Research Article

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Monosodium glutamate induces hypothalamic–pituitary–adrenal axis hyperactivation, glucocorticoid receptors down-regulation, and systemic inflammatory response in young male rats: Impact on miR-155 and miR-218

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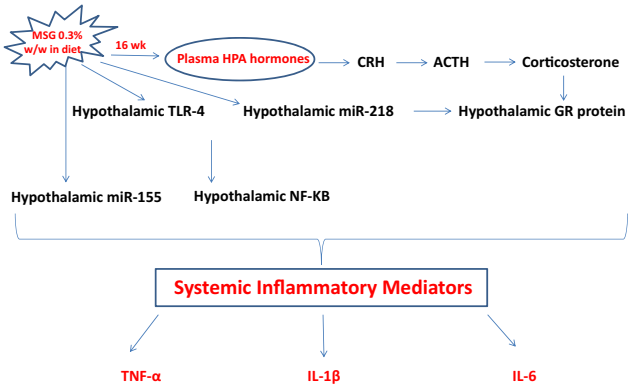
Abstract: Young children are attracted to flavored foods with enhancers, particularly monosodium glutamate (MSG). Experimental studies have proven that MSG can alter the hypothalamic–pituitary–adrenal (HPA) axis response in neonates. We, therefore, investigated the modulation of microRNAs (miRNAs) by dietary MSG and its association with the stimulation of the HPA axis and inflammatory response in young male rats. One-month-old male rats were fed chow enriched with MSG (3 g/kg) for 16 weeks. Feeding MSG to rats markedly up-regulated hypothalamic miR-218, Toll-like receptors-4, and nuclear factor-kB but down-regulated miR-155 and glucocorticoid receptors (GR). In addition, it triggered a remarkable elevation in adrenocortical lipid peroxidation and depletion of antioxidants. These changes were coupled with increased plasma levels of the HPA axis hormones, comprising corticotropin-releasing hormone, adrenocorticotropic hormone, corticosterone levels, and serum pro-inflammatory cytokines.



Graphical abstract

Taken together, current findings indicated that MSG caused an activation of the HPA axis, a down-regulation of GRs, and a systemic inflammatory response. These disturbances were associated with modulating hypothalamic miRNAs, encompassing miR-218 and 155.

Keywords: the HPA axis, glucocorticoid receptors, miR-155, NF-kB, flavor enhancers

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1 Introduction

Monosodium glutamate (MSG) (E621), an L-glutamate sodium salt, is a popular flavor enhancer in different foods [1]. The application of MSG is increasing to improve the taste and aroma of foods and flavoring products. Consequently, humans consume a considerable amount of this flavor enhancer daily [2,3]. The United States Food and Drug Administration (FDA) categorized MSG as a safe

substance in 1959. However, some reports indicated that MSG might cause mild symptoms. Therefore, the FDA asked an independent scientific group (FASEB) to inspect MSG safety in the 1990s. This group found that this flavor enhancer is safe but identified some slight temporary adverse effects in sensitive individuals, such as headache, drowsiness, allergic reactions, palpitations, and numbness after consumption of ≥3 g MSG with no food [4].

A recent publication has developed a highly sensitive and selective sensor towards MSG, detecting a low limit. The luminescence response is MSG-specific, allowing its accurate determination in food even if other substances exist [5].

Evidence suggests that MSG is risky for human health. Several clinical and experimental studies indicated that MSG, even at the lowest dose, is health-threatening. It can induce multi-organ damage, disrupting their functions. For example, MSG has been reported to cause Chinese restaurant syndrome, obesity, neurotoxicity, central nervous system (CNS) disorder, reproductive abnormalities, and liver damage [6–11].

Pregnant women are exposed to MSG as a result of increased consumption. Exposure to MSG during pregnancy can adversely impact fetal brain development and metabolism [12]. For example, rats subjected to MSG during pregnancy have been reported to cause autism-like behavior in their offspring via increasing brain glutamate and decreasing glutathione [13]. In addition, children are most vulnerable to MSG health hazards where consumption of this flavor enhancer could seriously impair their cognitive skills and learning abilities [3,14].

A recent review summarizes various existing studies on the disruptive impact of MSG on male reproductive health and function. It discusses how MSG exposure can induce a reduction in sperm count, motility, and testosterone levels. This review also highlights several potential mechanisms mediating MSG reproductive toxicity in males. These include hormonal changes, inflammation, oxidative stress, and direct effects on the testes [15]. Another novel experimental study suggests the potential protective impact of black garlic against MSG's adverse effects on reproductive health. It examines the influence of MSG and black garlic treatment on some reproductive parameters, encompassing gonadosomatic index (GSI), follicle-stimulating hormone (FSH) levels, and sperm quality. Results have shown that MSG exposure negatively affects reproductive parameters, encompassing GSI, FSH levels, and sperm quality, indicating reproductive toxicity. However, black garlic supplementation mitigates MSG's deleterious impact, improving the aforementioned reproductive health markers [16].

Interestingly, the adrenal gland is the most common endocrine target to different chemicals and toxins following chronic exposure, even at lower levels, inducing adrenocortical toxicity and disruption of endocrine function [17,18]. Plasma levels of corticosterone and its stimulus, adrenocorticotrophic hormone (ACTH), were elevated in rats and their body weights were gradually increased by the injection of glutamate into their third ventricles [19]. Additionally, it was found that chronic administration of MSG resulted in excessive accumulation of glutamate concurrently with greater stimulation of the hypothalamus leading to impairment of the hypothalamic–pituitary–adrenal (HPA) axis responsiveness [20]. Another study also demonstrated that treating newborn mice with MSG resulted in adrenal gland hypertrophy along with widening of the cortex, in particular, fasciculate cell enlargement and Cushing's obesity, compared with controls. These findings suggested that neonatal exposure to MSG caused disturbances in the HPA axis [21].

MicroRNAs (miRNAs) act as gene regulators in various biological systems, including the neuroendocrine system. They are also key contributors to stress responses [22]. For example, miR-18a could reduce glucocorticoid receptor (GR) expression *in vitro* [23]. MiR-218 also is an epigenetic modifier for susceptibility to stress [24]. Moreover, miRNAs are predominantly involved in CNS development and brain response [25]. Physiologically, these non-coding RNAs function as regulators for the expression of GR [26]. Numerous miRNAs, including miR-155, are also implicated in inflammatory and immune responses, which are controlled by glucocorticoids [27,28].

In this study, we, therefore, examined the alterations of some hypothalamic miRNAs, encompassing miR-218 and 155 in MSG-treated young male rats and their association with the HPA axis hyperactivation, GR modulation, and inflammatory response in these rats.

2 Materials and methods

2.1 Animals

The experimental protocol was concordant with the recommendations of the National Institutes of Health for animal handling with adherence to the guidelines of the local ethics committee. It has been reviewed and accepted by Zagazig University's ethics committee, ZU-IACUC/3/F/125/2024.

One-month-old male Wistar rats with an average weight of 170 ± 25 g were purchased from Zagazig University, Faculty of Agriculture. They were accommodated at 22 ±1°C in an air-conditioned and moderately humid animal unit with unrestricted access to food and water and a light–

dark cycle (12 h). The acclimatization was for 1 week. All rats were weighed before applying experimental interventions and then weekly until finishing. Additionally, diet consumption was recorded every day.

Selecting male rats to investigate the modulation of miRNAs by dietary MSG and its association with the HPA axis function and inflammatory response herein was to avoid estrous cycle-associated fluctuations in rats' female sex hormone levels that may influence uniformity and constancy of the results [29,30]. Moreover, previous researchers have frequently enrolled male rats in similar experiments [20,30–34]. Thus, using male rats can ensure controlling hormonal variability, better continuity, and direct comparability of current results with earlier findings.

Rat resembles human beings in numerous aspects, making comparisons between them reliable. Both species possess comparable biological systems, such as hormonal system regulation. Rats and humans also possess conserved inflammatory pathways and immune cells that control inflammatory responses to several stimuli. Furthermore, they have similar physiological and metabolic responses to dietary interventions and stressors. Indeed, rats share significant genetic, structural, and behavioral similarities with humans. Additionally, miRNAs are conserved across both species. These non-coding RNAs play comparable roles in controlling biogenesis and gene expression [35].

2.2 Experimental design and dietary manipulations

After acclimatization, rats were randomized and allocated into two groups (n = 10/group) in metabolic cages. The normal control group was maintained on a standard rat chow (Mazuri®, USA) containing 30% carbohydrate, 23% protein, 6.5% fat, 3% minerals, 2 % fiber, 1% vitamins, 0.3% choline, and 0.2% cysteine. Meanwhile, the MSG group was fed the same chow fortified with (0.3% w/w) MSG [36] for 16 weeks. This percentage represents the optimal amount of MSG, a flavor enhancer, indicated by taste panel studies.

2.3 Blood and tissue sampling

The animals were fasted overnight (for about 12 h) on the last day of the experiment before the induction of anesthesia or the collection of blood samples. They were then weighed and anesthetized. Blood and tissues were taken between 8 and 9 a.m. to account for diurnal rhythm. Blood samples were then isolated from the retro-orbital plexuses to obtain serum and plasma. They were kept at −20°C for further analyses of the HPA hormones and inflammation markers. Rats were decapitated; adrenal glands and brains were dissected. Adrenal glands were weighed; adrenal cortexes were then isolated and stored at −20°C to measure malondialdehyde (MDA) and total antioxidants. The hypothalamus was also isolated from the brain and stored at −80°C for later analysis of Toll-like receptors-4 (TLR-4) and nuclear factor-kappa B (NF-kB) proteins by ELISA as well as miR-218 and -155 by real-time polymerase chain reaction.

2.4 Measurement of plasma levels of the HPA hormones

The plasma levels of corticotropin-releasing hormone (CRH), ACTH, and corticosterone were measured using ELISA Kits (Cusabio, China). Procedures were consistent with the manufacturer's instructions.

2.5 Measurement of GR and inflammatory mediators

GR, TLR-4, and NF-kB were determined in the hypothalamus by ELISA kits (Cusabio, China) as instructed by the manufacturers. Likewise, commercially available ELISA kits were utilized for quantifying serum levels of inflammatory cytokines as follows: tumor necrosis factor-alpha (TNF-α, R&D Systems, USA), interleukin-1beta (IL-1β), IL-6 (USCN, China), and IL-8 (MyBioSource, USA). All procedures were followed as described by the manufacturers.

2.6 Measurement of adrenocortical lipid peroxide and antioxidants

Lipid peroxide (MDA) and total antioxidants were determined in adrenal cortex homogenates by colorimetric biodiagnostic kits (Egypt).

2.7 miRNAs expression assessment by real-time PCR

Total RNA from the hypothalamus was extracted by NucleoSpin miRNA kit (Germany) as indicated by the manufacturers. RNA level was determined spectrophotometrically. It was converted to complementary DNA by iScript Select synthesis kit (USA) and Applied Biosystems specific miRNA primers for miR-155, 218, and U6 snRNA (USA). qPCR reactions were performed to detect

the relative expression of these miRNAs by the $2^{-\Delta\Delta Ct}$ method using an internal control (U6 snRNA).

2.8 Statistics

Data descriptive statistics mean ± standard error of mean (SEM) and *t*-test were performed by Graph Pad Instat v, 5 (USA) at $P < 0.05$. The *t*-test is commonly used to compare between the two groups. It determines whether there is a significant difference in the mean values of biological measures between the two groups. Reasons for choosing this statistical tool are availability, simplicity, ease of implementation and interpretation of results, normal distribution of data for each rat group, approximately equal variance of the two compared groups, and independent observations of each other within each group due to random allocation of rats [37]. Additionally, previous experimental studies examining MSG toxicity utilized *t*-test to compare results between the two groups [31,32]. The association between parameters was assessed by using Pearson correlation at $P < 0.05$.

3 Results

3.1 Dietary MSG impact on food intake, body, and relative adrenal gland weights

Food intake, whole body weight, and relative adrenal gland weight were significantly ($P < 0.05$) increased by MSG compared to control, suggesting obesity and adrenal gland hypertrophy (Table 1).

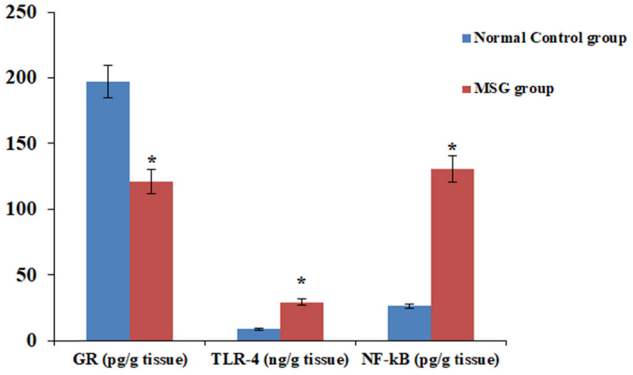

Figure 1: Impact of MSG (0.3% w/w) consumption in the diet on hypothalamic GR and pro-inflammatory cytokine stimulators, TLR-4 and NF-kB. Values are described as mean ± SEM of 10 animals/group. *Significant compared to the normal control group, $P < 0.05$.

3.2 Dietary MSG impact on plasma levels of the HPA axis hormones and hypothalamic GR and their regulator, miR-218

Long-term intake of MSG significantly ($P < 0.05$) increased plasma levels of the HPA axis hormones comprising CRH, ACTH, and corticosterone relative to control, implying stimulation of the HPA axis and impairment of its feedback inhibition by elevated levels of corticosterone in MSG-exposed rats (Table 2). However, MSG significantly decreased the protein level of GR and the expression of miR-218 in the hypothalamus, suggesting glucocorticoid resistance (Figures 1 and 2).

Table 1: Dietary MSG (0.3% w/w) impact on food intake, whole body weight, and relative adrenal gland weight

	Average daily food intake (g)	Body weight (g)	Relative adrenal gland weight (mg/100 mg bw)
Normal control group	12.06 ± 1.18	224.61 ± 1.87	0.79 ± 0.06
MSG group	15.33 ± 1.15*	291.43 ± 2.12*	1.32 ± 0.09*

Values are described as mean ± SEM of 10 animals/group.
*Significant compared to the normal control group, $P < 0.05$.

Table 2: Dietary MSG (0.3% w/w) impacts on plasma levels of the HPA axis hormones

	CRH (ng/ml)	ACTH (pg/ml)	Corticosterone (ng/ml)
Normal control group	3.24 ± 0.25	11.57 ± 1.08	68.32 ± 4.16
MSG group	7.96 ± 0.68*	44.12 ± 3.35*	116.51 ± 10.83*

Values are described as mean ± SEM of 10 animals/group.
*Significant compared to the normal control group, $P < 0.05$.

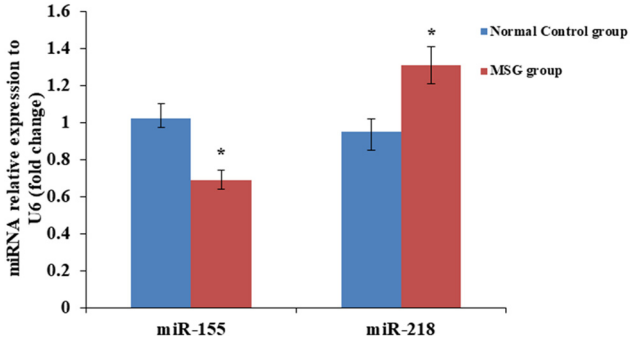

Figure 2: Impact of MSG (0.3% w/w) consumption in the diet on hypothalamic miRNAs: miRNA-155 and miRNA-218. Values are described as mean ± SEM of 10 animals/group. *Significant compared to the normal control group, $P < 0.05$.

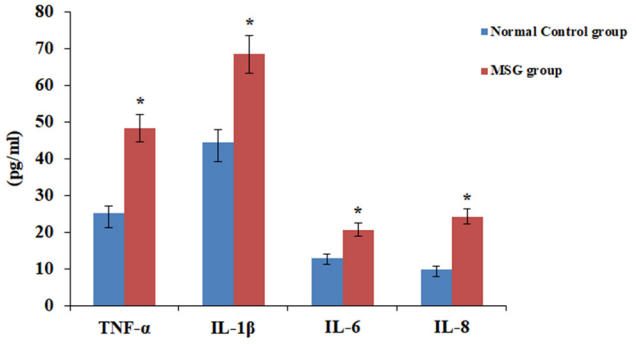

Figure 3: Impact of MSG (0.3% w/w) consumption in the diet on serum levels of pro-inflammatory cytokines: TNF-α, IL-1β, IL-6, and IL-8. Values are described as mean ± SEM of 10 animals/group. *Significant compared to the normal control group, $P < 0.05$.

3.3 Dietary MSG impact on systemic inflammation and hypothalamic miR-155

MSG caused a significant ($P < 0.05$) elevation in serum pro-inflammatory mediators, including TNF-α, IL-1β, IL-6, and IL-8, as well as their regulators, comprising hypothalamic TLR-4 and NF-kB compared to control. Conversely, it significantly ($P < 0.05$) down-regulated hypothalamic miR-155 (Figures 1–3). Although MSG enhanced corticosterone release, this anti-inflammatory hormone failed to suppress higher serum levels of inflammatory cytokines due to the down-regulation of hypothalamic GR.

3.4 Dietary MSG impact on oxidative stress

Dietary MSG markedly stimulated adrenocortical lipid peroxidation and depletion of total antioxidants in the adrenal cortex, suggesting adrenal oxidative damage (Table 3).

3.5 Pearson correlation analyses

There were significant and positive correlations among serum levels of inflammatory cytokines and each of the plasma HPA axis hormones, hypothalamic TLR-4 and NF-kB, and adrenal cortex MDA. Conversely, there were negative correlations among these cytokines and each of hypothalamic GR and miR-155 and adrenocortical total antioxidants (Table 4). Furthermore, hypothalamic miR-218 was inversely correlated with GR ($r = -0.77$, $p < 0.0001$).

4 Discussion

The rate of consumption of MSG has currently increased worldwide. Estimations refer to higher daily intake from the diet in different countries. For example, its average dietary intake is about 1 g in Europe, 4 g in Asia, and 10 g in Germany [38]. According to Solomon et al. [9], the mean daily intake of MSG is approximately 0.3–1 g. These levels may disturb neurons and adversely affect behavior. Despite MSG health threats, the public continues using large amounts of this flavor enhancer [39]. Furthermore, increased MSG consumption can seriously cause cognitive impairment in children [3,14]. Thus, this work aimed to unravel alterations in hypothalamic miRNAs and their association with deregulations of the HPA axis and systemic inflammation resulting from the consumption of MSG in the diet by young male rats.

Table 3: Dietary MSG (0.3% w/w) impacts on oxidative stress in adrenocortical tissues

	MDA (nmol/g tissue)	Total antioxidants (mmol/g tissue)
Normal control group	0.91 ± 0.14	2.68 ± 0.39
MSG group	2.07 ± 0.31*	1.12 ± 0.16*

Values are described as mean ± SEM of 10 animals/group.
*Significant compared to the normal control group, $P < 0.05$.

Table 4: Correlations of serum inflammatory cytokines with plasma HPA axis hormones, hypothalamic TLR-4, NF-kB, GR, and miRNA155, and adrenocortical MDA and total antioxidants in both groups

Inflammatory cytokines	Corticosterone	ACTH	CRH	TLR-4	NF-kB	GR	miR-155	MDA	Total antioxidants
TNF-α	$r = 0.99$*	$r = 0.99$*	$r = 0.99$*	$r = 0.99$*	$r = 0.99$*	$r = -0.88$*	$r = -0.83$*	$r = 0.99$*	$r = -0.85$*
IL-1β	$r = 0.99$*	$r = 0.97$*	$r = 0.98$*	$r = 0.97$*	$r = 0.97$*	$r = -0.82$*	$r = -0.76$*	$r = 0.99$*	$r = -0.78$*
IL-6	$r = 0.99$*	$r = 0.98$*	$r = 0.98$*	$r = 0.98$*	$r = 0.97$*	$r = -0.83$*	$r = -0.77$*	$r = 0.99$*	$r = -0.80$*
IL-8	$r = 0.99$*	$r = 0.99$*	$r = 0.99$*	$r = 0.99$*	$r = 0.99$*	$r = -0.90$*	$r = -0.85$*	$r = 0.98$*	$r = -0.88$*

ACTH: adrenocorticotropic hormone; CRH: corticotropin-releasing hormone; GR: glucocorticoid receptors; TNFα: tumor necrosis factor alpha; IL: interleukin; TLR-4: Toll-like receptor-4; NF-kB: nuclear factor-kappa B; miR: microRNA; MDA: malondialdehyde, *r*: correlation coefficient, *significant at $p < 0.0001$.

In concordance with previous findings [40], we found that chronic exposure to MSG resulted in hypercorticosteronemia coupled with an increase in the levels of ACTH and CRH. Current findings suggest activation of the HPA axis and functional deterioration of its feedback inhibition in these rats since even higher corticosterone levels failed to suppress ACTH secretion from the pituitary gland. Similarly, Torrezan et al. [33] have reported that the onset of MSG-induced obesity is coupled with disruption of the HPA axis central control.

Plasma levels of ACTH in MSG-treated animals were variable among previous studies. One study suggested lower levels [41], while another one indicated unaltered levels [42], and finally, another one showed higher levels of ACTH in MSG-treated animals [43]. However, these studies demonstrated elevated plasma levels of corticosterone in these animals [41–43]. Reduced corticosterone clearance from the liver [42,44], amplified adrenocortical sensitivity to ACTH, or secretion of other corticosterone secretagogues than ACTH during MSG treatment might account for hypercorticosteronmeia [41]. The hypothalamus is vulnerable to glutamate accumulation because, unlike the CNS, it lacks a blood–brain barrier. So, glutamate can enter the hypothalamus by passive transport, inducing neuronal injury [45]. As an excitatory neurotransmitter, glutamate can stimulate the HPA axis [46], elevating levels of ACTH and corticosterone [47]. It is a precursor of gamma-aminobutyric acid [48], an inhibitory and a major hypothalamic neurotransmitter in the paraventricular nucleus where neurons secreting CRH are located [49–51]. Thus, glutamate can stimulate the hypothalamic neurons of the paraventricular nucleus, releasing CRH that reaches the adenohypophyses through the hypophyseal portal circulation. In the anterior pituitary gland, CRH subsequently stimulates ACTH secretion into the circulation and cortisol release from the adrenal gland [52].

Similar to our result, a recent study has shown that MSG-treated rats displayed an increase in secretions and thickness of zona fasciculate cells of the adrenal gland cortex, leading to a significant increment in their body weight [53]. It has been suggested that MSG causes obesity in rats due to increasing plasma corticosterone and retention of salt and water [44,54]. Administration of MSG can also trigger neuroendocrine obesity via inducing hypothalamic lesions, chronic inflammation, and metabolic disorders, comprising insulin resistance and weight gain [55,56].

We noted for the first time that despite higher plasma corticosterone levels herein, they failed to suppress MSG-induced elevation of serum inflammatory cytokines. This effect could be due to the down-regulation of hypothalamic GR. Stress-related disorders are typically characterized by hypercortisolism and enhanced inflammation. These effects may be attributed to glucocorticoid resistance through impaired functioning of GR [57]. Monomers of these receptors can indirectly inhibit activator protein-1 and NF-kB transcriptional activity on pro-inflammatory molecules via protein–protein interactions [58]. *In vitro* studies supported the GR anti-inflammatory mechanism [59,60]. However, *in vivo* studies suggested that the GR and DNA binding might mediate GR's anti-inflammatory impact [61,62]. Several conditions characterized by glucocorticoid resistance and enhanced inflammatory status could result from increased glucocorticoid levels and impaired GR functioning [63].

There is extensive communication between the neuroendocrine and the immune systems [64]. The HPA axis is critically implicated in this communicated network during stress or infection [65]. Immunological effects involve releasing various pro-inflammatory mediators, such as TNF-α, IL-1β, and IL-6 [66]. These cytokines regulate the function of the central nervous system, contributing to observed changes during neurodegenerative and psychiatric disorders [67]. They act as mediators in the HPA axis, where their elevation activates the HPA axis, enhancing stress response [64,68].

Oxidative stress also seems to be involved in MSG toxicity [69,70]. In line with this, the current study showed that

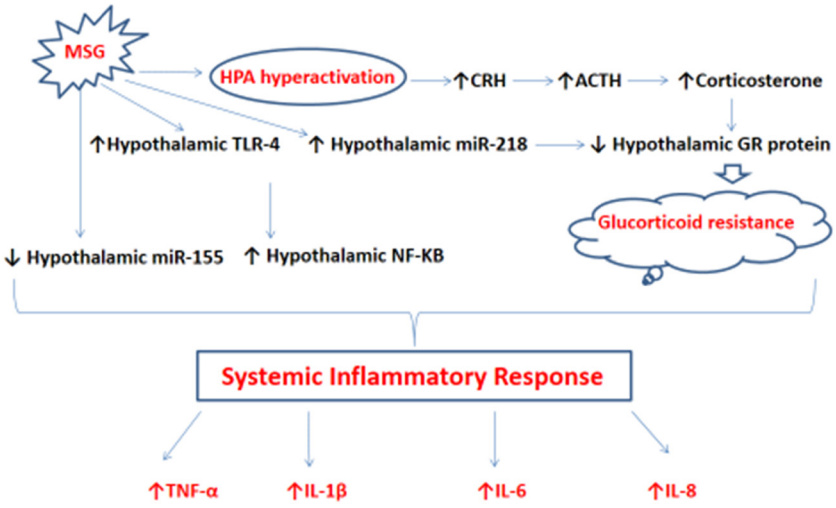

Figure 4: The putative mechanism of MSG-induced activation of the HPA axis and systemic inflammation.

dietary MSG resulted in adrenocortical lipid peroxidation due to the depletion of total antioxidants.

Notably, the current study indicated that MSG-triggered stimulation of the HPA axis was coupled with increased serum levels of pro-inflammatory cytokines. Based on these results, we hypothesized that MSG-induced deregulations in the HPA axis might lead to the observed inflammatory response in these animals. MSG inflammatory effect has been reported in the adrenal zona fasciculata. It increases the macrophage population [71]. Similar results were observed in the alveolar macrophages of MSG-treated rats [72]. MSG triggered immune and endocrine disorders, in particular, dysfunction of lymphocytes and macrophages [73]. In addition, inflammatory cytokines released by macrophages can directly stimulate corticosterone secretion independent of ACTH [74] or affect adrenal cortex steroidogenesis via medullary catecholamines [73]. It is noteworthy that Nakanishi et al. [75] also observed that the livers of long-term MSG-treated 12-month-old mice displayed portal and lobular inflammation and lymphocyte infiltration. Similarly, MSG treatment evoked a low significant increment in the relative mRNA expression levels but a high increment in the plasma protein levels of pro-inflammatory cytokines coupled with obesity in mice [76].

To investigate the underlying mechanism by which MSG resulted in systemic inflammatory milieu herein, we assessed the role of TLR-4 and NF-kB. Our results revealed that rats fed MSG displayed a remarkable increase in the protein levels of TLR-4 and NF-kB in the hypothalamus concurrently with an increase in serum levels of pro-inflammatory cytokines. TLR-4 stimulates inflammatory processes via the activation of NF-kB [77]. Meanwhile, NF-kB has also been found to up-regulate the genetic expression of pro-inflammatory cytokines [78]. This transcription factor might herein be activated by MSG-elicited oxidative stress. We further explored the modulation of hypothalamic miR-155 and 218 expression and its association with MSG-induced hyperactivation of the HPA, down-regulation of the hypothalamic GR, and systemic inflammation in MSG-fed rats. Of note, we reported for the first time a remarkable down-regulation of miR-155 and an up-regulation of miR-218 in the hypothalamus. Previous findings revealed that miR-132, 155, and 218 regulated GR in humans [79,80] and rodents [81] suffered from depression. The expression of inflammatory genes was found to be regulated by miR-155 [82]. In confirmation, we reported an inverse association between this miRNA and inflammatory mediators in MSG-fed rats. Furthermore, similar to current findings, overexpression of miR-218 expression has been reported to be associated with reduced GR expression in the brains of rats subjected to pharmacological depression [81].

5 Conclusion

Overall, current results revealed that chronic consumption of MSG caused an activation of the HPA axis, induction of hypercorticosteronemia, a down-regulation of GR receptors, and a systemic inflammatory response. These disturbances were coupled with a down-regulation of miR-155 and an up-regulation of TLR-4, NF-kB, and miR-218 in the hypothalamus, suggesting the implication of these modulated miRNAs and transcription factors in MSG-induced HPA deregulations. Despite higher plasma corticosterone levels, they failed to suppress MSG-induced elevation of serum inflammatory cytokines. This effect could be due

to the down-regulation of hypothalamic GR. These findings support the hazardous impact of MSG on human health. The putative mechanism of MSG-induced activation of the HPA axis and systemic inflammatory response is illustrated in Figure 4.

5.1 Limitations of the study

Although using male rats helps to control the hormonal variability, maintain consistency, and allow for a precise interpretation of the study's findings, it is necessary to acknowledge that findings from this study may not be directly generalizable to females due to potential sex differences. Thus, it is relevant to conduct follow-up studies in the future incorporating female rats or mixed-sex cohorts to provide a more comprehensive and complete understanding of the effects of dietary MSG on the HPA axis across both sexes.

Given that rats and humans differ in environmental exposures, physiology, and lifespan, current findings cannot be generalized or directly applied. Therefore, they can serve as a preliminary step, guiding further research in clinical trials before extrapolation to humans.

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Conflict of interest: The authors state no conflict of interest.

Ethical approval: All procedures for experimental animals were in line with NIH and local IACUC guidelines. They have been reviewed and got approval by local ethics committee, ZU-IACUC/3/F/125/2024.

Data availability statement: All data generated or analyzed during this study are included in this published article.

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