Endocr (2009) 36:510–517
DOI 10.1007/s12020-009-9263-2

ORIGINAL ARTICLE

Autonomic activity and glycemic homeostasis are maintained by precocious and low intensity training exercises in MSG-programmed obese mice

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Received: 23 March 2009 / Accepted: 18 September 2009 / Published online: 24 October 2009

Abstract Current research employed electrical records from superior vagus and sympathetic nerve branch that supply fat retroperitoneal tissue (RS nerve) to investigate whether very moderate swim training in obese-programmed mice would change sympathetic and parasympathetic autonomic nervous system activities. Neonatal mice were treated with monosodium L-glutamate (MSG), during their first 5 days of life, to induce obesity. Mice started training on weaning, comprising free swimming 3 days/week, 15 min/day for 10 weeks. After 12 h fasting, the nerve electrical signals of the 90-day-old mice were processed to obtain firing rates. Blood samples were collected to measure glucose and insulin levels. Adrenal catecholamine content was measured. MSG treatment caused obesity. Hyperglycemia and hyperinsulinemia in MSG-obese mice, without any change in food intake, were obtained. Vagus firing rates were higher in obese mice than those in lean ones. A decrease in RS nerve activity and lower adrenal catecholamine stores have been observed. Swimming normalized blood glucose and insulin levels and MSG-obesity onset was attenuated by exercise. Vagus activity from obese mice decreased, whereas RS nerve activity and adrenal catecholamine levels increased in trained ones. Results suggest that autonomic activity imbalance and metabolic dysfunctions observed in MSG-obese mice were inhibited by precocious and moderate exercise training.

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Keywords Mice · Parasympathetic activity · Sympathetic activity · Exercise training · MSG-obesity

Introduction

Metabolic diseases, such as type 2 diabetes, among others, are closely associated with uncontrolled body weight [1, 2]. Overweight and obesity are worldwide, including developing countries. It has also been shown that calorie amounts are not the only reason foregrounding the obesity epidemic [3]. Insufficient or lack of physical activity is another cause for uncontrollable increase in body weight [4]. All the pathophysiological mechanisms that underlie the onset of obesity are unfortunately unknown, although the central nervous system (CNS) has a predominant role in the control of body weight [5]. Indeed many pharmacological strategies for the treatment of obesity malaises have been targeted on CNS [6]. Body mass control is organized by many CNS areas. The hypothalamus concentrates neuron groups which, after receiving humoral and neural signals from periphery, change food intake and spend nutrient storage and/or energy stocks [7]. Lesions and injuries in some hypothalamic areas, such as ventromedial (VMH), arcuate (ARC), and paraventricular (PVN) areas, provoke obesity [8, 9]. Afferent and efferent signals, which allow brain to control body weight, are mostly conducted to the autonomic nervous system (ANS) [10, 11]. Experimental models of obesity involving humans and certain animals present high parasympathetic and low sympathetic activity [12, 13]. ANS imbalance that governs the dual behavior, or rather, the decreased activity of the parasympathetic nervous system (PNS) and the low activity of the sympathetic nervous system (SNS), foregrounds the

autonomic theory that explains the main metabolic features observed in obesity [14].

It has been known that exercise may improve clinical hallmarks shown by metabolic syndrome. Hyperglycemia, high insulin blood levels, hypertension, dyslipidemia, and tissue insulin resistance may be improved by physical activities [15, 16]. A short exercise is enough to reduce insulin resistance [17], even though this benefit is also rapidly reversible [18] if another exercise session is not undertaken. As a rule, exercise stimulates SNS which mobilizes fat reserves to supply the cell energy demand with fatty acids, mainly through the muscle tissue. Rats submitted to swimming exercises present high blood concentration of free fatty acid. Further, it has also been shown that fat mobilization is dependent on SNS activity and on adrenaline secretion from adrenal medullar cells [19–21]. Few data exist on the effect of long-term exercise on SNS activity. In addition, results of sympathetic activity are indirectly obtained by their action on fat tissue, for instance, free fatty acid blood concentration, catecholamine, adrenaline, and noradrenaline blood levels, and tissue noradrenaline turnover [22–24]. However, no data are extant that directly measure ANS nerve activity in obese animals or as an effect of exercise.

Current study investigated whether a moderate swimming program applied during the weaning period is able to change ANS activity in mice obese-programmed by neonatal treatment with L-monosodium glutamate (MSG). A branch of vagus and sympathetic nerves was used to register their electrical firing rate.

Results

Figure 1a shows that MSG treatment caused a 13.6% increase in mice's Lee index when compared to that in untreated ones ($P < 0.001$). Exercise provoked a Lee index reduction of 10.7% in MSG-mice when compared to that in sedentary MSG-mice ($P < 0.001$). Swimming program also caused a 6.6% decrease in the Lee index of MSG-untreated mice.

Figure 1b shows that MSG treatment induced a 32.0% enhancement of periepididymal fat pat weight when compared with that in untreated animals ($P < 0.001$). Retroperitoneal fat accumulation also increased threefold in MSG-treated mice when compared to that in untreated ones ($P < 0.05$), as Fig. 1c shows. Swimming training caused 41.0 and 44.0% decrease in fat accretion, respectively, on gonadal and retroperitoneal tissue of MSG-mice when compared to that in sedentary animals ($P < 0.05$). Swimming also reduced fat accumulation, respectively, by 58.0 and 46.2% in fat mass of periepididymal and retroperitoneal tissues in MSG-untreated mice when compared to that in sedentary ones ($P < 0.01$), as Fig. 1b and c shows, respectively.

Table 1 shows that MSG treatment does not change total chow intake. There was no change in food consumption in mice from both experimental groups submitted to swimming training.

MSG treatment provoked 24.0% increase in blood glucose levels when compared to those in untreated ones ($P < 0.05$). Table 1 shows that blood insulin concentration was also raised by 3.0 times by MSG treatment when compared with that in controls ($P < 0.05$). Although swimming program failed to modify fasting blood glucose and insulin levels in MSG-untreated mice, a decrease in glucose and insulin plasma concentrations, respectively, 11.3 and 44.9%, occurred in MSG-mice submitted to exercise training ($P < 0.05$), as Table 1 also demonstrates.

Figure 2 shows that vagus nerve firing rate in fasting conditions was increased by 78% in MSG-mice when compared to that in controls ($P < 0.05$). While exercise did not cause any significant modification on number of spikes of vagus nerve in control mice, a 53.20% decrease of vagus

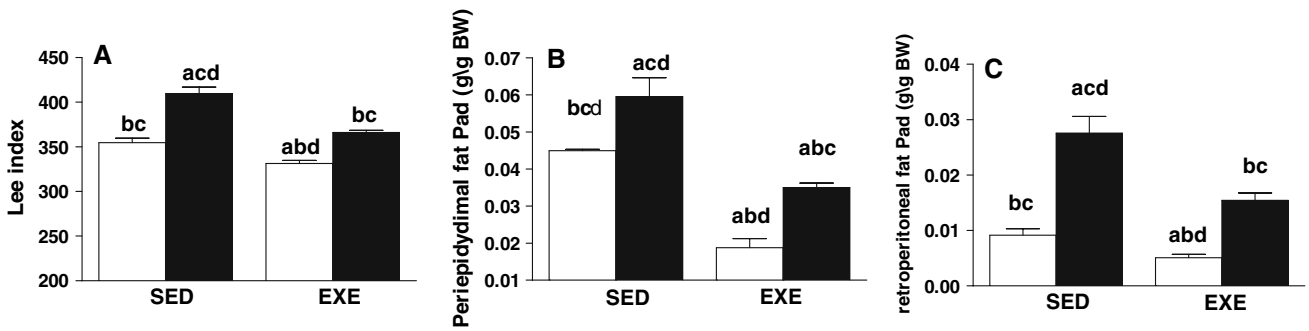

Fig. 1 Effect of MSG treatment and swimming training on Lee index, fat accumulation on epididymal and retroperitoneal tissues. *Bars* represent mean Lee index (**A**), epididymal (**B**), and retroperitoneal fat pads (**C**) obtained from 10–12 animals for each group; *open bars* represent control group and *filled bars* MSG group. *Lines* on bar top represent SEM. The *letters* over bars represent significant differences ($P < 0.05$) between *a*—control SED, *b*—MSG SED, *c*—control EXE, and *d*—MSG EXE

Table 1 Effect of MSG treatment and swimming training on food intake, blood glucose and insulin concentration

	SED		EXE	
	Control	MSG	Control	MSG
Glucose (mmol/l)	8.7 ± 0.2[b]	10.8 ± 1.4[acd]	8.9 ± 0.4[b]	9.6 ± 0.2[b]
Insulin (pmol/ml)	105.9 ± 20.4[b]	333.6 ± 41.6[acd]	85.2 ± 29.4[b]	183.9 ± 26.6[b]
Food intake (g)	81.2 ± 2.9	83.2 ± 5.1	77.8 ± 3.2	86.8 ± 2.0

Data represent the mean ± SEM of the area under the curve of the food intake, blood glucose, and insulin concentration obtained with 10–12 animals for each group, during 69 days, age between 21- and 90-days old. *Letters* over the data represents significant statistic differences ($P < 0.05$) between a—control SED, b—MSG SED; c—control EXE, and d—MSG EXE

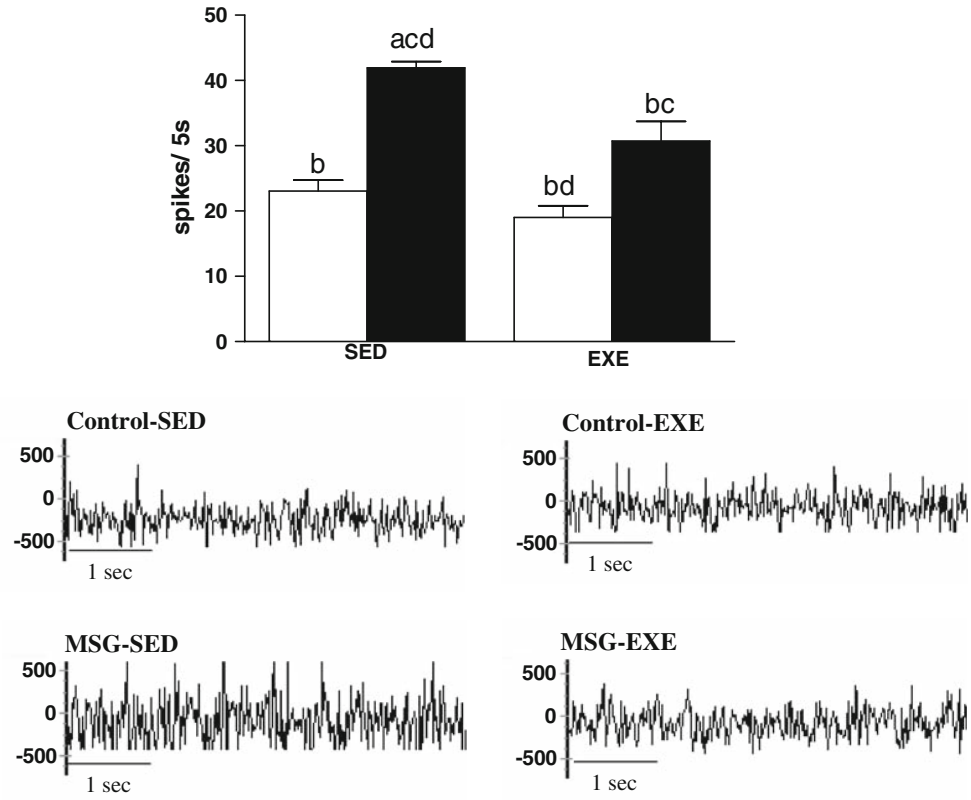

Fig. 2 Effect of MSG treatment and swimming training on vagus nerve electrical activity. *Bars* represent mean spike numbers obtained from 10–12 animals for each group; *open bars* represent control group and *filled bars* MSG group. *Lines* on bar top represent SEM. *Letters* over bars represent significant differences ($P < 0.05$) between *a*—control SED, *b*—MSG SED, *c*—control EXE, and *d*—MSG EXE. Representative records of nerve discharge from animals that illustrate data of each experimental group are given *beneath* the Figure

nerve electrical activity was reported in MSG-mice when compared to that in MSG-sedentary ones ($P < 0.05$).

A 60% decrease in electrical activity of the RS nerve was reported in adult mice due to MSG neonatal treatment when compared to that in untreated animals ($P < 0.05$). Figure 3 shows that swimming program increase by one- and fivefold the firing rate of RS nerve in control- and MSG-mice, respectively ($P < 0.05$).

Neonatal MSG-treated mice presented a 43.80% decrease in adrenal medulla catecholamine stores when compared to those in untreated animals ($P < 0.05$). Exercise induced an increase in adrenal catecholamine stocks by 44.30 and 57.70%, respectively, in control- and in MSG-mice when compared to those in sedentary animals ($P < 0.05$) (Fig. 4).

Discussion

Increment in rodent body's mass index and in its fat tissue shown in current paper confirms the fact that neonatal MSG treatment causes obesity in mice [25–27], without any increase in food intake. Other obesity animal models are somewhat dependent on food intake, such as VMH lesion [28], or on their genetic origin, such as ob/ob mice and Zucker rats [29, 30]. Results also show that MSG-obese mice are hyperglycemic and present high insulin blood levels, as has been reported by other authors [31, 32]. It has been reported that MSG-mice are tissue insulin resistant [33, 34]. In results not shown using insulin tolerance test, a low glucose disappearance rate in MSG-treated mice, which represents insulin resistance, has been

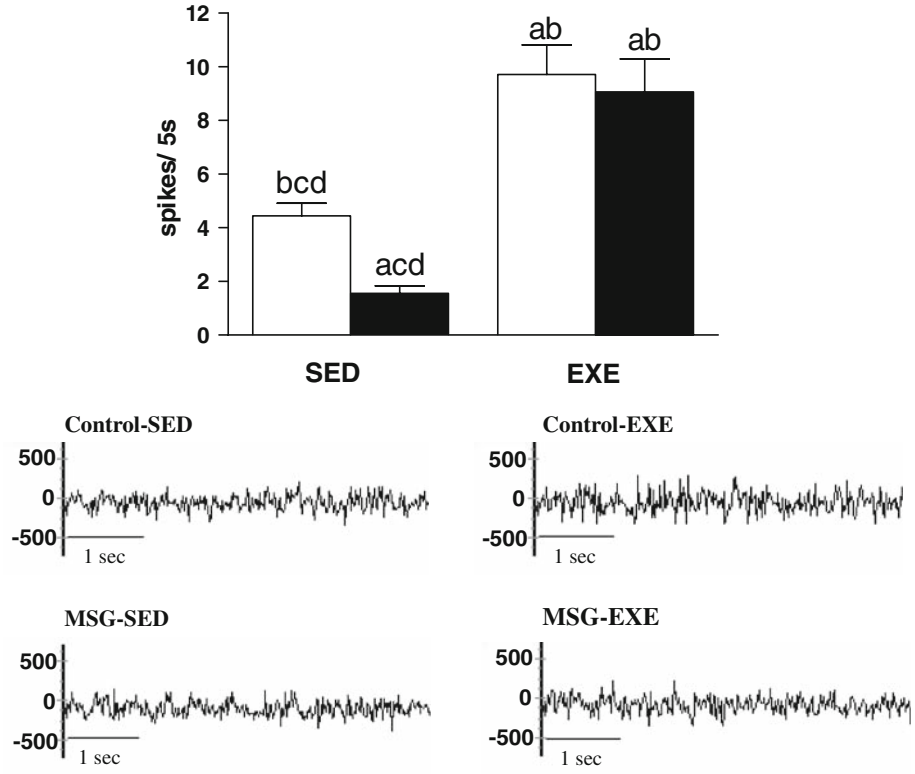

Fig. 3 Effect of MSG treatment and swimming training on RS nerve electrical activity. *Bars* represent mean spike numbers obtained from 10–12 animals for each group; *open bars* represent control group and *filled bars* MSG group. *Lines* on bar top represent SEM. *Letters* over bars represent significant differences ($P < 0.05$) between *a*—control SED, *b*—MSG SED, *c*—control EXE, and *d*—MSG EXE. Representative records of nerve discharge from animals that illustrate data of each experimental group are given *beneath* the Figure

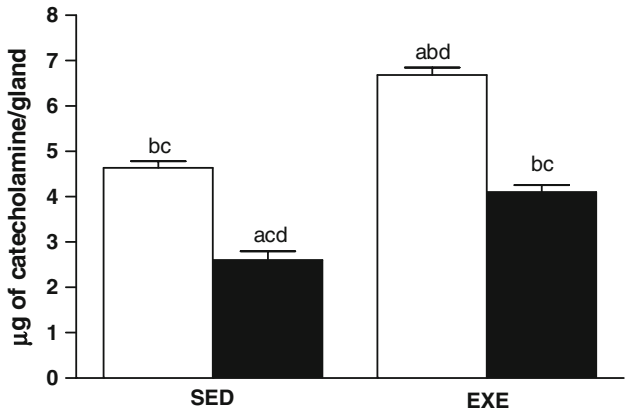

Fig. 4 Effect of MSG treatment and swimming training on total catecholamine contents from adrenal medullae. *Bars* represent mean total catecholamine contents from isolated adrenal medullae obtained from 10–12 animals for each group; *open bars* represent control group and *filled bars* MSG group. *Line* on bar top represents SEM. *Letters* over bars represent significant differences ($P < 0.05$) between *a*—control SED, *b*—MSG SED, *c*—control EXE, and *d*—MSG EXE

reported. Glucose homeostasis break observed in MSG obesity model has been attributed to the lesions on hypothalamic areas, such as ARC, that influence the body energy metabolism [35]. Although destruction of ARC neural circuits is expressed by alterations on autonomic functions [14, 25], the exact mechanisms are still unknown. However, evidence suggests that ANS is involved. Bilateral subdiaphragmatic vagotomy is able to attenuate or even block body weight gain, hyperinsulinemia, and insulin resistance, among other hallmarks dysfunctions, of rat obesity onset from different origins [36–39]. Stimulus on SNS, for instance, provided by acute swimming exercises, may improve or even correct insulin resistance, high insulin levels, hyperglycemia, and overweight [40].

Swimming exercise program was consistently able to diminish MSG-induced obesity. Exercise also imposed a significant reduction of fat tissue accumulation in MSG-untreated animals. The latter datum suggests that the group of animals considered lean is not actually lean, because exercise training reduces their fat storages. This fact shows that sedentary mice are overweight. It has been demonstrated that sedentary rats accumulated excessive fat on tissues [41]. Nevertheless, the swimming exercise was more effective in reducing fat deposition in MSG-treated than in MSG-untreated mice. It may be suggested that MSG-animals are metabolically disturbed whereas normal mice present a more balanced metabolism [38, 42]. When obesity risks are so evident, early intervention, such as physical activity, may change the destiny of people leading a Western life-style.

Recent results from our laboratory show that when very moderate swimming training is applied to weaned MSG-treated mice, their fat tissue accretion is drastically inhibited by the exercise, even after 5 weeks that exercise ceased [26]. These results were corroborated by Patterson et al., who registered inhibition of fat tissue deposits in voluntary

trained rats which were obese and genetically sensitive to high fat diet, after 10 weeks on end of training [43].

Exercise did not change blood glucose and insulin concentration in lean mice. However, fasting glycemia was normalized and hyperinsulinemia was drastically reduced in MSG-obese animals. Blocking of fat tissue gain and improvement of metabolism in MSG-treated and MSG-untreated mice were obtained with slight physical exercise. Low frequency, 3 times/week; short term, 15 min/day; and free swimming may be considered a light swimming program when compared to the moderate training program in which mice freely swim for 60 min, 5 days a week, during 18 weeks [44]. It has been observed that reduction of body weight gain is obtained by more aerobically vigorous exercise. This is due to high oxygen uptake (>70% of VO_{max}) and high session frequency, at least 5 sessions/week [45, 46]. Research in our laboratory recently showed that the same 30-day exercising program used in current study did not halt fat tissue accumulation when applied to sexually mature (post-puberty stage) 60-day-old mice [26]. It is well-known that health benefits from exercise training depend on time, frequency, and charge [47]. These comments indicate that exercise training used as protocols for the present study could not by themselves inhibit fat tissue accretion. In spite of the above, different programmed obesities onset, such as those in MSG-treated mice and in rats sensitive to rich fat diet, were respectively attenuated by moderate swimming training and voluntary exercise. Both exercises were applied early, or rather, immediately after weaning. These studies also show that if exercise is halted, effect on fat metabolism persists for at least the next 5–10 weeks [26, 48]. These results indicate that exercise has permanent influence on brain structures that control body weight. It has been known that development of CNS continues after birth and prolongs itself during lactation [49, 50]. Perinatal stress, including weaning phase, such as overeating or malnourishment, are able to injury certain hypothalamic areas, with a subsequent body weight dysfunction [51, 52]. It has already been reported that lesions on hypothalamic areas that contribute to satiety and hunger control, ventromedial (VMH) and lateral (LH) areas, respectively, change ANS activity [53].

While results cannot discriminate between afferent and efferent nerve signals, the increase and decrease of firing rate from vagus and retroperitoneal sympathetic nerves, respectively, caused by MSG-induced obesity, are shown for the first time. This fact supports the autonomic hypothesis on obesity [54, 55]. Autonomic imbalance showed by electric nerve activities in MSG-obese mice might have a strong relationship with the glycemic homeostasis rupture and high capacity to accumulate fat in tissues from obese animals. MSG-rodents and other obese animal models show insulin over-secretion, which has a close relationship with high vagus activity [38, 56]. Precocious bilateral subdiaphragmatic vagotomy was able to block hyperinsulinemia in MSG-rats [37, 38]. Although no parasympathetic nerve terminal in fat tissue was reported [57], vagus nerve indirectly plays a very important role on fat accretion. This is due to the fact that blood insulin quantity is also modulated by vagal activity in pancreatic islets. Acetylcholine released from vagus nerve terminals is bound to muscarinic receptors from beta cell plasma membrane and enhances glucose-stimulated insulin release [58, 59].

Exercise training has been able to equilibrate autonomic activity of MSG-treated mice; PNS, represented by vagus nerve activity, decreased, and SNS, represented by RS nerve activity, increased. It is known that high PNS activity stimulates anabolic pathways such as lipogenesis reported in obesity onset [60], while high SNS activity stimulates catabolic metabolism, such as lipolysis [61]. SNS stimulation has been frequently associated with exercise [62]. Current results show that MSG-obesity present low sympathoadrenal axis activity, as indirectly evaluated by total catecholamine content in adrenal medullae. In fact, MSG-obese mice present low protein expression of tyrosine hydroxylase (TH), which is a rate-limiting enzyme of catecholamine biosynthesis pathway [63]. Exercise provided adrenal medullar cells with high quantities of catecholamines. Other results (not shown) demonstrated that the same swimming training was also able to increase TH protein expression of MSG-mice and control-mice. These data coincide with increasing nerve sympathetic activity also induced by the swimming training described in current study.

All metabolic and autonomic changes observed in trained MSG-mice and normal mice were not dependent on oscillation of food intake. Recent study with high-fat diet-induced obese rats also showed that early voluntary exercise did not change food intake behavior, although fat tissue accumulation was inhibited [43]. These data suggest that energy consumption increases, while energy intake remains constant in MSG-treated or in MSG–untreated mice, submitted to early very moderate swimming training. This unusual pathway to metabolism may occur because exercise changes brain development. Weaning is a very vulnerable growth phase and any stress may provide permanent changes in CNS, including neuronal areas that control metabolism and body weight gain. Moderate swimming or running exercise training, or voluntary wheel exercise training applied on weaned rodents were able to increase proliferation of and decrease apoptosis in neurons of the hippocampus area. These trained animals presented better cognitive behavior than untrained ones [64, 65].

Metabolic changes observed in central obesity produced by neonatal treatment with MSG coincide with an imbalance in autonomic alteration. A very moderate exercise training applied at weaning is able to improve metabolism,

attenuate obesity onset and balance autonomic activity. Further studies using other obesity animal models and different exercise trainings are necessary to test whether obesity onset can be halted by early moderate exercise. It would also clarify the mechanisms underlying this process, including the ANS role. Further, early intervention to balance or to maintain the normal activity of the autonomic nervous system as soon as possible may prevent the progress of obesity and its complications, such as metabolic syndrome, cardiopathies, and type 2 diabetes. Early exercise training is a strong candidate.

Materials and methods

Animals

All animal protocols were approved by the Ethics Committee of the State University of Maringá, according to Brazilian Law for the Protection of Animals. Sets of four female and one male Swiss mice, 50 days old, were mated. On delivery, the litter was corrected to six to share milk amount among all pups [66]. During the first 5 days after birth, MSG (4 mg/g body weight) was injected subcutaneously in cervical area of the pup mice. Control animals were injected with saline solution. Males were selected and all females were discarded 1 day prior to weaning (21st day). Control and MSG-treated young males were randomly chosen for exercise. Mice received water and commercial diet chow (Nuvital, Curitiba, Brazil) ad libitum. During all protocols they were placed in an environmentally controlled room [23 ± 3°C and 12 h light/dark photocycle (07:00–19:00 h.)].

Swimming training

As described elsewhere [26], control and MSG-obese mice were trained by free swimming in a glass tank (30 × 35 × 30 cm), filled with water, at 32 ± 3°C. Mice swam over a period of 10 weeks (EXE), for 15 min a day, 3 days a week. Six mice from each group were placed simultaneously into the pool at 17:00 h. A lead weight, corresponding to 2.5% of their body weight, was attached to the tip of their tails to ensure that animals were in constant swimming activity. Some mice from treated- and untreated-MSG ones did not swim at all (SED). After each exercise session, mice were dried and returned to their respective boxes until the next swimming session.

Obesity

To evaluate obesity onset, all 90-day-old mice, trained or untrained, were anaesthetized by an intraperitoneal injection of sodium pentobarbital (5 mg/100 g body weight) and killed by cervical dislocation. Periepididymal and retroperitoneal fat pads were removed, washed, and weighed to estimate obesity induced by MSG treatment [26]. Body length and weight were taken to calculate the rodent body mass index or Lee index [67].

Blood glucose and insulin concentration

After 12 h of fasting, blood samples were collected from all experimental groups. Plasmas obtained were stored in freezer for posterior analyses of glucose by the glucose-oxidize method [68] and for insulin concentration by radioimmunoassay [69].

Food intake

After weaning, mice from all groups were weighed and food intake determined every week by non-ingested chow. Total area under the curve of food consumption versus time was calculated [70].

Parasympathetic activity

When 90 days old, a batch of mice from all experimental groups were anesthetized with thiopental (45 mg/Kg). Surgical longitudinal incision was made on the anterior cervical region. Under dissection microscope, the nerve bundle of the left vagus superior branch was severed from carotid artery close to trachea. The nerve trunk was pulled with a fine cotton line and a pair of recording silver electrodes (0.6 mm diameter), similar to a hook, were placed under the nerve. Nerve was covered with silicone oil to prevent dehydration. The electrode was connected to an electronic device (Bio-Amplificator, Insight®, Riberão Preto, Brazil) which amplified electrical signal up to 10,000 times, prior to low and high frequencies, 1–80 kHz, were filtered. The neural signal output was acquired by an Insight interface (Insight®, Riberão Preto, Brazil), viewed online and stored by a personal computer running a software developed by Insight (Insight®, Riberão Preto, Brazil). During all data acquisition, animals were placed into a Faraday cage to avoid any electromagnetic interference. Nerve activity was analyzed by amount of spikes during 5 s. After stabilization of signal during 5–10 min, 20 record frames of 15 s from each animal were randomly chosen for spike counting. Spikes were assumed as signals over 0 mV. Average spikes were used as nerve firing rate for each mouse.

Sympathetic activity

The sympathetic branch nerve from the lumbar plexus that innervates the retroperitoneal white fat tissue, which may be called the "retroperitoneal sympathetic (RS) nerve,"

was dissected from another batch of anesthetized mice from all experimental groups. The electrode was placed under the RS nerve, close to the retroperitoneal area. Firing rates from RS nerve were obtained as described for vagus nerve.

Adrenal total catecholamine content

Adrenal glands were removed and weighed. During handling, glands were maintained in standard Krebs-Hepes solution in an ice bath. Total adrenal gland catecholamines—epinephrine and norepinephrine—were quantified by the trihydroxyindole fluorescence method [71]. For total catecholamine content, right and left glands were homogenized in 350 µl acetic acid 10% using an ultrasonic processor, and centrifuged at $10{,}000 \times g$ for 1 min. Results were obtained by plotting the values on a linear regression of the standard epinephrine curve.

Statistical analysis

All results are presented as mean ± SEM. One-way ANOVA coupled to Bonferroni post-test was applied to all data from all mice groups, with $P < 0.05$ as statistically significant. Analyses were performed using GraphPad Prism version 5.0 for Windows (GraphPad Software, San Diego California USA).

Acknowledgments Current research was funded by the Brazilian Research Agencies: CNPq, CAPES, and Fundação Araucaria.

References

1. F. Bacha, R. Saad, N. Gungor, S.A. Arslanian, Diabetes Care **29**(7), 1599–1604 (2006)
2. G.A. Bray, T. Bellanger, Endocrine **29**(1), 109–117 (2006)
3. S.M. Phillips et al., Obes. Res. **12**(3), 461–472 (2004)
4. R.A. Winett, D.F. Tate, E.S. Anderson, J.R. Wojcik, S.G. Winett, Prev. Med. **41**(2), 629–641 (2005)
5. S.C. Woods, M.W. Schwartz, D.G. Baskin, R.J. Seeley, Annu. Rev. Psychol. **51**, 255–277 (2000)
6. T. Adage et al., J. Neurosci. **21**(10), 3639–3645 (2001)
7. T. Kishi, J. Horiguchi, Nihon Shinkei Seishin Yakurigaku Zasshi **23**(5), 197–203 (2003)
8. G.A. Tejwani, C.W. Richard III, NIDA Res. Monogr. **75**, 497–500 (1986)
9. J.W. Olney, Science **164**, 719–721 (1969)
10. G.A. Bray, Int. J. Obes. **11**(Suppl 3), 83–95 (1987)
11. L. Freire-Maia, A.D. Azevedo, Med. Hypotheses **32**(2), 91–99 (1990)
12. N. Atef, A. Ktorza, L. Picon, L. Penicaud, Am. J. Physiol. **262**(5 Pt 1), E736–E740 (1992)
13. F.S. Leigh, L.N. Kaufman, J.B. Young, Int. J. Obes. Relat. Metab. Disord. **16**(8), 597–604 (1992)
14. S. Inoue, G.A. Bray, Life Sci. **25**(7), 561–566 (1979)
15. R.J. Barnard, S.J. Wen, Sports Med. **18**(4), 218–228 (1994)
16. D.S. Hittel, W.E. Kraus, C.J. Tanner, J.A. Houmard, E.P. Hoffman, J. Appl. Physiol. **98**(1), 168–179 (2005)
17. T. Hayashi, J.F. Wojtaszewski, L.J. Goodyear, Am. J. Physiol. **273**(6 Pt 1), E1039–E1051 (1997)
18. J.J. Betts, W.M. Sherman, M.J. Reed, J.P. Gao, Obes. Res. **1**(4), 295–302 (1993)
19. H. Zouhal et al., Int. J. Sports Med. **19**(3), 172–176 (1998)
20. A.J. Scheurink et al., Am. J. Physiol. **256**(1 Pt 2), R161–R168 (1989)
21. A.J. Scheurink et al., Am. J. Physiol. **256**(1 Pt 2), R155–R160 (1989)
22. B. Stallknecht, M. Roesdahl, J. Vinten, K. Capito, H. Galbo, Acta Physiol. Scand. **156**(4), 465–473 (1996)
23. T.J. Bartness, C.K. Song, Lipid Res. **48**(8), 1655–1672 (2007)
24. B. Stallknecht et al., Am. J. Physiol. **259**(5 Pt 2), R998–R1003 (1990)
25. S. Lucinei Balbo, C. Gravena, M.L. Bonfleur, P.C. de Freitas Mathias, Horm. Res. **54**(4), 186–191 (2000)
26. D.X. Scomparin et al., Life Sci. **79**(22), 2151–2156 (2006)
27. K. Hamaoka, T. Kusunoki, J. Nutr. Sci. Vitaminol. (Tokyo) **32**(4), 395–411 (1986)
28. F. Rohner-Jeanrenaud et al., J. Physiol. (Paris) **78**(9), 833–837 (1982)
29. R.A. Batt, C.A. Wilson, D.L. Topping, Int. J. Obes. **2**(3), 303–307 (1978)
30. M.Y. Loh, W.P. Flatt, R.J. Martin, D.B. Hausman, Proc. Soc. Exp. Biol. Med. **218**(1), 38–44 (1998)
31. D.P. Cameron, L. Cutbush, F. Opat, Clin. Exp. Pharmacol. Physiol. **5**(1), 41–51 (1978)
32. D.P. Cameron, T.K. Poon, G.C. Smith, Diabetologia **12**(6), 621–626 (1976)
33. P. de Carvalho Papa, A.M. Vargas, J.L. da Silva, M.T. Nunes, U.F. Machado, Life Sci. **71**(16), 1917–1928 (2002)
34. U.F. Machado, I. Shimizu, M. Saito, Physiol. Behav. **55**(4), 621–625 (1994)
35. H. Inoue, S. Kohsaka, M. Otani, S. Toya, Y. Tsukada, Neurosci. Res. **3**(6), 555–567 (1986)
36. S.L. Balbo et al., Res. Commun. Mol. Pathol. Pharmacol. **108**(5–6), 291–296 (2000)
37. S.L. Balbo et al., Diabetes Metab. **28**(6 Pt 2), 3S13–3S17 (2002). discussion 13S108-112
38. S.L. Balbo et al., Endocrine **31**(2), 142–148 (2007)
39. T. Kiba, K. Tanaka, O. Endo, S. Inoue, Gastroenterology **104**(2), 475–484 (1993)
40. A.V. Astrup, N.J. Christensen, L. Simonsen, J. Bulow, J. Neurosci. Methods **34**(1–3), 187–192 (1990)
41. A.R. Carpinelli, U.F. Machado, R. Curi, Neurosci. Biobehav. Rev. **20**(2), 183–188 (1996)
42. R. Dawson Jr., Z. Annau, Brain Res. Bull. **15**(2), 117–121 (1985)
43. C.M. Patterson, B.E. Levin, Neuroendocrinology **87**(2), 65–70 (2008)
44. C. Napoli et al., Proc. Natl Acad. Sci. USA **101**(23), 8797–8802 (2004)
45. J.M. Jakicic, J. Nutr. **132**(12), 3826S–3829S (2002)
46. J.M. Jakicic, A.D. Otto, Am. J. Clin. Nutr. **82**(1 Suppl), 226S–229S (2005)
47. H.O. Chambliss, Clin. J. Sport Med. **15**(2), 113–115 (2005)
48. C.M. Patterson, A.A. Dunn-Meynell, B.E. Levin, Am. J. Physiol. Regul. Integr. Comp. Physiol. **294**(2), R290–R301 (2008)
49. C.D. West, T.L. Kemper, Brain Res. **107**(2), 221–237 (1976)
50. V.V. Prasad, Lipids **26**(7), 553–556 (1991)
51. A. Plagemann et al., Int. J. Dev. Neurosci. **17**(1), 37–44 (1999)
52. H. Davidowa, Y. Li, A. Plagemann, Zh. Vyssh. Nerv. Deiat. Im. I. P. Pavlova **53**(5), 663–670 (2003)
53. H. Yoshimatsu, Y. Oomura, T. Katafuchi, A. Niijima, Am. J. Physiol. **253**(3 Pt 2), R418–R424 (1987)

54. G.A. Bray, S. Inoue, Y. Nishizawa, Diabetologia **20**(Suppl), 366–377 (1981)
55. G.A. Bray, Int. J. Obes. **8**(Suppl 1), 119–137 (1984)
56. S. Inoue, G.A. Bray, Endocrinology **100**(1), 108–114 (1977)
57. T.J. Bartness, J. Clin. Invest. **110**(9), 1235–1237 (2002)
58. P.C. Mathias, L. Best, W.J. Malaisse, Cell Biochem. Funct. **3**(3), 173–177 (1985)
59. S. Grassiolli, C. Gravena, P.C. de Freitas Mathias, Eur. J. Pharmacol. **556**(1–3), 223–228 (2007)
60. J.A. Romijn, E. Fliers, Curr. Opin. Clin. Nutr. Metab. Care **8**(4), 440–444 (2005)
61. A. Giordano et al., Am. J. Physiol. Regul. Integr. Comp. Physiol. **291**(5), R1243–R1255 (2006)
62. B. Balkan, G. Van Dijk, J.H. Strubbe, J.E. Bruggink, A.B. Steffens, Am. J. Physiol. **262**(6 Pt 2), R981–R985 (1992)
63. A.C. Martins et al., Life Sci. **74**(26), 3211–3222 (2004)
64. N. Uysal et al., Neurosci. Lett. **383**(3), 241–245 (2005)
65. S.A. Neeper, F. Gomez-Pinilla, J. Choi, C.W. Cotman, Brain Res. **726**(1–2), 49–56 (1996)
66. A.S. Moura, A.R. Carpinelli, F.B. Barbosa, C. Gravena, P.C. Mathias, Res. Commun. Mol. Pathol. Pharmacol. **92**(1), 73–84 (1996)
67. L.L. Bernardis, B.D. Patterson, J. Endocrinol. **40**(4), 527–528 (1968)
68. R.M. Torrezan RG, M.L. Ferrarese, F.B. de Melo, A.M.D. Ramos, P.C.F. Mathias, D.X. Scomparin, Arq. Bras. Endocrinol. Metabol. **52**(9), 1489–1496 (2008)
69. V. Leclercq-Meyer, J. Marchand, O. Rebolledo, W.J. Malaisse, R. Leclercq, Diabetologia **11**(5), 419–425 (1975)
70. A.C.P. Martins, H.E. Borges, R.M.G. Garcia, S.R. Carniatto, P.C.F. Mathias, Neurosci. Res. Commun. **28**(1), 49–57 (2001)
71. K.L. Kelner, R.A. Levine, K. Morita, H.B. Pollard, Neurochem. Int. **7**, 373–378 (1985)