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Original Article

Modulatory Effects of Dietary Saturated Versus Omega-3 Fatty Acids on Cognitive Impairment, Depressive-Like Behavior, HPA Axis Dysregulation and Systemic Inflammation in Monosodium Glutamate-Treated Young Male Rats

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ABSTRACT

Background and Objectives: Children consume fast food rich in saturated fats and flavor enhancers like Monosodium Glutamate (MSG). These agents might alter Hypothalamic-Pituitary-Adrenal (HPA) axis activity, inducing behavioral deficits. Consequently, we hypothesized that adding saturated fat to MSG-treated rats' diet could exaggerate their HPA axis response, behavioral deficits and systemic inflammation whereas dietary polyunsaturated omega-3 fatty acids could alleviate MSG-induced detrimental effects by affecting hypothalamic Glucocorticoid Receptor (GR) and microRNAs. **Materials and Methods:** To test current hypotheses, young male rats were gavaged with 3 g/kg MSG and concurrently fed either a normal or a 10% palm oil- or a 10% fish oil-enriched diet for 4 months. **Results:** Feeding saturated fats to MSG-treated rats aggravated HPA axis activation and hypothalamic GR down-regulation. They also worsened MSG-triggered cognitive impairment and depressive-like behavior. Saturated fats also amplified systemic inflammation by up-regulating hypothalamic Nuclear Factor kappa B (NFkB) and Toll-Like Receptor 4 (TLR4). Conversely, dietary omega-3 fatty acids significantly alleviated MSG-induced HPA axis activation and hypothalamic microRNA218 (miRNA218) up-regulation. Additionally, these fatty acids markedly ameliorated GR down-regulation. They also protected MSG-treated rats against cognitive dysfunction, depressive-like behavior and systemic inflammatory responses. Their anti-inflammatory potential was coupled with down-regulation of NFkB and TLR4 and up-regulation of miRNA155 in the hypothalamus. **Conclusion:** Thus, it is highly recommended to replace saturated with omega-3 fatty acids in fast foods to prevent MSG harmful effects on the HPA axis, particularly in children, after performing broad-scale clinical trials.

Keywords: Fatty acids, Glucocorticoid receptors, miRNA218, TLR4.

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INTRODUCTION

Western diets have become increasingly popular in many countries. It often contains food flavors like glutamate, saturated fat, sugar and refined grains in more than recommended amounts. This dietary style increases the incidence of obesity, type 2 diabetes, neurodegenerative and cardiovascular diseases.[1-3]

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Free glutamate is a crucial excitatory neurotransmitter in the human body. Its increase can elevate cognitive impairment risk. Fortunately, human brains protect themselves from excessive glutamate intake via the Blood-Brain Barrier (BBB).[4,5] However, individuals suffering from cognitive dysfunction are susceptible to glutamate neurotoxicity through their free passage via BBB. Consequently, long-term Monosodium Glutamate (MSG) administration can lead to Hypothalamic-Pituitary-Adrenal axis (HPA) stimulation since the hypothalamus is BBB compromised.[6,7]

Evidence indicates the modulatory influence of dietary lipids on adrenocortical function. For instance, a saturated fat-rich diet elevates adrenocortical hormones. However, increasing the omega-3 polyunsaturated fatty acids content in the diet diminishes the adrenocortical response.[8] The HPA axis and associated glucocorticoids also play regulatory roles in physiology, metabolism and behavior. They can be greatly influenced by nutritional status, in particular, by dietary fatty acids. Hryhorczuk *et al*.,[9] indicated that prolonged intake of saturated fats resulted in hypercorticosteronomy, systemic inflammation and glucocorticoid resistance.

Previous studies demonstrated that postnatal dietary supplementation of high levels of omega-3 fatty acids rescued adult offspring from high-fat diet-induced glucocorticoid-programmed adiposity, hyperlipidemia, insulin resistance, hypertension and inflammation.[10-12] Additionally, oral intake of fish oil containing high levels of omega-3 fatty acids encompassing Eicosapentaenoic Acid (EPA) and Docosahexaenoic Acid (DHA) improved lipid profile and insulin sensitivity in MSG-induced experimental obesity and insulin resistance. Long-chain omega-3 fatty acids have several beneficial effects on clinical and experimental neuroendocrine dysfunctions and neurodegenerative diseases.[13-15] Moreover, they are involved in the treatment of some neuropsychiatric disorders like depression, anxiety, mood-related behaviors and fragile X syndrome by enhancing corticosterone secretion.[9,16-18] However, prolonged nutritional deficiency of omega-3 fatty acids causes alterations in emotional behavior via diminishing corticosterone secretion. Another study showed that omega-3 fatty acids protected the adrenal gland from electromagnetic fields.[19-21]

The current study assessed the differential contribution of dietary saturated and omega-3 polyunsaturated fatty acids to the modulation of MSG-induced HPA axis stimulation, Glucocorticoid Receptor (GR) downregulation and their associated behavioral deficits. Moreover, we investigated the underlying micro molecular mechanism by which these fatty acids modulate the HPA axis and GR. To test this hypothesis, we added saturated fat (10% palm oil) and polyunsaturated fat (10% fish oil) to MSG-treated rats' diet and tested their cognitive performance and depressive-like behavior. In addition, we measured HPA hormones, glucocorticoid receptors, inflammation mediators and some regulatory microRNAs (miRNAs).

MATERIALS AND METHODS

Animals

All animal and experimental procedures were consistent with institutional ethical guidelines to ensure the welfare of the animals. They have been revised and approved by the institutional committee, ZU-IACUC/3/F/126/2024. For testing the current hypothesis, 8-week-old Wistar male rats, weighing (168±21 g) were obtained from national center of research, Egypt. They were housed in optimal conditions in an animal unit with free access to water and food. The rats were acclimated for two weeks. Food consumption was daily computed.

Study design and diet

MSG solution was administered orally at 3 g/kg/day for 4 months.[7,22] Rats were then maintained on one of the following three diets for 4 months. Both control and MSG groups (*n*=8 rats/each) received standard rat chow for 4 months. Meanwhile, MSG+PO and MSG+FO groups (*n*=8 rats) received either a saturated fatty acid-enriched diet containing 10% w/w palm oil (PO, rich in palmitic acid) or a polyunsaturated fatty acid-enriched diet containing 10% w/w fish oil (FO, rich in omega-3 fatty acids)[8] along with MSG (3 g/kg/day, p.o) for 4 months. We ensured that the diets' overall fat content was the same for each group to reduce confounding variables. Diet ingredients (g/kg) are illustrated in Table 1. We have also taken steps to standardize overall dietary intake across all groups as much as possible by using metabolic cages. Daily consumed foods contain small amounts of MSG. This additive might also have different titles. Thus, it is very difficult to determine foods containing MSG. Humans are five times more sensitive to this additive than rats. This means that one-fifth MSG level used to induce adverse effects in rats will do the same in humans.[23] Previous studies indicated that 3 g/kg of MSG is slightly toxic.[22,24] Additionally, this dose equate MSG amount in a single sachet consumed by a human.[25] Additionally according to the FASEB evaluation of MSG adverse effects in 1995, some healthy individuals exposed to high MSG level of (≥3 g) manifested symptoms and adverse reactions within 1 hr.[26]

The duration of the study (4 months) may provide the potential for long-term effects associated with MSG intake. The effects of MSG can occur cumulatively over time with subsequent multiple exposures over a longer period leading to tissue injury and damage. Although 4 months duration may not directly reflect typical human consumption patterns, this extended exposure was mimic chronic dietary intake and its potential cumulative effects. This scenario is often observed in human dietary habits over time.

Really, the primary aim of utilizing a higher dose of MSG for prolonged duration herein was to exaggerate its potential biological effects in rats. Such design enables detecting biological changes as measurable outcomes to explore underlying mechanisms in a preclinical setting.

Behavioral assessment of cognitive performance and depression

After finishing the experimental period, testing behavior was performed on next days. Cognitive ability was evaluated using Y-maze test. The maze consists of three identical arms arranged in Y-shape. This test depends on the ability of a rat, placed in the center of the maze, to recognize arms and enter them consecutively. Spontaneous alternation % was computed as follows: [The number of successful entrance alternation/ the number of entries into all arms-2]x100. Anhedonia, a common feature of depression, was assessed by sucrose preference test. This test measured the percentage of sucrose consumption relative to sucrose and water.Indeed both tests were chosen to provide a focused examination and a valid measure of spatial working memory and cognitive flexibility as well as anhedonia and hedonic behavior, respectively, as initial indicators of cognitive and mood changes that aligned with primary research objectives.

Blood and tissue sampling

After finishing all experimental interventions, rats were anesthetized using isoflurane. Sera were isolated from withdrawn blood samples by centrifugation. They were frozen at -20°C and kept for analysis. Animals were then euthanised by exsanguination. Hypothalamic tissues were excised from sagittal cerebral sections. Tissues were kept at -80ºC for quantitation of Corticotropin-Releasing Hormone (CRH), Glucocorticoid Receptors (GR), inflammation inducers and miRNAs by ELISA and real-time PCR, respectively.

Hypothalamic pituitary adrenal (HPA) axis hormones and Glucocorticoid Receptors (GR) determination

MyBioSource rat ELISA kits were used to determine serum levels of corticosterone and Adrenocorticotropic Hormone (ACTH) and the hypothalamic contents of CRH and GR as directed by the manufacturer.

Inflammation markers analysis

Serum levels of pro-inflammatory cytokines, such as Tumor Necrosis Factor alpha (TNFα), Interleukin 1 beta (IL1β) and Interleukin 6 (IL6) were measured by Raybiotech ELISA kits for rats (Norcross, USA). Similarly, hypothalamic inflammation stimulators comprising Nuclear Factor kappa B P65 (NFkB P65) and Toll-Like Receptor 4 (TLR4) protein levels were quantified using Rat Cusabio ELISA kits (Cusabio, China) as instructed by the manufacturer.

MicroRNAs (miRNAs) relative expression detection by real-time PCR

The hypothalamic tissues were carefully lysed to extract total RNA along with miRNAs. Following purification, reverse transcription and amplification using specific primers, the relative expression of miRNA218 and 155 were detected in hypothalamic tissues by qRT-PCR. The $2^{-\Delta\Delta Ct}$ method was applied to compute fold change.[27]

Statistical Analyses

Results were depicted in the form of mean±standard deviation. Statistical tests, comprising ANOVA and Tukey Kramer were conducted using the SPSS program for comparing means among the studied groups at $p<0.05$. A Pearson correlation coefficient was also calculated between parameters at $p<0.05$.

RESULTS

Dietary fatty acids effect on MSG-triggered deterioration of cognitive function and depressive-like behavior

Monosodium glutamate induced deficits cognitive performance in rats as evidenced by the significant decline in spontaneous alternation % of Y maze test relative to the Control group (55.2±4.1 versus 72.6±5.8, $p<0.05$). MSG+PO group, received a saturated fatty acid-enriched diet, recorded greater decline in response to Y maze test than MSG group, suggesting a significant impairment of spatial cognition (37.5±2.6 versus 55.2±4.1, $p<0.05$). However, MSG+FO group, received dietary omega-3 fatty acids, was significantly protected against MSG-induced cognitive dysfunction as indicated by the significant improvement in spontaneous alternation % of Y maze test compared to MSG group (68.3±4.7 versus 55.2±4.1, $p<0.05$).

Similarly, MSG significantly ($p<0.05$) reduced sucrose intake% in rats with respect to their controls. Notably, feeding

Table 1: Diet ingredients.

Ingredients (g/kg diet)	Control diet	Saturated fat (Palm Oil, PO)-enriched diet	Omega-3 polyunsaturated fat (Fish Oil, FO)-enriched diet
Casein	200	200	200
Soybean Oil	100	--	--
Palm oil	--	100	--
Fish oil	--	--	100
Corn starch	500	500	500
Sucrose	100	100	100
Vitamins	10	10	10
Minerals	35	35	35
Fibers	50	50	50
Choline	3	3	3
Cysteine	2	2	2

saturated fats to MSG-treated rats markedly ($p<0.05$) enhanced MSG-elicited reduction in sucrose preference% relative to MSG group. In contrast, dietary intake of omega-3 polyunsaturated fatty acids significantly ($p<0.05$) ameliorated the reduction of sucrose consumption% in MSG-treated rats (Figure 1). Sucrose preference% is considered a behavioral index of depression.

Dietary fatty acids effect on MSG-triggered HPA axis stimulation and Glucocorticoid Receptors (GR) down-regulation

Prolonged exposure to MSG triggered significant hypercorticosteronomy, elevation in serum ACTH and hypothalamic CRH and down-regulation of hypothalamic GR relative to the control group ($p<0.05$). Likewise, feeding saturated fat to MSG-treated rats exaggerated HPA axis activation and hypothalamic GR down-regulation relative to the MSG group ($p<0.05$). Conversely, dietary polyunsaturated omega-3 fatty acids significantly alleviated MSG-elicited HPA axis stimulation and GR down-regulation compared to the MSG group ($p<0.05$). Additionally, these fatty acids markedly ameliorated hypothalamic miRNA218 overexpression relative to the MSG group ($p<0.05$) (Figures 2 and 3).

Dietary fatty acids effect on MSG-triggered systemic inflammation

Despite higher serum corticosterone levels in the MSG group, they failed to suppress MSG-induced systemic inflammation, suggesting impaired glucocorticoid response which was amplified by dietary saturated fat. This, in turn, markedly worsened MSG-induced systemic inflammation as evidenced by the significantly higher levels of serum pro-inflammatory cytokines such as TNFα and IL1β&6 than their levels in the MSG group ($p<0.05$). The systemic inflammatory response was coupled with dramatic increases in hypothalamic NFkB P65 and TLR4 in comparison with the MSG group ($p<0.05$). However, dietary omega-3 fatty acids significantly protected MSG-treated rats against systemic inflammation. They markedly decreased TNFα and IL1β&6 levels in serum through down-regulation of NFkB P65 and TLR4 proteins and up-regulation of miRNA155 in the hypothalamus compared to the MSG group ($p<0.05$) (Tables 2 and 3, Figure 4).

Correlations

Overall group data analysis revealed a significant negative association between serum levels of corticosterone and behavioral indices of cognitive ability and depression (r=-0.92 and -0.81, $p<0.0001$). Moreover, serum pro-inflammatory cytokines were directly correlated with HPA axis hormones, hypothalamic NFkB P65 and TLR4 proteins. However, they were inversely correlated with hypothalamic GR and miRNA155 (Table 4). In addition, hypothalamic GR and sucrose preference%, a measure of depressive-like behavior, were negatively associated with miRNA218 (r=-0.58 and -0.92, $p<0.0001$).

DISCUSSION

The main finding in the current work was that saturated fat in diet exaggerated MSG-induced cognitive dysfunction, depressive-like behavior, HPA axis hyperactivation, systemic inflammation and GR down-regulation. Conversely, dietary omega-3 polyunsaturated fat alleviated the behavioral and emotional deficits as well as HPA stimulation via modulating hypothalamic miRNAs. This study largely confirms previously known effects of saturated versus omega-3 fats on HPA axis function and inflammation.[8,9,28-31] However, the main novel aspect herein is exploring the biological impact of fat combination with MSG. Excessive intake of dietary fats via Western or fast food

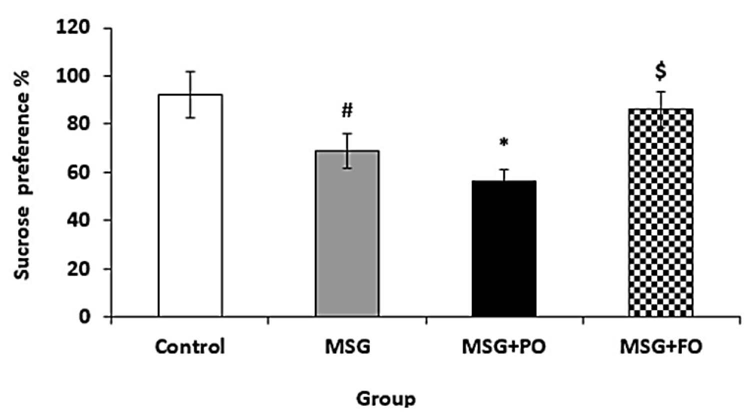

Figure 1: Four weeks consumption of dietary saturated fatty acids (10% w/w palm oil, PO) attenuated but omega-3 fatty acids (10% w/w fish oil, FO) improved sucrose preference% in Monosodium Glutamate (MSG)-treated rats. Statistics were done by ANOVA and Tukey Kramer using SPSS. Mean and standard deviation are used to depict each group values (*n*=8).

#significantly different from Control group, *significantly different from MSG group, $significantly different from MSG+PO group at $p<0.05$.

is increasing worldwide.[1,2] Furthermore, these foods contain flavor enhancers, attracting children. They can elicit several major complications, encompassing obesity, mood disturbances, diabetes, cardiovascular diseases and impaired stress response.[28] MSG, a flavor enhancer, influences HPA axis activity in neonates.[29,30] Likewise, overconsumption of dietary lipids and their saturation impacts HPA axis functionality, promoting behavioral anxiety.[8,31]

Really, the current study mainly focused on identifying candidate miRNAs accompanied with the observed behavioral and inflammatory findings, establishing a foundation for future mechanistic investigations.

Herein we explored the impact of adding saturated or omega-3 fatty acids to MSG-treated rats' diet on their behavioral indices of cognition and depression and HPA axis response. We found that dietary saturated fat exaggerated depressive-like behavior, HPA activation and inflammation, but severely reduced Y-maze test response and hypothalamic GR in MSG-treated rats, suggesting impairment cognitive function and glucocorticoid response. Contrariwise, dietary omega-3 fatty acids alleviated the adverse effects via suppressing hypothalamic NFkB, TLR4 and miRNA218 and up-regulating miRNA155.

In line with previous reports, current findings indicated that chronic MSG administration induced a remarkable elevation in HPA axis hormones and down-regulation of GR.[7,32] These effects could be due to the glutamate excitatory effect, activating the HPA axis.[33,34] Glutamate can also stimulate the hypothalamic paraventricular nucleus to secrete CRH. This elicits the adenohypophyseal release of ACTH and thereby the adrenal release of cortisol.[35]

Nutritional lipids regulate adrenocortical function. They change circulating free fatty acids, affecting HPA axis proximal components and modifying corticosteroid output. Moreover, enhanced lipolysis raises fatty acids in the circulation, influencing corticosteroid output.[36-39] Fatty acids may also be integrated with adrenocortical membranes, altering ACTH and corticosteroid signaling.[40] Additionally, fast changes in adrenal esterified lipids modulate cholesterol esters concentration and corticosterone dynamic response to different stressors.[41] Indeed, both naturally occurring and modified fatty acids impact adrenocortical cell function.[36,42-46] The mobilization of stored visceral fats causes

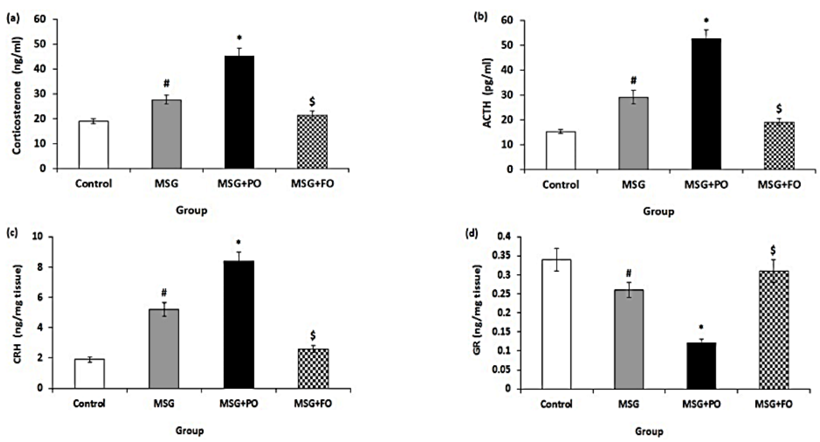

Figure 2: Four weeks consumption of dietary saturated fatty acids (10% w/w palm oil, PO) amplified but omega-3 fatty acids (10% w/w fish oil, FO) ameliorated Hypothalamic-Pituitary-Adrenal (HPA) axis response and hypothalamic Glucocorticoid Receptor (GR) downregulation in Monosodium Glutamate (MSG)-treated rats.

(a) Serum corticosterone level, (b) Serum adrenocorticotropic hormone (ACTH), (c) Hypothalamic corticotropin-releasing hormone (CRH), (d) Hypothalamic GR. Statistics were done by ANOVA and Tukey Kramer using SPSS. Mean and standard deviation are used to depict each group values (*n*=8). #significantly different from Control group, *significantly different from MSG group, $significantly different from MSG+PO group at $p<0.05$.

Table 2: Dietary saturated (10% w/w palm oil, PO) versus omega-3 fatty acids (10% w/w fish oil, FO) impact on systemic inflammation in Monosodium Glutamate (MSG)-treated rats.

	Control	MSG	MSG+PO	MSG+FO
TNF-α (pg/mL)	34.17±4.81	85.43±8.27[#]	201.76±21.33[*]	45.01±5.45[$]
IL1β (pg/mL)	84.21±7.79	192.69±18.78[#]	357.14±33.05[*]	92.48±8.19[$]
IL-6 (pg/mL)	56.32±6.63	140.15±9.86[#]	292.73±23.02[*]	70.69±7.21[$]

Statistics were done by ANOVA and Tukey Kramer using SPSS. Mean and standard deviation are used to depict each group values (*n*=8). [#]significantly different from Control group, [*]significantly different from MSG group, [$]significantly different from MSG+PO group at $p<0.05$. TNF-α: Tumor necrosis factor alpha; IL1β: Interleukin 1beta; IL-6: Interleukin 6

Table 3: Dietary saturated (10% w/w palm oil, PO) versus omega-3 fatty acids (10% w/w fish oil, FO) impact on hypothalamic inflammation regulatory factors in Monosodium Glutamate (MSG)-treated rats.

	Control	MSG	MSG+PO	MSG+FO
NF-kB P65 (pg/mg tissue)	0.43±0.04	0.98±0.1[#]	2.64±0.3[*]	0.52±0.05[$]
TLR4 (ng/mg tissue)	0.11±0.01	0.26±0.02[#]	0.49±0.05[*]	0.14±0.01[$]

Statistics were done by ANOVA and Tukey Kramer using SPSS. Mean and standard deviation are used to depict each group values (n=8). [#]significantly different from Control group, [*]significantly different from MSG group, [$]significantly different from MSG+PO group at p<0.05. NFkB P65: Nuclear factor kappa B P65; TLR4: Toll like receptor 4

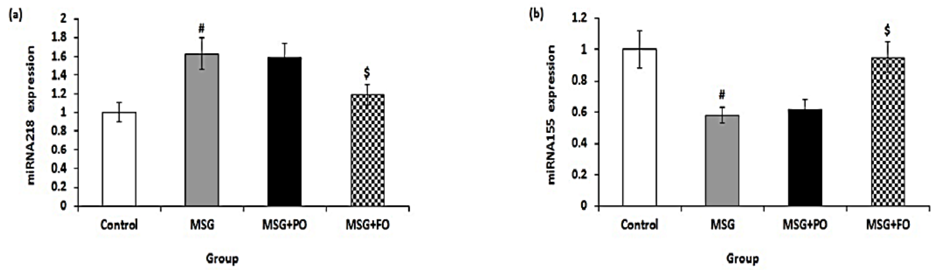

Figure 3: Four weeks consumption of dietary saturated fatty acids (10% w/w palm oil, PO) enhanced miRNA218 overexpression and miRNA155 downregulation whereas omega-3 fatty acids (10% w/w fish oil, FO) counteracted these disturbances in Monosodium Glutamate (MSG)-treated rats' hypothalamic miRNAs relative expression.

(a) miRNA218, (b) miRNA155. Statistics were done by ANOVA and Tukey Kramer using SPSS. Mean and standard deviation are used to depict each group values (n=8). #significantly different from Control group, *significantly different from MSG group, $significantly different from MSG+PO group at p<0.05. miRNA: microRNA.

certain fatty acids to be released and processed in the hepatic portal circulation, leading to their oxidation. These changes can alter adrenocortical function.[44,45] Furthermore, the category of fatty acids affects leptin levels, modifying adrenocortical function.[47-50]

This study provided evidence that prolonged intake of saturated fatty acids boosted HPA axis function and suppressed hypothalamic GR in MSG-treated rats. Confirming current notions, Carsia *et al.*,[8] referred to the stimulatory effect of dietary saturated fat on adrenocortical function by significantly elevating plasma levels of corticosterone and ACTH. They suggested that adequately high ACTH levels could raise corticosteroid levels. As a result of long-term intake of fatty acids, their free levels might increase, modulating HPA axis proximal components and corticosteroid secretion.[36] In concordance, another recent study has indicated that chronic overconsumption of saturated fat in diet induces hypercorticosteronemia and down-regulation of GR mRNA expression in the hypothalamus.[9] Tannenbaum *et al.*,[51] Cano *et al.*,[52] and McNeilly *et al.*,[53] reported also that rats chronically fed palm oil displayed increases nocturnal corticosterone plasma levels. The down-regulation of GR by a saturated fatty diet herein was similar to Noguchi *et al.*,[54] findings. It seems to be an adaptive mechanism to minimize hypercorticosteronomy detrimental impact on tissues by suppressing stress response.[9]

There is an association between HPA axis overstimulation and depression in humans and experimental animals. The HPA axis is also involved in induction of depression-like behavior following stress.[55,56] In addition, emergence of behavioral deficits comprising cognitive impairment and depressive-like behaviors is not only associated with dietary consumption of saturated fatty acids but also with deterioration of HPA-axis function and prolonged elevation of glucocorticoid levels.[57] Accordingly, current findings demonstrated an inverse correlation between corticosterone levels and each of spatial cognition and sucrose preference indices.

Dietary consumption of polyunsaturated fatty acids corrected chronic stress-induced behavioral deficits by attenuating glucocorticoid secretion. This might enable adequate physiologic response to stress.[58,59] In line with these findings, we reported that concurrent omega-3 fatty acids supplementation with MSG significantly ameliorated HPA axis hyperactivity. This was manifested by reduced serum levels of corticosterone, ACTH and hypothalamic CRH. Current observations are similar to those reported by Ferraz *et al.*,[58] and Choi *et al.*,[60] Long-chain polyunsaturated fats modulate adrenal cortex cellular function.[37,38] A previous study revealed that a diet rich in omega-3 fatty acids could dampen the adrenocortical response to ACTH-induced corticosteroid release.[8] Liu *et al.*,[61,62] indicated that fish oil, rich in omega-3 fatty acids, effectively alleviated HPA axis activation caused by the experimental lipopolysaccharide challenge. In line with this, fish oil significantly attenuated the lipopolysaccharide-induced increase in healthy volunteers' plasma ACTH and cortisol levels.[15] In addition, a previous clinical study proved that fish oil prevented mental stressors-induced adrenal gland activation by directly affecting the adrenal gland or indirectly affecting the central nervous system through crossing the BBB.[63] Ross[17] also found that omega-3 fatty acids alleviated anxiety symptoms and inhibited HPA axis activation. However, the deficiency of these fatty acids in rat offspring's diet during critical brain development exacerbated their HPA axis response to stress.[64] Furthermore, omega-3 fatty acids deficiency markedly induced corticosterone hypersecretion in mice. Nutritional deprivation of omega-3 fatty acids also significantly down-regulated GR protein expression in the prefrontal cortex.[18,65]

Despite the limited number of behavioral tests herein, we believe that our results contribute meaningful insights into the effects of dietary factors on cognitive and mood outcomes.

Glucocorticoid resistance is the reduction of immune cell sensitivity to glucocorticoids that typically stop inflammatory responses.[66,67] Although long-term MSG administration induced hypercorticosteronemia herein, it failed to suppress inflammatory cytokines, implying glucocorticoid resistance due to GR down-regulation. In the same way, glucocorticoid resistance results from chronic stressful events, disrupting corticosterone anti-inflammatory properties.[68] Thus, the inflammatory response in MSG-treated rats, as shown in this study, could be explained by HPA axis deregulation. MSG inflammatory impact was reported in earlier studies.[69-73] Interestingly, pro-inflammatory cytokines can also stimulate the HPA axis to secrete corticosterone in response to stress.[74,75] Consistently, we observed a positive correlation between serum pro-inflammatory cytokines and HPA axis hormones and a negative correlation between these cytokines and hypothalamic GR (Table 4).

Current findings are like those reported by Hryhorczuk *et al.*[9] They found that dietary saturated fats induced an inflammatory response and glucocorticoid resistance in MSG-treated rats. Both studies demonstrated an increase in inflammatory cytokines levels and down-regulation of hypothalamic GR. A saturated fatty diet induces obesity, enhancing metabolic impairments such as hypercortisolemia, peripheral inflammation and depression.[76-78]

Consistent with Choi *et al.*,[60] findings, we reported that dietary omega-3 fatty acids markedly increased hypothalamic GR but decreased miRNA218 in MSG-treated rats. In this study, correlation analysis also supports current and previous results. It revealed an inverse moderate association between hypothalamic miRNA218 and each of hypothalamic GR and sucrose preference (an index of depressive-like behavior). The miRNA regulates GR during depression.[79]

Long-chain omega-3 polyunsaturated fatty acids, including EPA and DHA, have immunomodulatory effects.[58,80] They display beneficial anti-inflammatory effects during inflammation and inflammatory diseases.[81,82] These polyunsaturated fatty acids inhibit pro-inflammatory cytokines overproduction.[58,83-85] Accordingly, concurrent supplementation of MSG-exposed rats with omega-3 fatty acids, in the current study, significantly ameliorated pro-inflammatory cytokines release, HPA axis activation and hypothalamic miRNA155 down-regulation. Therefore, these fatty acids can protect against MSG-induced neuroendocrine-immunological interactions. Current findings agree with Choi *et al.*,[60] Supporting these notions, fish oil was found to attenuate lipopolysaccharide-induced HPA axis activation in weaned piglets. These effects were associated with a marked reduction in brain pro-inflammatory cytokines. Thus, fish oil anti-inflammatory potential might contribute to its ameliorative effect on HPA axis activation.[62]

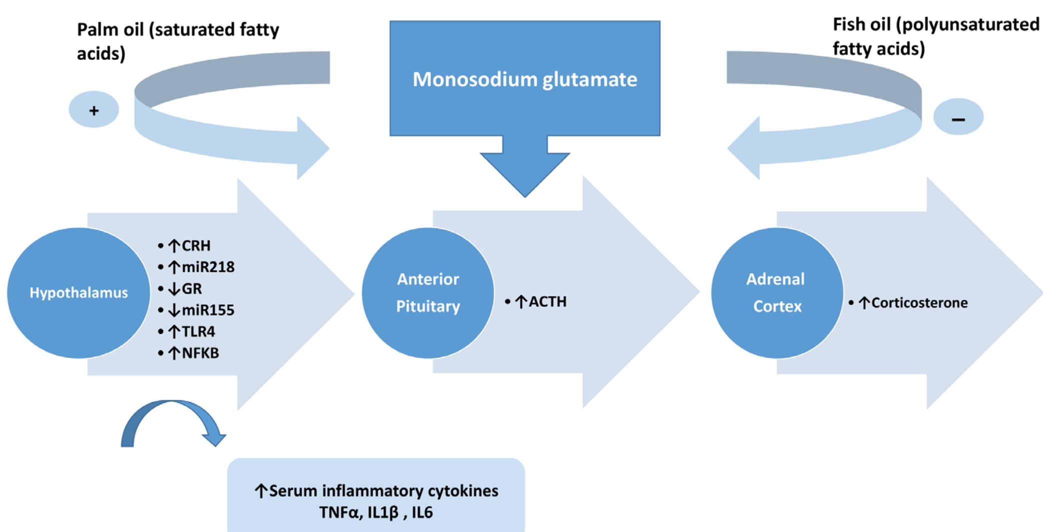

Figure 4: Suggested mechanism by which dietary saturated fatty acids exaggerated and omega-3 fatty acids ameliorated monosodium glutamate-triggered Hypothalamic-Pituitary-Adrenal (HPA) axis stimulation and systemic inflammation.

Table 4: Correlations of serum pro-inflammatory cytokines with each of serum HPA axis hormones, hypothalamic CRH, GR, NFkB P65, TLR4 proteins and miRNA155 in all studied groups including the Control, Monosodium Glutamate (MSG)-treated rats, MSG+PO and MSG+FO.

Inflammatory cytokines	Corticosterone	ACTH	CRH	NFkB P65	TLR4	GR	miRNA155
TNF-α	r=0.96 HS	r=0.98 HS	r=0.97 HS	r=0.99 HS	r=0.99 HS	r=-0.92 HS	r=-0.62 HS
IL1β	r=0.96 HS	r=0.98 HS	r=0.99 HS	r=0.98 HS	r=0.99 HS	r=-0.91 HS	r=-0.68 HS
IL-6	r=0.97 HS	r=0.98 HS	r=0.99 HS	r=0.99 HS	r=0.99 HS	r=-0.93 HS	r=-0.66 HS

PO: dietary saturated fat (10% w/w palm oil), FO: dietary omega-3 fatty acids (10% w/w fish oil); r: correlation coefficient, HS highly significant at $p<0.0001$. HPA: Hypothalamic-pituitary-adrenal; ACTH: adrenocorticotropic hormone; CRH: Corticotropin-releasing hormone; GR: Glucocorticoid receptors; TNF-α: Tumor necrosis factor alpha; IL1β: Interleukin 1beta; IL-6: Interleukin 6; NF-kB P65: Nuclear factor kappa B P65; TLR4: Toll like receptor 4; miRNA: microRNA.

To investigate the molecular mechanism by which omega-3 fatty acids alleviate MSG-induced HPA axis activation herein, we measured the levels of hypothalamic NFkB P65, TLR4 and miRNA155 and their correlation with systemic inflammatory cytokines. Activated NFkB up-regulates pro-inflammatory cytokines and cyclooxygenase 2.[86] Our results revealed that rats received omega-3 fatty acids concomitantly with MSG displayed a remarkable decrease in the levels of NFkB p65 and TLR4 in the HPA axis. In addition, they displayed a decrease in serum levels of pro-inflammatory cytokines. In the same manner, Liu *et al.*,[62] suggested that fish oil inhibitory effect on lipopolysaccharide-induced HPA axis stimulation in pigs might be mediated through the downregulation of activated NFkB, decreasing the production of pro-inflammatory cytokines. Moreover, EPA suppresses the synthesis of pro-inflammatory cytokines via up-regulation of miRNA-155 in leukemic human monocytes.[87,88] Accordingly, we found that systemic pro-inflammatory cytokines were directly correlated with hypothalamic NFkB P65 and TLR4 proteins but inversely correlated with hypothalamic miRNA155 (Table 4).

CONCLUSION

Overall, the present work indicated for the first time that dietary saturated fatty acids augmented MSG-induced cognitive impairment, depression-like behavior, HPA axis hyperactivation, systemic inflammation and GR down-regulation, but omega-3 fatty acids mitigated such disturbances by restoring hypothalamic miRNA218 and 155 (Figure 3). Thus, replacing saturated fats in fast foods with omega-3 fatty acids can mitigate MSG adverse effects on the HPA axis and behavior, particularly in children. Performing clinical trials is necessary to verify current findings. Additionally, the effect of other fatty acids for example monounsaturated fatty acids should be tested on MSG-triggered stimulation of HPA in the future. The impact of other fast-food components like fructose, coloring agents, etc. on MSG-treated rats should also be determined.

LIMITATIONS

The MSG dosing (3 g/kg) and duration (4 months) in rats may not accurately reflect human consumption patterns, limiting the direct translational value of current findings.

The study design lacks proper controls for dietary fat content. While it compares palm oil (saturated) vs fish oil (omega-3), it does not properly control total fat content or include other fatty acid types like monounsaturated fats, making it difficult to attribute observed findings specifically to fat saturation rather than total fat intake.

The behavioral assessments are limited to just two tests (Y-maze and sucrose preference), which may not comprehensively capture the full spectrum of cognitive and mood effects. Additional validated behavioral assays would strengthen the conclusions.

The mechanistic insights herein are relatively superficial. Although this study shows changes in miRNA218 and miRNA155, it doesn't fully elucidate the molecular pathways linking these changes to behavioral and inflammatory outcomes. The observed correlation-based findings are also suggestive and do not prove causation. Therefore, further research is warranted to explore the causative roles of miRNA-218 and miRNA-155 in modulating behavioral and inflammatory responses by deeper delving into the underlying molecular pathways linking miRNA changes to current outcomes.

FUTURE DIRECTIONS

Future studies should use dosing regimens and durations that better approximate real-human consumption of MSG to enhance the translational relevance of the results. Clinical trials should also be conducted to validate these findings in humans.

In upcoming research, we plan to incorporate additional control groups with matched total fat content and different fatty acid profiles, such as diets enriched with monounsaturated fats, to better elucidate the influence of other specific fatty acid types. We will also consider outcomes of including a broader range of dietary fat composition to better understand their individual contributions.

Additional validated behavioral assays could provide a more comprehensive evaluation of cognitive and affective domains. Future studies incorporating a broader range of behavioral assessments are needed to provide a more nuanced understanding of the effects of investigated dietary components on behavior.

Future studies should include functional assays to validate miRNA-target interactions. This involves gain- or loss-of-function experimental interventions, to directly assess miRNA functional roles in mediating behavioral and inflammatory changes. Moreover, downstream molecular targets of these miRNAs and key signaling pathways (e.g., NF-κB) could be investigated to provide a more comprehensive understanding of the underlying molecular mechanisms attributing to behavioral and inflammatory outcomes.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

ACTH: Adrenocorticotropic Hormone; **BBB:** Blood-Brain Barrier; **CRH:** Corticotropin-Releasing Hormone; **EPA:** Eicosapentaenoic Acid; **DHA:** Docosahexaenoic Acid; **FO:** Fish oil; **GR:** Glucocorticoid receptor; **HPA:** Hypothalamic-pituitary-adrenal; **Il:** Interleukin; **Mirna:** Microrna; **Msg:** Monosodium Glutamate; **NF-κB:** Nuclear Factor Kappa B; **Po:** Palm Oil; **TLR4:** Toll-Like Receptor 4; **TNF-α:** Tumor Necrosis Factor Alpha.

AUTHORSHIP CONTRIBUTION

Hebatallah Husseini Atteia, Amal F. Gharib, Mervat El-Sayed Askar designed experiments, selected biochemical parameters, performed statistical analyses, discussed results, wrote and revised the paper. Amal F. Gharib got a fund for this research. Manar Hamed Arafa, Gehan A. Ahmed performed animal interventions, wrote and revised the paper. Mervat El-Sayed Askar supervised experiments and biochemical analyses. Amr Tawfik Sakr carried out biochemical and statistical analyses, discussed results, wrote and revised the paper. All authors participated in writing and revising the manuscript.

SUMMARY

Children consume fast food rich in saturated fats and flavor enhancers like Monosodium Glutamate (MSG). These agents might alter Hypothalamic-Pituitary-Adrenal (HPA) axis activity, inducing behavioral deficits. This work therefore explored the impact of adding saturated fat or polyunsaturated omega-3 fatty acids to MSG-treated rats' diet on their HPA axis response, behavioral deficits and systemic inflammation and the underlying mechanism, focusing on hypothalamic Glucocorticoid Receptor (GR) and microRNAs. Feeding saturated fat to MSG-treated rats aggravated cognitive impairment, depressive-like behavior, HPA activation and hypothalamic glucocorticoid receptors down-regulation. This worsened MSG-triggered systemic inflammatory response. Conversely, dietary omega-3 fatty acids significantly alleviated these deleterious effects by decreasing hypothalamic miRNA218 and increasing miRNA155. These findings suggested that substitution of saturated with omega-3 fatty acids is highly recommended as a protective dietary intervention to minimize MSG deleterious effects on HPA axis and behavior, particularly in children.

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