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EFEITO DO EXERCÍCIO FÍSICO NO CONTROLE GLICÊMICO DE CAMUNDONGOS OBESOS-MSG

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EFEITO DO EXERCÍCIO FÍSICO NO CONTROLE GLICÊMICO DE CAMUNDONGOS OBESOS-MSG

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APRESENTAÇÃO DO TRABALHO

De acordo com as normas estabelecidas pela Coordenação do Programa de Pós-Graduação em Ciências Biológicas, esta dissertação de Mestrado foi redigida na forma de um artigo científico.

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À minha família, em especial meus pais e minhas irmãs pelo amor e compreensão...

Ao meu grande amor, por renovar minha vida e me fazer tão feliz.

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RESUMO

Introdução

O moderno estilo de vida, com abundância de alimentos e reduzida prática de exercício físico, resulta em um aumento dramático das taxas de sobrepeso e obesidade. Contudo, os mecanismos que causam a obesidade são desconhecidos. Em humanos obesos e modelos experimentais de obesidade, a disfunção metabólica resulta na deterioração da célula beta. A administração neonatal de glutamate L-monossódico (MSG) causa obesidade, hiperinsulinemia e hiperglicemia em roedores adultos, caracterizando resistência à insulina. A hiperinsulinemia de jejum na obesidade está relacionada com aumento na secreção de insulina, a qual é parcialmente atribuída à alta atividade do sistema parassimpático e baixa atividade simpática. Contudo, a vagotomia restabelece a insulinemia de jejum e parcialmente reduz o acúmulo de gordura em roedores obesos. Além disso, a ativação do sistema nervoso simpático pelo exercício físico aumenta o conteúdo de catecolaminas nas adrenais, reduz a hiperinsulinemia, a hiperglicemia e a massa adiposa. O objetivo desse trabalho foi avaliar os efeitos do exercício físico na normalização da função da célula beta e na melhora do controle glicêmico de camundongos obesos-MSG.

Material e Métodos

Camundongos receberam nos 5 primeiros dias de vida injeções diárias subcutâneas, na região cervical, de uma solução de MSG na dose de 4mg/g de peso corporal/dia. Os animais do grupo controle receberam salina isosmótica. Aos 21 dias de vida ocorreu o desmame e animais do grupo MSG e controle foram escolhidos ao acaso para iniciarem o

programa de natação. Os camundongos nadaram durante 8 semanas, 3 dias por semana, 15 minutos por dia. Após o desmame, o peso e o consumo alimentar dos camundongos foram avaliados semanalmente. Aos 90 dias de vida os animais foram sacrificados por deslocamento cervical após injeção intraperitoneal do anestésico pentobarbital de sódio (5mg/100g de peso corporal). O sangue foi coletado para posterior dosagem da glicemia e insulinemia plasmáticas pelos métodos da glucose-oxidase e radioimunoensaio, respectivamente. As glândulas adrenais foram removidas e pesadas. O conteúdo total de catecolaminas foi dosado pelo método espetrofluorimétrico do trihidroxindol. As gorduras periepididimais foram removidas, lavadas e pesadas. O teste de tolerância à glicose intraperitoneal (ipGTT) foi realizado após jejum de 12 horas com injeção de glicose na dose de 2g/Kg de peso corporal. As ilhotas pancreáticas foram isoladas pela técnica clássica da colagenase. Grupos de 4 ilhotas foram pré-incubados por uma hora com solução Krebs contendo 5.6 mM de glicose e, posteriormente, incubados com 16.7 mM de glicose em solução Krebs. Alíquotas das incubações foram utilizadas para dosagem de insulina por radioimunoensaio.

Resultados

O tratamento neonatal com MSG aumentou 44% o acúmulo de gordura periepididimal comparado ao controle. Entretanto, o exercício físico diminuiu 30.9% os estoques de gordura no grupo dos obesos e 37.9% no grupo controle. Camundongos obesos-MSG apresentaram significativo aumento na glicemia (45.2%) e insulinemia (137.9%). O programa de natação aboliu a hiperglicemia e a hiperinsulinemia nos animais obesos. O exercício não alterou os níveis plasmáticos de glicose e insulina dos camundongos controles. A glicemia dos camundongos obesos sedentários foi significativamente maior

durante o ipGTT comparado aos animais normais. No cálculo da área sob a curva da glicemia no ipGTT, os MSG sedentários tiveram um aumento de 39.6% em relação aos sedentários controles. A natação completamente restaurou a glicemia nos obesos exercitados. Em baixas concentrações de glucose, 5.6 mM, a secreção de insulina das ilhotas dos animais obesos foi 2.12 vezes maior que a observada nos animais controles. O exercício não causou alterações na secreção de insulina estimulada por 5.6 mM de glicose tanto nas ilhotas de animais obesos quanto de animais controles. Aumentando a concentração de glicose para 16.7 mM, as ilhotas respondem secretando mais insulina, 3.75 e 2.97 vezes comparado à secreção induzida por baixa concentração de glicose em camundongos controles e MSG, respectivamente. A natação provocou um aumento de 1.9 vezes na secreção de insulina estimulada por 16.7 mM nas ilhotas dos camundongos obesos; contudo, não houve alteração no grupo controle exercitado.

Conclusões

Os resultados revelam que o programa de treinamento físico iniciado logo no desmame é capaz de inibir consideravelmente a instalação da obesidade induzida durante o início da lactação em camundongos. Este efeito foi correlacionado a atividade do eixo simpatoadrenal. O exercício moderado também aboliu a hiperglicemia e recuperou o controle normal da secreção de insulina e a sensibilidade dos tecidos periféricos a ação da insulina dos animais que foram tratados com MSG.

Palavras-chaves: obesidade, glutamato L-monossódico, secreção de insulina, glicemia e exercício físico.

SUMMARY

Introduction

Modern lifestyle, with abundant nutrient supply and reduced physical activity, has resulted in dramatic increases in the rates of overweight and obesity. Unfortunately, the mechanisms that cause obesity, including early obesity onset, are not yet revealed; however, in obese human and experimental animals, it has been observed that metabolic dysfunction can be expressed by deteriorating pancreatic beta cell function. Postnatal administration of monosodium L- glutamate (MSG) induces obesity, hyperinsulinemia and hyperglycemia in adulthood rodents, thus suggesting the presence of insulin resistance. Fasting hyperinsulinemia in obesity is related with insulin oversecretion, which is partially attributed to a high parasympathetic activity, while sympathetic tonus is decreased. Vagotomy on obese rats restores fasting insulinemia and partially reduces tissue fat accumulation. Indeed, activation of sympathetic nervous system by exercise induces increase of catecholamine content and decreases hyperinsulinemia, hyperglycemia and fat accumulation. Therefore, the aim of this work was to investigated whether moderate swimming training, applied to MSG-weaned mice, besides the reduction of tissue fat accumulation, is able to normalize beta cell function and improve glycemic control.

Material and Methods

During the first 5 days after birth, MSG (4mg/g body weight) was injected subcutaneously on cervical area of the young mice. Control animals were injected with saline solution. The animals were weaned on the 21st postnatal day and control and MSG-treated males were randomly chosen for exercise. Mice swam for eight weeks, during 15

min a day, 3 days a week. One group of sedentary mice did not swim at all from 21 to 90 days old. After weaning, mice from all groups were weighed and the food intake determined every week from the not ingested chow. To evaluate obesity onset, all 90 days old mice trained or untrained were anaesthetized by an intraperitoneal injection of pentobarbital sodium (5mg/100g body weight) and killed by cervical dislocation. Total blood was collected to measure fasting plasma glucose and insulin by glucose-oxidase technique and radioimmunoassay, respectively. Adrenal glands were removed and weight. Trihydroxyndole fluorescence method was employed to measure total catecholamine content. Periepididymal fat pads were removed, washed and weighed. Intraperitoneal glucose tolerance test (ipGTT) was performed by injecting glucose (2g/kg body weight) intraperitoneally in overnight-fasted mice. Pancreatic islets were isolated by collagenase technique. Batchs of 4 islests were pre-incubated for 60 min in 5.6 mM glucose Krebs solution and then incubated in glucose 16.7 mM with Krebs. Aliquots from incubations were used to measure insulin concentration by radioimmunoassay.

Results

MSG treatment increased 44% periepididymal fat pad weight compared with control mice. However, exercise decreased 30.9% fatty tissue in MSG-animals compared to untrained; while, exercise reduced the fat tissue by 37.9% in MSG-untreated mice. MSG-obese mice presented a significant increase in plasma glucose (45.2%) and insulin (137.9%) concentration. Swim programming abolished both hyperglycemia and hyperinsulinemia in MSG-animals. Exercise did not change glucose and insulin blood levels in MSG-untreated mice. MSG-sedentary animals presented higher glycemia than MSG-untreated mice during ipGTT. Calculating the area under the curve of blood

glucose concentration throughout ipGTT sedentary MSG-mice present an increase of 39.6% compared to MSG-untreated ones. Swimming training completely restored the glycemic levels in MSG-mice. At low concentration of glucose, 5.6 mM, it was observed 2.12 fold increased insulin secretion to islets from MSG-mice when compared to untreated ones. Exercise did not caused any changes in the insulin secretion induced by 5.6 mM of glucose to MSG treated and untreated animals. Increasing glucose concentration to 16.7 mM, islets respond secreting more insulin; 3.75 and 2.97 times compared to secretion response to low glucose to sedentary MSG-untreated and -treated mice, respectively. Insulin secretion stimulated by 16.7 mM of glucose to islets from exercised MSG-untreated mice show no alteration when compared to sedentary ones. However, islets from 21-90 days-old exercised MSG-mice secreted 1.90 time more insulin than MSG-sedentary. Neonatal treatment with MSG caused a 42.6% reduction in the catecholamine content of the adrenal gland when compared to normal mice. However, MSG-mice which were submitted to swim training after weaning increased their catecholamine storage by 50% compared to sedentary MSG-mice. In normal animals during the same period, there was a 42.5% catecholamine increase.

Conclusion

In conclusion, our results show that moderate swim training, started at weaning is able to considerably inhibit the obesity onset induced by neonatal treatment with monosodium L-glutamate. The effect of exercise training was related to sympathoadrenal axis activity. Moderate exercise had also ability to abolish the hyperglycemia; insulin secretion control and tissue insulin sensitivity were restored in obese mice.

Key Words: obesity, monosodium L-glutamate, insulin secretion, glycemia and exercise.

Swim training applied at weaning improves glycemic control and inhibits monosodium L-glutamate-obesity onset in mice

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Abstract

Exercise has been recommended to reduce excessive weight gain and improved the metabolic disorders that accompanied overweight and obesity; while the mechanisms that underwent the obesity onset is not fully understood, nor are the effects of exercise on body weight control. A swim training applied to weaning pups, but not in young adult mice, was able to inhibit hypothalamic obesity onset and to recover sympathoadrenal axis activity. In the present work, swim training applied at early age was used to observe glycemic control in hypothalamic-obese mice produced by neonatal treatment with monosodium L-glutamate (MSG). MSG-treated and normal mice swam for 15 min/day, 3 days a week, from weaning up to 90 days old (EXE). Sedentary MSG and normal mice (SED groups) did not exercise at all. Animals were sacrificed at 90 days of age. MSG-obese mice presented fasting hyperglycemia and hyperinsulinemia and glucose intolerance. Beyond the inhibition of tissue fat accretion, exercise caused normalization of glycemic control. Pancreatic islets from obese-mice, who present failure to glucose-induced insulin secretion, were functionally recovered after swim program. We conclude that attenuation of MSG-hypothalamic obesity onset is caused, at least in part, to sympathoadrenal axis activity modulation imposed by early exercise, which may lead to glucose metabolism improvement.

Key words: MSG-obese mice, exercise, insulin secretion, pancreatic islets, glycemic control, sympathoadrenal axis

Introduction

Metabolic syndrome increases as obesity growing worldwide, scaring public health authorities. Although a number of different sets of diagnostic criteria have been proposed for this syndrome, insulin resistance, hyperglycemia, dyslipidemia, hypertension and central obesity are generally agreed to be the five key features [1]. More disaster from this pandemic is an increase of child metabolic syndrome. These young people can present obesity or overweight with insulin resistance, hyperinsulinemia, hypertension and hypercholinesteronemia; and some of them develop non insulin dependent diabetes mellitus (NIDDM) [2]. Epidemiologic findings indicate that there are a straight relationship between obesity in childhood and metabolic syndrome onset in adult life [3]. Unfortunately, the mechanisms that cause obesity, including early obesity onset, are not yet revealed; however, in obese human and experimental animals, it has been observed that metabolic dysfunction can be expressed by deteriorating pancreatic beta cell function [4]. Beta cells' functions are impaired and glucose-induced insulin release is enhanced in obesity, which contributes to hyperinsulinemia [5]. Peripheral insulin resistance observed in obesity demands an extreme effort to beta cell produce and release increased amounts of insulin, which should be allow to decrease high blood glucose concentration; however, without decrease hyperglycemia, beta cell loses their capacity to regulate insulin secretion, and high blood glucose levels still uncontrolled.

As glucose, other nutrients, such as aminoacids, fat acids and their metabolites stimulate also insulin secretion on pancreatic beta cells. These secretagogues induce an increase in cell metabolism and subsequent ATP production. Potassium ATP sensitive

channels (K_{ATP}) are inactivated by an increase in ATP/ADP ratio. Membrane depolarization and subsequently the activation of dependent Ca^{2+} channels-voltage induce a quick increase on intracellular calcium concentration. High cytosolic free calcium is an intracellular signal that triggers insulin secretion events. However, experimental evidences indicate that glucose may also be stimulating insulin secretion by alternative pathways to K_{ATP} channels [6]. Besides these mechanisms that involve stimulation of metabolism, beta cells are also submitted to neural control. Pancreatic cells are equipped with several receptors to neurotransmitters and neuropeptides. These receptors are stimulated by efferent signals from the central nervous system (CNS), which include autonomic nervous system (ANS), throughout their neural ends for pancreatic beta cells [7]. During glucose blood level oscillations, beta cells receive inputs from the parasympathetic and sympathetic systems to aid glycemia regulation. Overall, acetylcholine released from parasympathetic ends promotes potentiation of glucose-induced insulin secretion, whereas noradrenaline released from sympathetic terminals and adrenaline secreted from adrenomedulary cells inhibit it [8]. It has been observed that neural control of insulin secretion and glycemia is damaged in obesity [9]. An imbalance of ANS has been observed in obese human and rodents [10-12]. Fasting hyperinsulinemia in obesity is related with insulin oversecretion, which is partially attributed to a high parasympathetic activity, while sympathetic tonus is decreased [13-15].

Administrations of monosodium L- glutamate (MSG) to rodents' suckling pups promote the death of neurons in hypothalamic areas and induce changes in the development of the CNS [16,17]. Indeed, adult MSG-rats and mice exhibit disturbances in body weight, which lead to increased adiposity, hyperinsulinemia and insulin

resistance [15,18-20] Unlike other obese rodents, such as those from genetic origin or from other lesions in CNS, overeating in MSG-rodents does not occur [21,22]. While adult (90 days-old) MSG-rats are normoglycemic, adult mice who were neonatally treated with MSG present hyperglycemia [5,15]. Insulin oversecretion and altered ANS activity were registered in these MSG-obese rodents [13,15], as well as in other animal obese models [9,23,24]. Our laboratory has been showed that MSG-obese mice present also impairment in sympatoadrenal axis activity. Their chromaffin adrenal cells released low amounts of catecholamines and its biosynthesis is reduced [21,25].

Recently, we showed also that a moderated swimming program started at weaning was able to reestablish the catecholamine production on adrenal medullae cells and inhibited the MSG-obesity onset in adult mice [26]. Human and animals with metabolic syndrome submitted to physical exercise improved their blood insulin and glucose levels [27-29]. Whereas, it is known benefit of physical exercise, the mechanisms that are involved in the amelioration on metabolism still in debate, at least in part, the stimulation of sympathoadrenal axis activity is one candidate to be involved it [26]. The effect of physical exercise on metabolism is blocked in **lean** animals with adrenal demedullation [30]. Moderate swimming program applied at mice with 60 days-old, for 30 days, is not able to restore the sympathoadrenal axis function and to inhibit the MSG-obesity onset, as do when exercise is started at weaning, 21 days-old [26]. The current work was designed to verify whether moderate swimming training, applied to MSG-weaned mice, besides the reduction of tissue fat accumulation, is able to normalize beta cell function and improve glycemic control.

Methods

Animals: All animal protocols were approved by the Ethic Committee of the State University of Maringá. Sets of three female and one male Swiss mouse, 50 days old, were mated. After one week the pregnant mice were separated. On delivery the litter was corrected to six to assure the milk amount to all pups [31]. During the first 5 days after birth, MSG (4mg/g body weight) was injected subcutaneously on cervical area of the young mice. Control animals were injected with saline solution. The day before weaning (21^{st} day), males were selected and all females were discharged. Control and MSG-treated mice were randomly chosen for exercise. Animals received water and commercial diet *(Nuvital, Curitiba, Brazil)* ad libitum and during all protocol they were placed in an environmentally controlled room [23 ± 3 °C and 12 hour light / dark photocycle (07:00-19:00 h.)].

Swim training: Mice, untreated and MSG-treated were trained by free swimming in a glass tank (30 x 35 x 30cm) filled by taped water at 32±3 ^{0}C. Mice swam for eight weeks (EXE), during 15 min a day, 3 days a week. Six mice from each group were placed simultaneously into the pool at 17:00 hour. To ensure that animals were in constant swimming activity a plumb weight, which was corresponded to 2.5% their body weight was attached on the tail tips. One group of sedentary mice did not swim at all from 21 to 90 days old (SED). All groups comprise mice that were treated neonatally with MSG and control ones that were drug untreated. This swimming program should be consider as a moderate exercise because the animals present half from their maximal velocity of oxygen consumption, VO_{max} [32]. After each exercise session mice were dried with paper towel and placed back in their respective boxes until the next swimming session.

Obesity: To evaluate obesity onset, all 90 days old mice trained or untrained were anaesthetized by an intraperitoneal injection of pentobarbital sodium (5mg/100g body weight) and killed by cervical dislocation. Periepididymal fat pads were removed, washed and weighed to estimate obesity induced by MSG treatment [26].

Food intake: After weaning, mice from all groups were weighed and the food intake determined every week from the not ingested chow. The food intake was calculated by body weight from each animal at the time when the chow intake was measured. The total area under the curve of development of food consumption was calculated from weekly food intake [21].

ipGTT: Intraperitoneal glucose tolerance test (ipGTT) was performed by injecting glucose (2 g/kg) intraperitoneally in overnight-fasted mice. Blood glucose levels were determined prior (0) the injection and 30, 60, 90 and 120 min afterwards. Blood samples were obtained from the tail vein. Obtained plasmas were used to measure glucose concentration by glucose oxidase method (Kit-Bio Diagnostic Chemistry Industry®). The total area under the curve of ipGTT was calculated.

Glucose-induced insulin release in pancreatic islets: Collagenase technique was used to isolate pancreatic islets from mice described previously [33], with modifications. Briefly, intact mice from all groups, were deeply anesthetized. The abdominal wall was cut and open. The whole pancreas was isolated, washed with a Hank's buffered saline

solution (HBSS), weighted and quickly chopped. Supernatant solution was discharged after precipitation of pancreas peaces. Suspension was incubated with HBSS containing collagenase type IV (0.7 mg/ml of pancreas peaces suspension, Sigma Chemical CO., St. Louis, MO) at 37ºC. Suspension was then filtered with a 0.5mm metal mesh and washed with HBSS, containing 0.12% bovine serum albumin fraction V (BSA), in 5 continuous washings. Islets were collected with the aid of a microscope. At least 8 mice were used to obtain a pancreatic islet pool for each animal group. Groups of 4 islets placed on plastic cover slips were pre-incubated for 60 min in 1.0 ml of Krebs-Ringer bicarbonate-buffered solution containing 0.12% (wt/vol) bovine albumin (fraction V), and glucose 5.6 mM, pH 7.4. The solution was equilibrated against a mixture of CO_2 (5%) and O_2 (95%). After the adaptation to a low glucose concentration solution, islets were submitted to incubations for further 60 min in glucose 5.6 and 16.7 with Krebs-Ringer solutions. Islets used to observe insulin secretion were obtained from at least 8 different mice to each experimental group Aliquots from incubations were used to measure insulin concentration by radioimmunoassay [34].

Chemicals: Human Recombinant Insulin (Humalog) was purchased from Eli Lilly (Indianapolis, IN). [I^{125}] Human insulin was acquired from Pharmacia (São Paulo, BR). Routine reagents were purchased from Sigma, unless otherwise specified.

Statistical analysis: All results are presented as mean ± SEM. $P < 0.05$ was considered statistically significant. One-way ANOVA with Bonferroni post-test and

student's-t test were performed using GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego California USA).

Results

As shown in figure 1 (left panel), MSG treatment induced 44% enhancement of periepididymal fat pad compared to untreated animals ($p<0.001$). Swim training started at 21 days old induced 30.9% fall in the fat tissue at 90 days old MSG-animals compared to untrained ($p<0.001$); while, exercise reduced the fat tissue by 37.9% in MSG-untreated mice. Figure 1 show (middle and right panel) also that MSG treatment induced increases of 45.2% and 137.9% in glucose and insulin blood concentration, respectively, when compared to MSG-untreated ones ($p<0.001$); however, swim programming abolished fasting hyperglycemia, as well as the hyperinsulinemia. Exercise did not change glucose and insulin blood levels in MSG-untreated mice.

Mice from all groups were responded to glucose load during ipGTT, showing transient blood glucose concentration increases, as shown in upper panel of figure 2. MSG-sedentary animals presented higher glycemia than MSG-untreated mice; in addition, at the test end the blood glucose levels were 45% more elevated than those of fasting ($p<0.001$). Swimming program applied to MSG-mice reduced the glucose level oscillations to the same concentrations presented by MSG-untreated animals; whereas, no alterations were registered to normal mice submitted to exercise. Calculating the area under the curve of blood glucose concentration throughout ipGTT sedentary MSG-mice present an increase of 39.6% compared to MSG-untreated ones ($p<0.001$), as shown in the lower panel of figure 2. Swimming training completely restored the glycemic levels

in MSG-mice; while, in normal mice exercise did not alter the blood glucose concentration.

Figure 3 show that MSG treatment did not change the diet consumption. It is also show that swimming program not cause any alteration in food intake to MSG-treated or untreated mice.

At low concentration, 5.6 mM, glucose stimulated insulin release in islets from normal as well as from MSG-mice; while, it was observed 2.12 fold increased insulin secretion to islets from MSG-mice when compared to untreated ones ($p<0.001$). Exercise did not caused any changes in the insulin secretion induced by 5.6 mM of glucose to MSG treated and untreated animals. Increasing glucose concentration to 16.7 mM, islets respond secreting more insulin; 3.75 and 2.97 times compared to secretion response to low glucose to sedentary MSG-untreated and treated mice, respectively, as shown in the right panel of figure 3 ($p<0.001$). Insulin secretion stimulated by 16.7 mM of glucose to islets from exercised MSG-untreated mice show no alteration when compared to sedentary ones; however, islets from 21-90 days-old exercised MSG-mice secreted 1.90 time more insulin than MSG-sedentary ($p<0.001$).

Figure 5 shows the total catecholamine stores in mice adrenal glands. Neonatal treatment with MSG caused a 42.6% reduction in the catecholamine content of the adrenal gland when compared to normal mice ($p<0.001$). However, MSG-mice which were submitted to swim training after weaning increased their catecholamine storage by 50% compared to sedentary MSG-mice ($p<0.001$). In normal animals, exercise caused a 42.5% catecholamine increase ($p<0.001$).

Discussion

Neonatal treatment with MSG causes fast hyperinsulinemia in adult rodents [13,15,18]. Hyperinsulinemia was recorded in MSG-mice, accompanied for high blood glucose concentration. MSG treatment lesioned a significant number of neurons localized in the hypothalamic arcuate nucleus (ARC), disarranging an important area involved in body weight [35] and glycemic control [36]. Obese hyperinsulinemic rodents, who have a genetic defect relate to ARC function, such as Zucker rats, and ob/ob and db/db mice, presenting hyperphagia [22,35]; however, current data show that MSG-mice did not increase food intake, confirming other authors [37,38]. Whereas, presenting normophagia, MSG-mice accumulate increased fat in periepididymal pads, which leads to estimation total fat tissue content and to allow characterize the obesity, as showed in the current research and by other authors [19,26,35]. Present swimming program did not disturbed the food intake to MSG-treated or to untreated mice. It has been shown that exercise did change food intake [39]. Although any decrease in food consumption, exercise training caused decrease in fat tissue accumulation in both animal group; however, the fat reduction observed in exercised MSG-mice lead the fat accumulation to the same levels from normal sedentary mice. These data suggest that the swimming program is able to inhibit MSG-obesity onset; however, it can not conclude that this kid of exercise training cause complete blocking of obesity onset, because exercise was also able to reduce significantly the fat accumulation of MSG-untreated mice. We have

observed that adult MSG-mice (90 days old) who swam after weaning for 30 or 70 days presented less fat accretion than sedentary ones or MSG-mice who started swimming at 60 days of age [26].

Swimming program was also capable to normalize blood insulin levels. Hyperinsulinemic human and rodents submitted to different types of exercise training show reduction of blood insulin levels; while the magnitude of decrease is dependent on time and intensity of the training [40,41]. It can also suggest that decrease in fat tissue accumulation is related to normalization of fasting insulin levels. Insulin levels had been identified as an adiposity signal, like leptin [36]. Increases in fasting blood insulin concentration accompany rises in fat tissue content [42]. Fasting significant hyperinsulinemia, as showed in MSG-mice, can also indicate a failure in tissue insulin action, which leads to peripheral insulin resistance [42]. It was demonstrated a decreased of the stimulatory effects of insulin on glucose transport in adipocytes from MSG rats [18]. MSG rodents possess lower content of glucose transporter (GLUT4) protein in fat cells, skeletal and cardiac muscles and in brown adipose tissue [20,43]. However, during the development of obesity in MSG mice, the GLUT4 content was increased in white adipose tissue and it may play a key role in the fat tissue accumulation observed in MSG-mice [44].

Physical activity can reduce insulin resistance and improve glucose intolerance in obesity [30,45]. A single bout of exercise increases the rate of glucose uptake into the contracting skeletal muscles, a process that is regulated by the translocation of GLUT4 to the plasma membrane [46]. Furthermore, exercise training increases expression of GLUT4 protein in muscle [47]. Exercise and insulin action utilize different signaling pathways, both

allow activation of glucose transport, which perhaps explain why humans and experimental animals with insulin resistance can increase muscle glucose transport in response to an acute bout of exercise [48](Hayashi *et al*., 1997). A mechanism that participates on insulin-independent pathway by which muscle contractions stimulate glucose transport is the release of Ca^{2+} from sarcoplasmic reticulum; another one involves the AMP-activated protein kinase (AMPK) [49]. Swimming program was able to normalized fasting glycemia and improved glucose tolerance of MSG-mice. These data indicate that glucose uptake on peripheral tissues was activated by exercise training; however this effect can not be attributed only to insulin-independent pathway, because exercise also reduce insulin levels and fat tissue storage, which can suggest an increase in insulin tissue sensitivity. Indeed, in results not shown insulin resistance was observed in MSG-mice and it was abolished by swimming training.

There are several experimental evidences that MSG rodents present low sympathetic activity [14,26,50]. Studies in our laboratory have shown reduced secretory capacity of adrenal medullary chromaffin cells from MSG mice, coupled to low catecholamine stores and reduced expression of tirosine hydroxylase (TH), a limiting enzyme to catecholamine biosynthesis [21,25]. Acute and long term exercise training stimulate sympathetic driving. Catecholamines blood levels and their turnover are increased by exercise [51]. Moderated swimming program started at weaning reestablished the catecholamine production on adrenal medullae cells and inhibited the MSG-obesity onset in adult mice [26]. In results not shown the same exercise training was able to enhance the TH protein expression in MSG-mice. We also show, in the current research, that exercise training recovered catecholamine content from adrenal

gland of MSG mice and this may contributed to normalization "*in vivo*" of insulin oversecretion and it allow to restore normoinsulinemia. Epinephrine, released from adrenal medulla, and norepinephrine released from the sympathetic nerve endings, inhibits insulin release via stimulation of α_2-adrenergic receptors on the beta cells of the islets of Langerhans [24].

Our laboratory [15] showed that islets from MSG mice were able to increased the overall insulin secretion 14 times from 5.6 mM to 16.7 mM glucose perfusion, while islets from control mice increased to 28 times. The low secretion in response to high glucose concentration may indicate dysfunctions in pancreatic beta cells, which can lead to development of type 2 diabetes in these mice. Present research also observes failure in insulin secretion control of beta cells from MSG- mice; insulinotropic effect of high glucose concentration is low in MSG-obese mice compared to lean ones. Exercise training was able to induce in islets from MSG-mice an increase insulin release stimulate by high glucose dose greater than lean mice. These results indicate that training program induces a recovery of the ability to respond to glucose concentration changes in pancreatic beta cells from MSG-mice.

In conclusion, our results show that moderate swim training, started at weaning is able to considerably inhibit the obesity onset induced by neonatal treatment with monosodium L-glutamate. The effect of exercise training was related to sympathoadrenal axis activity. Moderate exercise had also ability to abolish the hyperglycemia; insulin secretion control and tissue insulin sensitivity were restored in obese mice. We suggest that exercise training may interfere on central control of energy expenditure by autonomic nervous system activity, among other mechanisms, which allow it to

reestablish the tissue insulin sensitivity and beta cell insulin secretion control; however; further studies must be conduct to clarify these mechanisms.

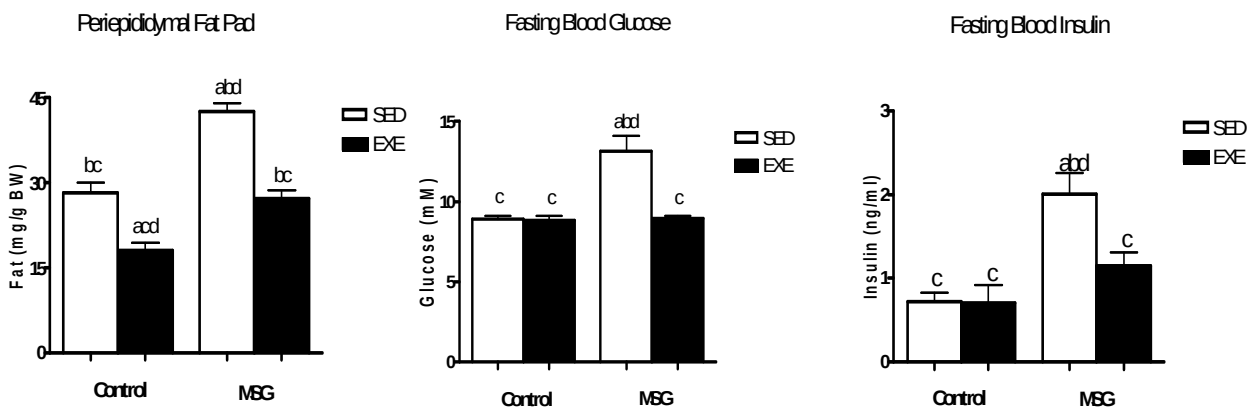
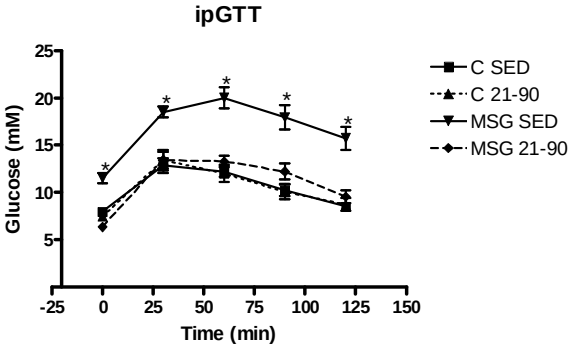

Periepididymal Fat Pad
Fat (mg/g BW)
45
30
15
0
bc
acd
abd
bc
Control
MSG
SED
EXE
Fasting Blood Glucose
Glucose (mM)
15
10
5
0
c
c
abd
c
Control
MSG
SED
EXE
Fasting Blood Insulin
Insulin (ng/ml)
3
2
1
0
c
c
abd
c
Control
MSG
SED
EXE

Fig 1


ipGTT
Glucose (mM)
Time (min)
C SED
C 21-90
MSG SED
MSG 21-90

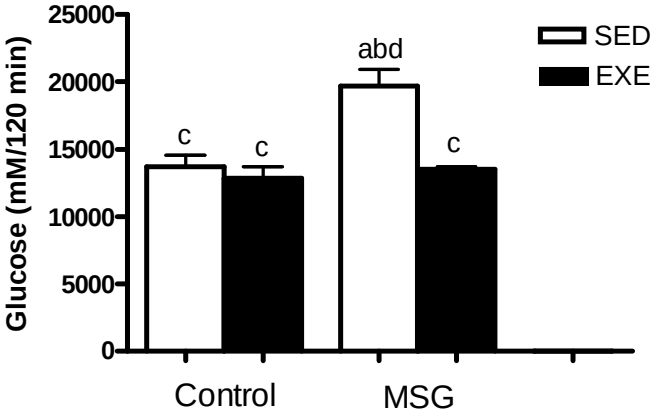
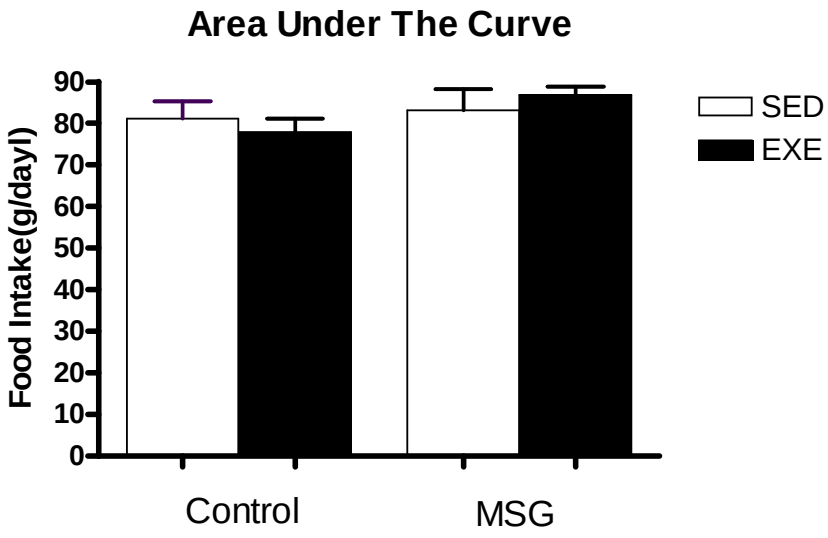

Glucose (mM/120 min)
SED
EXE
abd
c
c
c
Control
MSG

Fig 2

Fig 3


Area Under The Curve
Food Intake(g/dayl)
90
80
70
60
50
40
30
20
10
0
Control
MSG
SED
EXE

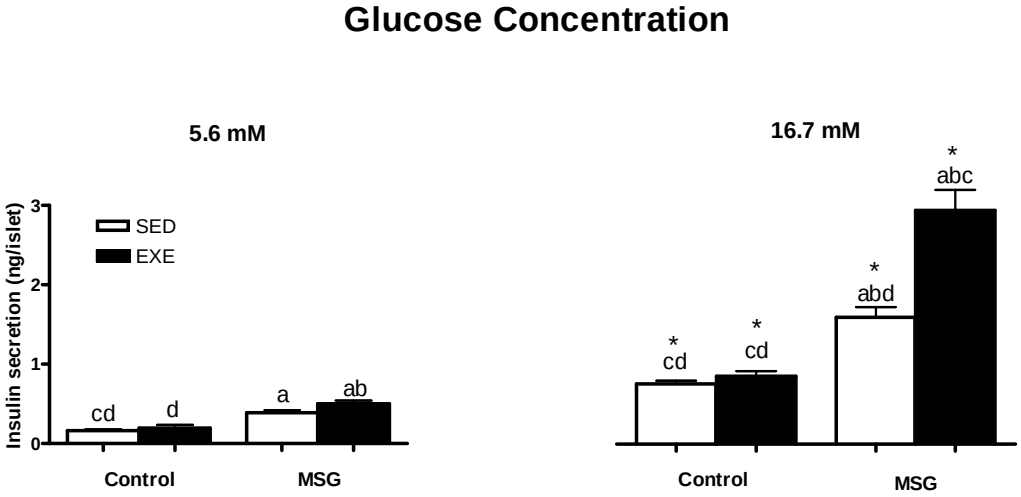
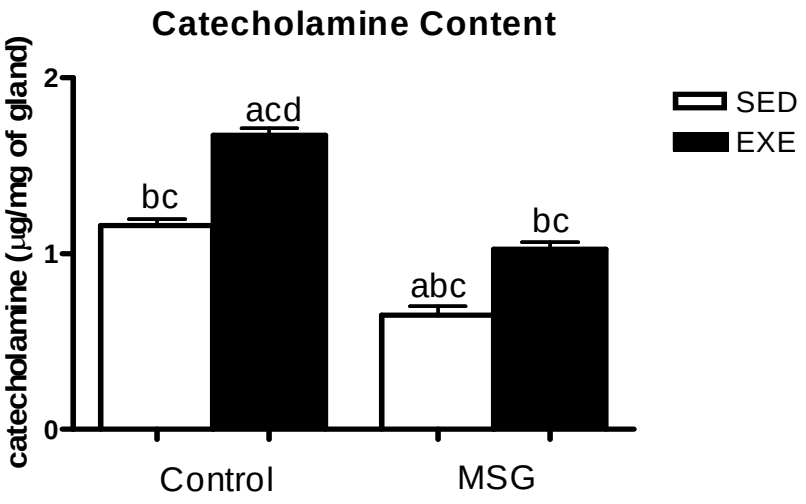

Glucose Concentration
5.6 mM
16.7 mM
Insulin secretion (ng/islet)
SED
EXE
cd
d
a
ab
*
cd
*
cd
*
abd
*
abc
Control
MSG
Control
MSG

Fig 4

Fig 5



Legends of Figures

Figure 1. Effect of MSG treatment and swim training on fat accumulation on epididymal pads (left panel), plasma glucose (middle panel) and insulin (right panel).

Bars represent the mean obtained with 10–12 animals for each group. Lines at the top of the bars represent the SEM. The letters over the bars represent significant differences with $p<0.05$ between groups: a- C SED; b-C EXE ; c-MSG SED and d-MSG EXE.

Figure 2. Effect of MSG treatment and swim training on ipGTT.

In the upper panel, at the left, symbols represent the mean of blood glucose concentration during ipGTT registered to 10-12 mice for each group. Lines on the symbols represent the SEM. In the lower panel, bars represent the mean of area under the curve of glycemia during all ipGTT period obtained with 10–12 animals for each group. Lines at the top of the bars represent the SEM. The letters over the bars represent significant differences with $p<0.05$ between groups: a- C SED; b-C EXE ; c-MSG SED and d-MSG EXE.

Figure 3. Effect of MSG treatment and swim training on food intake.

Bars represent the mean obtained with 10–12 animals for each group. Lines at the top of the bars represent the SEM.

Figure 4. Effect of MSG treatment and swim training on insulin secretion stimulated by low (5.6mM) and high (16.7mM) glucose concentration.

The islets were obtained from a pool of 10 mice for each group. Bars represent the mean obtained with 24 batches of islets. Lines at the top of the bars represent the SEM. The letters over the bars represent significant differences with $p<0.05$ between groups: a- C SED; b-C EXE ; c-MSG SED and d-MSG EXE.

Figure 5. Effect of MSG treatment and swim training on total catecholamine content from adrenal medullae

Bars represent the mean of catecholamine content from adrenal medullae isolated from 10–12 animals for each group. Lines at the top of the bars represent the SEM. The letters over the bars represent significant differences with $p<0.05$ between groups: a- C SED; b-C EXE ; c-MSG SED and d-MSG EXE.

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