it is indicated that the therapeutic conduct involving intermittent training must be practiced regularly to avoid the detraining condition, defined as the interruption of the, so far, regularly practiced physical training, when the benefits generated by the exercise are partially or totally lost (Kim et al., 2013).

The literature available lacks perspicuity regarding the effect of intermittent training followed by detraining on the body composition and hepatic tissue of eutrophic and obese rats, also considering the possible clinical aspects that may support new approaches for patients during the treatment of obesity and its comorbidities. Therefore, this study aims to evaluate the effect of an intermittent training program followed by detraining on the body composition and the liver tissue of rats subjected to a normolipidic or hyperlipidic diet.

**MATERIAL AND METHOD**

**Animals.** Sixty male Wistar rats (each 60 days old) were obtained and kept in shared plastic cages, groups of three animals per cage, in the vivarium under average temperature of 22 ± 2 °C and light/dark cycles of 12 h, with the light cycle commencing at 7 h, and fed standard feed and tap water, provided ad libitum. The animals body mass was taken three times a week, throughout the experimental period.

All procedures followed the Ethical Principles in Animal Experimentation adopted by the Brazilian Society of Science in Laboratory Animals (SBCAL). This study was approved by the Ethics in Animal Research Committee (CEUAS) from FCT/UNESP, filed under n° 004/2011. The 60 animals were randomly assigned, distributed as follows:

Initially, Control and Obese groups were each divided into two subgroups: Sedentary Control (SC, n = 10), Exercised Control (EC, n = 20), Sedentary Obese (SO, n = 10) and Exercised Obese (EO, n = 20). The groups SC and SO were divided once again in two subgroups: Sedentary Control (SC, n = 5), Sedentary Control euthanized at six months of age (SC(6), n = 5); Sedentary Obese (SO, n = 5), Sedentary Obese euthanized at six months of age (SO(6), n = 5).

Within the EC and EO groups the same subdivisions also occurred: Exercised Control (EC, n = 10), Deterained Control (CD, n = 10); Exercised Obese (EO, n = 10), Deterained Obese (DO, n = 10) (Fig. 1). This division in the groups was made because the groups SC, EC, SO and EO were euthanized after the intermittent training protocol period, 60 days after the experiment began, while groups SC(6), DC, SO(6) and DO were euthanized after the detraining protocol period, 120 days after the experiment began.

The animals from the groups SC, EC, DC and SC(6) were fed standard feed (Labina feed, from the brand Purina®), provided with tap water ad libitum. The groups SO, EO, DO and SO(6) followed a hyperlipidic diet (cafeteria diet) (Lamas et al., 2004; Panveloski-Costa et al., 2011) which consisted of: bacon, bologna, sausage, cookies, soda and standard feed, on a ratio of 2:2:2:1:1:1, respectively, in a composition of 28 % carbohydrate, 13 % protein and 59 % fat by the start of the training protocol, provided ad libitum and tap water ad libitum, resupplied every day.

**Intermittent training**

**Training Model.** The intermittent training was the "Jump Squat" model (Tamaki et al., 1992). The device allowed the animal to be placed on a metal platform while immobilized by a fitted vest. The jumps were performed by an electrical stimulation from a metallic clip attached to the tail end of the animal and connected to a Dualpex type stimulator 961, the Quarker®, with a frequency of 1 Hertz (Hz) and duration of 0.3 s, with 2 s intervals between each electrical stimulation and a charge ranging from 3 to 6 milliamperes (mA) (Fig. 2).

With this stimulation, the animals underwent the full movement extent of the leg (knee and ankle), lifting a load that was placed on the posterior part of the vest. The movement "jump squat" was tested and validated as an inducer of hypertrophy in animals, similar to the hypertrophy obtained in human weightlifters (Tamaki et al., 1997).

The training protocol was composed of three sets of 12 repetitions, with an interval of 60 s between sets and performed three times a week, with an interval of 24 h between each day of training, for a period of 60 days.

Three preliminary sessions were performed for adaptation to the training in the first two weeks, during which the animals underwent training without load increase. From the third week, a load equivalent to 50 % of the body mass of the animal was imposed for all experimental period, with adjustments or overload according to the variations of the weight of each animal. This training protocol was initiated in combination with a hyperlipidic diet when the animals reached 60 days old.
Surgical procedure. The animals of the groups SC, EC, SO and EO were euthanized 24 h after the last intermittent training session. The animals of the groups SC(6), DC, SO(6) and DO were euthanized 24 h after the last day of detraining protocol. The animals had their length and body mass measured to calculate the body mass gain (DM = final mass - initial mass). Then, a paramedian incision was made in the abdomen, folding the epithelial tissue for the exposure of the organs. Soon after the incision, the epididymal adipose tissue was removed and weighed on a precision weighing scale.

The animals’ hepatic tissue was also extracted and weighed on precision weighing scale. In all animals, only the right upper lobe of their livers was standardized for analysis and fixed in a 10 % formalin solution. Twenty-four h after the extraction of the liver’s right upper lobe, this part of the organ was transferred to vials with alcohol 70 %. This solution was replenished for three days, resulting in the subsequent removal of formalin residue (Camargo Filho et al., 2011).

Body Composition Assessment

Lee Index. The Lee Index was calculated in all animals using the relation between the cube root of body mass (g) divided by the snout-rump length (cm) and multiplied by 10 (Novelli et al., 2007).

Body Mass Index. The Body Mass Index (BMI) was calculated by: Body Mass (g) / [snout-rump Length (mm)\(\sum\)] (Sjögren et al., 2001).

Epididymal Adipose Tissue (EAT). After extraction, the epididymal adipose tissue (EAT) was weighed on a precision weighing scale. For the data analysis, both the percentage and total value of the tissue relative to the mass of each animal were determined by: [{[EAT (g) \times 100]} / Body Mass (g)]. This procedure was established as a way of correcting the difference in body mass between the animals (Shi et al., 2007).

Liver Tissue Evaluation

Tissue Morphology. After the fixation and permanence in alcohol 70 %, a procedure for the inclusion of the material in paraffin was
carried out, as well as the microtomy in the rotary microtome Microm® HM 325, which performed transversal cuts 6mm thick. The staining was performed by associating two stains: Hematoxylin and Eosin (HE) (Dal Pai, 1995). The HE-stained slides were used for kariometric and stereological analysis of the liver tissue.

**Kariometry and Stereology of the Liver Tissue.** The images of the liver tissue of animals were obtained through a Nikon® 50i optical microscope, with 1000x magnification and the coupling of a Nikon® Infinity 1 digital camera and the images were captured using the computer image analysis software NIS-Elements D 3.0 - SP7 - build 547, Nikon® for Windows®.

The kariometric analysis (Martins et al., 2010) was performed using the computer image analysis software Image-Pro Plus 4.5.0.29 For Windows® 98-NT-2000, Media Cybernetics Inc. In the images obtained were accounted 100 nuclei of the hepatocytes of each animal, in which two lines were traced, one on the largest diameter (major diameter (D)) and the other on the smallest diameter (minor diameter (d)) of each nucleus. Soon after, these values were put into equations by providing the kariometric parameters.

For the stereological study (Martins et al.) 20 images of each component group were obtained and the areas for analysis were delimited, using the computer image analysis software ImageJ® 1.47t for Windows® the National Institute of Health - USA (Fig. 3). The only counted test-points were the ones that touched the cellular structure of the hepatocytes, within an area. This counting system of test-points was performed in the cytoplasm, nucleus and extracellular matrix of the hepatocytes. The projection was performed in 20 different test-areas, with 100 points for each test-area, and a total of 2000 points per animal group. After these procedures, these values were put into equations and the stereological parameters was obtained (Figs. 4 and 5).

The unit of measurement used for the values was the micrometer (µm). The calculations was made using the computer software Excel 2013® from Microsoft Office 2013® for Windows®.

**Statistical Analysis.** For statistical comparison of the body composition, kariometric and stereological results between groups, the Levene’s test was used to verify the normality of the data, the ANOVA One Way test and the Tukey’s post-test were used for parametric data and the Kruskal-Wallis’ test and the Dunn’s post-test were used for non-parametric data. All procedures adopted the significance level of (p<0.05). The calculations were performed with the R version 3.5.2 software for Windows® and the Origin version 9.0® software for Windows® was utilized for the elaboration of charts.
RESULTS

Figure 6 shows the values of body mass gain of the studied groups on 2nd, 4th and 6th Month’s age of the animals during the experiment (start, middle and end, respectively).

Statistically significant difference can be seen in body mass gain value of the SO(6) in comparison to EC (p=0.04).

Table I shows the values for body composition and liver mass (LM) of all groups.

It was possible to see in Table I statistically significant differences (p<0.05) in the variable EAT (g) of the EC and DC compared to SO(6) and DO; in the EAT (%) of DC compared to SO(6) and DO; in the BMI of EC compared to SO(6) and DO; and in the LM between EC and DO, and between SO(6) and all other groups.

The kariometric values of the nuclei of the hepatocytes of all the groups are found in Table II.

In Table II, we can observe significant differences (p<0.05) between DC, EO and DO in the variables minor diameter, mean diameter, D/d, volume, area, shape coefficient and contour index.

The stereological values for liver tissue of all the groups are found in Table III.

In Table III, there were no statistical differences between the stereological variables found among the groups studied.
DISCUSSION

The main results of this study regarding the body composition of the groups were: lower body mass gain of EC compared to the SO(6); lower amount of EAT (g) in EC and DC in relation to SO(6) and DO; lower EAT (%) in DC compared to SO(6) and DO; lower BMI in EC compared to SO(6) and DO.

A study suggests that intermittent training can positively affect inflammation parameters, body mass, adipocyte areas and lipids concentration in mice (Speretta et al., 2012). In addition to the regular training, calorie intake restriction can contribute to the increase of systemic glucose tolerance, improve mitochondrial fatty acid oxidation and oxidative function of enzymes and suppress hepatic lipogenesis (Rector et al., 2011).

Such parameters corroborate the results of our study, the EC had lower body mass gain compared to the SO(6), in addition to the lower values of body composition, particularly the EC and DC in comparison to the SO(6) and the DO. Also, the values of body composition of DC do not show significantly different from EC, which can indicate that the detraining did not reflect any type of influence on the effects initially provided by the intermittent training.

In regards to the LM, animals receiving a hyperlipidic diet have higher gain in body mass, an increase in adiposity and IHTG (Leite et al., 2013). These variables may have influenced the increase in liver mass of SO(6) in relation to all groups, and of DO compared to EC.

As for the kariometric analysis of liver tissue, the main findings were that the animals of the DC had the nuclei of their hepatocytes with greater size and volume, and less deformation in comparison to animals of the SO(6) and DO. As for the stereological analysis, there were no significant differences between groups.

Nuclear size and shape changes in liver tissue of animals exposed to toxic substances are considered a phenomenon known as anisonucleosis, associated with the presence of immunomarkers of oxidative damage in the liver, which may point to an inflammatory process in the liver tissue (Jarrar & Taib, 2012).

A hyperlipidic diet may induce a decrease in size and change in the conformation of the nucleus of the hepatocytes of the animals. The hydropic degeneration, which occurs in the liver tissue of obese animals according to inflammatory processes generated by a macrovesicular steatosis, contributes to the anisonucleosis of the nucleus of hepatocytes (Accioly et al., 2013). The hepatocytes from SO(6) and DO are more exposed to the risk of increased IHTG concentration compared to DC.

A study found that the use of a hyperlipidic diet may affect the pathophysiology of NAFLD, since the excess saturated fat and fructose contained in food may stimulate the accumulation of IHTG, aggravating the hepatic steatosis (Berglund et al., 2011). This same study also shows that changes in the type of diet used may provide a preventive
### Table I. Values of total and percentage Epididymal Adipose Tissue (EAT) total and percentage (g and %), Lee Index (g/mm³), Body Mass Index (BMI, in g/cm²) and Liver Mass (LM, in g) of animals for comparison between groups

<table>
<thead>
<tr>
<th>Variables</th>
<th>SC (n = 5)</th>
<th>EC (n = 6)</th>
<th>SC(6) (n = 4)</th>
<th>DC (n = 9)</th>
<th>SO (n = 5)</th>
<th>EO (n = 9)</th>
<th>SO(6) (n = 4)</th>
<th>DO (n = 9)</th>
<th>SO(6) (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EAT (g)</td>
<td>2.46 (1.93)</td>
<td>3.50 (4.29)</td>
<td>2.26 (3.11)</td>
<td>2.30 (1.12)</td>
<td>0.83 (0.32)</td>
<td>2.28 (1.30)</td>
<td>0.93 (1.46)</td>
<td>0.78 (0.30)</td>
<td>2.74 (1.12)</td>
</tr>
<tr>
<td>EAT (%)</td>
<td>2.67 (0.44)</td>
<td>0.91 (1.46)</td>
<td>0.83 (0.32)</td>
<td>2.28 (1.30)</td>
<td>0.93 (1.46)</td>
<td>0.78 (0.30)</td>
<td>2.74 (1.12)</td>
<td>0.238 – 0.023</td>
<td></td>
</tr>
<tr>
<td>Lee index</td>
<td>11.14 (2.22)</td>
<td>134.76 (4.03)</td>
<td>134.76 (4.03)</td>
<td>11.14 (2.22)</td>
<td>134.76 (4.03)</td>
<td>134.76 (4.03)</td>
<td>11.14 (2.22)</td>
<td>134.76 (4.03)</td>
<td>11.14 (2.22)</td>
</tr>
<tr>
<td>BMI</td>
<td>0.71 ± 0.059</td>
<td>0.48 ± 0.048</td>
<td>0.71 ± 0.059</td>
<td>0.48 ± 0.048</td>
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<td>0.71 ± 0.059</td>
<td>0.48 ± 0.048</td>
<td>0.71 ± 0.059</td>
</tr>
<tr>
<td>LM</td>
<td>11.00 ± 1.949</td>
<td>9.57 ± 0.617</td>
<td>11.00 ± 1.949</td>
<td>9.57 ± 0.617</td>
<td>11.00 ± 1.949</td>
<td>9.57 ± 0.617</td>
<td>11.00 ± 1.949</td>
<td>9.57 ± 0.617</td>
<td>11.00 ± 1.949</td>
</tr>
</tbody>
</table>

### Table II. Values of kariometric analysis for major, minor and mean diameters (µm), volume (mm³), area (mm²), volume/area ratio, shape coefficient and eccentricity of liver tissue of the animals.

<table>
<thead>
<tr>
<th>Variables</th>
<th>SC (n = 5)</th>
<th>SC(6) (n = 4)</th>
<th>DC (n = 9)</th>
<th>SO (n = 5)</th>
<th>EO (n = 9)</th>
<th>SO(6) (n = 4)</th>
<th>DO (n = 9)</th>
<th>SO(6) (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Major D.</td>
<td>0.718 – 0.064</td>
<td>0.718 – 0.064</td>
<td>0.718 – 0.064</td>
<td>0.718 – 0.064</td>
<td>0.718 – 0.064</td>
<td>0.718 – 0.064</td>
<td>0.718 – 0.064</td>
<td>0.718 – 0.064</td>
</tr>
<tr>
<td>Minor D.</td>
<td>0.411 (0.04)</td>
<td>0.411 (0.04)</td>
<td>0.411 (0.04)</td>
<td>0.411 (0.04)</td>
<td>0.411 (0.04)</td>
<td>0.411 (0.04)</td>
<td>0.411 (0.04)</td>
<td>0.411 (0.04)</td>
</tr>
<tr>
<td>Mean D.</td>
<td>0.542 – 0.031</td>
<td>0.542 – 0.031</td>
<td>0.542 – 0.031</td>
<td>0.542 – 0.031</td>
<td>0.542 – 0.031</td>
<td>0.542 – 0.031</td>
<td>0.542 – 0.031</td>
<td>0.542 – 0.031</td>
</tr>
<tr>
<td>Perimeter</td>
<td>1.825 (0.20)</td>
<td>1.825 (0.20)</td>
<td>1.825 (0.20)</td>
<td>1.825 (0.20)</td>
<td>1.825 (0.20)</td>
<td>1.825 (0.20)</td>
<td>1.825 (0.20)</td>
<td>1.825 (0.20)</td>
</tr>
<tr>
<td>Volume/Area</td>
<td>0.875 (0.07)</td>
<td>0.872 (0.07)</td>
<td>0.875 (0.07)</td>
<td>0.872 (0.07)</td>
<td>0.875 (0.07)</td>
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<td>0.875 (0.07)</td>
<td>0.872 (0.07)</td>
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<tr>
<td>Contour I.</td>
<td>3.796 (0.18)</td>
<td>3.796 (0.18)</td>
<td>3.796 (0.18)</td>
<td>3.796 (0.18)</td>
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<td>3.796 (0.18)</td>
</tr>
<tr>
<td>Eccentricity</td>
<td>0.812 (0.08)</td>
<td>0.812 (0.08)</td>
<td>0.812 (0.08)</td>
<td>0.812 (0.08)</td>
<td>0.812 (0.08)</td>
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</tr>
</tbody>
</table>

### Table III. Stereological values of cytoplasmic volume (Cit. Vv. - mm³), nuclear volume (Nucl. Vv. - mm³), matrix volume (Mat. Vv. - mm³), cellular volume (Cel. Vv. - mm³), number of cells (Nv - no/mm³) and numerical density (Nv - no/mm³) of the liver tissue of the animals.

<table>
<thead>
<tr>
<th>Variables</th>
<th>SC (n = 5)</th>
<th>SC(6) (n = 4)</th>
<th>DC (n = 9)</th>
<th>SO (n = 5)</th>
<th>EO (n = 9)</th>
<th>SO(6) (n = 4)</th>
<th>DO (n = 9)</th>
<th>SO(6) (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cit. Vv.</td>
<td>0.718 – 0.064</td>
<td>0.718 – 0.064</td>
<td>0.718 – 0.064</td>
<td>0.718 – 0.064</td>
<td>0.718 – 0.064</td>
<td>0.718 – 0.064</td>
<td>0.718 – 0.064</td>
<td>0.718 – 0.064</td>
</tr>
<tr>
<td>Nucl. Vv.</td>
<td>0.411 (0.04)</td>
<td>0.411 (0.04)</td>
<td>0.411 (0.04)</td>
<td>0.411 (0.04)</td>
<td>0.411 (0.04)</td>
<td>0.411 (0.04)</td>
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<td>0.411 (0.04)</td>
</tr>
<tr>
<td>Mat. Vv.</td>
<td>0.542 – 0.031</td>
<td>0.542 – 0.031</td>
<td>0.542 – 0.031</td>
<td>0.542 – 0.031</td>
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<td>0.542 – 0.031</td>
<td>0.542 – 0.031</td>
<td>0.542 – 0.031</td>
</tr>
<tr>
<td>Cel. Vv.</td>
<td>3.796 (0.18)</td>
<td>3.796 (0.18)</td>
<td>3.796 (0.18)</td>
<td>3.796 (0.18)</td>
<td>3.796 (0.18)</td>
<td>3.796 (0.18)</td>
<td>3.796 (0.18)</td>
<td>3.796 (0.18)</td>
</tr>
<tr>
<td>Nv</td>
<td>0.812 (0.08)</td>
<td>0.812 (0.08)</td>
<td>0.812 (0.08)</td>
<td>0.812 (0.08)</td>
<td>0.812 (0.08)</td>
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</tbody>
</table>

Legend: SC = Sedentary Control; EC = Exercised Control; SC(6) = Sedentary Control euthanized at six months old; DC = Detrained Control; SO = Sedentary Obese; EO = Exercised Obese; SO(6) = Sedentary Obese euthanized at six months of age; DO = Detrained Obese. The ANOVA One Way with Tukey’s post-test (Mean ± Standard Deviation) and the Kruskal-Wallis’ test with the Dunn’s post-test (Median (interquartile range)) were used for comparison of the results. The significance values p<0.05 were adopted for all procedures. aStatistically significant difference of p<0.05 in comparison to the group EO. bStatistically significant difference of p<0.05 in comparison to the subgroup SO(6).
effect against NAFLD. This suggests that the diet may be an important variable in the development of NAFLD, and that less consumption of hyperlipidic diets may reduce the content of IHTG, thereby reducing its deleterious effects on the liver tissue.

Besides that, the addition of an intermittent training protocol minimizes the deleterious effects provided by a hyperlipidic diet consumption (Leite et al.). This occurs as a result of the increased activity of superoxide dismutase and catalase in the hepatic tissue of animals that underwent intermittent training, improving the effectiveness of the antioxidant system, thus reducing the markers of liver peroxidation (Botezelli et al., 2011).

The intermittent training results in the decrease of lipid content of EAT, serum lipid profile and decrease in IHTG in animals. Furthermore, the intermittent training can stimulate the release of hepatic glucagon through metabolic pathways, contributing to the reversal of fatty liver condition in rats (de Wit et al., 2012).

A combined intervention with intermittent training and a normolipidic diet can reduce the concentration of IHTG, serum leptin and of the size of adipose cells and may reflect in the reduction of body mass and also reduction of apoptosis markers in the liver tissue cells, avoiding liver tissue damage caused by IHTG (Kurosaka et al., 2011).

Therefore, the use of the normolipid diet in addition to intermittent training may have been an important factor why the animals in the EC and DC groups presented lower values of body composition compared to the animals of the SO(6) and DO groups, as well as for the animals in the DC group presented greater preservation effect on the hepatic tissue in relation to EO and DO.

This study analyzed the liver condition only by images, besides evaluating the body composition using EAT and variables derived from the length and body mass of the animals. Based on what has been presented, this study has some suggestions for future researches. It would be interesting to quantify the amount of intrahepatic fat and verify if this is a determining factor for the difference in LM of the obese groups mentioned. It would also be valid to compare the performance of other training protocols implemented in isolation and together with intermittent training, to calculate the food coefficient of the animals and to analyze the concentration of anti-inflammatory and proinflammatory cytokines, considering the effects of both types on the accumulation of lipids found in obesity and in NAFLD.

We conclude that the intermittent training showed better effects on the DC liver tissue relative to the EO and DO, and it showed better effects on the body composition of the EC and DC in relation to the SO(6) and DO. Detraining have no significant effects on intermittent training.

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Corresponding author:
Alan José Barbosa Magalhães
Rua Roberto Simonsen, 305, Centro Educacional Presidente Prudente – SP
BRASIL

Email: ajb_magalhaes@yahoo.com.br

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