



ORIGINAL ARTICLE

Impact of transient correction of increased adrenocortical activity in hypothalamo-damaged, hyperadipose female rats

G Moreno¹, M Perelló¹, G Camihort², G Luna², G Console², RC Gaillard³ and E Spinedi¹

¹Neuroendocrine Unit, Multidisciplinary Institute on Cell Biology (CONICET-CICPBA), La Plata, Argentina; ²Department of Histology and Embryology 'B', School of Medicine, UNLP-CICPBA, La Plata, Argentina and ³Division of Endocrinology, Diabetology & Metabolism, University Hospital (CHUV), Lausanne, Switzerland

Objective: To explore the effects of transient correction of enhanced corticoadrenal activity in monosodium L-glutamate (MSG)-damaged female rats on peripheral insulin sensitivity and *in vitro* retroperitoneal (RP) adipocyte function.

Designs: A dose of 4 mg/g body weight (BW) of MSG or vehicle (CTR) was i.p. injected, once every 2 days, between days 2 and 10 of age, in female rats. Intact and 21 day-operated (sham or adrenal enucleation (AE)) rats from both (CTR and MSG) groups were used for experimentation on day 120 of age. Circulating levels of several hormones, in basal and after i.v. high-glucose load conditions, and RP adiposity morphology and function were then evaluated.

Results: MSG rats developed increased adrenocortical function, hyperadiposity, hyperleptinemia, hyperinsulinemia and decreased peripheral insulin sensitivity. These characteristics were fully reversed after transient correction of corticoadrenal hyperactivity induced by AE. In addition, *in vitro* experimentation with isolated RP adipocytes indicated that cells from intact MSG animals displayed decreased sensitivity to insulin and dexamethasone stimulation of leptin secretion. Interestingly, adipocyte dysfunction in MSG rats was fully abrogated after AE-induced transient correction of insulinemia, leptinemia and adrenocortical activity. Importantly, the reversion of these metabolic abnormalities, induced by AE for 21 days, in MSG animals did occur, despite no significant changes in BW values.

Conclusion: Our results support that the changes in adipocyte characteristics and peripheral insulin resistance, developed in this pseudo-obese female rat model, are mainly due to increased glucocorticoid production. Importantly, appropriate correction of the enhanced adrenocortical activity fully reversed these abnormal functions.

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Introduction

Hypothalamic lesions induced in rats is a widely recognized tool for studies of changes in neuroendocrine functions. Monosodium L-glutamate (MSG) administration in neonatal rats is recognized to mainly damage hypothalamic arcuate nucleus (ARC) neurons.^{1–3} MSG-induced partial hypothalamic denervation alters several neuroendocrine-metabolic functions. It has been proposed that early loss of hypothalamic neurotransmitters induced by MSG, namely in the ARC,^{1,4,5} is responsible for such abnormalities. Among these, MSG animals develop a number of morphological, behavior-

al, and endocrine abnormalities.^{2,3,4,6,7} Also, the changes in hypothalamic neuronal function particularly enhance hypothalamo–pituitary–adrenal (HPA) axis activity.^{8–12} It is known that hypophagic,¹³ MSG-damaged rats are characterized by increased adiposity and insulin resistance;¹⁴ however, the exact mechanisms whereby these abnormalities develop are not fully understood.

These MSG-induced changes in HPA axis activity, a key function for maintaining homeostasis, have been attributed to dysfunctions of several hypothalamic peptidergic and monoaminergic systems^{15–17} driving pituitary function, and, in turn, modifying adrenal development and function,¹¹ thus resulting in enhanced corticoadrenal activity.^{8–12} The bi-directional crosstalk between HPA axis and adipose tissue activities is widely accepted.¹⁸ We recently demonstrated that the peripheral satiety signal leptin,¹⁹ a physiological inhibitor of ACTH-driven adrenal glucocorticoid production,²⁰ is several fold increased in the circulation of

Correspondence: Dr E Spinedi, Neuroendocrine Unit, IMBICE, PO Box 403, La Plata, BA 1900, Argentina.

E-mail: spinedi@imbice.org.ar

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MSG rats shortly after the end of treatment, and is even more pronounced at adulthood.²¹ Interestingly, the mRNA expression of its biologically active receptor (Ob-Rb) is reduced in the adrenal gland of MSG rats.²² Thus, in this animal model, enhanced HPA axis activity could be explained, at least in part, by its adrenal leptin resistance.²² Interestingly, adrenal leptin resistance characterizing MSG rats could be restored after normalization of leptinemia due to adrenal enucleation (AE),²¹ thus indicating that regenerating adrenocortical cells that have not been exposed for a long period of time to high concentrations of the adipokine did recover the normal inhibitory tone of leptin on ACTH-stimulated glucocorticoid secretion.

In spite of the above, and based on the hypothesis that enhanced HPA axis activity could be a key factor for the development of the pseudo-obese phenotype in MSG-damaged rats, the aims of the present study were to: (a) characterize adipose tissue morphology and function, and peripheral insulin sensitivity, and (b) determine whether AE-induced correction of enhanced adrenocortical activity could be relevant for amelioration of some abnormalities characterizing pseudo-obese rats.

Materials and methods

Animals and treatment

Adult male (300–330 g body weight (BW)) and female (240–280 g BW) Sprague–Dawley rats were allowed to mate in colony cages in a light (lights on from 0700 to 1900 h)- and temperature (22°C)-controlled room. Rat chow and water were available *ad libitum*. Pregnant rats were transferred to individual cages. Beginning on day 2 after parturition, female newborns were injected i.p. with either 4 mg/g BW MSG (Sigma Chemical Co., St Louis, MO, USA) dissolved in sterile 0.9% NaCl or 10% NaCl (litter-mate controls; CTR) once every 2 days up to day 10 of age.²³ Rats were weaned at 21 days of age; daily BW and food intake of rats were recorded up to the experimental day (120 days of age). MSG-injected animals were screened for effectiveness of treatment by decreased hypothalamic NPY mRNA expression and macroscopic observation of degeneration of the optic nerves at the time of killing. In each experiment, CTR and MSG female rats were members of the same litters; however, when accumulating experiments, each different experiment was performed with (CTR and MSG) animals from different litters. As MSG rats had an abnormal estrous cycle, the microscopic observations of their daily vaginal smears showing a constant diestrous stage, we used rats from CTR litters for experimentation after ascertaining by screening that they were at the diestrous stage of their estrous cycle.

Our Institutional Animal Care Committee approved all experimental designs. Animals were killed by decapitation, according to the protocols for animal use, in agreement

with NIH guidelines for care and use of experimental animals.

Experimental designs

Experiment 1. Intact (nonoperated) CTR and MSG animals (10–12 rats per group) were killed by decapitation (between 0830 and 0915 h) in nonfasting condition. Trunk blood was collected into plastic tubes containing 0.2 ml EDTA (10% w/v) for further measurement of different metabolites in plasma samples (see below). Immediately after killing of rats, freshly dissected retroperitoneal (RP) fat pads were weighed and used for either histological or adipocyte function studies.

For histological studies of adipocytes, adipose (RP) tissues were fixed in Bouin's fluid and embedded in paraffin. Sections of 4 μm were obtained, at different levels of the blocks, and stained with hematoxylin–eosin. Adipose tissue sections were incubated for 1 h at room temperature with the primary serum anti-leptin (rabbit polyclonal, Santa Cruz Biotechnology Inc., USA), diluted 1:200. Thoroughly washed sections were treated for 30 min with a ready-to-use EnVision reaction system (Dako, CA, USA). Diaminobenzidine was used as the peroxide-sensitive chromogen. The specificity of the primary antiserum was monitored by ability to block the immunocytochemical reaction by either pre-absorption of the antibody with an excess of leptin or replacement of the specific antibody by, similarly diluted, normal rabbit serum. Morphometric studies were performed as previously reported in detail.²⁴ Briefly, measurements of adipocyte parameters were made with an image analysis system (Imaging Technology, Optimas 5.2). Each field of immunostained cells in the reference area (RA; area scanned where adipocytes were scored) was measured for an average of 10 micrographs taken from two levels (e.g. a and b). These measurements were recorded and processed automatically, and the following parameters calculated afterwards: volume density ($\text{VD} = \Sigma \text{adipocyte area} / \text{RA}$), cell density ($\text{CD} = \text{number of adipocytes} / \text{RA}$) and cell size (CS; expressed in μm^2). Then, with the sum (Σ) of the individual areas (A) of adipocytes, referred to as RA, we obtained VD, which indicates cell mass. The number of cells (CD) was calculated by dividing the immunostained area of the adipocyte population by the mean individual cell area. For this parameter, 100 adipocytes were recorded.

For studies on adipocyte function, RP fat pads were used for isolation of adipocytes as described previously.²⁵ Briefly, preweighed fat pads ($n = 2\text{--}3$ rats per group per experiment) were slightly minced and placed in KREBS-MOPS medium (Sigma-Aldrich Co., St Louis, MO, USA) containing 1% BSA and 0.1% collagenase (Sigma-Aldrich, Type 1) in a ratio of 3 ml of solution per gram of fat tissue. Tissues were gently shaken at 37°C for 50 min in polypropylene flasks in a 95% air–5% CO_2 atmosphere inside a metabolic incubator. At the end of this period, cell suspensions were filtered through one layer of nylon cloth (100 μm), transferred to conical polypropylene tubes and centrifuged at 100 g for 20 min at room

temperature. Cells were then washed three times with medium alone to eliminate the stromavascular fraction and collagenase. Adipocytes were then counted and diluted with DMEM (Sigma-Aldrich)-1% BSA medium, pH 7.4, to the necessary volume to obtain approximately 4×10^5 adipocytes per 1.4 ml of medium. This volume was distributed into each 15 ml conical polypropylene tube containing 0.1 ml of medium either alone (basal) or with different concentrations of insulin (Sigma-Aldrich; 0.2 and 5.0 nM).²⁵ At least six tubes (replicates) per experimental condition were run in each experiment. Tubes were incubated by shaking at 37°C for 2 h in a 95% air–5% CO₂ atmosphere. At the end of incubation, tubes were centrifuged for 20 min at 100 g; the infranatant was separated from adipocytes and kept frozen (–20°C) until measurement of medium leptin concentration.

Experiment 2. Effects of bilateral AE on peripheral insulin sensitivity – AE²¹ is a surgery carried out by the dorsal approach and under light ketamine anesthesia, exposing the adrenals and making a puncture in the capsule; thereafter, the parenchyma is extruded by squeezing the gland very gently with forceps and leaving the capsule intact. AE animals, once recovered, were maintained by drinking 0.9% (w/v) NaCl solution up to day 4 after surgery. Thus, AE induces a transient decrease in adrenal glucocorticoid production that in turn triggers adrenal regeneration, a process which is normally complete 21 days after AE.²¹ Thus, on day 21 before experimentation, CTR and MSG were subjected to either sham surgery (performed by exposing the glands but without touching the glands or their pedicles; sham-CTR and sham-MSG groups) or AE (AE-CTR and AE-MSG groups). Metabolic responses to high glucose load were evaluated in groups of 24-h-fasting CTR and MSG rats subjected to either sham operation or AE (bearing an indwelling i.v. cannula, implanted 48 h before the experiment). Animals ($n=8–10$ rats per group/condition) were bled, on day 21 after surgery, before (sample time zero) and at several times (5, 15, 30, 60 and 90 min) after glucose (2 g per kg BW) i.v. administration.²⁶ Plasma samples, split into aliquots, were kept frozen (–20°C) until determination of glucose, insulin and leptin concentrations.

Experiment 3. Effects of bilateral AE on RP adipocyte function – On day 120 of age, different groups of rats (sham-CTR, sham-MSG, AE-CTR and AE-MSG; $n=2–3$ rats per group/condition per experiment) were weighed and rapidly killed (between 0830 and 0915 h, in nonfasting condition) by decapitation. Trunk blood, collected into plastic tubes containing 0.2 ml EDTA 10%, was immediately centrifuged for further determination of different metabolites in plasma samples (see below). Preweighed RP fat pads from different group/condition were processed for isolation of adipocytes as described in Experiment 1. Then 4×10^5 adipocytes per 1.4 ml of medium were incubated, as described above, with 0.1 ml of medium alone (basal) or containing insulin (Sigma-Aldrich, 0.2 and 1.0 nM) or dexamethasone (DXM; Sigma-

Aldrich; 25 and 50 nM).²⁷ At least six tubes (replicates) per experimental condition were run in each experiment. At the end of the incubations, tubes were processed as described above and the concentration of leptin released into the medium was then evaluated.

Metabolite determination

Plasma glucose concentrations were determined by an enzymatic–colorimetric assay from Weiner Lab. (Argentina). Circulating ACTH concentrations were measured by a previously validated immunoradiometric assay²⁸ from our laboratory; the standard curve ranging between 15 and 3000 pg/ml and intra- and interassay coefficients of variation (CVs), respectively, of 2–3 and 6–8%. Leptin circulating levels were determined by a specific radioimmunoassay (RIA) developed in our laboratory and validated for mouse and rat leptin against a commercial kit from Linco Res. Inc.;²⁹ the standard curve ranged between 0.2 and 25 ng/ml, with intra- and interassay CVs of 5–8 and 10–12%, respectively. Insulin circulating levels were determined by a specific rat RIA previously developed in our laboratory;³⁰ the standard curve ranged from 0.1 to 10 ng/ml, intra- and interassay CVs were 3–7 and 8–11%, respectively. Finally, plasma free corticosterone (FB) concentrations were measured as earlier reported.³¹ Briefly, a 50 μ l steroid-stripped serum sample appropriately diluted in assay buffer was incubated overnight (4°C) with graded concentrations of cold B (200 μ l) in the presence of ³H-B (New England Nuclear; approximately 10 000 c.p.m. in 50 μ l of assay buffer); we separated bound from free hormone fractions by adding 0.1 ml charcoal (1 g%)–dextran T 70 (0.1 g%). Bound radioactivity was determined in a Tracor Analytic Scintillation System. Analysis of binding data for further determination of the concentration and affinity of corticosteroid-binding globulin (CBG) and circulating FB levels were carried out as reported previously.³¹

Data analysis

Data were expressed as the mean \pm s.e.m. Mean values were compared by ANOVA or ANOVA with repeated measures (when appropriate), followed by *post-hoc* comparisons with Fisher's test.³² Morphometric data were analyzed by the least significant difference test for multiple comparisons.³²

Results

Body characteristics and circulating hormone levels in the MSG female rat

MSG rats have daily eaten, since weaned, a significantly lower amount of food than CTR litters (not shown). As previously found,²² 120-day-old MSG female rats displayed a 60% (approximately) reduction in hypothalamic NPY mRNA expression and optic nerve degeneration when examined immediately after the killing (not shown). Moreover, as

shown in Figure 1, at age 120 days, animals have developed the classical phenotype of hyperadiposity and stunted growth; characteristics associated with a significantly ($P < 0.05$ vs CTR rats) higher RP adipose tissue mass, despite significantly ($P < 0.05$) lower BW than their normal counterparts (CTR) (Table 1).

Results from circulating concentrations of several hormones determined in intact 120-day-old female CTR and MSG rats, at the trough time of day³³ and in nonfasting condition, are included in Table 1. These data indicate that basal plasma ACTH concentrations were similar in both groups examined. However, MSG rats displayed significantly ($P < 0.05$) higher circulating levels of FB, insulin and leptin than CTR animals.

Histological, immunohistochemical and morphometric characteristics of adiposity in CTR and MSG-damaged rats

Owing to the increased RP fat mass and leptinemia displayed by MSG-treated rats, we explored whether these characteristics were associated with qualitative/quantitative modifications of the adipogenic process. Figure 2 shows representative fields of RP adipose tissue from intact CTR

(panels a and c) and MSG (panels b and d) animals, either hematoxylin–eosin-stained (panels a and b) or specifically immunolabeled for leptin (panels c and d). The MSG-damaged rats presented large adipocytes that showed an ochre cytoplasmic rim labeled for leptin (Figure 2d).

Morphometric analyses of RP unilocular adipocytes in both experimental groups indicate that MSG rats displayed a significant ($P < 0.01$) increase in cell VD (Figure 2e) vs CTR adipocytes, and consequently a significant ($P < 0.001$) decrease in CD (Figure 2f). These morphometric characteristics

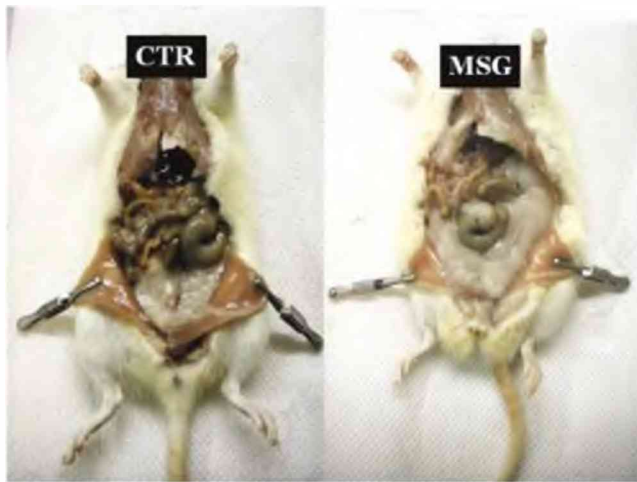


Figure 1 Characteristic adult (120-day-old) hyperadipose phenotype of the MSG female rat (CTR: age-matched normal female rat).

Table 1 Basal circulating levels of several parameters, BW values and RP fat mass in nonoperated, CTR and MSG female rats at 120 days of age

Parameter	CTR	MSG
ACTH (pg/ml)	29.09 ± 2.61	30.98 ± 3.54
FB (ng/dl)	46.01 ± 9.03	95.77 ± 14.11 [†]
Leptin (ng/ml)	3.94 ± 0.72	33.52 ± 8.34 [†]
Insulin (ng/ml)	0.61 ± 0.11	0.92 ± 0.13 [†]
BW (g)	223.4 ± 7.48	174.6 ± 10.8 [†]
RP fat mass (g/100 g BW)	1.85 ± 0.41	4.01 ± 0.51 [†]

Values are the mean ± s.e.m., $n = 10-12$ rats per group. [†] $P < 0.05$ vs CTR values.

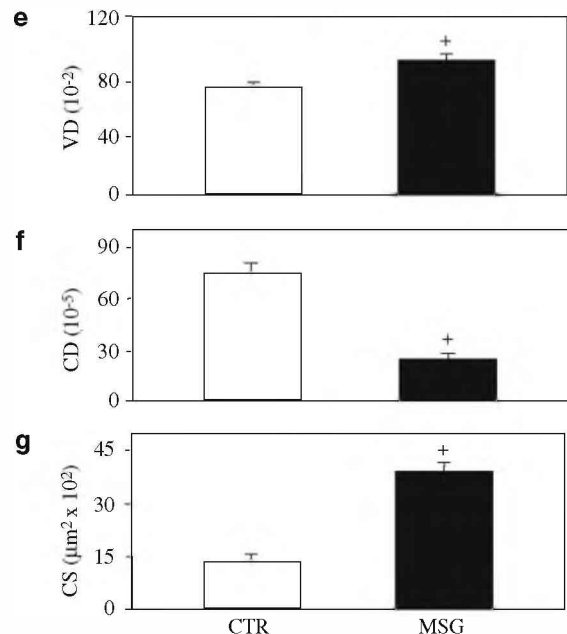
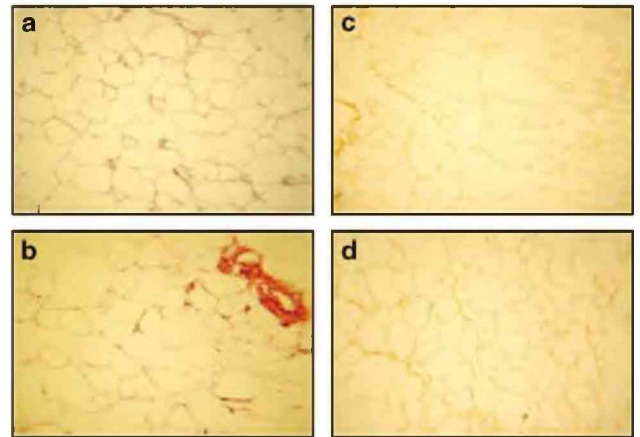


Figure 2 Representative fields of RP adipose tissue from CTR (a, c) and MSG (b, d) rats. RP adipocytes were stained with hematoxylin–eosin (a, b) and immunolabeled for leptin (c, d); they all show the cytoplasmic rim of mature unilocular adipocytes. Micrographs were taken with an × 20 objective. Morphometric analyses (e–g) of RP adipocytes (e–g) from CTR and MSG animals. VD: volume density, CD: cell density and CS: cell size. Values are the mean ± s.e.m. ($n = 6$ rats per group). [†] $P < 0.01$ vs the respective CTR values.

were associated with a significant ($P < 0.001$) increase in CS (Figure 2g).

Effect of neonatal MSG treatment on RP adipose tissue function in vitro

Figure 3 shows the results of leptin release into the medium by isolated RP adipocytes (from intact CTR and MSG rats) incubated in the absence (basal) or presence of several concentrations of insulin (0.2–5.0 nM). Basal leptin release by MSG adipocytes was significantly ($P < 0.05$) greater than that spontaneously secreted by CTR cells. As depicted, CTR

adipocytes significantly ($P < 0.05$ vs basal values) respond, in a concentration-related fashion, to all insulin concentrations assayed. As depicted, whereas the two lower concentrations (0.2 and 0.5 nM) of insulin tested were equipotent for releasing leptin, 1 and 5 nM insulin were able to elicit higher responses ($P < 0.05$) than that observed with 0.2 nM insulin. Conversely, adipocytes from MSG rats were significantly ($P < 0.05$) less sensitive to insulin stimulation, regardless of the insulin concentration used. As depicted, 1 nM or higher insulin concentration was needed to significantly ($P < 0.05$) enhance leptin release over the respective baseline.

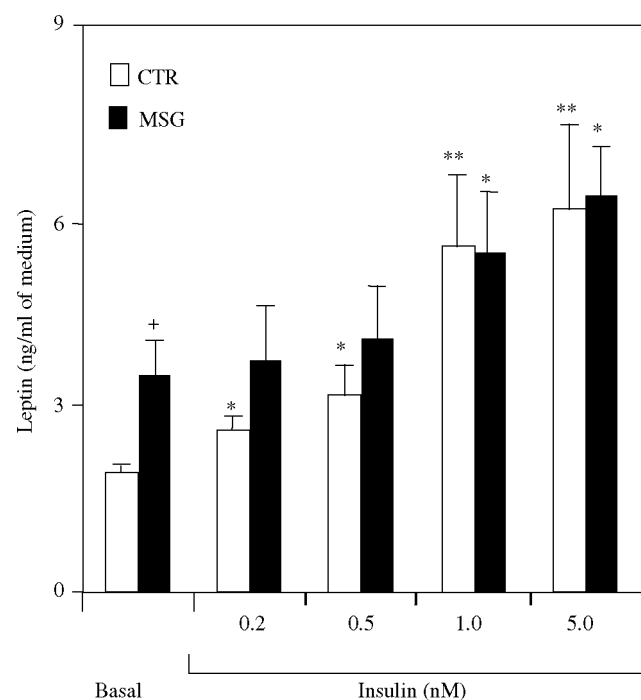


Figure 3 Leptin release into the medium by isolated RP adipocytes from CTR and MSG rats ($n = 2-3$ animals per experiment), incubated *in vitro* in the absence (basal) or presence of several concentrations of insulin (0.2–5.0 nM). Values are the mean \pm s.e.m. ($n = 3$ different experiments, 5–6 replicates per condition in each experiment). $^{\dagger}P < 0.05$ vs MSG values in similar condition. $^*P < 0.05$ vs basal values in the respective cell group. $^{**}P < 0.05$ vs 0.2 nM insulin values in the respective cell group.

Effects of AE on basal circulating hormone levels and BW characteristics

Table 2 shows the results of several parameters measured, in basal conditions, in CTR and MSG subjected to different surgeries. No differences were found in any of these parameters between intact (Table 1) and sham-operated rats from both groups (Table 2). However, while CTR rats displayed normal plasma ACTH levels on day 21 post-AE, AE-MSG showed significantly ($P < 0.05$ vs AE-CTR values) higher circulating concentrations of ACTH. Table 2 also shows that AE in MSG rats was able to normalize circulating FB levels and, interestingly, insulin and leptin plasma concentrations. Finally, although group differences in BW values remained, we found a significant ($P < 0.05$) decrease in RP fat pad mass in both groups of AE rats (Table 2).

Effect of AE on metabolic responses to acute i.v. high glucose load

Figure 4 shows the circulating levels of several parameters in basal condition (time zero) and at different times after i.v. administration of glucose (2 g/kg BW) in 24-h-fasting, CTR and MSG, rats either sham operated (panels a–c) or AE (panels d–f). Figure 4a shows glucose circulating levels, before and several times after high glucose load, in both sham-operated groups. As depicted in Figure 4a, despite similar basal levels, circulating glucose concentrations significantly ($P < 0.05$) peaked, in a similar fashion, in both groups 5 min post-glucose administration; however, plasma glucose levels, at 15 and 30 min, were significantly ($P < 0.05$) higher in sham-MSG than in sham-CTR rats. Sham-MSG rats

Table 2 Basal circulating levels of several parameters, BW values and RP fat mass in sham and AE, CTR and MSG female rats at 120 days of age

Parameter	Sham-CTR	Sham-MSG	AE-CTR	AE-MSG
ACTH (pg/ml)	27.88 \pm 3.13	35.88 \pm 9.75	36.77 \pm 7.59	69.79 \pm 9.96 †
FB (ng/dl)	45.77 \pm 7.01	99.12 \pm 8.85 †	55.08 \pm 5.14	65.02 \pm 7.09
Leptin (ng/ml)	4.13 \pm 1.09	31.97 \pm 4.41 †	2.81 \pm 0.75	4.38 \pm 1.99
Insulin (ng/ml)	0.63 \pm 0.13	0.98 \pm 0.16 †	0.54 \pm 0.38	0.61 \pm 0.22
BW (g)	228.8 \pm 5.1	211.8 \pm 7.9 †	233.2 \pm 8.9	207.9 \pm 9.9 †
RP fat mass (g/100 g BW)	1.76 \pm 0.08	3.43 \pm 0.18 †	1.18 \pm 0.14 *	2.36 \pm 0.23 $^{\dagger, \#}$

Values are the mean \pm s.e.m., $n = 10$ rats per group/surgery. $^{\dagger}P < 0.05$ vs CTR values in similar condition. $^*P < 0.05$ vs sham-CTR values. $^{\#}P < 0.05$ vs sham-MSG and AE-CTR values.

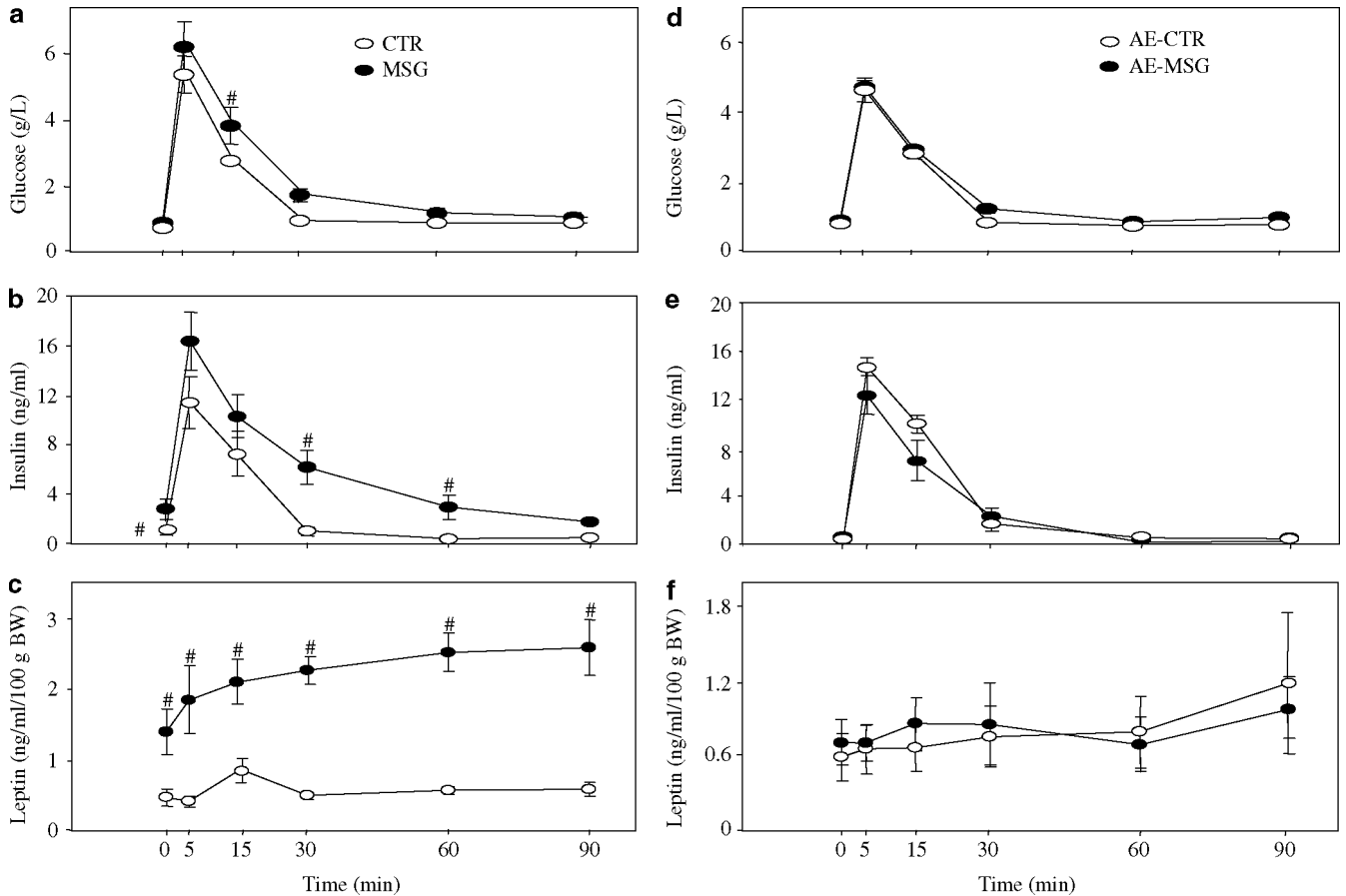


Figure 4 Effect of sham operation or AE on changes in circulating metabolite concentrations, before and at several times after i.v. high glucose load, in normal (CTR) and hyperadipose (MSG) rats. Values are the mean \pm s.e.m. ($n=7-9$ rats per group/surgery). # $P<0.05$ vs MSG values in similar condition (ANOVA repeated measures).

displayed (Figure 4b) basal hyperinsulinemia (vs sham-CTR rats), and glucose load induced a statistically similar increase in insulin values in both groups, 5 and 15 min post-glucose; however, sham-MSG rats displayed higher insulinemia (vs sham-CTR values), 30 and 60 min post-glucose. Finally (Figure 4c), 15 min after i.v. high glucose load, circulating leptin levels (expressed in ng/ml/100g BW) were significantly ($P<0.05$) enhanced (over time-zero values) in sham-CTR rats; thereafter, plasma leptin concentrations were back to baseline at 30 min post-glucose load. Although sham-MSG rats did also respond to high glucose administration by increasing (over the baseline) circulating leptin levels, this occurred in a significant ($P<0.05$) fashion later than in sham-CTR animals (30–90 min post-stimulus).

Interestingly, those significant differences found between sham-operated, CTR and MSG, rats were fully abrogated after AE of animals. In fact, the patterns of glucose (Figure 4d) and insulin (Figure 4e) levels, before and after high glucose load, were similar in both groups of AE rats examined. AE also was able to fully reverse hyperleptinemia in MSG rats (Figure 4f),

and high glucose load did not modify circulating leptin levels throughout the period examined, regardless of the group.

Impact of AE on adipocyte activity in vitro

Additional experimentation was performed to study the impact of transient correction of the enhanced circulating concentrations of hormones known to modify adipocyte function, for example, FB, insulin and leptin. For this purpose, a similar number of RP adipocytes from sham-operated and AE rats, from both groups, was incubated in the absence (basal) or presence of either insulin (0.2 and 1 nM) or DXM (25 and 50 nM), stimuli known to operate through different mechanisms. The results indicated that RP adipocyte function (both basal and insulin-elicited leptin output) in sham-operated (Figure 5a) and intact (Figure 3) rats was essentially similar, regardless of neonatal treatment. In addition, while both DXM solutions tested were able to induce a significant ($P<0.05$ vs respective basal values)

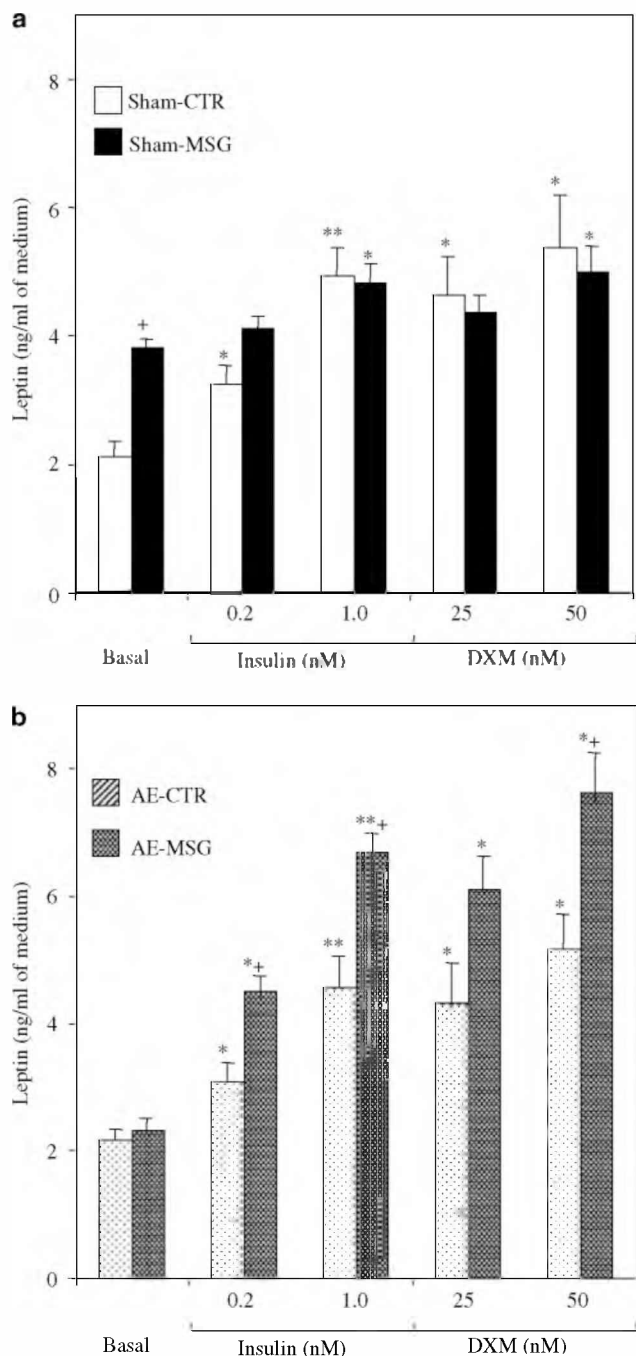


Figure 5 Effect of AE on RP adipocytes from CTR and MSG rats ($n=2-3$ animals per experiment), responses to several concentrations of insulin or DXM. Values are the mean \pm s.e.m. ($n=3$ different experiments, 5-6 replicates per condition in each experiment). [†] $P<0.05$ vs MSG values in similar condition. ^{*} $P<0.05$ vs basal values in the respective cell group. ^{**} $P<0.05$ vs 0.2nM insulin values in the respective cell group.

increase in leptin secretion in sham-CTR cells (Figure 5a), conversely, only 50 nM DXM induced a significant ($P<0.05$ vs the respective baseline values) increase in leptin release by sham-MSG adipocytes (Figure 5a). It should be mentioned

that the decreased sensitivity of sham-MSG cells to these DXM concentrations could be related to their intrinsically higher spontaneous leptin output; thus, it should not be excluded that DXM concentrations >50 nM could still induce higher leptin secretion. As found in experiments with adipocytes from intact MSG rats (Figure 3), adipocytes from sham-MSG spontaneously released more ($P<0.05$) leptin than adipocytes from sham-CTR rats, and these cells were sensitive for releasing leptin only after incubated with the highest concentration of both stimuli tested (Figure 5a). The pattern of leptin secretion by adipocytes from AE-CTR rats (Figure 5b) was similar to that developed by sham-CTR adipocytes (Figure 5a) after incubation in similar conditions. Interestingly, AE in MSG rats was able to significantly ($P<0.05$ vs sham-MSG basal values) reduce basal leptin secretion by isolated RP adipocytes (Figure 5b). In addition, AE in MSG rats enhanced RP adipocyte sensitivity. In fact, both concentrations of insulin and DXM tested significantly ($P<0.05$ vs the respective basal values) enhanced leptin release into the medium, in a fashion even more pronounced ($P<0.05$) than AE-CTR cells did (Figure 5b).

Discussion

Our study strongly supports glucocorticoid dependency in the development of several characteristics of this hypothalamo-damaged, pseudo-obese phenotype in rats. In fact, we have demonstrated that hyperleptinemia, hyperinsulinemia, peripheral insulin resistance and adiposity characteristics, mass and function, can be fully reversed by the transient correction of corticoadrenal hyperactivity (normalization of circulating FB levels due to AE) in the MSG female rat model. It should be stressed that the correction of insulin resistance in MSG rats subjected to AE appeared without significant changes in rat BW (Table 2), although AE-MSG rats remain lighter than their normal enucleated counterparts.

In previous studies from our laboratory, we found that AE is a very precise tool to demonstrate that enhanced corticoadrenal function is responsible for MSG-induced adrenocortical leptin resistance²¹ and hyperadiposity.³⁰ Thus, our previous and current studies strongly support that this nongenetic, pseudo-obese phenotype developed with enhanced adrenocortical activity. Other authors demonstrated that adrenalectomy (ADX) abolished the *ob/ob* mouse phenotype.³⁴ However, these results are predictable because of both a full lack of the orexigenic effect exerted by glucocorticoids³⁵ and the increase in hypothalamic CRH-ergic activity, a potent anorectic signal,³⁵ after ADX. The main difference between our and that study, beside models, is that 21-day AE-induced transient correction of circulating glucocorticoid did not modify food intake (data not shown), although body fatness and insulin sensitivity became normal in MSG rats. Conversely, spontaneous restoration of high glucocorticoid production (e.g. 35 days after AE) in MSG rats

resulted in augmented body fat mass,³⁰ thus clearly suggesting a direct glucocorticoid dependency in body fatness development, regardless of food intake.

Although we found no significant change in basal circulating levels of ACTH in intact MSG rats, this observation tallies with previous findings for adult male MSG rats.²¹ Conversely, circulating FB concentrations were significantly higher in MSG than in CTR rats. Additionally, reduced corticosterone clearance rate^{9,10} and increased fasciculata to glomerulosa width ratio¹¹ have been reported in MSG rats. Thus, these additional abnormalities could contribute to the high glucocorticoid levels characterizing this animal model.

Our study also shows morphological RP adipose tissue characteristics of the adult female MSG rat, such as enlarged adiposity and clearly enhanced adipogenic activity. It is accepted that the target white adipose tissue (e.g. RP fat) is characterized by the presence of adipocytes with a unique lipid inclusion (unilocular) and eccentric nucleus. Fat mass depot results from the balance between lipogenesis and lipolysis.³⁶ While lipogenesis encompasses lipid synthesis, adipogenesis is a process consisting in the differentiation of pre-adipocyte to mature adipocyte. The development of target adipose tissue occurs rapidly after birth as a result of an increment in both adipocyte size and number.³⁷ Even in adult life, the potential for generating new adipocytes persists, and differentiation and metabolism are regulated by a variety of endocrine and paracrine factors.³⁸ In MSG rats, RP adipocytes displayed a significant increase in VD at the expense of an increase in CS, a parameter clearly supporting adipogenesis. Moreover, enlarged adipocyte size has been directly associated with hyperleptinemia.³⁹ In addition, the fact that RP MSG adipocytes displayed *in vitro* insulin resistance could partly contribute to development of reduced lipolysis.⁴⁰

AE is known to be a surgical approach that decreases (on days 2–5 post-surgery) glucocorticoid production; as a result, enhanced hypothalamo-corticotrope activity develops because of the transient loss of glucocorticoid negative feedback.³³ The fall in glucocorticoid production, 2 days post-AE,²¹ enhances ACTH secretion in plasma, thus allowing the adrenal cortex to fully regenerate. This process, though completed 21 days post-AE in normal rats, is delayed up to 35 days in MSG animals.²¹ The transient decrease in circulating glucocorticoid levels after AE represents a diminished peripheral, glucocorticoid, adipogenic signal.⁴¹ It is also important to note that enhanced ACTH levels after AE will impact adipocytes inhibiting leptin secretion^{17,42} and probably adipogenesis (unpublished observation from our laboratory). Thus, these two signals contribute to AE-induced reduction in adipose tissue mass and leptin secretion. Moreover, we found that among changes induced by AE, in this pseudo-obese phenotype, included the normalization of insulinemia, leptinemia, peripheral insulin sensitivity, and RP adipocyte function and morphometric characteristics (not shown). Therefore, the decrease in circulating levels of insulin, another adipogenic signal,^{43,44}

could also contribute to the changes observed in the AE-MSG female rat.

The development of hyperadiposity and insulin resistance in MSG-damaged rats has been described previously.^{14,45,46} Although we previously described³⁰ that, in male MSG rats, AE is able to reduce both enhanced body fatness and hyperleptinemia, to our knowledge, these data are the first showing that transient correction of enhanced adrenocortical function, in the adult AE-MSG female rat, resulted in: (a) full reversion of peripheral insulin resistance and (b) normalization of, *in vivo* and *in vitro*, adipocyte function. It has been proposed that the stimulatory effect of insulin on adipocyte glucose transport is diminished in adult MSG rats,⁴⁶ an effect probably dependent on both low adipocyte insulin binding, unrelated to TNF α overproduction,⁴⁷ and reduced GLUT4 protein content. However, other authors also found that cells from several insulin-sensitive tissues of MSG rats display increased protein insulin receptor-tyrosine phosphatase 1B interaction and impaired downstream insulin signaling.⁴⁸ The inhibitory effect of glucocorticoid on insulin action in rat adipocytes is a well-recognized mechanism.^{49,50} However, these alterations have been attributed to alterations in insulin-mediated glucose transport,^{49,51} not to changes in adipocyte insulin binding.⁵¹ Also, high leptin circulating levels are known to alter insulin action.^{52,53} For instance, human hepatic cells exposed *in vitro* to leptin, at concentrations comparable with those present in obese individuals, display attenuation of several insulin-induced activities. Two of these are tyrosine phosphorylation of the insulin receptor substrate-1 (IRS-1) and gluconeogenesis.⁵³ Therefore, the deleterious effect of an excess of glucocorticoid, due to enhanced production and/or reduced urinary excretion,^{9,10,54} on insulin sensitivity could be further affected by the hyperleptinemia characterizing the MSG animal model.

In summary, our study strongly supports that AE-induced transient correction of increased adrenal cortex activity in pseudo-obese MSG female rats: (a) decreases leptinemia and insulinemia and (b) restores normal peripheral insulin sensitivity and adipocyte function. Thus, enhanced adrenocortical activity seems to be the key factor in the development of this phenotype of hypothalamic hyper-adiposity and, what is important in a therapeutic perspective, the correction of adrenocortical hyper-function reversed these abnormalities.

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