Effects of the association of experimental neuroendocrine and exocrine obesity on tail blood pressure and glucose metabolism in Wistar rats

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Abstract

Objective: To study two different models of obesity, exocrine and endocrine, and its association on tail arterial pressure (TAP), body weight (BW), glucose metabolism and visceral fat content. **Methods:** Male Wistar rats were studied. The MSG group was composed by rats that received of MSG in neonatal period. At the 3rd month of life, part of these animals received cafeteria diet. Animals received saline control in the neonatal period. In the 12 weeks of study, body weight and blood pressure were measured twice a week. In the end of this period on, Oral Glucose Tolerance Test (OGTT) was performed and the Insulin Sensitivity Index (ISI) was calculated, also the left Relative Ventricular Weight (RLW) and Relative Epididimal Fat Weight (REFW) were obtained. **Results:** No changes on BW and TAP were verified. The obesity induced by MSG and CAF, individually, let to increases on insulin resistance (WST = 23,25 ± 9,31; CAF = 15,92 ± 9,10*; MSG = 13,41 ± 3,84* mg-1mU-1, p < 0,05 *vs* WST) and relative epididimal fat content (WST = 6,20 ± 0,57; CAF = 8,27 ± 1,53*; MSG = 8,23 ± 1,98* g/100 g, *p < 0,05) when these rats were compared to control rats. An enhanced effect upon these parameters was observed with the association of both obesity models (MSG+CAF = 9,34 ± 5,77 mg-1mU-1, p < 0,05 *vs* MSG and CAF) and visceral fat content (MSG+CAF = 11,12 ± 3,85 g/100g, p < 0,05 *vs* MSG and CAF). **Conclusion:** The association of these two experimental models of obesity aggravates insulin resistance that probably is due at least in part to the increase of visceral fat content.

Keywords: obesity; blood pressure; insulin resistance; metabolic syndrome X; rats, Wistar.

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Introduction

Obesity is one of the major problems of public health in developed and developing countries, regardless of the economic and social conditions. Evidence has suggested that the prevalence of overweight and obesity has increased at an alarming rate.[1]

In human beings and experimental animals, obesity results from the unbalance between energy intake and expenditure for maintaining vital processes. Thus, obesity can be triggered when there is excessive calorie intake without a corresponding increase in energy expenditure, or when, even in the presence of normal calorie intake, metabolic disorders occur in the use of substrates.[2]

The high incidence of obesity and its association with risk factors, such as diabetes mellitus, dyslipidemia, and arterial hypertension, increase mortality due to cardiovascular diseases.[3]

The coexistence of arterial hypertension and obesity increases the risk of cardiovascular disease, and is frequently associated with dyslipidemia and glucose intolerance, making the possibilities of angina pectoris, myocardial infarction, heart failure, stroke, and sudden death even more frequent.[4]

Human obesity is known to be a multifactorial disease, thus, controlling each variable involved in its etiology is

impossible. Experimental models allow the controlled study of each variable involved in the physiopathology of obesity.

The experimental models of obesity comprise the induced models that imitate the western diet with high calorie intake (called cafeteria diet) and those with neonatal administration of monosodium glutamate (MSG), a neuroexcitatory amino acid that destroys neurons of the arcuate nucleus of the hypothalamus.[5] Other experimental models, in which obesity can be studied in obese rats and mice, are the genetic models based on the reduced leptin action.

In those induced models, an increase in body weight and in insulin resistance has been observed. However, studies assessing blood pressure alteration in those experimental models are still controversial. Insulin resistance and hyperinsulinemia are factors that can cause an increase in blood pressure.[6]

To assess the effect of those two models of obesity on blood pressure, we studied rats receiving monosodium glutamate in the neonatal period and cafeteria diet for 12 weeks and assessed tail blood pressure, body weight, glucose metabolism, and visceral fat content. This study also aimed at assessing the effect of the association of those two models of obesity on the parameters studied.

Methods

This study used male Wistar rats, kept in cages (maximum of five animals per cage) at the Universidade Federal de São Paulo. The protocol of this study was reviewed and approved by the Committee on Ethics of the Universidade Federal de São Paulo – Escola Paulista de Medicina (protocol nº 0310/07).

On the day following birth, the animals were separated and part of them began to receive subcutaneous injections of 2 mg/kg/day of MSG up to the 11th day of life. The other animals received the same volume of saline solution. Upon completion of three months of life, part of the animals receiving the neonatal MSG injections had their standard food preparation replaced by cafeteria diet (MSG+CAF, n = 10). The other MSG animals continued to receive the standard diet (MSG, n = 9). Part of the animals receiving saline solution in the neonatal period also received cafeteria diet (CAF, n = 14), while others continued to receive the standard diet (WST, n = 8). The cafeteria diet is composed of standard food preparation (37.5%), corn crackers (12.5%), toasted peanuts (25%), and granular chocolate (25%). All groups were followed up for 12 consecutive weeks.

All groups had their tail blood pressure, by use of the oscillometric method, and body weight measured twice a week. Prior to inclusion in the study, two baseline measurements of tail blood pressure and body weight were taken. The animals with baseline tail blood pressures greater than 140 mm Hg were excluded from the study. The mean values of two tail blood pressure and body weight measurements per week were used as representative of that week.

After a 12-week follow-up of tail blood pressure and body weight measurements, the animals underwent an oral glucose tolerance test, for which they were anesthetized and a polyethylene catheter was inserted in the left femoral artery. That catheter traveled along the subcutaneous tissue and was fixed on the animal's dorsum.

After a 12-hour fasting, approximately 1.0 milliliter of blood was collected from each animal through the arterial catheter for measuring fasting glycemia and serum insulin level. After that, the rats received 68 mg/kg of anhydrous glucose solution through gavage. New blood samples were collected at 15, 30, 60, 90, and 120 minutes after glucose administration for determining serum insulin level and glycemia.

Plasma glucose was measured by use of a glucose meter (Accu-Chek – Advantage) and appropriate test strips (Accu-Chek Advantage II). For measuring serum insulin level, the blood samples collected in Eppendorf tubes were centrifuged (Eppendorf Centrifuge S403) at 3,000 rpm for 10 minutes and stored at -70° C in the freezer (Revco Scientific Inc. Asheville, NC, USA).

After the glucose and insulin measurements were taken, the areas under the curves for glucose and insulin were calculated (AUCG and AUCI, respectively) by determining the weighted average of glycemia and serum insulin level throughout the entire oral glucose tolerance test. Then, the insulin sensitivity index (ISI) was calculated by dividing the factor 10,000 by the product of the areas under the curves for glucose and insulin. The result is expressed as $mg^1.mU^1$.

After the oral glucose tolerance test, the animals were once again anesthetized and the right and left ventricles, as well as the epididymal fat, were dissected and weighed in an analytical scale (Gehara, BG440). The relative weight of the organs was calculated by dividing the organ mass by the body weight.

Statistical analysis was performed by use of repeated-measures analysis of variance (One Way RM ANOVA) for comparing the temporal variations of tail blood pressure and body weight with baseline values. Analysis of variance was used (One Way ANOVA) for comparing the means of four sets of observations for which a parametric test is applicable: absolute weights of heart, left ventricle, and epididymal fat, glycemia, serum insulin level. Non-parametric analysis of variance (ANOVA ON RANKS) was used to compare the means of two sets of observations for which a non-parametric test is applicable: AUCG, AUCI, ISI, relative weights of the heart, left ventricle, and epididymal fat, and delta variation of body weight and tail blood pressure. In all tests, the significance level of 5% was adopted ($p < 0.05$).

Results

Body weight (Table 1)

The analysis of body weight in all experimental groups showed a significant increase at the 12th week as compared with body weight at baseline. When comparing the values of body weight at the 12th week, the MSG group showed the greatest gain in body weight, which was significantly greater than the one obtained in MSG+CAF rats. The weight gain of WST and CAF rats was similar.

Tail blood pressure (Table 1)

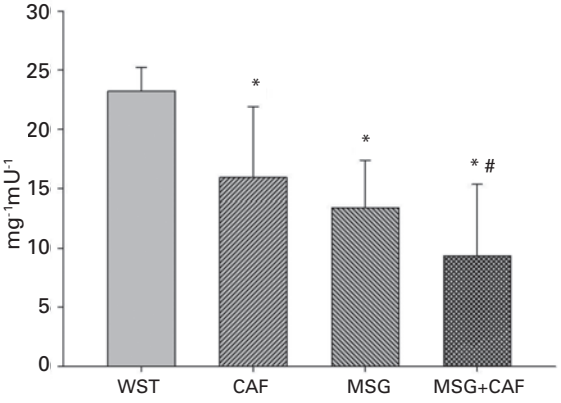
The comparison between initial and final tail blood pressure in the Wistar Control (WST), Wistar MSG (MSG), Wistar Cafeteria (CAF), and Wistar MSG Cafeteria (MSG+CAF) groups showed no statistical differences.

Table 1 Absolute values of body weight (BW, grams), tail blood pressure (TBP, mmHg), insulin sensitivity index (ISI, mg-1mU-1), areas under the curves for glucose (AUCG, mg/dL) and insulin (AUCI, mU/L), and relative epididymal fat (REF, g/100g) in the groups studied

	WST	CAF	MSG	MSG+CAF
BW (baseline)	226 ± 10.13	285.63 ± 16.73	220.79 ± 6.40	273.75 ± 29.6
BW (12 weeks)	294.38 ± 15.86	343.14 ± 29.53	363.75 ± 18.98	403.50 ± 46.55*
TBP (baseline)	119.38 ± 7.76	120.79 ± 3.02	121.75 ± 5.78	120.00 ± 11.28
TBP (12 weeks)	122.25 ± 7.03	123.93 ± 2.89	118.00 ± 5.81	120.17 ± 5.08
ISI	23.25 ± 9.31	15.92 ± 9.10*	13.41 ± 3.84*	9.34 ± 5.77*#@
AUGC	120.87 ± 19.68	191.45 ± 71.03*	143.59 ± 32.97*	231.60 ± 61.97*#@
AUCI	3.96 ± 0.96	5.22 ± 3.73*	5.58 ± 1.07*	5.96 ± 2.87*
REF	6.20 ± 0.57	8.27 ± 1.53*	8.23 ± 1.98*	11.12 ± 3.85*@

Values shown as mean ± standard deviation. *$p < 0.05$ *vs.* WST, #$p < 0.05$ *vs.* CAF and @$p < 0.05$ *vs.* MSG.

Figure 1. Values of mean ± standard error of the insulin sensitivity index (mg^{-1} mU^{-1}) of the groups studied. *$p < 0.05$ *vs.* WST; @$p < 0.05$ *vs.* MSG and #$p < 0.05$ *vs.* CAF.



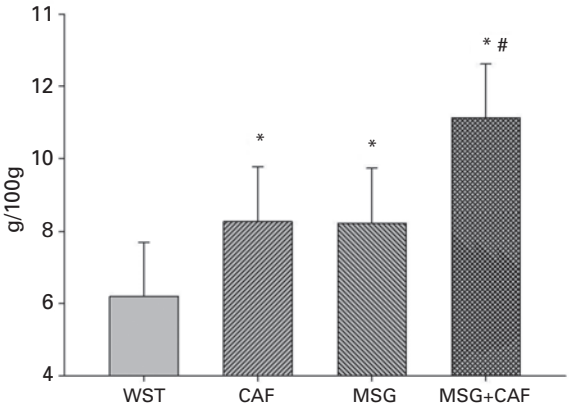
Area under the curve for glucose (AUCG) and area under the curve for insulin (AUCI) (Table1)

The induction of obesity produced a significant increase in the areas under the curve for glucose and insulin in the CAF, MSG and MSG+CAF groups as compared with those of WST rats. In MSG+CAF animals, a significant increase in the AUCG was observed when those animals were compared with CAF and MSG rats separately. No difference was observed in the AUCI between the groups of animals treated.

Insulin sensitivity index (ISI) (Table 1, Figure 1)

The administration of the cafeteria diet caused a significant decrease in ISI in the MSG, CAF, and MSG+CAF

Figure 2. Values of mean ± standard error of the relative epididymal fat (grams/100grams of the rat) of the groups studied, @p < 0,05 *vs*. MSG.



groups. The association between neuroendocrine and exocrine obesity produced a synergic reduction in the ISI of the MSG + CAF group (MSG + CAF = 9.34 ± 5.77 $mg^{-1}mU^{-1}$ & p < 0.05 *vs*. CAF, *p < 0.05 *vs*. MSG).

Absolute weight of epididymal fat (Table 1, Figure 2)

All obese groups showed a significant increase in epididymal fat when compared with the control WST group. Even when corrected by body weight, the epididymal fat continued increased in MSG+CAF rats.

Left ventricular weight

The analysis of the absolute left ventricular weight showed no variation in the groups (WST = 0.63 ± 0.06; MSG = 0.63 ± 0.07; CAF = 0.70 ± 0.07; MSG + CAF = 0.54 ± 0.07). A reduction in the absolute ventricular weight of MSG+CAF rats is observed, due to the greater body weight in that group.

Discussion

This study assessed the effect of producing obesity and insulin resistance by use of two experimental models of neuroendocrine and exocrine obesity, and the effect of the association of the models on glucose metabolism and tail blood pressure of normotensive Wistar rats. Separately, both experimental models caused an increase in insulin resistance, and, when associated, they produced a synergic effect on insulin resistance. The increase in insulin resistance in those animals may be correlated with an increase in the amount of visceral fat. None of the experimental groups showed any alteration in tail blood pressure.

When analyzing the body weight of the animals, some important aspects should be stressed. In the first 12 weeks of the follow-up period, the greatest body weight gain was observed in MSG rats (+65%), followed by the rats receiving MSG+CAF (+47%). The administration of the cafeteria diet did not cause additional body weight gain as compared with that of the standard diet group. Data referring to body weight alterations in rats induced to obesity by the neonatal MSG administration are controversial[7,8] and previous studies by this research group have evidenced no weight gain alteration in those animals. However, MSG animals are known to have a reduced secretion of growth hormone[9] and an increased secretion of cortisol,[10] and, thus, the weight gain in MSG and MSG+CAF animals is mainly due to the increase in visceral fat. Although the animals receiving the hypercaloric diet did not have an increase in body weight, they also had an increase in visceral fat.[11] This is mainly due to the fact that animals receiving a hypercaloric diet reduce their daily food intake, but, because of the high amount of carbohydrates and fat contained in the cafeteria diet, visceral fat builds up.[12]

The increase in cardiovascular risk is more associated with the distribution of visceral fat than with the mere increase in body weight.[3] Studies on the oriental population have already evidenced that patients with increased visceral fat, despite their normal body weight, had an increased prevalence of cardiovascular diseases.[3] In this study, all experimental animals had an increase in epididymal fat and the greatest increase in that parameter occurred in the group associating the two models of obesity: exocrine and neuroendocrine (MSG+CAF group).

The increase in visceral fat results in a series of metabolic alterations, such as the production of adipokines[13,14] and/or decrease in the metabolism of cortisol,[15] which can culminate in an increase in blood pressure and in peripheral resistance to insulin action.[16] Hyperleptinemia secondary to the increase in visceral fat could activate the sympathetic nervous system, with higher secretion of catecholamines, leading to an increase in blood pressure and insulin resistance.[17] Nevertheless, our animals had no alterations in blood pressure. It is worth emphasizing, however, that Wistar animals receiving MSG during the neonatal period increase

their adrenergic activity, since a previous study of ours has already shown that those animals have an increase in heart rate and in total peripheral resistance.[10] Studies reporting an increase in thermogenesis of rats receiving the cafeteria diet support the fact that those animals also have an increase in sympathetic activity.[1] The lack of alteration in blood pressure in our rats could be attributed to the short duration of the study.

However, the increase in the sympathetic activity associated with an increase in visceral fat has determined metabolic alterations that culminate in increased insulin resistance.[18] When the large amount of free fatty acids, originating from visceral fat, arrive at the liver, it causes an increase in gluconeogenesis, a decrease in muscle glucose uptake, and a reduction in the liver metabolism of insulin. It is worth emphasizing that the experimental models, when studied separately, showed an increase in epididymal fat and insulin resistance. When both stimuli were associated, a greater deposition of visceral fat and a synergic effect on insulin resistance were observed. The experimental model used contributes to the study of the metabolic repercussions that occur when human beings, who already have a genetic/neuroendocrine component, undergo an exocrine stimulus, or the administration of a hypercaloric diet, a condition that has become increasingly common with modern life.

It is worth stressing that the decrease in the ventricular mass observed in MSG+CAF rats can be attributed to the body weight of those animals at the end of the study. Maybe the use of a more reliable parameter for that situation, such as correction by the shin bone, would be required to confirm that value.[10]

Conclusions

In conclusion, this study shows that the administration of a cafeteria diet potentiates the effect of the neonatal administration of monosodium glutamate on insulin resistance and that increase could be attributed to the great increase in visceral obesity observed in the animals.

References

1. Pereira LO, Francischi RP, Lancha AH. Obesidade: hábitos nutricionais, sedentarismo e resistência à insulina. Arq Bras Endocrinol Metab 2003; 42(2):111-27.
2. Denadai RC, Sigulem DM, Vítolo MR, Fisberg M, Dâmaso AR. Efeitos da atividade motora sobre a composição corporal, taxa metabólica basal e diária de adolescentes obesos. Rev Paul Pediatr 1996; 14(4):163-8.
3. Carneiro G, Faria AN, Ribeiro F *et al*. Influência da distribuição da gordura corporal sobre a prevalência de hipertensão arterial e outros fatores de risco cardiovascular em indivíduos obesos. Rev Assoc Med Bras 2003; 49(3):306-11.
4. Dâmaso AR. Obesidade na infância e adolescência. In: Teixeira LR. Educação Física escolar adaptada: postura, asma, obesidade e diabetes na infância e adolescência. São Paulo: EEFUSPSP/EFP, 1993, pp. 127-38.
5. Rose PA, Weick RF. Effects of anterior hypothalamic deafferentation and neonatal monosodium L-glutamate treatment on pulsatile LH secretion in the castrated rat. Neuroendocrinology 1986; 43(1):12-7.
6. Pagliassotti MJ, Gayles EC, Podolin DA, Wei Y, Morin CL. Development stage modifies diet-induced peripheral insulin resistance in rats. Am J Physiol 2000; 278(1):66-73.
7. Mancini CM, Halpern A. Aspectos fisiológicos do balanço energético. Arq Bras Endocrinol Metab 2002; 46(3): 230-48.
8. Habitante CA. Efeitos da lipectomia e exercício crônico sobre o metabolismo lipídico de ratos obesos. [dissertação]. São Paulo (SP): Universidade Federal de São Paulo. Escola Paulista de Medicina, 2004.
9. Braga LR, Mello MAR, Gobatto CA. Exercício contínuo e intermitente: o efeito do treinamento e do destreinamento sobre a gordura corporal de ratos obesos. Arch Latinoam Nutr 2004; 54(1): 58-65.
10. Voltera AF, Cesaretti MLR, Ginoza M, Kohlmann O. Efeito da indução da obesidade neuroendócrina sobre a hemodinâmica sistêmica e a função ventricular esquerda de ratos normotensos. Arq Bras Endocrinol Metab 2008; 52(1):47-54.
11. Souza F, Marchesini JB, Campos ACL *et al*. Efeito da vagotomia troncular em ratos injetados na fase neonatal com glutamato monossódico: estudo biométrico. Acta Cir Bras 2001; 16(1):32-45.
12. Polacow VO, Lancha AHJ. Dietas hiperglicídicas: efeito da substituição isoenergética de gordura por carboidratos sobre o metabolismo de lipídeos, adiposidade corporal e sua associação com a atividade física e com o risco de doença cardiovascular. Arq Bras Endocrinol Metab 2007; 51(3):389-400.
13. Costa JV, Duarte JS. Tecido adiposo e adipocinas. Acta Med Port 2006; 19(3):251-6.
14. Rothenbacher D, Brenner H, März W, Koenig W. Adiponectin, risk of coronary disease and correlations with cardiovascular risk markers. European Heart Journal 2005; 26(16):1640-6.

15. Matos AFG, Vieira AR, Coutinho W *et al*. A obesidade estaria relacionada ao aumento do volume das adrenais? Arq Bras Endocrinol Metab 2000; 44(1): 21-9.
16. Ribeiro Filho FF, Mariosa LS, Ferreira SRG, Zanella MT. Gordura visceral e síndrome metabólica: mais que uma simples associação. Arq Bras Endocrinol Metab 2006; 50(2): 230-9.
17. Galvão R, Kohlmann O. Hipertensão arterial no paciente obeso. Rev Bras Hipertens 2002; 9: 262-7.
18. Foley JE. Rationale and application of fatty acid oxidation inhibitors in treatment of NIDDM. Diabetes 1992; 15(6):773-82.