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PAPER

Decreased lipolysis and enhanced glycerol and glucose utilization by adipose tissue prior to development of obesity in monosodium glutamate (MSG) treated-rats

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OBJECTIVE: To determine the metabolic alterations that lead to the neonatal administration of monosodium glutamate (MSG), which results in arrested growth and obesity.
ANIMALS AND DESIGN: Wistar rats were injected 5 times, every other day, with 4 g of MSG/kg b.w. or with hyperosmotic saline (controls), within the first 10 days of life, and were studied at the age of 30 days.
RESULTS: Body weight was lower, whereas adipocyte lipid content, cell diameter, surface area and volume were higher in MSG rats than in controls. Plasma glucose, insulin, NEFA, glycerol and triglyceride levels, and *in vitro* production of NEFA by lumbar fat pad pieces incubated under basal conditions or in the presence of epinephrine and epinephrine plus glucose in the media were lower in MSG than in control rats. In the same fat pad pieces, the conversion of 1-^{14}C-glycerol into fatty acids was always enhanced and its conversion into glyceride glycerol was enhanced when incubations were carried out in the presence of epinephrine or glucose. Both the hormone sensitive lipase activity and mRNA expression were lower in adipose tissue from MSG rats. Besides, the number of insulin receptors, lipid synthesis from U^{14}C glucose, ^{3}H-2-deoxy D-glucose uptake and cellular GLUT4 translocation index were higher in adipocytes from MSG rats than from the controls.
CONCLUSION: It is proposed that an enhanced insulin sensitivity in 1 month old MSG rats is responsible for the decreased lipolytic activity and enhanced glucose uptake. In addition, the enhanced lipogenesis and glycerol reutilization seen in their adipose tissue, disturbs the normal balance between fat depots breakdown and accumulation in favor of the latter.
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Keywords: monosodium glutamate obesity; lipolysis; hormone sensitive lipase; glycerol; GLUT4

Introduction

Obesity, which is defined as body fat excess, usually develops slowly as a result of long-term alterations in energy balance. In general, when food intake exceeds energy expenditure, the retained energy is deposited as fat. Neonatal administration of monosodium glutamate (MSG) to rodents destroys 80–90% of the arcuate nuclei neurons[1,2] and injures other central structures[3] resulting in several neuroendocrine and metabolic abnormalities, including arrested growth and obesity.[3,4] Unlike obesity induced by gold thioglucose or bipiperidyl mustard which are associated with hyperphagia, obesity in neonatal MSG-treated rodents, is not a result of overeating. In mice, MSG obesity is associated with a doubling of metabolic efficiency,[5] secondary to the high level of corticosterone.[6] In MSG-treated rats high levels of corticosterone have also been described[7,8] and this is probably related to the central (via sympathetic nervous system) or directly suppresive effect of glucocorticoids on brown adipose tissue (BAT) activity.[9,10] In most types of obese animals, BAT is in a thermogenically quiescent and relatively atrophied state. Recently, reduced GLUT4 in BAT was found in both 30 and 90 day old MSG-treated rats.[11] The inactivity of BAT results in a deficit in energy expenditure for thermogen-

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esis and it is believed to contribute to the high metabolic efficiency and obesity of these animals. We have previously shown that MSG-rats had lower carcass protein and higher carcass lipid content than controls.[12] Exhaustion of lipid stores was almost complete after 10 days of fasting in control rats (83% loss), while the MSG rats lost only 45% of their initial stores, indicating a lower rate of energy expenditure.[12] Indeed, adipose tissue preparations from 3 month-old neonatally MSG-treated rats showed decreased responsiveness to lipolytic agents.[12,13] Otherwise, 90 day old MSG treated rats showed increased lipogenesis[14] associated with a decreased GLUT4 content.[15] Therefore, the massive accumulation of adipose tissue in these animals seems to be related to an imbalance between the signals which control both the final lipolytic response and the lipogenic activity.

Although under normal conditions the capability of adipose tissue to utilize glycerol is very low[16] due to negligible glycerokinase activity,[17,18] the activity of the enzyme has been shown to be elevated in the adipose tissue of different forms of genetic obesities,[19] which may contribute to an enhanced capability of the tissue to reesterify the released fatty acids, and therefore promote net fat accumulation. On the other hand, hormone sensitive lipase (HSL) is the key enzyme that controls the break-down of endogenous triglycerides. Conditions like fasting,[20] pregnancy [21] and hibernation[22] have been shown to modify the activity of this enzyme in adipose tissue, therefore contributing to the respective amount of fat depots.

In an attempt to gain further insights into the development of MSG-induced obesity, lumbar adipose tissue cellularity, *in vitro* lipolysis and glycerol and glucose utilization, as well as HSL activity and mRNA expression and GLUT4 content in lumbar adipose tissue were studied in 1 month old neonatally MSG-treated rats, when net fat accumulation has not yet developed.

Material and methods

Animals

Male offspring Wistar rats were injected 5 times subcutaneously, every other day, with 4 g/kg b.w. MSG (monosodium glutamate, from Sigma, USA) or hyperosmotic saline (1.25 g/kg b.w., controls) within the first 10 days of life. Pups were weaned on the 21st day of life and kept in plastic cages of 40 × 30 × 18 cm (10 per cage). They were maintained under controlled light (0700 to 1900 h) and temperature (23 ± 1°C) conditions and had free access to food and water. At the 30th day of age, animals were decapitated and trunk blood was collected in ice-chilled heparinized tubes. Plasma was separated by centrifugation at 15 000 × *g* at 4°C and kept at 20°C until analysis for glycerol,[23] NEFA,[24] tiglycerides,[25] cholesterol[26] and insulin by a rat-specific radioimmunoassay kit (Novo Industry A/S, Copenhagen, Denmark). Glucose was assayed by a glucose oxidase kit (Boehringer Mannheim, Mannheim, Germany).

Lipid carcass content

After shaving and removing the gastrointestinal tract, carcasses were warmed overnight at 45°C. Softened carcasses were then homogenized in a blender and lipids extracted from 5 g aliquots with petroleum ether and determined gravimetrically.

In vitro lipolytic activity and glycerol utilization

Pieces of lumbar fat pads (around 10 mg) were placed in 20 ml vials containing 0.5 ml of Krebs–Ringer bicarbonate (KRB) supplemented with 20 mg/ml of free fatty acids free bovine serum albumin. 0.5 ml of KRB without bovine albumine but containing 1-^{14}C-glycerol (0.5 μCi, 20 μM) (from the Radiochemical Center, Amersham, UK) and supplemented or not with 10 mM D-glucose and/or 0.1 μg epinephrine bitartrate were pipeted into each vial. They were sealed, gassed with O_2/CO_2 (95 : 5) for 5 min and incubated for 90 min at 37°C in a Dubnoff metabolic shaker set at 100 cycles/min. The incubation was stopped by placing the vials on ice. Aliquots of the media were placed in 10% $HClO_4$ in the cold and supernatants were neutralized with saturated $KHCO_3$ to measure glycerol.[23] Other aliquots of the medium were used to measure NEFA.[24] The tissue pieces were thoroughly rinsed with saline and placed in chloroform-methanol (2 : 1, by vol) for lipid extraction[27] and fractionation.[28] Radioactivity values were normalized by considering 10^6 dpm the total radioactivity present in each vial at the onset of the incubations.

Hormone-sensitive lipase mRNA expression and activity

Total cellular RNA was isolated from lumbar fat adipose tissue by following a single-step acid guanidinium thiocyanate-phenol-chloroform extraction method[29] and Northern blot analysis for HSL mRNA levels were done as previously described.[21] HSL activity was measured in infranatants of adipose tissue samples homogenized at 4°C in 3 vol of 0.25 M sucrose containing 1 mM EDTA, 1 mM dithioerythreitol, 10 μg/ml leupeptin, and 10 μg/ml antipain, at pH 7.4, and centrifuged at 110 000 × *g* in a Beckman TL-100 centrifuge for 45 min at 4°C, with a phospholipid-stabilized emulsion of a dioleoylglycerol ether analogue, 1(3)-mono[^{3}H]oleyl-2-O-oleoylglycerol as substrate.[30] Protein was determined according to the Bradford method.[31]

Determination of GLUT4 in plasma membranes

Subcellular membrane fractions of lumbar adipose tissue were prepared as described by Oka *et al*[32] taking pooled tissue samples for homogenization, instead of fat cell suspension. To prepare each membrane fraction, lumbar adipose tissue from 4 to 6 rats were homogenized in 7 wt/vol buffer (10 mM Tris-HCL, 1 mM EDTA and 250 mM sucrose, pH 7.4) and centrifuged at 3000 × *g* for 15 min. Fat cakes were discarded, and the infranatant fat-free extract was

submitted to a second centrifugation at 12000 $\times g$ for 15 min to precipitate the plasma membrane fraction (PM). Supernatant was centrifuged at 28000 $\times g$ for 15 min and the resulting supernatant was centrifuged at 146000 $\times g$ for 75 min. The final pellet was resuspended and referred to as the microsomal fraction (M).

Western blotting

A 12 amino acid peptide of GLUT4, synthesized according to the deduced carboxy-terminal sequence of this glucose transporter (kindly provided by Dr J Utsumi, Toray Basic Research Laboratory, Fujisawa, Japan), was coupled to key-hole, and used for the immunization of male New Zealand rabbits. Serum was collected 2 months after injection and used for immunoblotting at 1:100 dilution. The specificity of anti-GLUT4 antibody was checked in membrane fractions of human red blood cells (HRBC) which contain a high concentration of GLUT1. No GLUT4 immunoactivity was detected in the HRBC.

Membrane samples (40 µg protein) were solubilized in Laemmli's sample buffer, subjected to SDS-PAGE (10%) and electrophoretically transferred to nitrocellulose paper. After blocking with bovine serum albumin, the sheets were incubated with the antiserum raised against GLUT4, with a subsequent washing and incubation with [^{125}I] protein A (Amersham Life Sciences). Blot densities were quantified through the densitometry using imagemaster® software (Imagemaster® 1D, Pharmacia Biotech). The GLUT4 translocation index was calculated using the total cellular content of PM and M GLUT4 following PM $\times$100/(PM + M).

Isolation and cellularity of adipocytes

The lumbar fat pads were excised and minced, and incubated for 50–55 min in plastic cups with 4 ml of a medium containing Dulbecco's modified eagle's medium (DMEM) (9.6 g/l), 25 mM HEPES, 40 mg/ml BSA, 2.0 mg/ml collagenase type II, pH 7.4 at 37°C, according to Rodbell.[33] Cells were filtered through a fine nylon mesh and washed three times with 25 ml of HANKS, 20 mM HEPES, 1% BSA (HANKS-HEPES-BSA buffer, HHB), pH 7.4 at 37°C. They were resuspended in the same buffer until a 5% lipocrit was reached, and their volume and number determined.[34]

Glucose transport assay

475 µl aliquots of the 5% lipocrit adipocyte suspension were incubated in a shaking water-bath at 37°C for 30 min. A 2-deoxy-[2,6-^{3}H]-D-glucose (^{3}H-2DG) pulse (25 µl) (0.12 µCi/tube and 0.1 mM, final concentration) was added at the end of the 30 min period of pre-incubation and the transport reaction was interrupted exactly 3 min after centrifuging 200 µl aliquots of the reaction mixture through silicon oil. The cell pellet on top of the oil was dipped into a scintilation cocktail for aqueous samples. In a parallel tube, the same procedure was repeated except that a [1-^{14}C]-L-glucose pulse (0.1 µCi/tube) was used instead of ^{3}H-2DG, in order to assess the non-specific uptake.

Insulin receptor number assay[35]

The isolated adipocytes were washed three times in HHB buffer, pH 7.8 at 16°C, and resuspended to achieve 10% cell concentration (v:v). Aliquots (450 µl) from this cell suspension were pipetted into test tubes containing ^{125}I-monoiodo-A_{14}-insulin (Amersham Co., UK)(10000 cpm/tube, 25 µl) and cold insulin (25 µl) in increasing concentrations (0, 25, 50, 100, 250, 1000, 2500, 10000, 10^6 nM) and the mixture was incubated for 3 h at 16°C (since at this low temperature the insulin internalization process is negligible). At the end, 200 µl aliquots of the mixture were collected and transferred to microfuge tubes (400 µl capacity) previously filled with 100 µl of silicone oil (Dimethylsilicone fluid, Thomas Scientific, Swedesboro, NJ, USA) and centrifuged for 6 seconds in a Beckman microfuge E (Beckman Instruments Inc., USA). The cell pellet on the top of the oil layer was collected and the incorporated radioactivity measured in a gamma counter (Abbot). The tube containing 10^6 nM cold insulin was used to determine the non-specific radioactive tracer trapping, which was discounted from the remaining tubes. Scatchard data analysis was performed to determine the insulin receptor number and results were normalized per 10^5 cells.

Glucose oxidation

The isolated adipocytes were first washed and then resuspended to a 5% lipocrit (as described above) in KRB buffer, pH 7.4 at 37°C with 10 mg/ml BSA and 2 mM glucose, previously gassed for 15 min with 95% O_2/5% CO_2.

[U-^{14}C]-D-glucose oxidation was assayed using a modification of the protocol described by Rodbell.[33] In brief, 450 µl aliquots of the 5% lipocrit cell suspension were transferred to 17 $\times$ 100 mm plastic tubes containing 50 µl of D-[U-^{14}C]-glucose (0.1 µCi/tube and 2.0 mM final concentration). The vials were gassed with 95% O_2/5% CO_2, tightly capped, and incubated for 60 min at 37°C in an orbital shaking water-bath (180 rpm). At the end of the incubation, 150 µl of 8N H_2SO_4 was added to the mixture and the $^{14}CO_2$ released was collected by adsorption onto a Whatman No. 2 filter paper previously impregnated with 200 µl ethanolamine and coiled in a small plastic well fixed on top of the tube. The filter papers were then transferred to scintillation vials for radioactivity measurement.

Glucose incorporation into total lipids

After the collection of $^{14}CO_2$ in the above assay, lipids from cell lysates were extracted with Dole's reagent (isopropanol: heptane 4:1 v/v)[36] and the heptane fraction of the extracted lipids was transferred to scintillation vials for determination of ^{14}C incorporated.

Statistical analysis of the data

Results are expressed as means ± s.e. Analysis of variance was used to test the significance of the differences between groups. Where differences were statistically significant, they were tested by Tukey and Rsquo's test. Differences between two groups were analyzed by Student's unpaired test.

Results

As shown in Table 1, 30 day old rats given MSG when neonates showed a significantly lower body weight than their controls, whereas their percentual fat carcass content and lumbar fat pad weight did not differ when comparing the two groups. However, as also shown in Table 1, lipid content per cell, adipose cell diameter, surface area and volume appeared significantly higher in MSG rats than in controls. Otherwise, cell number was lower in MSG rats than in controls.

As shown in Table 2, plasma glucose, insulin, NEFA, glycerol and triglyceride concentration were significantly lower in MSG compared to control rats whereas plasma cholesterol did not differ between the two groups.

In vitro production of both glycerol and NEFA was studied in pieces of lumbar fat pads incubated under different conditions. As shown in Table 3, when incubated without glucose and epinephrine in the medium, the release of glycerol into the medium presented no difference between tissues from both control and MSG rats, while the production of NEFA was significantly lower in tissues from MSG rats compared to the controls. Epinephrine significantly enhanced the production of both glycerol and NEFA in tissues from both groups incubated in the absence of glucose, although the increase in NEFA found in the tissues from MSG rats (92%) was smaller than the one seen in those from control rats (160%), and the difference between the groups remained statistically significant. When glucose was present in the medium, the production of both glycerol and NEFA in the tissues incubated under basal conditions (that is, in the absence of epinephrine) was lower than in those studied in the absence of glucose, and no differences between MSG and control rats could be detected. In the presence of glucose, epinephrine enhanced the production of glycerol and NEFA in tissues from both groups although the effect on NEFA but not on glycerol was smaller in MSG rats (141%) than in control rats (303%).

In order to check whether adipose tissue from MSG and control rats differed in their capability to reutilize the released glycerol, incubations of pieces from lumbar fat pads were carried out in the presence of tracer amounts of 1-^{14}C-glycerol and its conversion into tissue lipids was measured. As shown in Table 4, when incubations were carried out in the absence of glucose, the appearance of radioactivity in tissue lipids mainly corresponded to the glyceride glycerol fraction and did not differ when comparing the control and MSG rats, although the amount of radioactivity present in fatty acids was higher in the latter group. In the absence of

Table 1 Body weight, carcass lipid content and white adipose tissue cellular characteristics from 1 month-old MSG treated-rats

	Control	*MSG*
Body weight (g)	95.8 ± 1.6	72.9 ± 1.5*
Lipid carcass content (%)	12.4 ± 1.4	14.4 ± 1.1
Fat pad weight (g)	0.25 ± 0.01	0.23 ± 0.02
Lipid content per cell (ng)	40.4 ± 1.6	61.3 ± 2.2*
Cell diameter (μm)	43.9 ± 0.5	50.4 ± 0.6*
Cell surface (μm^2)	6056 ± 149	7985 ± 180*
Cell volume (pl)	44.4 ± 1.6	67.3 ± 2.2*
Cell number ×10^5/ml	11.3 ± 1.8	7.4 ± 0.9*

*$P < 0.001$ compared to controls.
$n = 6$ rats/group.

Table 2 Plasma level of metabolites and insulin in 1 month-old MSG-treated rats

	Control	*MSG*
Glucose (mmol/l)	7.4 ± 0.1	6.3 ± 0.1*
Insulin (pmol/l)	387 ± 19	292 ± 9*
NEFA (mM)	284 ± 32	173 ± 13*
Glycerol (μM)	232 ± 8	114 ± 5*
Triglycerides (mg/dl)	134.0 ± 11.8	87.5 ± 4.1*
Cholesterol (mg/dl)	87.8 ± 2.9	90.9 ± 3.7

*$P < 0.001$ compared to controls.
$n = 6$ rats/group.

Table 3 *In vitro* glycerol and NEFA release by lumbar fat pad pieces from 1 month-old MSG-treated rats

	Glucose (5 mM)	−	−	+	+
	Epinephrine (0.5 μg/ml)	−	+	−	+
Glycerol (nmol/100 mg)	Control	220 ± 30^a	376 ± 43^b	65 ± 7^c	738 ± 106^d
	MSG	237 ± 24^a	416 ± 42^b	60 ± 17^c	890 ± 60^d
	P	NS	NS	NS	NS
NEFA (nmol/100 mg)	Control	415 ± 52^a	1078 ± 120^b	220 ± 27^c	886 ± 92^b
	MSG	257 ± 23^a	493 ± 20^b	152 ± 27^c	366 ± 40^b
	P	< 0.05	< 0.001	NS	< 0.01

$n = 4-6$ rats/group.
Superscript letters indicate the statistical comparisons between the different incubation conditions within each group (different letters = $P < 0.05$) whereas *P* values correspond to the comparison between the two groups (NS = Not significant, $P > 0.05$).

glucose the presence of epinephrine in the medium greatly decreased the appearance of radioactivity in tissue total lipids, the effect being smaller in tissues from MSG rats (50%) than from controls (71%). Total lipids, fatty acids and glyceride glycerol showed significant difference between MSG and controls although nonsaponifiable lipids were not. In the presence of glucose in the incubation medium but in the absence of epinephrine, tissues from MSG rats incorporated more radioactivity in total lipids than control rats, the effect corresponding to all the lipidic fractions (Table 4). In the presence of glucose, the addition of epinephrine to the medium greatly reduced the conversion of labelled glycerol into tissue lipids, but still under this condition, tissues from MSG rats showed a higher amount of radioactivity in total lipids than those from controls, and this effect mainly corresponded to the label appeared in fatty acids.

As shown in the left part of Figure 1, HSL activity was significantly lower in the adipose tissue of MSG rats than in control rats. Northern hybridization techniques were used to explore the effect on HSL mRNA extracted from lumbar adipose tissue and the results are illustrated in the right part of Figure 1. The insert of this figure shows a representative auto radiogram of the hybridization of the cDNA probe for HSL mRNA in both experimental groups. Lumbar adipose tissue from MSG rats showed decreased HSL mRNA in comparison to control animals.

The adipocyte functionality related to glucose utilization was evaluated and values are shown in Table 5. When incubated in the presence of U-^{14}C-glucose both the incorporation of ^{14}C into total lipids and into CO_2 were significantly higher in adipocytes from MSG than from control rats. The uptake of ^{3}H-2-deoxy-D-glucose was also higher in adipocytes from MSG than from control rats, whereas plasma membrane GLUT4 content did not differ in the two groups, the microsomal GLUT4 content was higher in controls than in MSG rats, also causing a higher GLUT4 translocation index in the latter. The amount of insulin receptors in adipocytes from 1 month-old MSG rats was significaty higher (4.92 ± 0.63 fm/10^5, $n=6$) than that from control rats (1.76 ± 0.09, $n=6$, $P<0.001$).

Discussion

The present study shows that already at the age of 30 days neonatally MSG-treated rats weigh less than control rats, which agrees with the information found in previous reports,[13,37] but percentual carcass lipid content remains constant. This finding also agrees with the fact that mice neonatally injected with MSG do not become obese until puberty.[4] Decreased body weight in these animals may be associated to decreased growth hormone (GH) levels, which was previously found in 30 day old MSG-treated rats.[38,39] Decreased plasma levels of both NEFA and glycerol as well as decreased adipose tissue lipolysis and HSL activity found in our MSG-treated rats also support such decreased GH levels, since these changes have been reported to occur in GH deficient conditions in adults.[40]

However, adipose tissue cellularity shows that adipocytes in our 1 month old MSG-rats are already enlarged, indicating that they are initiating the development of obesity of the hypertrophic type, as has also been shown to occur in MSG-treated mice.[41] The present findings also show that at this early stage of development, the adipose tissue of the MSG-rats is already greatly functionally modified. Adipose tissue lipolytic activity is clearly decreased in these animals, as shown by the decreased *in vitro* release of NEFA under basal and epinephrine-stimulated conditions, the low activity and mRNA expression of HSL seen in this tissue as well as the

Table 4 Tissue radioactivity in pieces of lumbar adipose tissue from 1 month-old MSG-treated rats incubated *in vitro* in the presence of 1-^{14}C-glycerol

		Corrected DPM/100 mg tissue			
	Glucose (5 mM)	−	−	+	+
	Epinephrine (0.1 μg/ml)	−	+	−	+
Total Lipid	Control	235375 ± 43734^{a}	69106 ± 6943^{b}	121861 ± 19694^{c}	41272 ± 2921^{d}
	MSG	206956 ± 43396^{a}	97483 ± 5314^{b}	205406 ± 12494^{a}	65067 ± 7218^{d}
	P	NS	<0.01	<0.01	<0.01
Fatty acids	Control	2310 ± 610^{a}	597 ± 265^{b}	42399 ± 6565^{c}	7139 ± 1259^{b}
	MSG	4503 ± 163^{a}	3106 ± 113^{b}	67573 ± 688^{c}	18805 ± 4087^{d}
	P	<0.01	<0.001	<0.01	<0.05
Nonsaponifiable Lipids	Control	949 ± 43^{a}	831 ± 25^{b}	943 ± 49^{a}	521 ± 20^{c}
	MSG	918 ± 27^{a}	813 ± 28^{b}	2405 ± 307^{c}	761 ± 14^{b}
	P	NS	NS	<0.001	<0.001
Glyceride-glycerol	Control	228711 ± 45193^{a}	66305 ± 6576^{b}	74568 ± 10320^{b}	30239 ± 3898^{c}
	MSG	194985 ± 36821^{a}	97445 ± 6076^{b}	135428 ± 18655^{a}	45501 ± 9928^{c}
	P	NS	<0.01	<0.05	NS

$n=4-6$ rats/group.
Superscript letters indicate the statistical comparisons between the different incubation conditions within each group (different letters = $P<0.05$) whereas *P* values correspond to the comparison between the two groups (NS = Not significant, $P>0.05$).

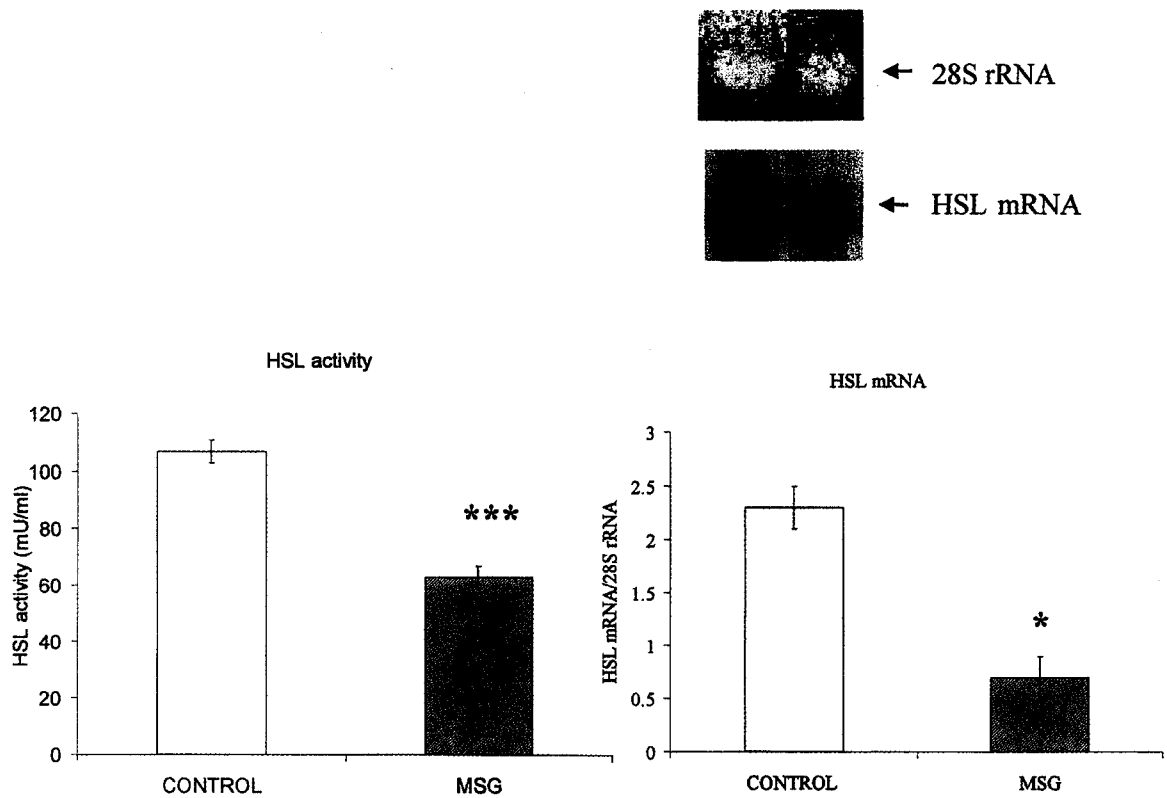

Figure 1 HSL activity and mRNA in adipose tissue of 1 month old neonatally MSG treated rats. Total HSL, activity (left side of the figure) and HSL mRNA (right side of the figure) were measured as described in the Materials and methods section. The insert of the figure shows a representative autoradiogram of hybridation of cDNA probes for HSL mRNA and a photograph of the gel showing the band corresponding to the 28S rRNA. Statistical comparison between the two groups are shown by asterisks: * = $P < 0.05$, *** = $P < 0.001$. $n = 6$ rats/group.

Table 5 Incorporation of U-^{14}C-glucose into total lipids and $^{14}CO_2$, ^{3}H-2-deoxy-D-Glucose uptake and GLUT4 content in adipocytes from lumbar adipose tissue of 1 month-old MSG-treated rats

	Control	*MSG*
^{14}C-lipid from ^{14}C-glucose (pmoles $\times h^{-1} \times 10^{-5}$ cells)	42.2±4.7	115.7±23.1*
$^{14}CO_2$ production from ^{14}C-glucose (pmoles $\times h^{-1} \times 10^{-5}$ cells)	22.9±3.4	59.6±14.8*
^{3}H-2-deoxy-D-Glucose uptake (pmoles $\times 10^{-5}$ cells $\times 3$ min)	53.8±5.1	151.6±12.0*
Plasma membrane GLUT4 (PM) (cpm/μg protein)	33.7±4.2	36.8±5.6
Microsomal GLUT4 (M) (cpm/μg protein)	54.9±2.1	39.4±2.28*
Index of cellular GLUT4 translocation (PM × 100/(PM+M))	46.6±4.6	63.7±6.4*

*$P < 0.01$ compared to controls.
$n = 6$ rats/group.

decreased plasma NEFA and glycerol levels. A decreased availability of lipolytic products to the liver could also explain the decreased plasma triglyceride levels found in the 1 month old MSG-rats. This is the first time that such decreased adipose tissue lipolytic activity is reported in these animals, and although this change contrasts with the decreased plasma insulin level seen in these animals, an enhanced insulin sensitivity condition may occur as will be discussed below. An inverse relationship between plasma insulin and adipose tissue lipolytic activity has also been reported in NZO mice.[42]

In vitro studies using epididymal adipose tissues from 3 month old MSG-rats have found both basal and isoproterenol stimulated lipolytic response diminished.[12,13] A decreased tissue NE content and slow rates of NE turnover have been reported in both the interscapular brown adipose tissue (IBAT) of 3-weeks old pre-obese MSG mice[43,44] and white adipose tissue of 12–18 week old MSG rats.[13,45] It may therefore be possible that in our 1 month old MSG-rats, the sympathetic regulation of lumbar adipose tissue activity is impaired, being then responsible for the modified lipolytic response to the epinephrine stimulus.

The present findings clearly show that adipose tissue of 1 month old MSG rats have an enhanced capability to reutilize the lipolytic released glycerol for both fatty acids and glyceride glycerol synthesis, the effect being specially manifested when pieces of tissue were incubated in the presence of epinephrine and/or in the presence of glucose. This enhanced conversion of labeled glycerol to lipids under conditions

where the amount of cold glycerol in the medium did not differ between the two groups, indicates that the effect is not an artefact due to the different dilution of the tracer. Utilization of glycerol by adipose tissue is normally very low[16,17] due to its low glycerolkinase activity,[18] but it may be acutely modulated by insulin, catecholamines, glucose and metabolic inhibitor agents.[28,46–48] Besides, it has also been shown that glycerolkinase activity is enhanced in certain types of obesity,[19] and therefore, it may represent a key step in the capability of the tissue to reesterify fatty acids, controling its balance between triglyceride breakdown and accumulation. The enhanced capability of adipose tissue from our neonatal MSG-rats to reutilize the lipolytic released glycerol, may therefore contribute to their development of obesity.

Besides the decreased adipose tissue lipolytic activity and enhanced glycerol reutilization, an increase in glucose uptake and its conversion into lipids could also be contributing to the enhanced adipocyte lipid content present in the 1 month old MSG-rats, and this change could be a consequence of the increased index of cellular GLUT4 translocation seen here in their adipocytes. This finding agrees with the fact that in adipocytes of pre-obese fatty rats both GLUT4 mRNA and protein levels have been shown to be more than twice those of controls,[49] whereas a decreased glucose transporter (GLUT4) content was found in epididymal adipose tissue of adult obese aurothioglucose and MSG-treated mice[15] and MSG rats.[50] Although not directly measured *in vivo*, the decrease in both glycemia and insulinemia, the enhanced incorporation of U-^{14}C-glucose into lipids and the increased ^{3}H-2-deoxy-D-glucose uptake as well as the increased number of receptors found here in adipocytes of 1 month old MSG-rats would suggest an enhanced insulin sensitivity condition. The decreased HSL activity and mRNA expression, found in adipose tissue of our MSG-treated rats also supports this conclusion, since conditions of hyperinsulinemia and decreased insulin sensitivity, like that taking place during late pregnancy, are known to have the opposite effect on these variables.[21] Enhanced insulin sensitivity in 1 month old MSG-rats may occur despite hyperinsulinemia and insulin resistance being reported in adult MSG-rats,[51] since a similar transient insulin hypersensitivity followed by decreased insulin responsiveness in white adipose tissue has been reported to occur in both VMH-induced obesity[52] and obese Zucker rats.[53]

The present findings do not allow us to establish the order of the different factors contributing to the later development of obesity in the MSG rats. The results reported here however indicate that the enhanced insulin sensitivity present in 1 month old MSG-rats, could contribute, and even be the inductive factor of several of the metabolic alterations taking place in their adipose tissue, which clearly must contribute to the development of obesity: a decreased lipolytic activity and an enhanced glucose uptake, lipogenesis and glycerol reutilization, which would disturb the normal balance between the breakdown of fat depots and the accumulation in favor of the latter.

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