Physiol. Res. 54: 57-65, 2005

Lipid Metabolism of Monosodium Glutamate Obese Rats after Partial Removal of Adipose Tissue

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Received September 4, 2003
Accepted February 19, 2004

Summary

We analyzed the effects of partial fat pad removal on retroperitoneal and epididymal fat depots and carcass metabolism of control (C) and MSG-obese (M) rats. Three-month-old C and M male Wistar rats were submitted to either partial surgical excision of epididymal and retroperitoneal fat tissue (lipectomy, L) or sham surgery (S) and studied after 7 or 30 days. Retroperitoneal and epididymal tissue re-growth after lipectomy was not observed, as indicated by the low pads weight of the L groups. The lipolysis rate was stimulated in LC7 and LM7, probably due to surgical stress and low insulin levels. In LM7, but not in LC7, *in vivo* lipogenesis rate increased in retroperitoneal and epididymal fat tissue, as did the diet-derived lipid accumulation in epididymal fat tissue. Although these local increases were no longer present in LM30, this group showed a large increase in the percentage of small area adipocytes in both pads as well as increased carcass lipogenesis rate. The present data showed that the partial removal of fat depots affected the metabolism of control and MSG-obese rats differently. In the obese animals only, it stimulated both local and carcass lipogenesis rate as well as adipocyte differentiation, i.e. responses likely to favor excised tissue re-growth and/or compensatory growth of non-excised depots.

Key words

Lipectomy • MSG rats • Adipocyte area • Lipogenesis rate • Lipolysis rate

Introduction

Adipose tissue plays a role in energy storage and insulation from environmental temperature and trauma and also functions as an endocrine organ. In the long run, white fat mass reflects the net balance between energy expenditure and energy intake. The adipocytes store energy in the form of triacylglycerols. Fat storage occurs both by the direct uptake of circulating lipoprotein triacylglycerols, which are hydrolyzed by lipoprotein lipase to non-esterified free fatty acids, and also by local lipogenic pathways, i.e. the “de novo” synthesis from glucose and other precursors (Pénicaud *et al.* 2000). On the other hand, this tissue can release both free fatty acids and glycerol, providing circulating substrates for other tissues, according to their energy needs.

In addition to serving as an energy store site, adipocytes secrete hormones (e.g. leptin, adiponectin, resistin) that regulate energy balance, metabolism, and neuroendocrine response to altered nutrition (Arner 2003). The hormone leptin stimulates energy expenditure and inhibits food intake by acting *via* hypothalamic

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receptors, and also has peripheral effects, such as inhibition of liver and white adipose tissue lipogenesis rate and lipolysis stimulation in adipocytes (Siegrist-Kaiser *et al.* 1997, Bryson *et al.* 1999). The expression of leptin in adipocytes and its plasma concentration are both positively correlated with total adiposity.

The neonatal administration of monosodium glutamate (MSG) to rodents destroys 80-90 % of the arcuate nuclei neurons and damages other central structures, resulting in several neuroendocrine and metabolic abnormalities (Dolnikoff *et al.* 2001). As adults, these animals develop an obesity syndrome characterized by excess fat deposition and reduced lean body mass, which occurs, in the absence of hyperphagia (Dawson *et al.* 1989), as a function of reduced metabolic rate (Poon and Cameron 1978). Low plasma levels of GH and IGF-1 (Kubota *et al.* 1994, Pinterová *et al.* 2001) also contribute to the stunting and decreased lean body mass.

Additionally, decreased adipose tissue lipolytic activity (Dolnikoff *et al.* 2001), increased adipocyte and liver lipogenesis rate (Macho *et al.* 2000, Nascimento Curi *et al.* 1991) and lipoprotein lipase activity (Nascimento Curi *et al.* 1991) have been demonstrated in MSG-obese animals. Hyperinsulinemia (Sartin *et al.* 1985), insulin resistance (Machado *et al.* 1994), adipocyte hypertrophy and decreased cellularity (Ochi *et al.* 1988, Zorad *et al.* 1997) as well as hyperleptinemia (Morris *et al.* 1998) and hypercorticosteronemia (Ribeiro *et al.* 1997, Macho *et al.* 1999) have also been described in this obesity model.

It has been shown that, after partial lipectomy, or surgical removal of body fat, there is a compensatory lipid deposition (Weber *et al.* 2000, Harris *et al.* 2002), which occurs in a fat pad specific manner (Mauer *et al.* 2001). However, the metabolic reactions leading to this response are not completely understood. The aim of this study was thus to analyze retroperitoneal and epididymal fat as well as whole body lipid metabolism, 7 or 30 days after the partial removal of these fat depots, in control or MSG-obese rats.

Methods

Animals

The Experimental Research Committee of the São Paulo Federal University approved the following experimental procedures.

Male Wistar pups received a subcutaneous injection of 4.0 g/kg monosodium glutamate (M group) or hyperosmotic saline (1.25 g/kg, controls, C) every 2nd day, for the first ten days of life. The pups were weaned at 21 days of age and had free access to rat commercial chow and water during the whole experimental period. They were maintained in a room at 23±1 ºC with lights on from 07.00 to 19.00 h. On the 90th day of life, each group (C and M) was divided into lipectomized groups (LC and LM) and sham-lipectomized groups (SC and SM).

The animals were killed by decapitation 7 or 30 days after the surgery for measurement of adipocyte lipolysis rate and adipocyte area; ^{14}C-triolein uptake, lipogenesis rate in retroperitoneal (RET) and epididymal (EPI) white adipose depots, and serum triglycerides, insulin, and leptin concentrations.

Surgical and experimental procedures

Surgery was performed under ketamine and xylazine (66.6 and 13.3 mg/kg, respectively) intraperitoneal anesthesia. A 20-mm linea alba incision was made, allowing the partial removal of the epididymal (EPI) and retroperitoneal (RET) fat pads. Approximately 1.2 g of RET and 2.0 g of EPI from LC and 1.6 g of RET and 2.5 g of EPI from LM were removed. In the sham surgery, an identical incision was made but the fat pads were left intact (groups SC and SM). Body weight and the food intake were measured weekly during the whole experimental period.

Measurements of the lipogenesis rate and lipid content

Rats were killed by decapitation, 1 h after the intraperitoneal administration of 0.111 GBq $^{3}H_2O$. Samples of RET, EPI, and the carcass were saponified and the fatty acids extracted by the method of Stansbie *et al.* (1976). Tissue lipogenesis rate was expressed as µmol of $^{3}H_2O$ incorporated into lipid/h. g of the tissue (Robinson and Williamson 1978). Lipid content was measured as described by Oller do Nascimento and Williamson (1988) and expressed as mg of lipid/100 mg of tissue.

Measurement of ^{14}C-lipid absorption from intestine and accumulation in RET and EPI

Seven or 30 days after surgery, the rats received an intragastric load of [1-^{14}C]triolein (about 0.5 g; 0.3 mCi per rat). After 4 h, the rats were killed by decapitation. The whole intestinal tract and 1 g samples of RET and EPI were withdrawn and homogenized. The lipids were saponified and extracted by the method of

Stansbie *et al.* (1976).

The extracted fatty acids were dissolved in 5 ml of scintillation liquid for determination of the ^{14}C-labelled lipid accumulated in the tissues and the amount of ^{14}C-labelled triolein remaining in the intestinal tract. The absorption of ^{14}C-triolein was determined by subtracting the radioactivity remaining in the intestinal tract from the amount administered.

Determination of lipolysis rate "in vitro"

Tissue fragments (about 100 mg) of RET and EPI were used for "*in vitro*" determination of glycerol release, an index of lipolytic rate (Arner and Engfeldt 1987). The samples were minced into small fragments and incubated for 1 h at 37 °C under continuous shaking in Ca^{2+}-free Krebs-Henseleit solution containing 2 % (w/v) bovine albumin (fraction V – essentially fatty acid-free), pH 7.4. Lipolysis was interrupted by placing the vials on ice. The tissue fragments were then removed and the glycerol content in the medium was determined enzymatically by the method of Eggstein and Kreutz (1966). The results were expressed as µmol of glycerol released/ h.100 mg of tissue.

Adipocytes area determination

Fragments (50 mg) of RET and EPI were fixed in 0.2 M-collidine buffer, pH 7.4, containing 2 % (w/v) OsO_4 at 37 ºC. After 48 h, they were washed with warmed saline (9 g NaCl/l), as described by Hirsch and Gallian (1968). The adipocyte area was measured using an image analysis software (Image Tool 2.00; UTHSCSA, TX, USA) and expressed as μm^2.

Leptin, insulin, and triglycerides

We used commercial radioimunoassay kits to determine the serum concentration of insulin (Coat-A-Count DPC MedLab, CA, USA) and leptin (Linco Research, Inc, MO, USA). Serum triglyceride levels were determined spectrophotometrically using a commercial diagnostic kit (Labtest Diagnostics SA, Brazil).

Statistical analysis

The results are expressed as mean ± S.E.M. Statistical inter-group comparisons were carried out by three-way ANOVA (C x M; S X L; 7 x 30). In the analysis of body weight and food intake we used two-way ANOVA. Significance was accepted at the $p<0.05$ level. Post hoc analysis (Duncan test) was performed on significant ANOVA results.

Results

The M groups had lower initial body weight than the C ones. Seven days after surgery the body weight varied similarly among the 4 groups. However, thirty days after surgery the percentage increment of body weight was higher in the M groups compared to the C groups. In both C and M rats, the partial fat pad removal decreased body weight gain. The relative food intake was lower in the LM7 than in the LC7 group (Table 1).

Table 1. Body weight and food intake of control (C) or MSG obese (M) animals, lipectomized (L) or sham operated (S) sacrificed at 7 or 30 days after surgery.

	SC	LC	SM	LM
	Body weight			
Initial	323.2 ± 7.43	314.4 ± 8.23	213.7 ± 6.87^{+}	226.0 ± 5.15^{+}
7 days (% of initial)	100.7 ± 0.97	91.38 ± 1.38*	101.30 ± 1.72	94.91 ± 1.26*
30 days (% of initial)	110.7 ± 0.99	105.5 ± 1.64*	119.10 ± 2.33 +	111.3 ± 1.87*$^{+}$
	Food intake (g/100 g body weight/24 h)			
7 days	8.34 ± 0.21	8.92 ± 0.23	8.18 ± 0.23	7.63 ± 0.42^{+}
30 days	8.56 ± 0.14	8.85 ± 0.25	8.30 ± 0.33	9.03 ± 0.40

The initial body weight was measured before surgery. Values are mean ± S.E.M. The number of rats used was 8. $^{+}$ Significantly different from C, * significantly different from S, $^{\#}$ significantly different from 7 days; $p<0.05$.

As shown in Table 2, the carcass weight of C groups was higher than that of M groups. However, lipid content was significantly lower in the C than in the M rats. The partial removal of fat pads did not modify these parameters, in either C or M groups. The carcass lipogenesis rate was higher in the M groups than in the C ones. It was also higher in LM30 as compared to both LM7 and SM30. The M30 groups, but not the M7 groups, accumulated more diet-derived lipid in the carcass than the respective C groups. The comparison between 7 and 30 days showed a decrease in diet-derived lipid accumulation in groups SC30, LC30 and SM30, but not in LM30.

The RET weight was higher in the M groups than in the C ones. In EPI, this was observed only in the sham-operated groups. The partial removal of RET and EPI decreased the weight of these fat pads in LM7, LM30, and LC30. In the group LC7, the weight decreased significantly in EPI only. The EPI lipid content of LC7, LM7, and LM30 was lower than that of the respective sham groups. In RET, similar results were observed in LC7, LM7, and LC30 (Table 3).

Table 2. Carcass weight (g), lipid content (g/100g), lipogenesis rate (µmol of ^3H_2O incorporated into lipids/g tissue.h) and accumulation of ^{14}C-lipid (% of absorbed lipid/ g of tissue or per total tissue) from control (C) or MSG obese (M) animals, lipectomized (L) or sham operated (S) sacrificed at 7 or 30 days after surgery.

	SC7	LC7	SM7	LM7	SC30	LC30	SM30	LM30
Carcass weight	218.4 ±4.45	210.8 ±4.67	179.3 ±3.16^{+}	165.8 ±4.84^{+}	246.3 ±9.30$^{\#}$	244.8 ±9.23$^{\#}$	206.7 ±3.65$^{\#+}$	205.7 ±2.19$^{\#+}$
Lipid content	1.46 ±0.14	1.23 ±0.08	6.28 ±0.18^{+}	5.53 ±0.29^{+}	2.04 ±0.08	1.84 ±0.22	8.47 ±0.41$^{\# +}$	8.47 ±0.45$^{\#+}$
Lipogenesis rate	0.48 ±0.02	0.65 ±0.02	0.86 ±0.07^{+}	0.71 ±0.03	0.49 ±0.02	0.58 ±0.02	0.91 ±0.10^{+}	1.22 ±0.19$^{\# +*}$
^{14}C-lipid/g g of tissue	0.058 ±0.004	0.065 ±0.008	0.069 ±0.005	0.080 ±0.005	0.030 ±0.001$^{\#}$	0.041 ±0.006$^{\#}$	0.051 ±0.003$^{\#+}$	0.065 ±0.003^{+}
^{14}C-lipid/ total tissue	11.45 ±0.82	12.36 ±1.30	10.36 ±0.84	10.12 ±0.86	7.48 ±0.49$^{\#}$	9.08 ±1.60$^{\#}$	9.80 ±0.65	12.24 ±0.73^{+}

Values are mean ± S.E.M. The number of rats used was 8. $^{+}$ Significantly different from C, * significantly different from S, $^{\#}$ significantly different from 7 days; $p<0.05$.

The *in vivo* lipogenesis rate of LM7 showed a 27-fold increase in EPI and a 7.5-fold increase in RET, in relation to SM7. This increase was no longer observed 30 days after surgery. In RET, the partial fat pad removal decreased the percentage of ^{14}C-lipid accumulation/g tissue in M7 and C30 groups as compared to the respective C7 groups. In EPI, this percentage increased in the LM7 group in relation to SM7.

The EPI lipolysis rate increased in LM7 as compared to SM7 and LC7 groups, and decreased 30 days after the surgery in all studied groups in relation to that observed 7 days after the surgery. The RET lipolysis rate was higher in the M7 groups than in the C7 groups. The same was seen in the comparison between LC7 and SC7 as well as LM7 and SM7. Thirty days after surgery, RET lipolysis rate was lower in LC30, SM30 and LM30 as compared to LC7, SM7 and LM7, respectively (Table 3).

The adipocyte area of the SM groups was higher than the respective SC groups in both the RET and EPI. The partial removal of fat pads reduced this parameter in EPI in both LM7 and LM30, as compared to the corresponding sham groups. In RET, this effect was observed only in LM30 vs. SM30. In both EPI and RET from M groups, the percentage of adipocytes with an area greater than 14 x 10^3 µm^2 was increased, in relation to the C groups. The partial removal of fat pads increased the percentage of adipocytes smaller than 6 x10^3 µm^2 in the EPI of the LC7, LM7, and LM30, and in the RET of LM30 (Table 4).

Serum triglyceride levels increased in LC7, LM7 and LC30 as compared to respective sham-operated groups, and in LC30 vs. LC7, LM30 vs. LM7 and SM30 vs. SM7. However, the serum triglyceride level was lower in LM30 than in SM30. The partial removal of fat pads decreased serum insulin levels in the LM7 group as

compared to SM7 group. The insulinemia was higher in LC30 and LM30 as compared to LC7 and LM7, respectively. Serum leptin level was increased in all M groups as compared to the C groups, and in LM30 and SM30 in relation to the LM7 and SM7 groups.

Table 3. Epididymal and retroperitoneal white adipose tissue weight (g), lipid content (g/100 g), lipogenesis rate (µmol of $^{3}H_2O$ incorporated into lipids/g tissue.h), accumulation of ^{14}C-lipid (% of absorbed lipid/g of tissue or per total of tissue) and lipolysis rate (µmol of glycerol released/ h.100 mg of tissue from control (C) or MSG obese (M) animals, lipectomized (L) or sham operated (S) sacrificed at 7 or 30 days after surgery.

	SC7	LC7	SM7	LM7	SC30	LC30	SM30	LM30
Epididymal white adipose tissue								
Weight	2.23 ±0.11	1.38 ±0.11*	4.04 ±0.23^{+}	1.37 ±0.09*	4.41 ±0.34$^{\#}$	2.47 ±0.14$^{\#}$*	5.48 ±0.45$^{\#+}$	2.07 ±0.27*
Lipid content	80.4 ±1.2	75.1 ±1.5*	86.2 ±0.6^{+}	80.3 ±1.0^{+}*	81.2 ±2.0	81.4 ±1.8$^{\#}$	92.9 ±0.4$^{\#+}$	89.2 ±0.9$^{\#+}$*
Lipogenesis rate	1.31 ±0.14	1.21 ±0.11	2.11 ±0.47	57.27 ±14.54^{+}*	2.69 ±0.58	1.39 ±0.08	1.94 ±0.19	1.62 ±0.22$^{\#}$
^{14}C-lipid /g of tissue	0.74 ±0.06	0.67 ±0.06	0.48 ±0.08^{+}	0.69 ±0.08*	0.34 ±0.03$^{\#}$	0.37 0.07$^{\#}$	0.38 ±0.04	0.41 ±0.02$^{\#}$
^{14}C-lipid / total tissue	1.59 ±0.22	0.70 ±0.06*	1.49 ±0.29	0.60 ±0.06*	1.04 ±0.20$^{\#}$	0.53 ±0.07*	1.78 ±0.13^{+}	0.86 ±0.07*
Lipolysis rate	0.05 ±0.007	0.06 ±0.006	0.07 ±0.011	0.12 ±0.012^{+}*	0.02 ±0.003$^{\#}$	0.03 ±0.005$^{\#}$	0.04 ±0.004$^{\#}$	0.04 ±0.004$^{\#}$
Retroperitoneal white adipose tissue								
Weight	0.94 ±0.14	0.70 ±0.12	3.92 ±0.23 $^{+}$	2.20 ±0.35^{+}*	3.30 ±0.37$^{\#}$	1.88 ±0.17$^{\#}$*	6.85 ±0.36$^{\# +}$	3.56 ±0.71$^{\#+}$*
Lipid content	78.71 ±3.68	60.70 ±3.85*	90.0 ±0.5^{+}	74.0 ±2.4^{+}*	80.2 ±1.5	72.0 ±4.4$^{\#}$*	93.7 ±0.8^{+}	90.6 ±1.3$^{\#}$
Lipogenesis rate	1.87 ±0.37	2.48 ±0.43	4.11 ±1.06	30.83 ±7.23^{+}*	1.71 ±0.19	1.76 ±0.17	1.83 ±0.14	1.59 ±0.34$^{\#}$
^{14}C-lipid /g of tissue	1.12 ±0.26	1.20 ±0.27	0.49 ±0.08^{+}	0.46 ±0.08^{+}	0.30 ±0.03$^{\#}$	0.27 ±0.04$^{\#}$	0.40 ±0.04	0.45 ±0.02
^{14}C-lipid / total tissue	1.19 ±0.28	0.45 ±0.03*	1.97 ±0.43^{+}	0.62 ±0.08*	0.62 ±0.11	0.41 ±0.11	2.24 ±0.26^{+}	1.65 ±0.12$^{\# +}$
Lipolysis rate	0.05 ±0.012	0.08 ±0.007*	0.13 ±0.019^{+}	0.16 ±0.015^{+}*	0.02 ±0.006	0.03 ±0.003$^{\#}$	0.03 ±0.002$^{\#}$	0.03 ±0.004$^{\#}$

Values are means ± SEM. The number of rats used in each determination was 8. $^{+}$ Significantly different from C, * significantly different from S, $^{\#}$ significantly different from 7 days; $p<0.05$.

Table 4. Area of adipocytes (μm^2) and percentual distribution by size of epididymal and retroperitoneal adipocytes from control (C) or MSG obese (M) rats, lipectomized (L) or sham operated (S) sacrificed at 7 or 30 days after surgery.

	SC7	LC7	SM7	LM7	SC30	LC30	SM30	LM30
Epididymal white adipose tissue								
Area x10³	5.69±0.60	4.80±0.47	13.7±2.01^{+}	7.88±0.75*	5.04±0.16	4.04±0.09	12.74±1.52^{+}	6.72±0.95*
% distribution by size								
<2 x10³ µm²	7.2±4.6	7.3±2.4	0	0.7±0.7	10.0±1.6	15.9±4.5	1.7±0.7^{+}	40.4±6.1$^{\#+}$*
2 - 6x10³ µm²	46.5±7.2	73.3±6.5*	9.6±2.9^{+}	29.7±4.9^{+}*	55.9±2.9	67.2±5.0	12.8±2.6^{+}	11.4±0.6^{+}
6 - 10x10³ µm²	40.6±5.6	16.4±4.2*	25.4±3.5^{+}	45.5±3.1^{+}*	32.0±1.4	16.6±2.6*	24.2±2.7	17.5±2.4$^{\#}$
10 - 14x10³ µm²	5.7±1.6	3.0±0.9	23.6±2.1^{+}	20.6±3.6^{+}	2.2±0.6	0.3±0.2	24.1±2.1^{+}	14.2±1.7^{+}*
>14x10³ µm²	0	0	41.4±12.2^{+}	3.6±2.8*	0	0	37.2±9.6^{+}	16.5±5.9^{+}*
Retroperitoneal white adipose tissue								
Area x10³	5.20±0.46	3.30±0.37	17.3±2.4^{+}	15.70±1.64^{+}	6.29±0.61	4.86±0.41	16.34±2.14^{+}	5.53±0.20$^{\#}$*
% distribution by size								
<2 x10³ µm²	5.1±2.1	18.3±7.8	2.1±2.1	0 $^{+}$	3.2±2.1	11.8±2.5	8.6±2.5	57.7±6.6#$^{+}$*
2 - 6x10³ µm²	62.9±7.7	76.3±5.2	7.6±2.6^{+}	6.2±1.8^{+}	44.8±5.5$^{\#}$	58.4±5.9$^{\#}$	13.5±3.2^{+}	15.9±1.4^{+}
6 -10x10³ µm²	25.8±5.7	5.4±1.6	9.6±2.5	17.4±3.4	42.0±3.4$^{\#}$	26.8±3.6$^{\#}$	8.7±1.5^{+}	6.4±1.0^{+}
10 - 14x10³ µm²	6.2±2.3	0	14.1±3.0	19.3±2.2^{+}	9.9±2.4	3.1±1.5	12.5±1.8	3.9±0.7$^{\#}$
>14x10³ µm²	0	0	66.7±12.1^{+}	57.2±11.6^{+}	0	0	56.7±9.6^{+}	16.1±5.3$^{\#}$*

Values are means ± SEM. The number of rats used in each determination was 8. $^{+}$ Significantly different from C, * significantly different from S, $^{\#}$ significantly different from 7 days; $p<0.05$.

Table 5. Serum triglyceride (mg/dl), insulin (µg/ml) and leptin (ng/ml) concentrations from control (C) or MSG obese (M) animals, lipectomized (L) or sham-operated (S) sacrificed at 7 or 30 days after surgery.

	SC7	LC7	SM7	LM7	SC30	LC30	SM30	LM30
Triglycerides	73.1±6.2	115.0±7.3*	61.0±3.4	87.2±6.8^{+}*	120.7±7.4$^{\#}$	149.0±7.0$^{\#}$*	138.3±11.3$^{\#}$	117.4±11.6$^{\#+}$
Insulin	14.74±0.86	11.09±1.35	18.56±1.90	13.40±0.90*	19.27±2.64	21.30±2.34$^{\#}$	18.43±1.17	19.54±1.27$^{\#}$
Leptin	0.67±0.04	0.66±0.06	6.81±0.50+	6.13±0.56^{+}	2.77±0.19	2.19±0.38	12.33±1.13$^{\#+}$	11.85±1.44$^{\#+}$

Values are means ±SEM. The number of rats used in each determination was 8. $^{+}$ significantly different from C, * significantly different from S, $^{\#}$ significantly different from 7 days; $p<0.05$.

Discussion

In agreement with previous reports (Nascimento Curi *et al.* 1991, Macho *et al.* 2000, Racek *et al.* 2001) the MSG-treated rats developed obesity, with low body weight and normal food intake and increased carcass lipid content, fat pads weight, and serum leptin.

Although not affecting food intake, the partial removal of adipose tissue lowered body weight, perhaps due to a higher stress level induced by the lipectomy, in comparison with the sham surgery (Michel and Cabanac 1999).

While reviewing the consequences of partial removal of specific fat pads, Mauer *et al.* (2001) pointed out the importance of distinguishing between re-growth of the excised pad and compensation at non-excised sites, the latter being the most common response. The authors also mentioned that the ability of fat pads to exhibit compensation is not uniform and depends on the fat pad that is excised.

In the present experiments, we failed to observe effective re-growth of RET and EPI. However, the carcass lipid content of the lipectomized groups did not differ from that of the sham groups. This indicates the possible occurrence of a compensatory reaction, a suggestion corroborated by other present observations. The accumulation of diet-derived lipids tended to increase in the lipectomized groups studied 30 days after the surgery. Importantly, only in the LM30 rats, a significant increase in the carcass "*in vivo*" lipogenesis rate had also occurred. This observation suggests that the obese animals are probably more prone to accomplish an increase in body lipid accretion after lipectomy.

In the LM7 group, we observed an increase in the percentage of diet-derived lipid accumulation in EPI and in the lipogenesis rate of RET and EPI. In LM30, these reactions were no longer present but there was a large increase in the percentage of small area adipocytes in both pads. It is possible that the high local lipid synthesis and accumulation represented a stimulus for adipocyte differentiation. In the long run this response would favor the reposition of the lost adipocytes and restoration of the fat pad removed. The same suggestion does not seem to apply to the LC animals, since they failed to exhibit stimulation of lipogenesis. Moreover, a conversion of larger (6 to 10 x 10^3 μm^2) into smaller (2 to 6 x 10^3 μm^2) cells, rather than adipocytes differentiation, probably occurred at 7 days, being abolished at 30 days.

In accordance with the present data, Harris *et al.* (2002) demonstrated an increase of RET adipocytes number and percentage of small cells, in obese ob/ob mice but not in the wild-type mice, after 6 weeks of lipectomy.

Recently, it has been demonstrated that adipocytes from ob/ob mice and mice in which obesity was induced with a high-fat diet, expressed the forkhead transcription factor Foxa-2. This factor has been identified as an obesity-induced gene mediating a negative feedback on adipocyte differentiation (Wolfrum *et al.* 2003). Thus we could suggest that partial removal of adipose tissue would decrease the Foxa-2 expression and lead to increase adipocyte differentiation.

The adipose tissue has an important protein secretory function. Cytokines, hormones, pro-hormones, and enzymes are secreted from adipocytes and act in an endocrine or paracrine way, regulating the white adipose and other tissues metabolism (Trayhurn and Beattie 2001).

It has been reported that angiotensin II produced by large adipocytes inhibits the recruitment of preadipocytes (Sharma *et al.* 2002). It is thus reasonable to suggest that the partial removal of fat pads would decrease the production of angiotensin II, favoring preadipocytes differentiation.

The lipolysis rate in RET was elevated by partial removal of fat pads in LM7 and LC7, and in the EPI of LM7, and this was accompanied by a decrease in serum insulin concentration. Insulin is the most important lipolysis inhibitor, decreasing the hormone-sensitive lipase enzyme (HSL) activity and triglyceride hydrolysis (Siegrist-Kaiser *et al.* 1997). The increase of lipolysis rate in the L7 groups may be partially explained by weaker inhibition of HSL.

Additionally, the surgical stress-induced stimulation of adrenergic hormone secretion and sympathetic activity would stimulate the adipocytes beta-adrenergic receptors which elevate the lipolysis rate (Axelrod and Reisine 1984). Accordingly, seven days after surgery, all groups showed an increase in the lipolysis rate compared to the corresponding 30-day groups.

The increase of triglyceridemia observed in the LC7, LC30 and LM7 groups may have been induced, at least in part, by a low triglycerides clearance by the adipose tissue, as indicated by the low diet-derived lipid uptake by the total tissue and the decreased tissue lipid content. Conversely, in the LM30 rats, the high number of newly formed adipocytes probably contributed to prevent an impairment of lipid clearance.

In conclusion, the present data showed that the partial removal of fat depots affected the metabolism of control and MSG-obese rats differently. In the obese animals only, it stimulated both local and carcass lipogenesis rate as well as adipocytes differentiation. These responses likely favor excised tissue re-growth and/or compensatory growth of non-excised depots.

List of abbreviations

SC7 / SC30 – sham operated control rats sacrificed 7 or 30 days after surgery

LC7 / LC30 – lipectomized control rats sacrificed 7 or 30 days after surgery

SM7 / SM30 – sham operated monosodium glutamate obese rats sacrificed 7 or 30 days after surgery

LM7 / LM30 - lipectomized monosodium glutamate obese rats sacrificed 7 or 30 days after surgery

RET – retroperitoneal white adipose tissue

EPI – epididymal white adipose tissue

MSG – monosodium glutamate

Acknowledgements

We thank Dr Silvia S M Ihara for the help in the measurement of adipocyte area. This research was supported by CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) and FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo) and CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnologico).

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