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The mechanisms involved in the increased adiposity induced by interruption of regular physical exercise practice

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ABSTRACT

Aims: We investigated the effects of physical detraining on lipogenesis/lipolysis and cellularity (apoptosis/adipogenesis) in rat subcutaneous (inguinal; SC) and visceral (retroperitoneal; RP) white adipose depots.
Main methods: Three groups of male Wistar rats (6-wk old) were studied: (1) (T) trained for 12 weeks; (2) (D) trained for 8 weeks and detrained for 4 weeks; and (3) (S) age-matched sedentary. Training consisted of treadmill running sessions (1 h/day, 5 days/week, 50–60% maximal race capacity).
Key findings: Physical detraining increased glucose oxidation, lipogenesis, and adipocyte size in the SC and RP depots. The number of apoptotic SC adipocytes was reduced by 53% in the T ($p < 0.0001$) and by 43% in the D ($p < 0.001$) as compared with S. RP adipocyte apoptosis in the T and D was 9.48% and 10.9% greater compared to the S, respectively ($p < 0.05$). In the SC stromal vascular fraction (SVF) of D rats, adiponectin, sterol regulatory element binding protein (SREBP)-1c, Peroxisome proliferator-activated receptor gamma (PPARγ), and Perilipin A mRNA expressions were more pronounced than S group, suggesting a more intense adipogenesis. This putative adipogenic effect was not observed in the RP depot. The physical detraining promoted rapid increase in the SC and RP depots however not through the same mechanisms.
Significance: Physical detraining induced fat cell hypertrophy (increase of lipogenesis) in both SC and RP whereas hyperplasia (increase of adipogenesis and reduction of apoptosis) was found in SC only. These results indicate the mechanism associated with obesogenic effects of detraining varies with the fat depot.

1. Introduction

The prevalence of obesity grows worldwide and is associated with high risk of death, hypertension, coronary artery disease, stroke, type 2 diabetes mellitus (T2DM), and cancer [1,2] and the use of non-pharmacological techniques in the control of obesity and associated diseases has increased in clinical practice, among them food control and physical exercise. [3–6].

Several effects of physical exercise on adipose tissue (AT) are known such as increase in lipolytic activity and improvement of insulin responsiveness [7,8]. These effects generally have repercussions on triacylglycerol (TAG) mobilization and oxidation rate resulting in reduction of fat mass and are dependent on the intensity, duration, and frequency of training sessions [9]. On the other hand, little is known about the effects of physical detraining on its metabolism, mainly on the balance between lipolysis and lipogenesis. Moreover, less understood is the influence of both physical training and detraining on AT cellularity (balance between apoptosis and adipogenesis) whose disruption may lead to hypertrophy or hyperplasia of total fat mass. Exercise training suppresses the ability of stromal vascular fraction (SVF) cells to differentiate into adipocytes since was observed reduction in the peroxisome proliferator-activated receptor γ (PPARγ) gene expression and increase in the preadipocyte factor-1 (Pref1) gene expression in periepididymal fat pad, and that underlying mechanisms involve the upregulation of hypoxia-inducible factor-1α (HIF-1α) expression [10]. Similarly, physical exercise does not stimulate adipogenesis in the

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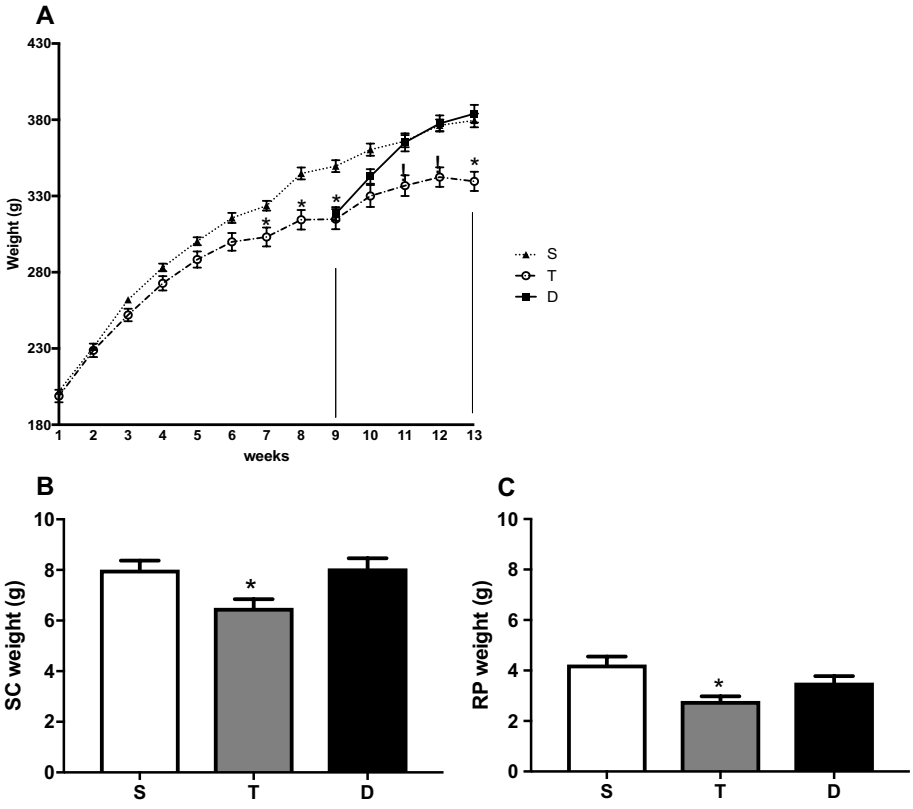

Fig. 1. Progress of body and fat pad weights. A: Variation of body weight (g). Week 1 - initial body weight; week 9 - body weight after 8 weeks of training (beginning of detraining); week 13 - final body weight. * $p < 0.01$ T vs S and D; B: SC fat pad weight (g) 12 weeks. * $p < 0.05$ T vs S and D; C: RP fat pad weight (g) 12 weeks. * $p < 0.05$ T vs S and D. n = 10 per group. Data is expressed as means ± s.e.m.

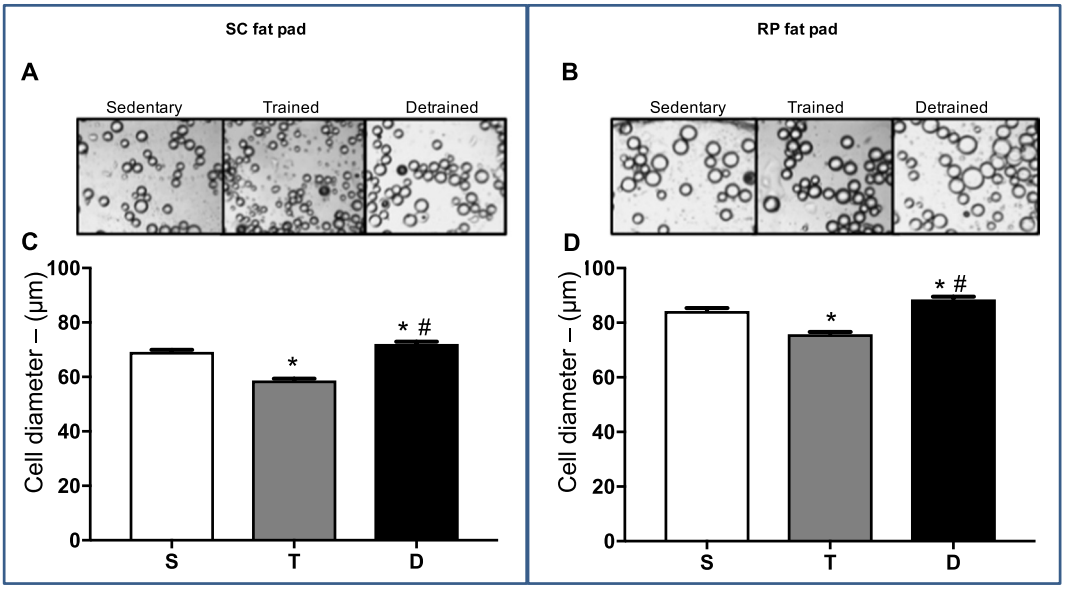

Fig. 2. Cell diameters. SC and RP adipocyte diameters. A and B: Representative photographs of isolated adipocytes from the SC and RP depots, respectively. The cells were isolated by the method of collagenase, fixed in 4% formaldehyde and photographed at 100× amplification in an optical microscope. The cell diameter was calculated from arithmetic average of 100 cells per animal. C: Representation of SC cell diameter (µm) 12 weeks. * $p < 0.05$ T vs S and D, # $p < 0.05$ D vs S and T. D: Representation of RP cell diameter (µm) 12 weeks. * $p < 0.05$ T vs S and D, # $p < 0.05$ D vs S and T. n = 10 per group. Data is expressed as means ± s.e.m.

periepididymal adipose tissue, but when training is interrupted a strong adipogenic stimulus occurs [11].

The rise in the number of adipocytes occurs through several events involving proliferation and differentiation of preadipocytes. Adipogenic transcription factors, including peroxisome proliferator-activated receptor gamma (PPARγ), sterol responsive element binding protein 1c (SREBP-1c), and CCAAT (CCAAT/enhancer binding protein, C/EBPs) play a key role in the transcriptional cascade that occurs during adipogenesis, with PPARγ being the most important transcriptional factor involved [12].

In contrast the reduction of the adipocyte number is strongly linked to the phenomenon of apoptosis (programmed cell death). One of the morphological characteristics of apoptosis is the chromatin condensation [13]. The extrinsic pathway is stimulated by activation of death receptors, Fas (CD95/Apo1) and TNFR1. These molecules have the ability to recruit caspase-8 that activates caspase-3, and induce cell

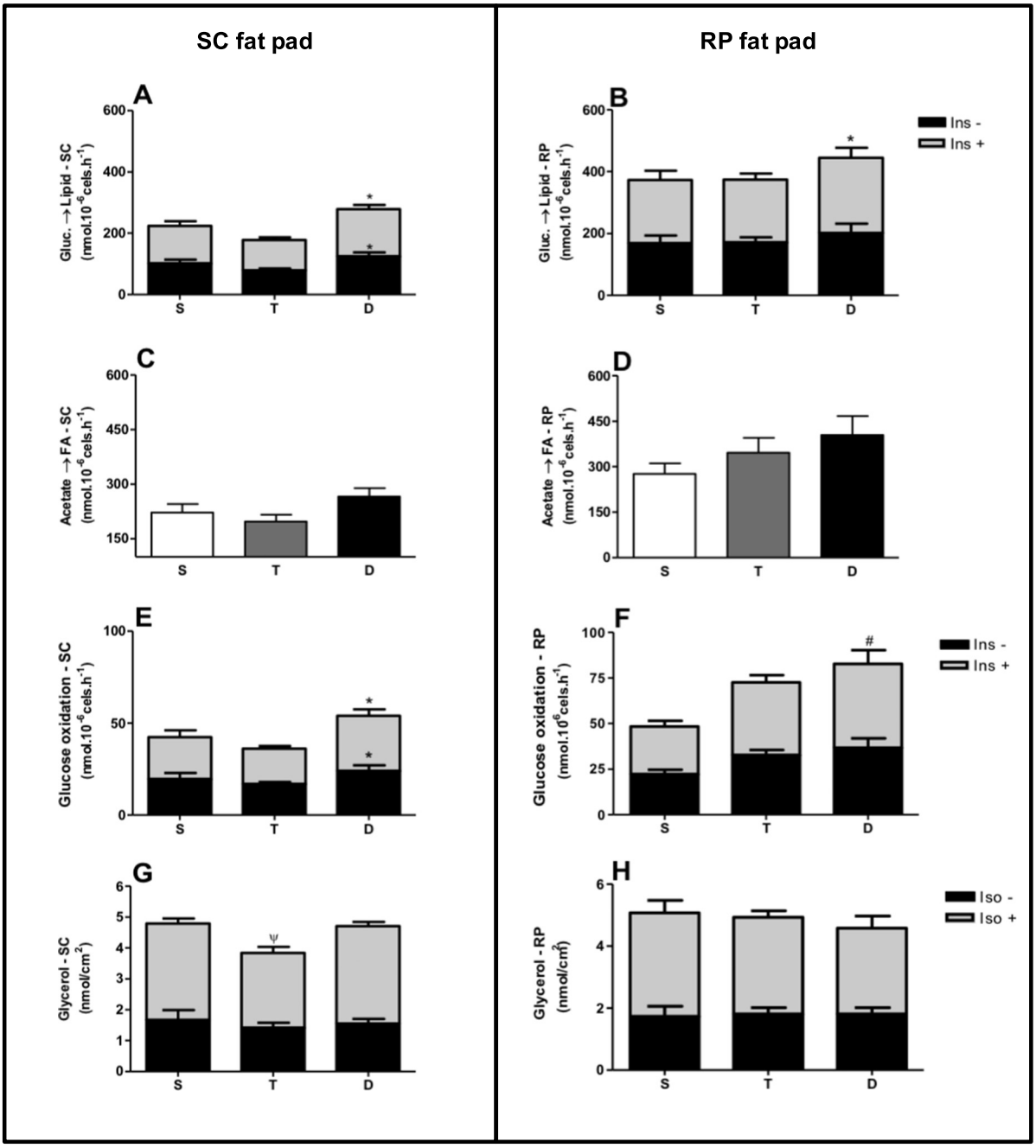

Fig. 3. Metabolic capacities of isolated adipocytes: lipogenesis, glucose oxidation and lipolysis. A and B: Lipogenesis – incorporation of glucose into triacylglycerol. A: Lipogenesis SC. *p < 0.05 D vs S e T (Ins- and Ins+); B: Lipogenesis RP. *p < 0.05 D vs S e T (Ins+). C and D: Lipogenesis – incorporation of acetate into fatty acid – no difference was observed between the groups. E and F: Glucose oxidation. E: Oxidation SC: *p < 0.05 D vs S e T (Ins- and Ins+); F: Oxidation RP. #p < 0.05 D vs S (Ins+). G and H: Lipolysis – G: Lipolysis SC. $^{\Psi}$p < 0.05 T vs S and D (Iso+). n = 10 per group. Data is expressed as means ± s.e.m.

death by apoptosis [14]. Intrinsic apoptotic signals lead to the release of cytochrome *c* that binds to Apaf-1 (apoptotic protease activating factor-1) in the cytosol [15]. Apaf-1 oligomerized proteins recruit caspase-9 [16].

Therefore, the objective of the present study was to investigate the metabolic and cellular mechanisms that are involved in the accelerated fat accretion observed after the interruption of the physical training that lead to the total recovery of the corporal weight in 4 weeks and how this interruption contributes in a long term to the genesis of obesity.

2. Material and methods

2.1. Ethical approval

The present study was per formed according to protocols approved by the Ethics Committee of the University of São Paulo (CEUA 78/21/3).

2.2. Animals and physical exercise training schedule

Male Wistar rats (45 days old/200 g) from the Animal Resources Center of the Institute of Biomedical Sciences of the University of São Paulo were used. The animals were divided into 3 groups (10 animals

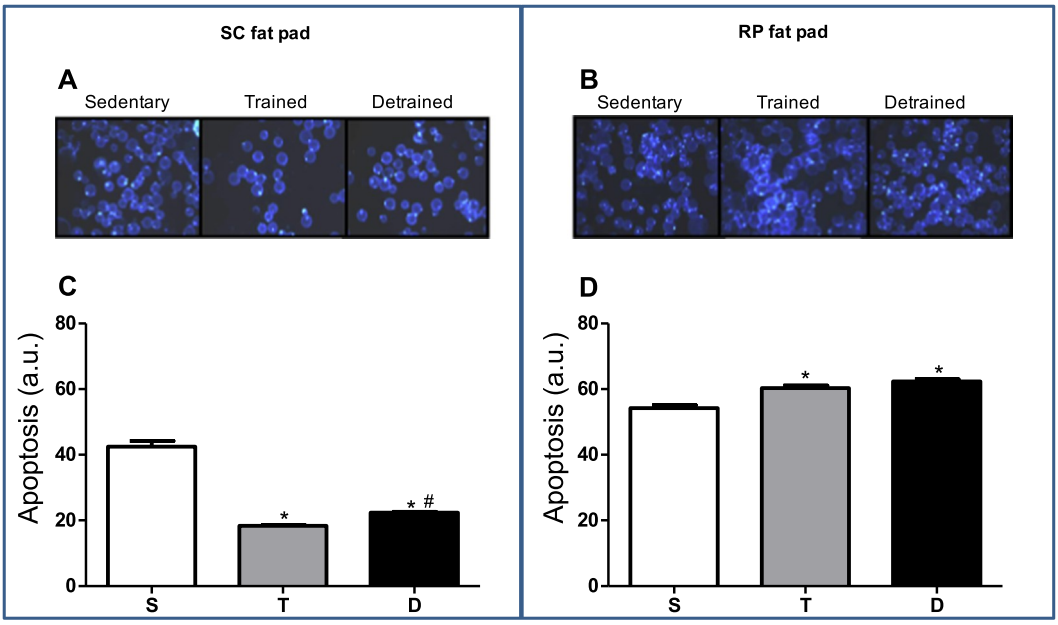

Fig. 4. Chromatin condensation. Analysis of chromatin condensation and measurement of caspase by ELISA. A and B: Representative photographs of chromatin condensation from the SC and RP fat pads. **C**: Mean + SEM of the fluorescence intensity obtained using fluorescence microscopy in SC adipocytes. *p < 0.001 S vs T &D, #p < 0.05 D vs T; D: Mean + SEM of the fluorescence intensity obtained using fluorescence microscopy in RP adipocytes. *p < 0.001 S vs T&D. n = 10 per group. Results are expressed as means ± s.e.m.

per group): 1) T, trained for 12 weeks; 2) D, trained for 8 weeks and detrained for 4 weeks; and 3) S, sedentary age-matched, as described in our previous study [11]. After the euthanasia, samples of subcutaneous (SC) and retroperitoneal (RP) fat pads were removed. This experimental model was previously tested [11] and validated by increasing the Citrate Sintase activity in the solear muscle.

2.3. Adipocyte isolation and morphometric cell analysis

The adipocytes were then isolated according to Rodbell (1964) [17]. The isolated adipocytes (7–8 × 10^5 cells/mL) were suspended in EHB buffer (Earle's salts, 20 mM HEPES, 1% bovine serum albumin, 2 mM sodium pyruvate and 4.8 mM sodium bicarbonate), pH 7.4, at 37 °C. Cell size and number were determined as previously described [11].

2.4. Incorporation of D-[U-^{14}C]-glucose and ^{14}C-acetate into lipids

Adipocyte suspension (at 10%), in Krebs/Ringer/phosphate buffer at pH 7.4 with 1% bovine serum albumin and 2 mmol/L glucose, were transferred to polypropylene test tubes containing 5 µL (1850 Bq/tube) of D-[U-^{14}C]-glucose or ^{14}C-acetate and incubated at 37 °C and saturated with a CO_2 (5%)/O_2 (95%) gas mixture in the presence or absence of insulin (10 nmol/L). The samples were then incubated (final volume = 500 µL) for 1 h in a water bath at 37 °C. The tubes had a rubber stopper, and the atmosphere inside was enriched with CO_2 (5%)/O_2 (95%). At the end of the incubation, the stopper was removed, 0.2 mL of H_2SO_4 (8 N) was added, and a 4-mL scintillation vial, containing a piece (2 × 4 cm) of filter paper moistened with 0.2 mL of ethanolamine, was immediately placed (mouth-to-mouth) on the top of the reaction tube. Next, the connection point between the tubes was sealed with a band of plastic film. The assembled tubes were incubated for an additional 30 min. At the end of this incubation, the scintillation vial with the filter paper was filled with 3 mL of scintillation cocktail (EcoLume, ICN Pharmaceuticals, Costa Mesa, CA) to measure the adsorbed radioactivity. Finally, the reaction mixture remained in the test tube was treated with 2.5 mL of Dole's reagent (isopropanol:*n*-heptane:sulfuric acid, 4:1:0.25 vol/vol/vol) for lipid extraction. This mixture was vortexed three times during the next 30 min and the tubes were then filled with 1.5 mL of heptane and 1.5 mL of miliQ water. The tubes were decanted and 0.5 mL of the upper phase was collected in duplicate for the determination of ^{14}C incorporation into lipids. The radioactivity was read in a scintillation counter (1450 LSC Counter - Microbeta Trilux, PerkinElmer). The results were expressed as nanomoles/10^6 cells/h. Similar procedure was used in our previous study [11].

2.5. ^{14}CO2 production from D-[U-^{14}C]-glucose by isolated adipocytes

The scintillation vials, placed on top of the reaction tubes containing the filter paper moistened with ethanolamine described above, were filled with scintillation cocktail and the radioactivity was counted to determine the $^{14}CO_2$ derived from ^{14}C-glucose. The radiation emitted was determined as described in [11].

2.6. Measurement of lipolysis

The rates of basal and isoproterenol-stimulated lipolysis were measured in the isolated adipocytes of rats according to the following protocol: aliquots (40 µL) of cell suspension (EHB buffer containing 5 mM of glucose) were transferred to microtubes (0.6 mL) and incubated with 0.3 mM adenosine for 30 min. Next, 20 µL of adenosine deaminase (ADA, Sigma, 0.2 U/mL in EHB buffer, pH 7.45) was added for 30 min at 37 °C to allow the adenosine to degrade [18]. After this period, the cells were incubated for 60 min at 37 °C with or without 10 µL of isoproterenol (10^{-5} M) in a total volume of 200 µL. At the end of the incubation, the reaction was stopped by transferring the tubes to an ice bath, and centrifuged (5000 × *g*) for 5 min at 4 °C to pellet the cells in the reaction medium. Samples of the incubation medium were collected and the amount of glycerol released from adipocytes was determined using an enzymatic-colorimetric method (Sigma) which was used as an index of the lipolysis rate. The results were expressed in nmol/cm^2. Similar procedure was used in our previous study [11].

2.7. Analysis of chromatin condensation using fluorescence microscopy

This technique was used to determine the degree of cell apoptosis.

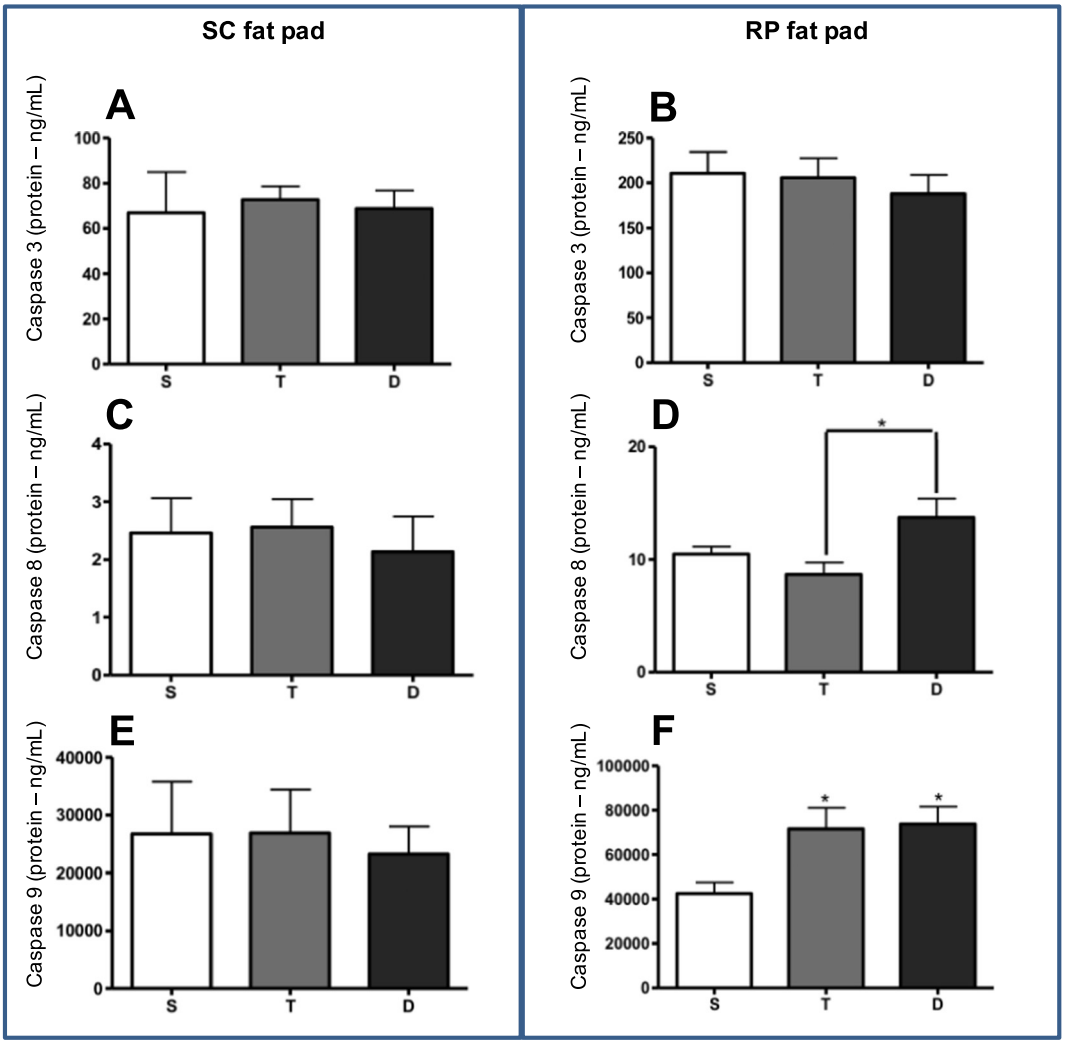

Fig. 5. Measurements of caspase proteins by Elisa. D: analysis of caspase 8 in the RP fat pad. * $p < 0.05$ D vs T; F: analysis of caspase 9 in the RP fat pad. * $p < 0.05$ S vs T and D rats. A, B, C and E there were no statistical difference. n = 10 per group. Results are expressed as means ± s.e.m.

The analysis was performed under fluorescence microscopy to highlight the expression of adipocyte-specific genes. The adipocytes in the sample (1 g of tissue) were isolated and resuspended in 20 µL of Hoechst 33342 (Molecular Probes) at 0.01 mg/ml in saline (NaCl 0.9%). After 10 min of incubation in the dark, 10 µl of sample was transferred to a glass slide and viewed with a fluorescence microscope with a 365/80 nm filter. This stain distinguishes nuclei with condensed chromatin in apoptosis from those with uncondensed chromatin in viable cells. The cells were analyzed using the program KS 300 (Carl Zeiss Vision).

2.8. Measurement of caspase content by ELISA

This assay employs the quantitative sandwich enzyme immunoassay technique. An adipose tissue sample (100 mg) was rinsed with 1× PBS, homogenized in 1 ml of 1× PBS and stored overnight at −20 °C. After performing two freeze-thaw cycles to break the cell membranes, the homogenates were centrifuged for 5 min at 5000 × *g*, 2–8 °C. The supernatant was removed and assayed immediately. In a 96-wells microplate pre-coated with anticaspase antibodies, aliquots (100 µL) of standards and samples are pipetted into the wells where any target protein present is bound by the immobilized antibody. After removing unbound substances, a biotin-conjugated antibody specific for the protein is added to the wells and avidin conjugated Horseradish Peroxidase (HRP) is added thereafter. Following a wash to remove any unbound avidin-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of specific protein bound in the initial step. The color development is stopped and the color intensity is read at 450 nm and measured.

2.9. Measurements of ADD1/SREBP1c, PPARγ, β2M, adiponectin and perilipin a gene expressions in the stromal-vascular cell fraction

RT-PCR was performed to quantify mRNA levels of sterol regulatory element binding protein (SREBP)-1c, Peroxisome proliferator-activated receptor gamma (PPARγ), Beta 2 Microglobulin (β_2M), Adiponectin and Perilipin A. Total RNA extraction was carried out using TRIzol reagent (Invitrogen, Carlsbad, CA) and RNA Purification Kit (Ambion, Carlsbad, CA) and the concentration and quality of RNA was spectrophotometrically confirmed at 260 nm. SuperScript™ III Reverse Transcriptase (Catalog number: 18080-093, Invitrogen – EUA) was used to reverse transcribe 2 µg of total RNA isolated using a 0,03 µg of the Random Primer in a total reaction volume of 100 µL. Quantitative analysis of mRNA expression was performed using StepOnePlus® Real Time PCR System (Applied Biosystems™) with TaqMan$^{\hat{a}}$ Gene Expression Assays (Applied Biosystems™) in a total volume of 10 µL. The relative amount of each target gene was analyzed using the comparative Ct (cycle threshold) method [19]. The Ct value is the estimated number of cycles during which the emitted fluorescence signal is significantly above baseline levels. Primers used and reference numbers: PPARgamma - Rn 00440945_m1; SREBP1c - Rn 00566440_ml;

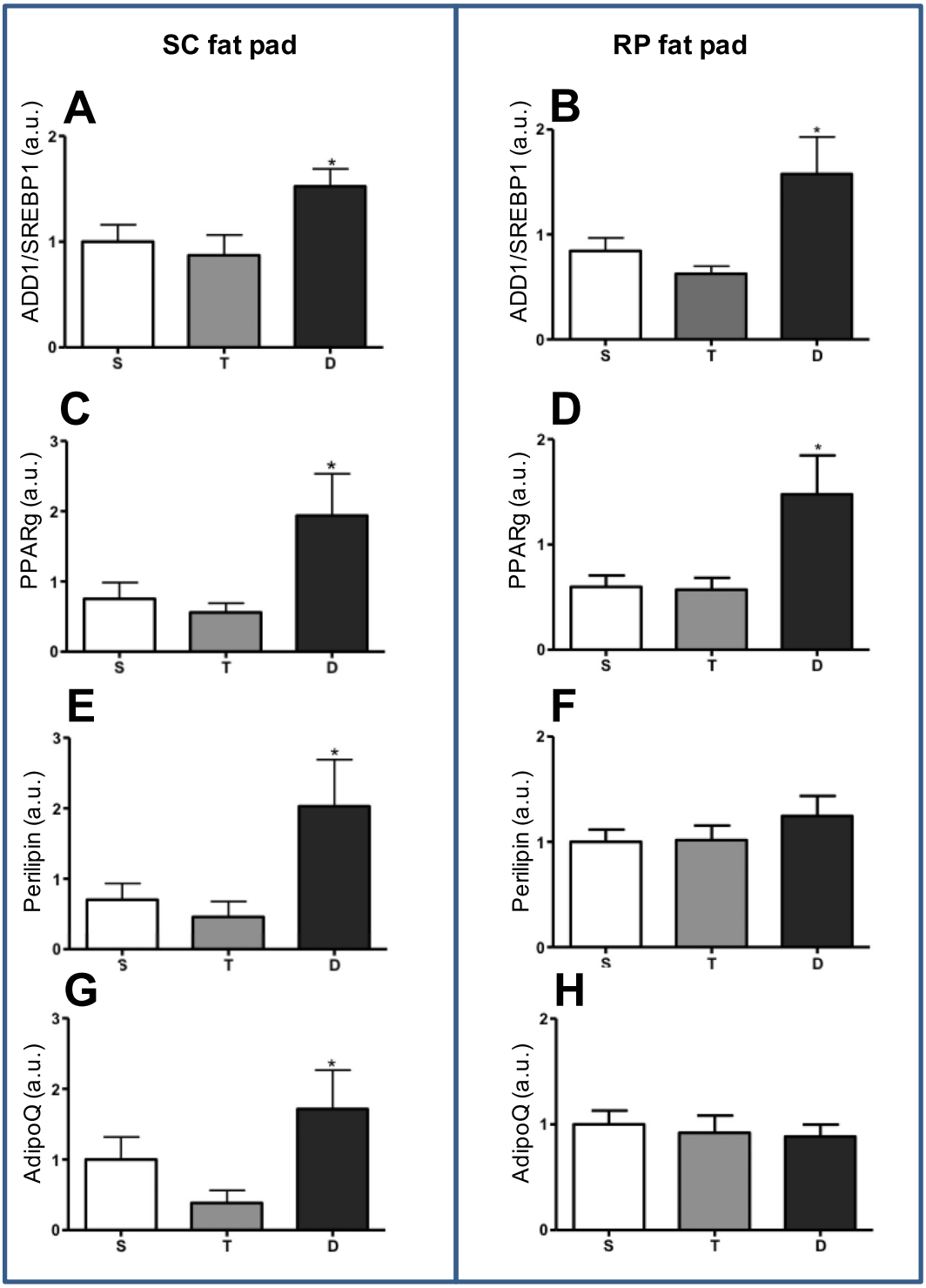

Fig. 6. Adipogenesis. Representative quantification of ΔΔ CT of ADDI/SREBP1c, PPARgamma, Perilipin and Adiponectin gene expressions in the stromal-vascular cell fraction of SC and RP fat pads. A: ADDI/SREBP1c in SC fat pad. * $p < 0.05$ D vs S and T; C: PPARγ in SC fat pad. * $p < 0.05$ D vs S and T; E: Perilipin in SC fat pad. * $p < 0.05$ D vs S and T; G: AdipoQ in SC fat pad. * $p < 0.05$ D vs S and T; B: ADDI/SREBP1c in RP fat pad. * $p < 0.05$ D vs S and T; D: PPARγ in RP fat pad. * $p < 0.05$ D vs S and T. n = 10 per group. Results are expressed as means ± s.e.m.

Adiponectin - Rn 00595250_m1; Perilipin - Rn 00558672_m1; B_2M - Rn 00560865_m1. (Font: http://www.appliedbiosystems.com).

2.10. Statistical analysis

The means ± SEM of the results from each group were obtained and analyzed using one way-ANOVA. Bonferroni post-test analysis was used for comparisons between groups. The upper limit of significance for rejection of the null hypothesis was established at 5% ($p < 0.05$).

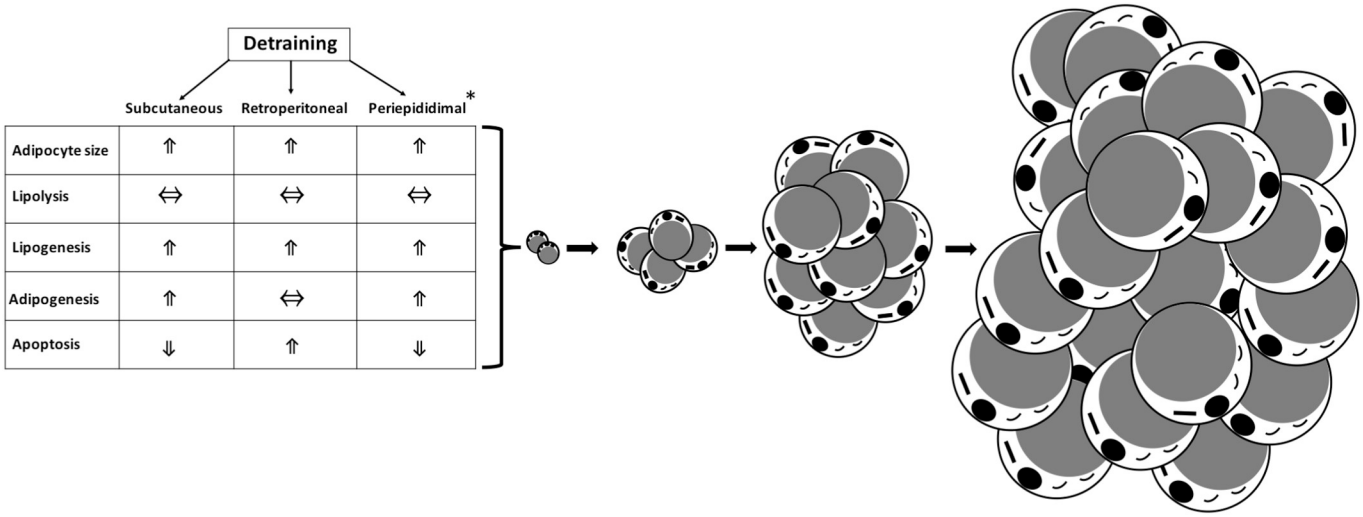

Fig. 7. Representative illustration of the adaptations that occur in the SC, RP and PE fat pads with the interruption of the regular practice of physical exercises. In general, adiposity gain with physical detraining is directly related to hypertrophy and hyperplasia. * data published in Sertie et al., 2013 [11].

3. Results

3.1. Bodyweight, fat pad weights and cell diameters

S and T rats were randomly distributed in such a way that the two groups began the training protocol with the same mean body weight (204.7 ± 1.82 g and 201.3 ± 2.05 g; S and T respectively). After 8 weeks of the physical training program, T rats had a body weight gain significantly lower than the S group (353 ± 4.85 g and 307 ± 5.11 g; S and T respectively, Fig. 1A). After 4 weeks of exercise interruption (detraining), the newly formed group D showed a much higher body weight gain than the other groups (81 g of weight gain against 33 g and 12 g for S and T rats, respectively), catching up the weight difference brought about by exercise and reaching a final body weight of sedentary animals (final body weight (g) −386.4 ± 2.7, 319.3 ± 3.02 and 388.8 ± 2.03; S, T and D respectively, Fig. 1A).

At the end of the 12 weeks of exercise training, the SC and RP fat pads of the T group were significantly lower than those of the S and D groups: SC fat pad mass (g) 12 weeks – 8.014 ± 0.35, 6.5 ± 0.33 and 8.06 ± 0.39 (S, T and D respectively); RP fat pad mass (g) 12 weeks – 4.23 ± 0.31, 2.19 ± 0.18 and 3.52 ± 0.24 (S, T and D respectively). As the masses of fat remained lower in T compared to S rats, the experimental protocol of exercise training proved its effectiveness to mobilize the fat reserves of the animals, as depicted in Fig. 1A. On the other hand, after 4 weeks of detraining, the differences in fat masses completely disappeared in the SC and RP fat pads of the D group when compared with the S group (Fig. 1B and C, respectively).

In isolated adipocytes (Fig. 2), a significant reduction in the cell diameter in both SC and RP fat pads after 12 weeks of exercise training (15.22% and 10.13% respectively) compared to S rats was observed. Also, after 4 weeks of exercise detraining, both SC and RP adipocytes of the D group had significantly larger cell diameters compared with the S groups (4.24% and 5.0%, respectively), suggesting strong correlation between exercise detraining and fat cell hypertrophy (SC cell diameter (μm) 12 weeks: 69.22 ± 0.77, 58.68 ± 0.69 and 72.16 ± 0.82; S, T and D, respectively. RP cell diameter (μm) 12 weeks: 84.33 ± 1.07, 75.78 ± 0.83 and 88.55 ± 1.02; S, T and D, respectively).

3.2. Evaluation of the metabolic capacities (lipogenesis, glucose oxidation and lipolysis) in isolated adipocytes

Subcutaneous fat cells of detrained rats showed higher ability to convert glucose into TAG as compared to the S and T groups, both in presence and in absence of insulin (Fig. 3A). RP adipocytes (Fig. 3B) from detrained animals had higher lipogenic capacity only when stimulated by insulin as compared to the other groups.

The use of ^{14}C-acetate to investigate lipogenesis is because the only metabolic route of acetate to lipid synthesis is through the so called lipogenesis de novo, i.e., the only way acetate can be incorporated into TAG is its conversion into the fatty acid moiety. By means of this strategy (Fig. 3C and D), no significant differences were observed between the three groups.

Since lipogenesis is an endergonic reaction we evaluated the glucose oxidation as a parameter of mitochondrial activity for ATP production. Part of this ATP will serve to maintain the cell activity, and part will be used to join the molecules of fatty acid and glycerol. SC adipocytes of D rats had a higher capacity to oxidize glucose, with and without insulin stimulation, when compared to the S and T groups (Fig. 3E). In the RP fat, exercise detraining increased the oxidative capacity of adipocytes only when compared to S rats and when maximally stimulated by insulin (Fig. 3F).

The basal and maximally isoproterenol-stimulated rates of lipolysis in isolated RP adipocytes were not significantly different among the groups (Fig. 3H). However, in the SC adipocytes, the exercise training reduced the maximal lipolytic response when compared to the S and D groups. The exercise detraining did not alter the lipolytic ability of adipocytes as compared to S animals in both fat pads (Fig. 3G).

3.3. Apoptosis: analysis of chromatin condensation and measurement of caspase proteins by ELISA

Chromatin condensation was used as an indicator of the occurrence of cell apoptosis. Exercise training sharply reduced the apoptosis in SC isolated adipocytes and exercise detraining reversed in part this reduction but it still remained lower than in sedentary rats (Fig. 4A). In RP fat, the apoptotic process occurred in an opposite way, i. e., exercise training increased it and exercise detraining did not reverse it (Fig. 4B). When the protein expression of caspases 3, 8 and 9 were evaluated, no significant differences were observed in the SC fat pad (Fig. 5A, C and E). In RP fat pad, a significant increase in protein expression of Caspase 8 was detected in the D compared to the T group (Fig. 5D). Still, the analysis of protein expression of caspase 9 in RP revealed that exercise training stimulated the increase of its expression and that the exercise detraining was not able to change such a high expression caused by the training program (Fig. 5F).

3.4. Adipogenesis: measurements of ADDI/SREBP1c, PPARgamma, perilipin and adiponectin

The vascular stroma of SC and RP fat pads were analyzed in order to investigate differences in the amount of newly differentiated adipocytes and the possible association of this finding with the growth of fat mass (Fig. 6). By Real Time PCR we studied the gene expression of the Add1, PPARγ, Adiponectin and Perilipin A. The exercise detraining considerably increased the gene expressions of Add1 and PPARγ in SC vascular stroma of detrained animals when compared to the S and the T groups (Fig. 6A and C). The gene expressions of perilipin A and adiponectin in SC fat pad of detrained animals were significantly increased (Fig. 6E and G). In the RP fat pad, similar results were observed for the nuclear transcription factors Add1 and PPARγ, which showed a significant increase in vascular stroma of detrained animals when compared to the other two groups (Fig. 6B and D). However, when we assessed the specific genes of newly differentiated adipocytes in the RP fat pad vascular stroma no significant differences were observed among the three groups (Fig. 6F and H).

4. Discussion

The results confirmed that exercise training caused a reduction in adipocyte size (attenuating weight gain – Fig. 1) and detraining promoted its hypertrophy in both fat pads (Fig. 2). Physical detraining in rats subjected to a high-fat diet was previously shown to increase the ability to store fat in the urogenital, mesenteric and retroperitoneal cushions and this expansion of fat stores has been attributed to many factors including the elevation of insulin sensitivity and lipoprotein lipase activity [20–22]. In our case, the incorporation of acetate into total lipids (TAG) is possible only by its incorporation into the free fatty acid moiety of TAG (Fig. 3C and D) and this maneuver indicated that the de novo lipogenesis process (DNL), that is the synthesis of long chain fatty acids from acetyl-CoA, is not the preferred route for lipogenesis (TAG synthesis) in our experimental model, but the generation of glycerol-P (Fig. 3A and B). This same phenomenon of cellular hypertrophy also occurs in the periepididymal fat pad (Fig. 7).

We also observed the increase in glucose oxidation rate in SC and RP fat pads stimulated (or not) by insulin in adipocytes of D group (Fig. 3E and F). As Coyle et al. [23] have shown, the intensification of the energy demand by exercise resulted in the increase of lipid and glucose oxidation. Rats swimming for 4 months showed a mitochondrial respiratory boost through cytochrome C oxidase subunit IV (COX IV) and Cytochrome C oxidoreductase subunit I (CORE I) [23] The increase in glucose oxidation seen here in exercised rats could be a mechanism to offer extra energy for esterification of fatty acids and glycerol. If this effect was maintained during the detraining process, it could lead the detrained animals to recover their adipose mass. This possibility, however, needs a further experimental confirmation. Furthermore, the gene expression of peroxisome proliferator-activated receptor-γ coactivator-1α (PGC1-α, greater regulator of mitochondrial biogenesis) and mitochondrial transcription factor A (TFAM - transcriptional factor that precedes PGC1-α) were also increased, suggesting that physical training increased the amount and activity of mitochondria in AT [24]. However, our finding that this phenomenon was superstimulated in the detraining period is unprecedented. We are assuming that these mechanisms were sustained after training cessation. Thus, this adaptive mechanism could be providing a favorable condition for the oxidative generation of ATP the limiting step for the formation of the TAG molecule and promoting complete recovery of fat mass of the animal. Another possibility could be the involvement of AMPK, favoring the substrate flow toward the mitochondria, since the lipogenesis de novo was not a preferential pathway for TAG synthesis as demonstrated by the results of ^{14}C-acetate incorporation into lipids.

One of the reasons for adipose mass reduction is the hypotrophy of mature adipocytes, which may be associated with an increased chronic lipolysis [4,25]. The absence of changes in the lipolytic rates in SC and RP fat pads of D group (Fig. 3G and H) associated with increased lipogenic capacity justifies the weight gain and adipocyte hypertrophy observed. One of the adaptations to regular exercise is the upregulation of adipocyte triglyceride lipase (ATGL). Moreover, the exercise-induced lower levels of insulin also correlates with higher levels of ATGL in primary adipocytes [26]. In our study, however, the recovery of adipose SC and RP masses after detraining was not due to changes in lipolytic capacity but to the increase in lipogenic capacity which was increased and could be the dominant effect. As shown in Fig. 7 this adaptation is not site specific and also occurs in the periepididymal (PE) fat pad.

Another important aspect is the balance between apoptosis/adipogenesis, whose disruption may also result in increase (or decrease) in fat mass. Among the morphological characteristics present in apoptotic cells, chromatin condensation can be detected early and it occurs before the disruption of nuclear membrane [27,28]. We observed that the physical training reduced the chromatin condensation in adipocytes of SC fat pad of T group and the physical detraining was not able to reverse this reduction (Fig. 4A and C). As a consequence, a reduced loss of mature adipocytes in SC fat pad was observed, contributing to the expansion of fat mass. We know that low-intensity exercise reduces oxidative stress and apoptosis in cardiomyocytes of streptozotocin-induced diabetic rats, showing a protective effect of exercise on cardiomyocytes [29,30], but this observation in adipocytes has been recently described only by our group. Once the exercise more intensely mobilizes fatty acids for the increased energy demands, we can infer that the preservation of mature adipocytes by reducing apoptosis may be a mechanism to maintain and sustain the fat stores. However, when detraining takes place this process of fat cell preservation would favor the rapid increment of fat mass.

In RP fat pad the exercise training increased the chromatin condensation and even after 4 weeks of detraining the phenomenon remained significantly elevated (Fig. 4B and D). Additionally, the determination of caspase protein 8 and 9 contents in this fat pad demonstrated that this pathway was activated (Fig. 5D and F). In adipose tissue of rats, immunofluorescence examination demonstrated that activation of apoptotic pathways is associated with increased caspase 8 and 9 [31–33]. On the other hand, incubation of isolated adipocytes with TNF-alpha (20 ng/ml) for 5 h enhanced the expression of the *inhibitor apoptosis protein 2* (IAP2) gene without any enhancement of caspase-3 activity in both the sedentary control and exercise-trained groups, but the ability of TNF-alpha to enhance IAP2 gene expression was significantly greater in exercise-trained grups than in control rats [34]. Furthermore, apoptosis is site specific in adipose tissue. For example, while oleoylestrone inducing apoptosis by highly active caspase 3, 8 occurs in the mesenteric adipose tissue, nothing happens in both SC and RP adipose tissues [35].

Moreover in SC fat pad the adipogenesis was stimulated which is confirmed by the elevated gene expression of the transcription factors ADD1\SREBP1c and PPARγ in the stromal vascular fraction in D group (Fig. 6A and C). Adipogenesis is modulated by a cascade of transcription factors including the PPARγ and ADD1\SREBP1c that are crucial for the induction of adipocyte phenotype [36]. These factors together promote the differentiation of preadipocytes into adipocytes, activating the expression of the adipocyte-specific genes [37,38]. In in vitro experiments, the ADD1/SREBP1c increased the transcriptional activity of PPARγ, raising the proportion of cells subjected to differentiation process [39]. It was detected in the vascular stroma of SC fat pad a significant increment of perilipin A and adiponectin mRNA content, two genes that are highly expressed only in adipocytes (Fig. 6E and G). Thus, we can infer that there is a greater number of newly differentiated adipocytes in the vascular stroma of SC fat pad of detrained animals.

In RP fat pad the gene expression of PPARγ and ADD1\SREBP1c followed the same tendency observed in SC fat (Fig. 6B and D); however there was no rise in the number of newly differentiated adipocytes in the vascular stroma of detrained animals since we did not detect

increase in perilipin A and adiponectin gene expression (Fig. 6F and H). Considering these aspects we speculate that a specific protective mechanism against hyperplasia is taking place in RP fat pad, thus preventing the excessive elevation of visceral fat mass, probably because excess visceral fat is responsible for the development of chronic diseases such as dyslipidemia, diabetes, insulin resistance, cardiovascular disease and hepatic steatosis [40]. On the other hand SC fat acts as a metabolic buffer, accumulating the energy excess in the form of TAG [41]. This fat gain via deconditioning was attributed as a 'catch-up' fat mechanism [42]. Physical detraining also reduces apoptosis and increases adipogenesis in the PE fat pad (Fig. 7), corroborating for total recovery of fat mass observed with physical detraining.

5. Conclusion

In conclusion, the physical detraining strongly induced a fat mass gain in SC and RP fat pads due the increase in the lipogenic rates. In the case of SC fat pad it also led to the increment in cellularity. This hyperplasia of fat cells may be important to attenuate the higher TAG accumulation in the RP deposits and to allow a redistribution of fat toward the SC regions.

Conflict of interest statement

There are no conflicts of interest.

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