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Adrenalectomy improves MSG-induced obesity in rats by increasing UCP-1 levels in interscapular brown adipose tissue

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ABSTRACT. The administration of monosodium glutamate (MSG) to neonates has been shown to result in hypothalamic damage and development of metabolic syndrome. The objective of this study was to investigate whether bilateral adrenalectomy (ADX) could alter sympathetic activity in adult MSG rats, with the potential to contribute to the improvement of metabolic syndrome by interfering with intermediary metabolism. Following a period of 99 days, the rats that received the control treatment and MSG were subjected to bilateral adrenalectomy (ADX). The following experimental groups were established, and designated as follows: CTL-SHAM, CTL-ADX, MSG-SHAM, and MSG-ADX. The subjects were 109 and 110 days-old at the time of the experiment. The assessment included murinometric and biochemical parameters, an intravenous glucose tolerance test (IVGTT), sympathetic nerve activity in interscapular brown adipose tissue (iBAT), and protein content of β3-adrenergic receptor (β3-ADR) and uncoupling protein 1 (UCP-1). The MSG rats presented metabolic syndrome, which was associated with lower sympathetic activity and a decreased content of the β3-adrenergic receptor when compared to the controls in iBAT. We can conclude that the lower β3-adrenergic receptor contributed to the maintenance of obesity in the MSG rats model. Although it has an adverse effect on the glucose tolerance of CTL-ADX animals, the typical features of metabolic syndrome in adult MSG rats were mitigated by ADX. These effects included an enhancement of UCP-1 in iBAT, a reduction in fat accumulation, and an improvement in glycemia, thereby contributing to an improvement in metabolic syndrome.

Keywords: MSG rats; fructosamine; iBAT; adipose tissue harvesting; TyG index; β3 - adrenergic receptor pathway.

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Introduction

Monosodium glutamate (MSG) is widely used as a food additive to enhance the flavor of foods (Banerjee, Mukherjee, & Maji, 2021). Glutamate is the most abundant excitatory neurotransmitter in the Central Nervous System (CNS), and in neonate rodents, it can cross the blood brain barrier (BBB) and cause neurotoxicity (Blood, Oser, & White, 1969). In fact, high levels of MSG are neurotoxic for the hypothalamic arcuate nucleus (ARC). Since ARC regulates the body mass and energy metabolism, MSG induces obesity in neonate rats (Hernández Bautista, Mahmoud, Königsberg, & López Díaz Guerrero, 2019).

MSG-induced obesity in rats is an experimental model of obesity characterized by many features also present in humans, like decreased sympathetic activity, hyperinsulinemia, severe visceral fat accumulation and other components of metabolic syndrome (Miranda et al., 2016; Torrezan et al., 2019). Despite exhibiting increased adiposity, MSG rodents display a low body weight phenotype in adulthood, indicating greater energy efficiency.

Furthermore, the surgical removal of the adrenal glands from MSG rats resulted in alterations to metabolic homeostasis, attributed to modifications in the insulinotropic response and the concentration of muscarinic receptors (M3AChR) within pancreatic islets (Miranda et al., 2016b). However, the implications of the β3-adrenergic receptor and uncoupling protein 1 in iBAT, and their consequences in the development of metabolic syndrome, remain under-researched. Moreover, evidence indicates that alterations in autonomic

activity and β3-adrenergic receptors may contribute to the pathogenesis of obesity (Miranda et al., 2016; Mirrakhimov et al., 2011). The sympathetic nerves modulate thermogenesis in the interscapular brown adipose tissue, thereby enhancing energy expenditure (Morrison & Madden, 2014).

Adrenalectomy mitigates signs of metabolic syndrome in humans and rats with hypercortisolism (Ferraù & Korbonits, 2018) and reduces the parasympathetic activity (Miranda et al., 2016). It was thus hypothesized that bilateral adrenalectomy would be beneficial in improving metabolic syndrome in MSG rats, with a particular focus on the upregulation of uncoupling protein 1 in brown adipose tissue, which is thought to occur through the β3-adrenergic receptor pathway.

Material and methods

Animals and experimental design

All the experiments were performed as recommended by the ARRIVE guidelines and the standards of the Brazilian Association of Animal Experimentation (COBEA) (Percie du Sert et al., 2020). All experimental procedures to obtain the results of this study were approved by the Ethics Committee in Animal Research of the *Universidade Estadual de Maringá* (UEM-PARANÁ), (protocol 6653070318). The obesity was induced by injecting 4 mg g^{-1} day^{-1} MSG intradermally in the cervical area of newborn rats from the first to the fifth day of life. Control animals were injected with saline solution (0.9% NaCl). At weaning (at 21th postnatal day - PN 21), only male rats were housed (4 rats $cage^{-1}$) for the experiments. Rats received water and commercial chow (Nuvital ®, Curitiba, Brazil) *ad libitum* and during all protocol stages they were placed in an environmentally controlled room (23 ± 3 ºC and 12h/12h light/dark photocycle). At PN 99 (n = 40 animals per group), control (CTL) or MSG rats were randomly chosen and were distributed into subgroups, sham-operated (CTL/SHAM, n = 25 and MSG/SHAM, n = 25) or adrenalectomized rats (CTL/ADX, n = 25 and MSG/ADX, n = 25). The analyses occurred at PN109 and PN110 day, as shown in Figure 1.

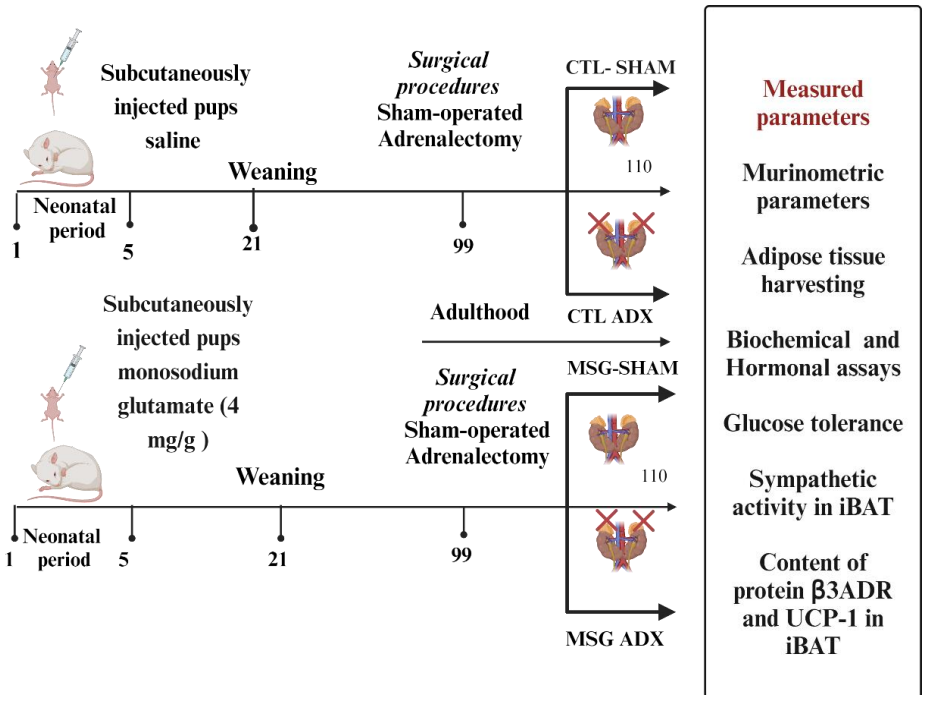

Figure 1. Experimental design of the study. Created with BioRender.com

Bilateral adrenalectomy

At PN 99 day, animals were anesthetized with thiopental (45 mg kg^{-1} BW ip.) and underwent bilateral ADX. SHAM rats were operated under the same conditions but keeping the glands intact. ADX rats were maintained on 0.9% NaCl to correct the aldosterone insufficiency until the experiments (Su et al., 2015). At the end of surgery, animals of all groups received an anti-inflammatory analgesic (meloxicam, 0.4 mg kg^{-1}, i.v.) and an antibiotic (enrofloxacin 5 mg kg^{-1}, i.v), and were then transferred to an appropriate environment for recovery (Cicero, Fazzotta, Palumbo, Cassata, & Lo Monte, 2018).

Final body weight and adipose tissue harvesting

At PN99, PN109 and PN110, the final body weight of all rodents in each experimental group was measured, for a final total of 46 animals. By the end of the experiment, we had a 10% loss of animals. The absolute weight loss was calculated as the difference between the final and baseline weight (BW final – BW initial), where final weight refers to the weight 10 days after sham or ADX surgery, and baseline weight was measured before surgery. Following the conclusion of the experiment, the animals were euthanized, and the adipose tissue depots (periepididymal, retroperitoneal, and iBAT) were excised, weighed, and the values were expressed as grams per 100 grams of body weight. Subsequently, the adipose tissues were stored at – 80 °C. To quantify the relative adipose weight loss in CTL and MSG rodents subjected to SHAM (sham-operated) or ADX (adrenalectomy) procedures on PN109 and PN110, the difference between the initial body weight, measured at PN99, and the final body weight was calculated. This difference was then analyzed for each rat to assess the changes in body weight associated with the procedures performed.

Sympathetic activity in iBAT

At PN 109 (n=10 rats per group), MSG and CTL either SHAM or ADX, were anesthetized as previously described and subsequently the sympathetic nerve branch that stimulates the iBAT was also recorded (Almeida et al., 2022). The nerve trunk was stimulated and the electrical signals were amplified using silver electrodes and a bio amplifier. To mitigate the risk of dehydration, the nerve was protected with a silicone oil coating. The neuronal activity was filtered between 1 and 80 kHz and subsequently recorded for subsequent analysis. During the acquisition of data, the animals were maintained within a Faraday cage in order to minimize the influence of electromagnetic noise. The analysis of nervous electrical impulses was based on the counting of peaks of the voltage exceeding 0 mV in 5-second intervals. Following a two-minute stabilization period, 20 15-second recording intervals were randomly selected for the purpose of determining the average firing rate of the nerves. At PN 109 rats from both groups were anesthetized with a combination of ketamine and xylazine (55 and 8 mg kg^{-1} BW, respectively), and subsequently euthanized via rapid decapitation.

Intravenous glucose tolerance test (ivGTT)

At PN 110 animals of all groups (n = 8 animals per group) were anesthetized and a Silastic cannula was implanted into the right jugular vein. After an overnight fast (12 hours), a glucose load (1 g kg^{-1} BW) was administered into the cannula. Blood samples were collected in heparinized syringes at 0 (before glucose administration), 5, 15, and 30 minutes for serum glucose determination using commercial kits according to the supplier's instructions (Gold Analisa®, Belo Horizonte, Minas Gerais State, Brazil). Additionally, fasting blood samples devoid of anticoagulant (serum) were obtained.

Biochemical and hormonal assays

The plasma obtained from each of the eight animals in each (IVGTT) test group was stored at – 20 °C for subsequent determination of glucose, total cholesterol, triglycerides, HDL-c, fructosamine, and total protein using the method described above. The TyG index was used as a predictor of insulin resistance (IR) and cardiometabolic syndrome, which proved to be a more reliable indicator than HOMA-IR. The mathematical formula for the TyG index is as follows: TyG Index = Ln [Fasting triglycerides (mg dL^{-1}) × Fasting glucose (mg dL^{-1})/2] (Araújo et al., 2022). Corticosterone concentration was measured using commercial enzyme-linked immunosorbent assay (ELISA) kit (Enzo Life Sciences, Plymouth Meeting, PA, USA).

Western blot analyses

At 110 days of age, iBAT samples (120 mg) were collected from SHAM or ADX rodents (n = 8 rats per group), homogenized in RIPA lysis buffer containing protease inhibitors, and centrifuged at 10,000 × g for 5 minutes. The supernatants were diluted in lysis buffer and combined with Laemmli buffer and 30 µg of proteins were applied in wells of a 10% gel for SDS-PAGE. Proteins were transferred to PVDF membranes. These membranes were blocked in a TBS-T containing 5% (w/v) skimmed milk powder for 1h at room temperature. Subsequently, the membranes were washed with TBS-T and incubated overnight at 4°C with primary antibodies: anti-β3-ADR (1:500) and anti-UCP1 (1:1000) (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA). Bands were detected by chemiluminescence (SuperSignal™ West Pico PLUS Chemiluminescent Substrate, Thermo Scientific, Waltham, MA, USA) after incubation with an appropriate horseradish peroxidase-conjugated secondary antibody, and bands were visualized using ImageQuant LAS 500 (GE Healthcare Life Sciences,

Chicago, IL, USA). Band intensities were analyzed using the software ImageJ®. GAPDH was used as a loading control for all the proteins analyzed.

Statistical analysis

The results were expressed as mean ± standard error of the mean (SEM). We used GraphPad Prism Software (version 8.0) to carry out the statistical analysis. We evaluated the statistical significance of the data using Student's t-test or two-way ANOVA followed by Tukey post hoc. We considered the results significantly different when $p < 0.05$.

Results

Murinometric parameters

The MSG-SHAM rats presented a lower final body weight (Figure 2A; $p < 0.05$), increased periepididymal (Figure 2C; $p < 0.05$), retroperitoneal (Figure 2E; $p < 0.05$) iBAT (Figure 2G; $p < 0.05$) adipose tissue harvesting, when compared to CTL-SHAM rats. Adrenalectomy decreased body weight (Figure 2A; $p < 0.05$), MSG-ADX rats exhibited a more pronounced reduction in body weight (Figure 2B; $p < 0.05$) and retroperitoneal fat loss (Figure 2F; $p< 0.05$) lower retroperitoneal (Figure 2E; $p < 0.05$;) and iBAT (Figure 2G; $p < 0.05$) a greater reduction in fat mass was observed in the iBAT (Figure 2H; $p < 0.05$) in the MSG- ADX animals. In CTL-ADX rats, adrenalectomy reduced body weight (Figure 2A; $p < 0.05$) and iBAT mass (Figure 2D; $p < 0.05$). The remaining parameters exhibited no discernible variation. The administration of MSG in neonatal rats resulted in an accumulation of adipose tissue, particularly in the white and brown adipose depots. Conversely, the removal of the adrenal glands led to a reduction in body weight, overall adiposity, and consequently an improvement in the obese phenotype.

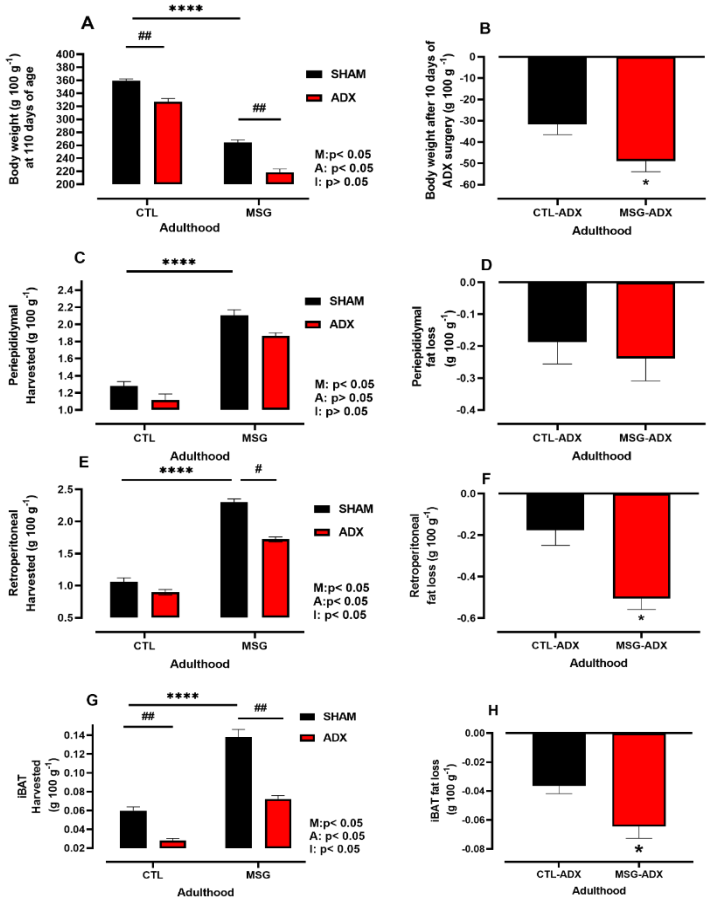

Figure 2. Final body weight (A); BW loss 10 days after adrenalectomy (B); Periepididymal harvesting (C); Periepididymal fat loss (D) Retroperitoneal harvesting (E) Retroperitoneal fat loss (F) iBAT harvesting (G) iBAT fat loss (H). Results expressed a mean ± SEM (n = 8 rats per group) from at least four different litters for weight and fat pad per group of PN 109 and PN 110. The symbols represent significant differences (Two-way ANOVA) followed by *post-hoc* Tukey or T-Student test. Differences between CTL and MSG are indicated by asterisks (*), while hash marks (#) indicate differences between sham-operated (SHAM) and adrenalectomized (ADX). M, Factor MSG; A, Factor adrenalectomy; I, Interaction between factors. Significance: $p < 0.05$.

Biochemical and hormonal assays

MSG-SHAM showed hypercortisolism (Figure 3A; $p < 0.05$) in comparison with CTL-SHAM. Adrenalectomy was effective in reducing such hormone in both CTL-ADX (Figure 3A; $p < 0.05$) and MSG-ADX (Figure 3A; $p < 0.05$). As shown in Figure 3, fasting glucose was higher in MSG-SHAM than in CTL-SHAM (Figure 3B; $p < 0.05$). Adrenalectomy reduced the fasting blood glucose in the MSG-ADX group compared with MSG-SHAM (Figure 3A; $p < 0.05$). MSG-SHAM rats showed hypertriglyceridemia (Figure 3C; $p < 0.05$) compared to CTL-SHAM. The ADX caused a reduction in triglyceridemia in both CTL-SHAM (Figure 3C; $p < 0.05$) and MSG-SHAM rats (Figure 3C; $p < 0.05$). The MSG-SHAM group exhibited hypercholesterolemia when compared to the CTL-SHAM group (Figure 3D; $p < 0.05$). Furthermore, a pronounced decline in total cholesterol was observed in the MSG-ADX cohort when contrasted with the MSG-SHAM animals (Figure 3D; $p < 0.05$). Obese MSG rats exhibited a statistically elevated TyG index (Figure 3E; $p < 0.05$) in comparison to CTL-SHAM rats. In contrast, ADX treatment resulted in the suppression of the TyG predictor in MSG-ADX and CTL-ADX rats (Figure 3E; $p < 0.05$). Furthermore, both ADX groups exhibited a reduction in HDL-c (Figure 3F; $p < 0.05$) compared to the MSG-SHAM and CTL-SHAM groups, respectively. Data indicate that the reduction in fructosamine levels in the MSG-SHAM group was significantly greater than that observed in the CTL-SHAM group (Figure 3G; $p < 0.05$). Conversely, the CTL-ADX group exhibited a decline in fructosamine levels (Figure 3G; $p < 0.05$), while the MSG-ADX group showed an increase in the same (Figure 3G; $p < 0.05$). These findings suggest a strong interaction effect between obesity and adenectomy. The removal of the adrenal glands resulted in a notable decline in total protein levels in both the CTL-ADX and MSG-ADX groups, as evidenced by statistical analysis (Figure 3H, $p < 0.05$).

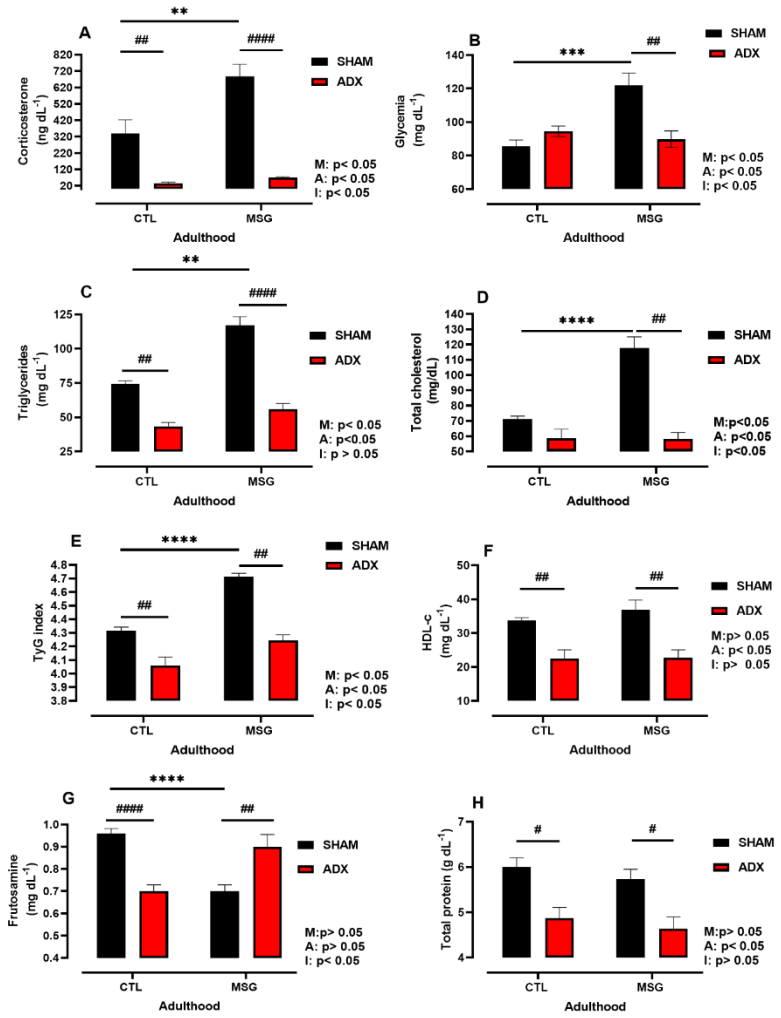

Figure 3. Effect of obesity and adrenalectomy on fasting glycemia (a) Corticosterone (b) Triglycerides (c) Total Cholesterol (d). The values are the mean ± SEM (n = 8 rats per group) at PN 110. The symbols represent significant differences (Two-way ANOVA) followed by *post-hoc* Tukey. Differences between CTL and MSG are indicated by asterisks (*), while hash marks (#) indicate differences between sham-operated (SHAM) and adrenalectomized (ADX). M, Factor MSG; A, Factor adrenalectomy; I, Interaction between factors. Significance: $p < 0.05$.

Intravenous Glucose Tolerance Test (ivGTT)

The glucose levels, observed during glucose tolerance test, are shown in Figure 4. The area under the curve values (inset in Figure 4, AUC) showed significant differences between the groups CTL-ADX and CTL-SHAM ($p < 0.05$), without differences across the other groups.

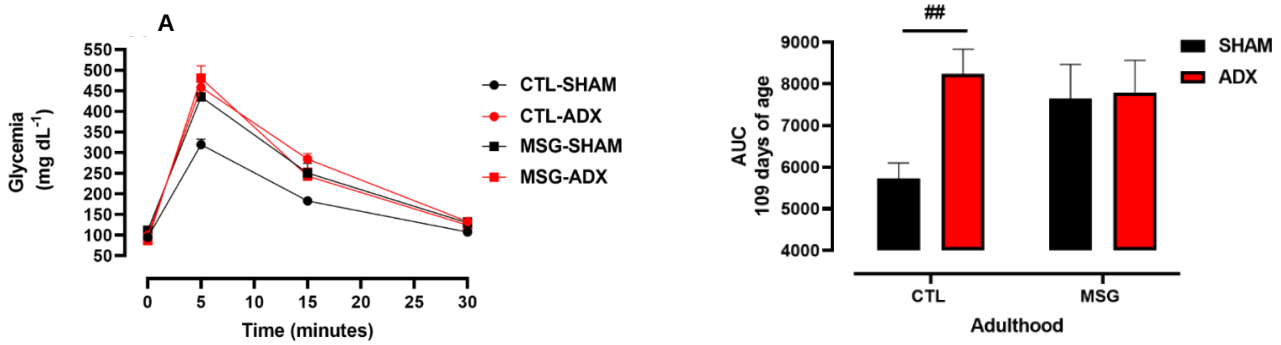

Figure 4. Plasma glucose concentrations during the intravenous glucose tolerance test. The bar graph at the top represents the area under the curve (AUC). Data are expressed as the mean ± SEM (n = 8 rats per group) from at least three different litters at PN 110. To determine differences in glucose over time, the analyses included repeated measures: The symbols represent significant differences (Two-way ANOVA) followed by Tukey *post-hoc*. # $p < 0.05$ SHAM *versus* ADX. M, MSG; A, Adrenalectomy.

The electrophysiology of the sympathetic nerve, the expression of the protein content of the adrenergic receptor subtype (β3-ADR), and uncoupling protein (UCP-1) in interscapular brown adipose tissue (iBAT)

We found sympathetic hypoactivity in the MSG-SHAM group compared to CTL-SHAM. The CTL-ADX and MSG-ADX animals are clearly not different from their respective controls, CTL-SHAM and MSG-SHAM. This research generated some remarkable discoveries. MSG-SHAM showed a reduction in β3-ADR expression in iBAT (Figure 5B; $p < 0.05$) compared to CTL-SHAM, proving without a doubt its involvement in maintaining obesity. There was no difference between the CTL-SHAM and MSG-SHAM in UCP-1 protein expression. Adrenalectomy increased UCP-1 expression in both groups CTL-ADX and MSG-ADX (Figure 5B; $p < 0.05$).

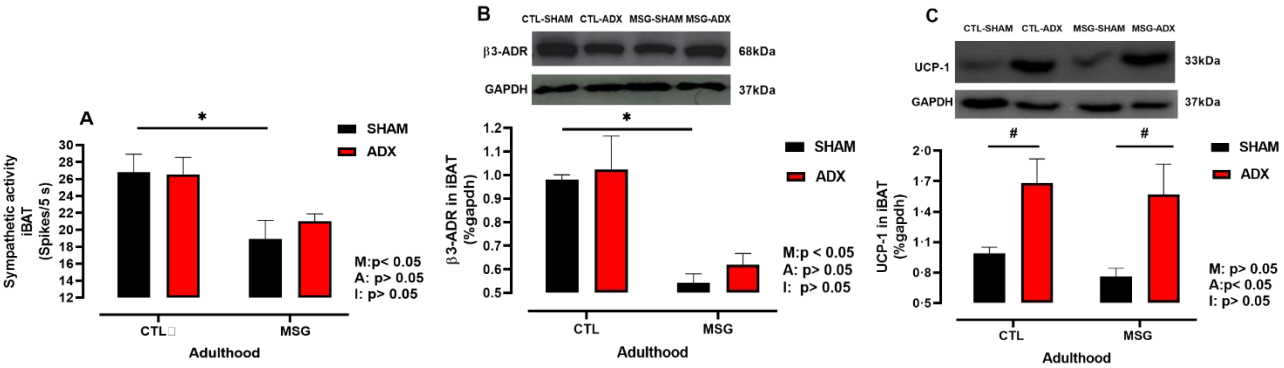

Figure 5. Electrical activity of iBAT sympathetic nerves from SHAM and ADX rats at PN 109, 10 days after surgery (n = 10 rats per group). Representative records of nerve discharges for each experimental group are shown above the bars. Values are represented by the mean (A). The protein content (B) of the adrenergic receptor subtype (β3-ADR) and (C) uncoupling protein (UCP-1) involved in the regulation of thermogenesis of interscapular adipose tissue in adult rats (n = 8 rats per group) in the PN 110. The groups are as follows: CTL-SHAM, MSG-SHAM, CTL-ADX and MSG-ADX. Results expressed a mean ± SEM. The symbols represent significant differences (two-way ANOVA) followed by Tukey *post-hoc*. Differences between CTL and MSG are indicated by asterisks (*), while hash marks (#) indicate differences between sham-operated (SHAM) and adrenalectomized (ADX). M, Factor MSG; A, Factor adrenalectomy; I, Interaction between factors. Significance: $p < 0.05$.

Discussion

Our findings revealed that subcutaneous injections of MSG during neonatal period induce hypercortisolism, which contributes to increased adiposity accumulation, dyslipidemia, dysglycemia, confirm the ability of inducing metabolic syndrome in adult life.

Additionally, the results show that MSG-SHAM phenotype exhibit hypofructosaminemia, despite no discernible differences in total serum protein levels. Fructosamine is a diagnostic marker for type 2 diabetes

mellitus (Danese, Montagnana, Nouvenne, & Lippi, 2015). Recently reported findings suggest that increased adiposity in obese individuals is positively correlated with reduced fructosamine levels (Vergès et al., 2021). They suggest that fructosamine formation may be impaired, given that MSG-SHAM animals are glucose intolerant (Yamazaki et al., 2011). Furthermore, corticosterone was observed to reduce the number of GLUT 4 receptors in MSG-induced conditions, thereby diminishing insulin-stimulated glucose uptake and promoting insulin resistance in skeletal muscle (Dolnikoff, Martín-Hidalgo, Machado, Lima, & Herrera, 2001). The TyG index was found to be elevated in MSG-SHAM rats, resulting from hypertriglyceridemia and dysglycemia. This confirms the TyG index as a reliable predictor for measuring peripheral insulin resistance and cardiometabolic disorders (Araújo, Juvanhol, Bressan, & Hermsdorff, 2022). Therefore, the TyG index may be considered a viable alternative to the HOMA-IR (Dundar, Terzi, & Arslan, 2023).

Our findings showed that ten days following adrenalectomy, MSG rats displayed a reduction in fat accumulation, with retroperitoneal visceral fat and iBAT exhibiting the most pronounced oxidation after adrenalectomy. In opposition, epididymal fat showed a similar profile between the CTL-ADX and MSG-ADX groups. It has been demonstrated that five weeks following adrenalectomy, insulin sensitivity is restored in patients with pheochromocytoma, as evidenced by the hyperinsulinemic-euglycemic clamp technique (Wiesner, Blüher, Windgassen, & Paschke, 2003).

In this study, adrenalectomy resulted in a rapid reduction in glucocorticoids in MSG-ADX rodents, which was associated with a decline in fasting blood glucose levels, hypertriglyceridemia, hypercholesterolemia, and HDL-c, while also showing an increase in UCP-1 in iBAT. On the other hand, serum fructosamine levels increased in MSG-ADX animals, while CTL-ADX rodents revealed a decrease. Although the increase in fructosamine is associated with hyperglycemia, the normalization of glycemia in obese adrenalectomized animals may be related to this increase in fructosamine. Surgical removal of the adrenal glands enhancers the process of muscle protein degradation. Additionally, it has been observed that this procedure promotes a reduction in body weight, along with a decrease in both liver and muscle tissue mass (Glasser & Izzo, 1962).

MSG rats exhibit sympathetic hypoactivity and decreased protein content of the β3-ADR in the iBAT. Scientific researchers have shown that, mice treated with an obegenic diet have experienced inflammation in sympathetic neurons expressing neuropeptide Y. The administration of MSG to rodents has been demonstrated to result in damage to the hypothalamus (Miranda et al., 2016). This finding provides evidence that NPY, which is expressed in iBAT sympathetic neurons, may be limited, thereby substantiating the observed increase in weight and whitening of this tissue in our MSG-treated rodents (Zhu et al., 2024). Downregulation of β3-ADR can be a consequence of obesity as described in different obesity models, such as hypercaloric diet-induced obesity (Valentine et al., 2022) and genetic obesity (fa/fa Zucker rats) (Holt & York, 1989). Adrenalectomy does not reestablish sympathetic activity and β3-adrenergic receptor content in the iBAT.

In humans, mirabegron, a selective β3-adrenergic receptor agonist, has shown to stimulate lipolysis and thermogenesis in brown adipocytes (Cero et al., 2021). Moreover, it has been demonstrated that mice treated with mirabegron exhibit enhanced glycemic and insulin regulatory functions. This treatment also stimulates UCP1 expression and induces the browning of white adipocytes (Hao et al., 2019). In these obesity models, administration of this drug may serve as an alternative means of improving metabolic control by increasing energy expenditure.

The glucocorticoids downregulate UCP-1 expression (Soumano, 2000). This effect can be confirmed with the administration of the glucocorticoid receptor antagonist RU 486, that stimulates UCP-1 expression in brown adipocytes (Rodriguez & Palou, 2004). The adrenalectomy is able to increase the protein content of the UCP-1 in both, CTL-ADX and MSG-ADX. We found that the absence of corticosterone in this model may be enhancing thermogenesis. We suggest that the corticosterone levels may be an important factor to control the energy expenditure since it regulates UCP-1 expression.

Conclusion

Adrenalectomy in MSG rats increased UCP-1 iBAT, which is essential to mitigate metabolic syndrome.

Acknowledgements

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