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ORIGINAL PAPER

White adipose tissue re-growth after partial lipectomy in high fat diet induced obese Wistar rats

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Abstract The effects of partial removal of epididymal (EPI) and retroperitoneal (RET) adipose tissues (partial lipectomy) on the triacylglycerol deposition of high fat diet induced obese rats were analyzed, aiming to challenge the hypothesized body fat regulatory system. Male 28-day-old wistar rats received a diet enriched with peanuts, milk chocolate and sweet biscuits during the experimental period. At the 90th day of life, rats were submitted to either lipectomy (L) or sham surgery. After 7 or 30 days, RET, EPI, liver, brown adipose tissue (BAT), blood and carcass were obtained and analyzed. Seven days following surgery, liver lipogenesis rate and EPI relative weight were increased in L. After 30 days, L, RET and EPI presented increased lipogenesis, lipolysis and percentage of small area adipocytes. L rats also presented increased liver malic enzyme activity, BAT lipogenesis, and triacylglycerol and corticosterone serum levels. The partial removal of visceral fat pads affected the metabolism of high fat diet obese rats, which leads to excised tissue re-growth and possibly compensatory growth of non-excised depots at a later time.

Keywords Partial lipectomy · High fat diet · Obesity · Adipose tissue · Triacylglycerol deposition

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Introduction

Increased food intake and decreased energy expenditure have contributed to the increasing prevalence of obesity observed worldwide. In the USA and United Kingdom, for example, the prevalence of obese adults has trebled during the last two decades [1, 2]. Obesity is considered as a disease, and it is often accompanied by abnormalities in carbohydrate and lipid metabolism [3, 4].

A high-fat intake is one of the key factors for the development of human obesity and has been addressed extensively in animal models. A useful model for experimental obesity has been represented by hyper-caloric diets, enriched with lipids and or carbohydrates [5, 6].

The white adipose tissue is the most important extra-hepatic site regulating the lipid metabolism. Tissue triacylglycerol deposition results from both in situ de novo fatty acid synthesis and fatty acid uptake from lipoproteins of dietary or hepatic origin, while mobilization results from lipolysis. The adipose tissue is also considered as an endocrine organ; white adipocytes secrete major factors involved with several physiological processes like energy balance, carbohydrate metabolism, vascular haemostasis and inflammation. These factors are increasingly considered to be directly linked to the pathologies associated with obesity, and their levels vary according to the level of fat mass in the body [3, 7, 8].

Lipectomy, namely the surgical removal of fat pads, is a means of challenging the hypothesized body fat regulatory system. Many studies have demonstrated compensatory increases of body fat in animals after lipectomy [9–15]. In the majority of studies that conducted lipectomy, the body fat content reduction was accomplished by removing whole fat pads or as much of it as possible. In the present study, the experimental model used consisted of partial removal of fat pads. In this way, the metabolic effects caused by this intervention, here denominated as partial lipectomy, are not completely understood.

The effects of partial lipectomy on the lipid metabolism of wistar rats fed a standard control diet have been demonstrated [16, 17]. In that experimental model, lipectomy did not modify carcass, retroperitoneal or epididymal white adipose tissue lipid content or lipogenesis rate. An experimental model of hypothalamic obesity, caused by the neonatal administration of monosodium glutamate, has also been studied [16]. It has been reported that the metabolic changes related to triacylglycerol deposition caused by partial lipectomy are much more dramatic in the hypothalamic obese rats, as compared to the changes observed in the standard diet fed rats. As the model of hypothalamic obesity is not related to hyperfagia [18] and can be induced even with animals fed a standard diet, we questioned whether lipectomyzed wistar rats chronically fed a high fat diet, which mimics a behavioral-related type of obesity, could also present altered triacylglycerol deposition. Thus, the aim of this study was to verify in male wistar rats chronically fed a diet enriched with palatable, inexpensive and energy dense ingredients, the effects of partial removal of white fat pads after 7 or 30 days of surgery. Parameters of the lipid metabolism related to triacylglycerol deposition were studied in the liver, retroperitoneal (RET) and epididymal (EPI) white adipose tissues, brown adipose tissue (BAT) and carcass.

Materials and methods

Animals

The Experimental Research Committee of the São Paulo Federal University approved all the procedures for the care of the animals used in this study. Seventy-two recently weaned (28-day-old) male wistar rats were obtained from CEDEME—Centro de Desenvolvimento de Modelos Experimentais—Sao Paulo Federal University, and kept at the animal house of the Department of Physiology, under controlled conditions of light (light:dark periods of 12 h) and temperature (22 ± 2°C). The animals had free access to water and the high fat diet during the whole experimental period, and were maintained in individual plastic cages.

High fat diet

A standard commercial rat chow (Nuvilab CR-1; Paraná, Brazil) was ground and enriched with dry-roasted peanuts without salt (Hikari, Brazil), milk chocolate (dairy milk; Nestlé, Brazil), and plain sweet biscuits (Maizena® Tostines Nestlé) in the proportion 3:2:2:1 w/w. This diet has been proved to cause obesity in male wistar rats [19].

All the ingredients were ground and mixed. Butylated hydroxytoluene (0.01%) was added as antioxidant. Lukewarm water was added to obtain the consistency necessary to allow homogenization of the mixture. After homogenization, the mixture was passed through a milling machine for the production of pellets, which were subsequently dried in a forced ventilation oven at 60°C for 24 h. The diet was stored in plastic containers at −20°C and offered to the animals in standardized portions, replaced every second day and the leftovers weighed and discharged.

Samples were sent to the Laboratory of Bromatology and Microbiology of Foods of the Department of Physiology, Sao Paulo Federal University, for the analysis of macronutrients. The diet caloric density was determined with an adiabatic calorimeter (IKA-C400). The total energy value of the high fat diet was 20.2 kJ/100 g, and consisted of lipids 24.0%, protein 20.0%, mineral residues 5.0%, carbohydrates 41%, and fiber 5.9%.

Surgical procedure

On the 90th day of life, the animals were randomly sorted into two groups: 36 lipectomyzed (L) and 36 sham operated (S). All animals had similar body weight before the surgery (data not shown) and were fasted overnight.

Surgery was performed under combined ketamine and xylazine (66.6 and 13.3 mg/kg, respectively) intraperitoneal anaesthesia. After being anesthetized, the animals' abdomens were shaved throughout, washed with lukewarm water and soap, and disinfected by 70% alcohol solution followed by thiomersal solution (49% mercury by weight). A 30-mm incision along the ventral midline was made on the skin and subcutaneous tissue, exposing the *linea alba* and muscular wall. A second incision was made into the abdominal cavity along the *linea alba*, exposing the viscera. The intestines were retracted to the side to allow access to the RET fat pad. Approximately 0.75 g of each RET was removed. The EPI fat pad was reached from the abdominal cavity, gently pulling this depot through the inguinal canal. Care was taken to not twist the testicle and related vases. Approximately 1.25 g of the distal portion of each EPI was removed. The amount of fat removed represents on average half of the volume of that fat pad, determined by visual inspection prior to incision, as standardized previously [16]. In the sham surgery, an identical

incision was made and the entire procedure was identical but the fat pads were left intact. During all interventions, care was taken to preserve all adjacent tissues, and any bleeding was stopped with electrocauterization. The abdominal wall was sutured with absorbable thread (MonoPlus®; BBraun, Brazil), and skin with sterile cotton thread. Twenty-four hours after surgery, all animals received prophylactically a single injection of 50,000 UI benzathine benzylpenicillin (300,000 UI/mL; Eurofarma Laboratories, Brazil) intramuscularly (*biceps femoris*).

Samples

The animals were kept in the animal house after surgery receiving the high fat diet ad libitum. Body weight gain and food intake was recorded. Half the animals from each group (S and L) were sacrificed 7 days after surgery and the other half sacrificed 30 days after. The animals were sacrificed by decapitation without sedation. All animals were sacrificed early in the morning to avoid chronobiological variations. Animals were acclimatized in a quiet room next to the laboratory under subdued light for 1 h, and taken to the laboratory individually to minimize stress.

Trunk blood was collected, immediately centrifuged at 4°C and frozen at −70°C. The abdominal cavity was inspected before sampling, and if it presented any abnormal occurrence, like visceral adhesions, for example, the animal was not used. Liver, BAT, RET and EPI were dissected and weighed. The carcass was eviscerated and stored at −20°C.

Measurement of lipid content

Lipid content was measured as described by Stansbie et al. [20]. Briefly, the carcass (excluding gastrointestinal tract) was autoclaved at 120°C for 90 min and homogenized with the double weight of water. Triplicate aliquots of this homogenate (1 g) and samples from other tissues (liver, RET, EPI and BAT) were weighed and placed in hermetically capped tubes with 3.0 mL of 30% KOH and kept for 15 min at 70°C. After that, 3.0 mL of 100% ethanol were added and tubes were kept at 70°C for additional 2 h. After cooling, 2.0 mL of 12 N H_2SO_4 were added, and the solution was washed three times, under constant shaking for 10 min, with petroleum ether for lipid extraction. The obtained solution was transferred to previously weighed tubes, and left at room temperature until complete evaporation of petroleum ether, and weighed again. Results are expressed as g lipid/g tissue.

Measurement of lipogenesis rate

Rats received an intraperitoneal administration of 0.111 GBq ^3H_2O and were left in their respective cages with no access to food for 1 h, being immediately sacrificed afterwards. The lipid residues of the samples used for lipid content measurement (described above) were dissolved in scintillator liquid and the radioactivity was assayed. Radioactivity of 20-μL serum samples was quantified for the determination of specific activity. Tissue lipogenesis rate was expressed as μmol of ^3H_2O incorporated into lipid/h g of tissue, according to Robinson and Williamson [21].

In vitro determination of lipolysis rate

Tissue fragments (approximately 100 mg) were used for the in vitro determination of glycerol release, an index of lipolysis rate [22]. Samples of RET and EPI were removed immediately after decapitation, weighed, minced into small fragments, placed in hermetically capped tubes and incubated for 1 h at 37°C under continuous shaking in Ca^{++}-free Krebs–Henseleit solution containing 1% (w/v) fatty acid-free bovine albumin, pH 7.4 in atmosphere gassed with 95% O_2 5% CO_2 mixture (White Martins Praxair, Brazil), as standardized by Gaiva et al. [23].

Lipolysis was interrupted by quickly cooling down the tubes in a melting ice bath. Tissue fragments were removed and the glycerol content was determined enzymatically by the method of Eggstein and Kreutz [24]. For each donor rat, two sets of incubation vials were prepared in duplicate, one with and the other without the addition of 100 mM noradrenaline (Arterenol; Sigma Chemical, USA) to the incubation medium. The results were expressed as μmol glycerol released/h per 100 mg tissue.

Adipocytes area determination

Triplicate adipose tissue samples (50 mg) of RET and EPI were fixed in 0.2 M collidine (2,4,6-trimethylpyridine) buffer, pH 7.4, containing 2% (w/v) OsO_4 (Sigma Chemical) at 37°C for 48 h, as described by Hirsch and Gallian [25] and standardized by Gaiva [23]. The samples were rinsed with warmed 0.9% saline solution, placed in glass slides and left to dry at room temperature in a dust-free cabinet. Three hundred and fifty cells from each tissue from each donor rat were individually photographed with a digital camera (Kodak) coupled to a microscope (Nikon). The cells were individually measured as surface area using an image analysis software (Image Tool 3.00; UTHSCSA, TX, USA). The average cell size values are expressed in μm^2.

Subsequently, the cells were segregated according to their size in five ranges of area (smaller than 2×10^3 μm^2; 2–6 $\times 10^3$; 6–10 $\times 10^3$; 10–14 $\times 10^3$ and larger than 14 $\times 10^3$ μm^2) and the number of cells in each range was counted. This result is presented as percentage distribution of cells by range size.

Biochemical and hormonal serum analysis

The serum concentrations of insulin and leptin were determined by radioimmunoassay, with a Coat-A-Count MedLab kit for human insulin (DPC, CA, USA), and a kit from Linco (Linco Research, USA) for rat leptin. Plasma corticosterone was determined according to Guillemin et al. [26]. Serum triglyceride, glucose, total cholesterol and HDL-cholesterol levels were determined spectrophotometrically (Labtest Diagnostics, Brazil).

Malic enzyme activity

Malic enzyme, a cytosolic lipogenic enzyme that responds to dietary and hormonal factors [27], catalyzes the reaction of malate into pyruvate through the reduction of NADP+. Malic enzyme activity was measured according to the method established by Newsholme and Williams [28] and standardized by Gaiva [23]. Briefly, samples of liver were quickly removed immediately after decapitation, frozen in liquid nitrogen and stored at −70°C. Samples of 0.5 g were homogenized with chilled extraction buffer (20 mM Tris–HCl, 1 mM $MgCl_2$, 100 mM KCl, 250 mM saccharose, 3 mM beta mercaptoethanol, pH 7.6) in the proportion of 1:5 (w/v), using a electric homogenizer (Polytron Ultra-Turrax). The tissue homogenate was transferred into a 1.5-mL sterile universal tube, and centrifuged at 12,000*g* for 2 min at 4°C for clarification. Care was taken to avoid the supernatant (fat) and the pellet (cell sediments). Aliquots of 50 µL were added into 850 µL of assay mix (50 mM Tris–HCl, 100 mM KCl, 1% v/v triton X100, 0,2 mM NADP, 20 mM $MmCl_2$), followed by the addition of 50 µL of 40 mM malate. In the control tube, 50 µL of distilled water was added instead. Enzyme activity was measured in plastic cuvettes for 10 min in a multi-cuvette spectrophotometer (Beckman, USA) at 340 nm at 37°C. Values are expressed as µmol/min/100 mg liver. All the chemicals used were obtained from Sigma Chemical.

Statistical analysis

All results are presented as means ± standard error of the mean (SE). The statistically significant differences among the four groups studied (S7, L7, S30, L30) were assessed by two-way ANOVA followed by Duncan test. Differences were considered as significant where $p < 0.05$.

Results

The body weight gain was lower in L7 as compared to S7, and similar between S30 and L30 rats. The same pattern was observed in the energy intake. The carcass lipid content and lipogenesis rate were similar between the four groups (Table 1).

The liver relative weight and lipid content were not altered by partial lipectomy. The lipogenesis rate was increased in L7 as compared to S7 and the malic enzyme activity was higher in L30 as compared to S30 (Table 1). The BAT relative weight and lipid content were not modified by lipectomy, but an important increase in the lipogenesis rate was found in L30 as compared to S30 (Table 1).

Concerning the effects of lipectomy on white adipose tissues, the total relative weight (amount removed at surgery added to the amount obtained at sacrifice) was increased in EPI after 7 days. After 30 days, this difference subsided and no significant differences were found in RET. The lipogenesis rate was increased by partial lipectomy after 30 days of surgery in both RET and EPI. The RET basal lipolysis rate was increased in L30, while the noradrenaline-stimulated lipolysis rate was increased in L7. The EPI noradrenaline-stimulated lipolysis rate was increased in both L7 and L30 as compared to their respective sham groups (Table 1).

The adipocytes area analysis revealed that partial lipectomy did not change the adipocytes mean area (Table 1) nor the distribution of adipocytes within area ranges after 7 days of surgery (Fig. 1a, b). However, after 30 days, lipectomy reduced the adipocytes mean area in S30 (Table 1), decreased the percentage of adipocytes with area larger than 14×10^3 µm^2 and increased the percentage of adipocytes with area smaller than 2×10^3 µm^2 (Fig 1c). In the EPI, partial lipectomy did not modify the adipocytes mean area (Table 1), but increased the percentage of adipocytes with area smaller than 2×10^3 µm^2 (Fig. 1d).

Partial lipectomy caused elevation in serum concentrations of triglyceride and corticosterone after 30 days of surgery. No alterations were found in animals sacrificed after 7 days (Table 2).

Discussion

The prevalence of obesity is increasing worldwide, and behavioral aspects play major causal roles. Among many influences, the percentage of energy consumed as fat in the diet is decisive. In this study, we have used an experimental model of obesity caused by the ingestion of high fat food, which causes obesity in rodents [5, 6, 29] and humans [30, 31]. This diet utilizes palatable, inexpensive and densely caloric ingredients, resembling a type of high calorie high fat diet for humans, and it has been proved to cause obesity in male wistar rats [19].

The effects of partial lipectomy on the metabolism of rats receiving a control diet have already been

Table 1 Lipid metabolism parameters of high fat diet induced obese rats, sham operated (S) or lipectomyzed (L), sacrificed 7 or 30 days after surgery

	S7	L7	S30	L30
Body weight gain (%)	5.08 ± 0.83 a	0.59 ± 0.72 b	19.90 ± 1.27 c	17.64 ± 2.29 c
Energy intake (kJ)	3194 ± 179.9 a	2577 ± 152.9 b	3659 ± 65.3 c	3698 ± 83.5 c
Carcass				
Relative lipid content	3.2 ± 0.42 a	3.0 ± 0.30 a	3.6 ± 0.22 a	3.0 ± 0.36 a
Lipogenesis rate	0.91 ± 0.05 a	0.92 ± 0.11 a	0.78 ± 0.05 a	1.07 ± 0.22 a
Liver				
Relative weight	3.42 ± 0.07 a	3.47 ± 0.08 a	2.81 ± 0.09 b	3.06 ± 0.13 b
Relative lipid content	3.55 ± 0.15 a	3.76 ± 0.19 a	4.67 ± 0.24 b	4.19 ± 0.26 ab
Lipogenesis rate	7.58 ± 0.75 a	19.78 ± 2.18 b	6.76 ± 1.29 a	7.36 ± 0.79 a
Malic enzyme	0.25 ± 0.02 ab	0.27 ± 0.02 ab	0.15 ± 0.01 a	0.31 ± 0.02 b
BAT				
Relative weight	0.08 ± 0.006 a	0.09 ± 0.003 a	0.11 ± 0.004 b	0.11 ± 0.006 b
Relative lipid content	47.14 ± 0.99 ab	51.75 ± 2.37 a	43.92 ± 3.39 b	48.40 ± 1.57 ab
Lipogenesis rate	7.28 ± 1.10 a	6.69 ± 0.53 a	8.32 ± 1.09 a	12.08 ± 0.75 b
RET				
Total relative weight	1.09 ± 0.05 a	1.31 ± 0.20 a	1.34 ± 0.25 a	1.32 ± 0.16 a
Lipogenesis rate	4.42 ± 0.65 a	5.09 ± 1.34 a	1.18 ± 0.12 b	2.36 ± 0.45 c
Lipolysis rate	0.09 ± 0.02 a	0.18 ± 0.08 ab	0.17 ± 0.02 a	0.32 ± 0.04 b
Nor-lipolysis rate	0.12 ± 0.05 a	0.29 ± 0.14 b	0.29 ± 0.07 b	0.32 ± 0.05 b
Adipocyte area	8,678 ± 2,279 a	7,764 ± 1,011 a	13,005 ± 1,775 b	6,694 ± 726 a
EPI				
Total relative weight	1.01 ± 0.08 a	1.40 ± 0.13 b	1.22 ± 0.16 ab	1.27 ± 0.09 ab
Lipogenesis rate	4.42 ± 0.95 ac	5.52 ± 1.45 a	1.09 ± 0.08 b	2.55 ± 0.36 c
Lipolysis rate	0.04 ± 0.01 a	0.10 ± 0.03 ab	0.19 ± 0.02 b	0.20 ± 0.04 b
Nor-lipolysis rate	0.06 ± 0.02 a	0.14 ± 0.04 b	0.15 ± 0.02 b	0.32 ± 0.05 c
Adipocyte area	5,767 ± 605 a	6,777 ± 672 a	8,102 ± 614 a	6,972 ± 904 a

Body weight gain (% at surgery) and energy intake (total kJ at the 1st or 4th week after surgery); carcass relative lipid content (g/100 g tissue) and lipogenesis rate (μmol of $^{3}H_2O$ incorporated into lipid/g of tissue); liver relative weight (g/100 g body weight), relative lipid content, lipogenesis rate and malic enzyme activity (μmol/min/100 mg liver); BAT relative weight, relative lipid content and lipogenesis rate; RET and EPI total relative weight (removed at surgery plus at sacrifice), lipogenesis rate, lipolysis rate (μmol of glycerol release/100 mg of adipose tissue), noradrenalin-stimulated lipolysis rate, adipocyte mean area (μm^2)

All results are presented as means ± standard error of the mean. The number of animals varied from 6 to 10. Values with different letters are significantly different from one another at $p < 0.05$

demonstrated [16, 17]. An experimental model of hypothalamic obesity, caused by the neonatal administration of monosodium glutamate [18, 32], has also been studied [16]. It has been verified that the metabolic alterations caused by partial lipectomy were more intense in the hypothalamic obese rats, in comparison to those found in the normal ones. Among other effects, increased carcass and white adipose tissue lipogenesis rates and also increased lipolysis rate were found in obese animals, but not in control diet fed ones. It is known that the model of hypothalamic obesity is accompanied by major neuroendocrine alterations [32], and having faced the dramatic changes in the metabolism of hypothalamic obesity wistar rats caused by partial lipectomy, we questioned whether the same effects can happen in a model of obesity caused by the ingestion of a high fat diet, which represents an evident behavioral approach to challenge the hypothesized body fat regulatory system.

Seven days after surgery, both body weight gain and energy intake were reduced in L animals as compared to S. Thirty days after surgery, no significant differences were found (Table 1). The recovery of body weight after lipectomy has been reported [33]. In agreement with these observations, lipectomy has been shown not to stimulate food intake, and it is suggested to reduce energy expenditure, in order to provide calories for the replacement of removed fat pads [12, 34].

In the liver, the relative weight and lipid content were similar between S and L. However, the lipogenesis rate was increased in L7 as compared to S7 and the malic enzyme

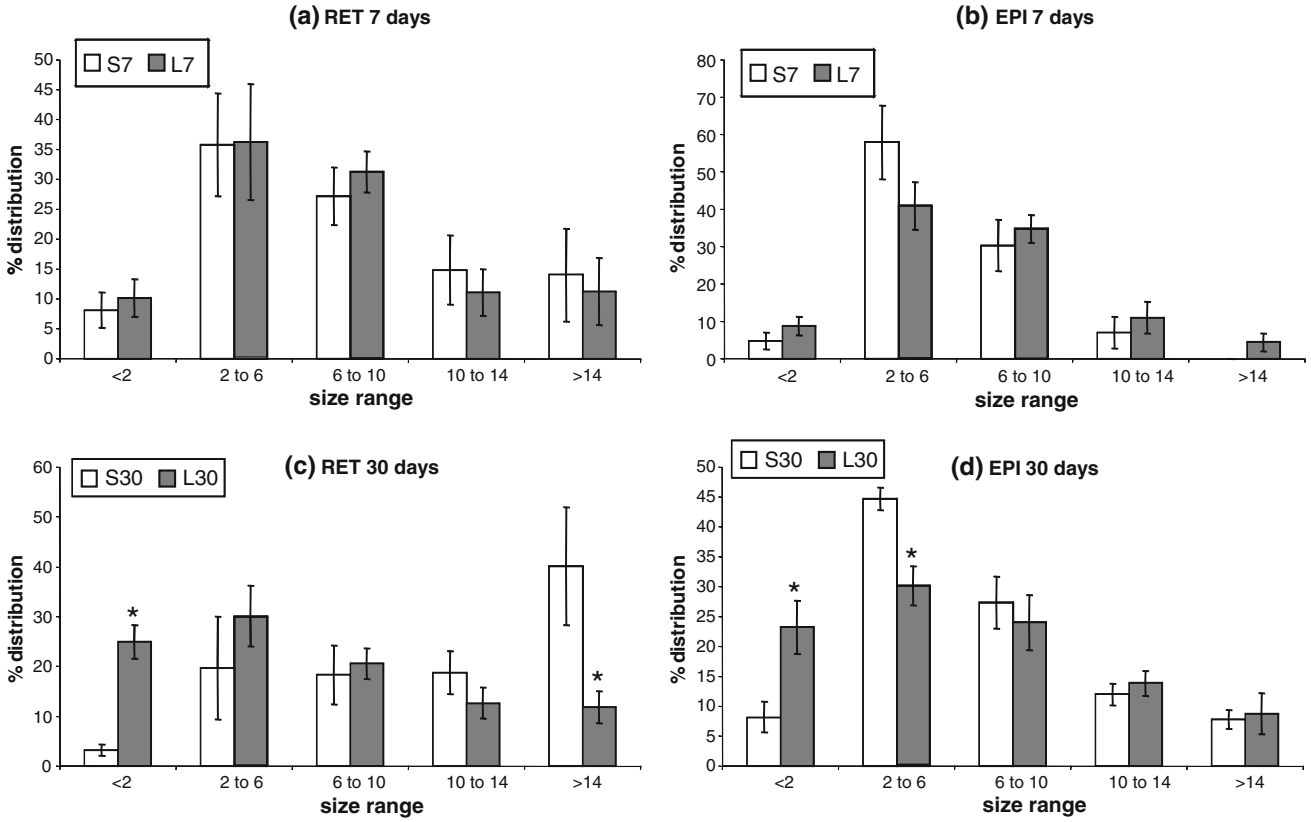

Fig. 1 Distribution of adipocytes, as percentage distribution, according to their size within area ranges ($\times 10^3$ μm^2), of retroperitoneal (*RET*) and epididymal (*EPI*) white adipose tissues of high fat diet induced obese rats, sham operated (*S*) or lipectomyzed (*L*), sacrificed 7 or 30 days after surgery. The number of animals ranged from 6 to 8. *Statistically significant difference between S and L at $p < 0.05$

Table 2 Serum glucose (mg/dL), insulin (μg/mL), leptin (ng/mL), corticosterone (μg/dL), triglyceride (mg/dL), total cholesterol (mg/dL) and HDL-cholesterol (mg/dL) concentrations of high fat diet induced obese rats, sham operated (S) or lipectomyzed (L), sacrificed at 7 or 30 days after surgery

	S7	L7	S30	L30
Glucose	116.4 ± 5.0 ab	113.1 ± 4.5 a	126.2 ± 3.2 b	116.8 ± 3.1 ab
Insulin	18.6 ± 1.6 a	22.9 ± 1.6 a	23.4 ± 1.97 a	23.9 ± 3.12 a
Leptin	2.83 ± 0.3 a	3.02 ± 0.40 a	3.26 ± 0.36 a	3.13 ± 0.25 a
Corticosterone	9.65 ± 1.59 a	9.72 ± 1.09 a	16.46 ± 2.94 a	25.09 ± 3.71 b
Triglycerides	100.7 ± 12.6 a	110.2 ± 6.4 a	111.8 ± 8.7 a	147.4 ± 7.4 b
Total cholesterol	116.6 ± 7.1 ab	120.8 ± 3.7 a	100.4 ± 6.2 b	104.4 ± 5.7 ab
HDL-cholesterol	50.9 ± 2.6 a	48.9 ± 2.7 a	50.7 ± 1.9 a	44.0 ± 2.3 a

All results are presented as means ± standard error of the mean. The number of animals ranged from 10 to 14. Values with different letters are significantly different from one another at $p < 0.05$

activity was higher in L30 as compared to S30 (Table 1). These results suggested that partial lipectomy increases the liver in situ de novo fatty acid synthesis, and the excess of lipid is most likely being exported.

Lipoprotein lipase enzyme (LPL) is responsible for the hydrolysis of circulating triglycerides from plasma. Brito et al. [35] and West et al. [36] have reported similar basal LPL activities between RET and EPI. Recuperation of RET and EPI relative weight has been observed after 30 days of surgery in our study, and also an effective re-growth in EPI after 7 days.

In this study, lipectomy increased BAT lipogenesis rate while the relative weight and lipid content were unchanged after 30 days of surgery (Table 1). Strack [37] verified that corticosterone administration in rats increased the lipid content of BAT, and hypercorticosteronemia has actually been observed in our study. It is possible to suggest that the accumulation of lipids in the BAT of lipectomyzed rats is a

process still in evolution, and perhaps the relative weight may increase in these animals at a later stage. This suggestion is also based on previous studies regarding thermogenesis. Shi et al. [15] reported a marked decrease in interscapular BAT norepinephrine turnover 3 weeks post lipectomy in Siberian hamsters. These authors suggest that this reduction is associated with decreased BAT thermogenesis, which diverts the energy losses as heat to rebuilt adipose tissue mass. Indeed, Hausman et al. [14] verified in male wistar rats a small but significant decrease in heat production 4 weeks post-lipectomy.

Lipectomy caused a dramatic reduction in the adipocytes average area of RET after 30 days of surgery. This reduction is due to an increase in the number of small cells within the total of analyzed cells during that period. No differences were found after 7 days. Similar results were found previously in a model of hypothalamic obesity in wistar rats, but not in control diet ones [16]. This observation of proliferation of new adipocytes in both hypothalamic and high fat diet induced models of obesity, but not in control animals, may lead us to suggest that there is a potential mechanism of body fat regulatory system involved with adiposity recovery after long-term lipectomy.

Some studies suggest that fat pads differ in their capacity to generate new adipocytes. According to Faust et al. [33], RET, unlike EPI, can increase adipocytes number by many-fold in response to the consumption of high fat diets. Other studies [38, 39] found that precursor cells in RET have larger replication rate and capacity to differentiate into mature adipocytes than those in EPI. Correspondingly, in our study, both RET and EPI presented increased lipogenesis rate and higher percentage of small area adipocytes in lipectomyzed rats after 30 days. The occurrence of adipocytes smaller than 2×10^3 μm^2 was approximately eight times higher in the RET, while the increase in the EPI was only close to three times higher after 30 days, but no differences were found after 7 days.

Increased serum corticosterone levels were observed in lipectomyzed rats in this study after 30 days of surgery. No effects have been observed after 7 days. It has been demonstrated that glucocorticoids stimulate the activity of peroxisome proliferator-activated receptor gamma [40, 41], an important transcription factor that stimulates adipocyte differentiation [42] and lipogenesis [43]. Despite the increased corticosterone serum concentrations, glucose serum levels in L30 tended to be nearly 10% lower than those of S30. It was previously demonstrated that small adipocytes take up much more glucose and have higher conversion of glucose into fatty acid than large adipocytes [44]. We believe that these new, small adipocytes are keeping the glucose levels lower, even under the hyperglycaemic effect of glucocorticoids.

Insulin is a hormone that, among many other functions, stimulates the activity of lipogenic enzymes [45, 46]. Although we did not observe alterations in serum insulin concentration, it is justifiable to speculate that lipectomy increases the sensitivity of RET and EPI insulin receptors, promoting a more intense signal for lipogenesis in those tissues. It was recently reported that lipectomyzed male wistar rats receiving a high fat diet present increased insulin sensitivity as compared to a control group, as assessed by homeostasis model of assessment index (HOMA) [47].

In this study, the RET basal lipolysis rate was increased in L30 and the noradrenaline-stimulated lipolysis rate was increased in L7. In the case of EPI, only the noradrenaline-stimulated lipolysis rate was increased in both L7 and L30 (Table 1). One of the several mechanisms that controls the accumulation and mobilization of lipids by the adipose tissue is through its innervations by the sympathetic nervous system [48]. One undeniable criticism to lipectomy is the fact that it is an invasive procedure, which causes great stress to the organism. Furthermore, Michel and Cabanac [49] suggested that lipectomy is more traumatic than the sham surgery, thus elevating the concentration of corticotrophin release hormone. The sympathetic activation in both RET and EPI may explain the increased triacylglycerol mobilization rate found in lipectomyzed rats. This could also help to explain the initial reduction in body weight and energy intake after 7 days of surgery but tendency to normalization after 30 days, in line with the findings of much higher lipolysis rate between S7 versus L7 than S30 versus L30.

Conversely, insulin is an important lipolysis inhibitor, decreasing the hormone sensitive lipase enzyme (HSL) activity and consequently triglyceride hydrolysis [50]. Based on the findings of new adipocytes within the excised tissues, lowered glycaemia and increased in situ lipogenesis rate found in lipectomyzed rats after 30 days of surgery, actions directly stimulated by insulin, confronted by the increased lipolysis rate found in these animals probably due to stress, we speculate that the lipid turnover in these fat pads tends to anabolism, favoring the storage of lipids in the long run.

Plasma triglyceride levels were increased in lipectomyzed rats after 30 days of surgery, but no differences were found after 7 days. These metabolic alterations could well be related to the increased corticosterone levels, once it had been described [51, 52] that dexamethasone increases the liver synthesis and secretion of triglycerides.

In a previous study about the effects of partial lipectomy on tissue triacylglycerol deposition of animals receiving a control diet and in a model of hypothalamic obesity, dramatic alterations were found in the obese ones [16]. In the present study, which makes use of a model of obesity

caused by the ingestion of a palatable, inexpensive, high fat diet, aiming to correlate with a typical model of human obesity, major alterations were also found. The alterations observed extended to organs such as liver and brown adipose tissue. Based on these findings and on the literature, one can suggest an important effect of the adiposity levels on the metabolic alterations trigged by partial lipectomy. According to the hypothesized body fat regulatory system, these alterations will most likely lead to the recovery of the lost adiposity.

In conclusion, the present study showed that, in high fat diet obese wistar rats, the partial removal of epididymal and retroperitoneal fat depots caused an increase in adipocyte differentiation and lipogenesis rate after 30 days of surgery, alterations to which tissue re-growth is related. Also, the increased corticosterone levels found in lipectomyzed animals sacrificed 30 days after surgery are likely to be associated with adipocyte differentiation and elevation of serum triglycerides observed. These results could contribute to partially explain how the body fat regulatory system works to influence parameters of the lipid metabolism related to triacylglycerol deposition, aiming at the re-growth of excised fat depots in diet induced obese rats.

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Conflict of interest The authors of this research disclose any potential conflict of interest.

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