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The Effect of High Carbohydrate and High MSG Intake on Body Weight and White Adipose Tissue

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Abstract. Asians often consume foods high in carbohydrates with the addition of MSG as a flavor enhancer. Foods with high carbohydrates and MSG are predicted to be obesogen. This study aims to explore the effects of high carbohydrate (HC), high MSG (HMSG), and a combination of high carbohydrate and MSG (HCHMSG) intake with the onset of obesity. Seven-week-old male Swiss Webster strain mice (*Mus musculus*) (n = 40) were divided into four groups. The control group consisted of 57% carbohydrates, 9% fiber, and 6.11% fat. The HC group was given food intake consisting of 71% carbohydrate, 4.62% fiber, and 1.81% fat for 14 weeks; the HMSG group's diet consisted of 57% carbohydrates, 9% fiber, 6.11% fat with an additional 10% MSG and group HCHMSG diet consisted of 71% carbohydrates, 4.62% fiber and 1.81% fat with an additional 10% MSG. Significant weight gain ($p < 0.05$) began at the fourth week in HC and HCHMSG groups and the HMSG group in the sixth week. After 14 weeks of study the HC group (BW 45.07 ± 4.41; $p < 0.05$) and the HCHMSG group were obese (42,817 ± 7,149; $p > 0.05$). WAT increases significantly in both HC and HCHMSG groups.

Keywords: Obesity, Carbohydrates, and MSG

INTRODUCTION

Since 1975, the obesity rate has tripled, with over 1.9 billion adults as young as 18 years old being overweight and 650 million obese. This is quite troubling because the prevalence also occurs in children under five years old, with an increase of 14% in 1975-2016 overweight and obese [1].The modern era that occurs resulted in rapid urbanization and economic development that resulted in the transition of nutrition in society. This condition is compounded by lifestyle changes and unhealthy behaviors [2-3]. The transfer of macronutrients consumed from fat to carbohydrates increases the prevalence of obesity [4]. Monosodium glutamate (MSG) consumption has risen dramatically over three decades [5]. People in Asia often eat foods that contain high carbohydrates [6,7] with the addition of MSG as a flavor enhancer [8–14]. MSG consumption in Southeast Asia is 2-3 times more than people in Europe [13]. Carbohydrates and MSG can trigger obesity or so-called obesogen [4-5,14-15]. Obesogens are chemicals that interfere with the body's physiological weight regulation by increasing the mass of white adipose tissue (WAT) and thus increasing body weight [16-19]. WAT is one type of adipose tissue that stores energy. Lipolysis will occur in WAT when the body needs energy, such as fasting and exercise. Fatty acids obtained from dietary metabolism are converted into triglycerides (TG) when the energy is obtained from excess food–especially carbohydrates [20–22]. TG is obtained from 2 sources: de novo lipogenesis, synthesized in the intestine and liver, then circulated and stored in lipid droplets [21].

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A high intake of carbohydrates has a high glycemic index [15,16]. Food with a high glycemic index is digested and absorbed quickly, resulting in hyperglycemia and leading to an individual feeling hungry quickly [3,16-17]. Meanwhile, the intake of MSG induces obesity by generating overeating effects [23]. High intake of MSG will acutely increase glucagon-like peptide-1 (GLP-1) secretion but chronically lowers the secretion of GLP-1 [11]. This happens due to the dysfunction of enteroendocrine cells in the epithelial intestinal [12,19]. GLP-1 is the main incretin that stimulates insulin secretion from the pancreas; the GLP-1 barrier will cause hyperglycemia [20-22,24–25]. Both intakes of HC and HMSG will result in hyperglycemia and building up adipose tissue mass due to increased lipogenesis [23,26–31]. Increased fat mass will increase body weight [17,32,33]; an increase in free fatty acids concentrations will happen as a result [34–36]. Not only that, but an increased body weight can also result in a decreased expression of mRNA adiponectin [35,37,38], increased concentration of leptin [33-37], and an increase in proinflammatory cytokines produced by fats that would cause a decrease in insulin sensitivity [37–39], and induce type 2 diabetes mellitus [33,36,40-41]. This research is the first study that looks into the effects of 71% carbohydrate intake, 10% MSG, and a combination of 71% carbohydrates and 10% MSG on body weight that results in obesity.

RESEARCH METHODS

Animal Experiments and Experimental Procedures

The Research Ethics Committee of the University of Surabaya, Indonesia has declared that this research design was ethical on September 1, 2020 (No: 138/KE/VIII/2020). Mice were selected by the criteria of a 5-week-old male, weighed 20-30 grams, was Swiss Webster's strain, and had no physical disabilities. The adaptation period is two weeks. Four groups of animals consisting of ten mice each: the control, the high carbohydrate intake (HC), the high MSG intake group (HMSG), and a combined group of high carbohydrate and high MSG (HCHMSG) was made. The control group's diet consisted of 57% carbohydrates, 9% fiber, and 6.11% fat. The HC group's diet consisted of 71% carbohydrates, 4.62% fiber, and 1.81% fat. The HMSG group's diet consisted of 57% carbohydrates, 9% fiber, 6.11% fat with an additional 10% of MSG, and the HCHMSG group's diet consisted of carbohydrates 71%, fiber 4.62%, fat 1.81% with an additional 10% of MSG.

The meal ingredients consist of the same ingredients from the beginning to the end of the research, and feed content was analyzed at the beginning of the study. The dietary intake was given *ad libitum* and can be accessed at any time. Animals were placed solitary with one cage containing one mouse. The researcher periodically weighs the feed before replacing it with a new batch of feed. Room temperature is set at 24-26 °C with attention to the dark cycle of light. After 14 weeks of animal treatment, the mice are euthanized for WAT retrieval.

TABLE 1. Sample Characteristics

	C	HC	HMSG	HCHMSG
N	9	10	10	10
Age (weeks)	±7	±7	±7	±7
Gender	Male	Male	Male	Male
Early Weight	29,8325 ± 1,73	29,916 ± 4,0	29,499 ± 3,646	29,27 ± 3,325
Final Weight	37,8138 ± 1,856	45,07 ± 4,41	40,357 ± 3,734	42,817 ± 7,149

Statistical Analysis

SPSS statistics 25 is used to analyze the results. The Shapiro-Wilk test was used to determine normality ($p > 0.05$), and the Levene's test was used to determine homogeneity ($p > 0.05$). The normally distributed data and homogeneity of variance are analyzed with a One-Way ANOVA Test and continued with Bonferroni Post Hoc test ($p < 0.05$). Researchers use GraphPad Prism version 9 to process the data analysis from SPSS to a graph.

RESULT

The weight, age, and sex of mice used in each group were similar at the start of this study (Table 1). Weight is measured periodically every two weeks. The HC and HCHMSG groups developed diabetes at the 14th week of treatment.

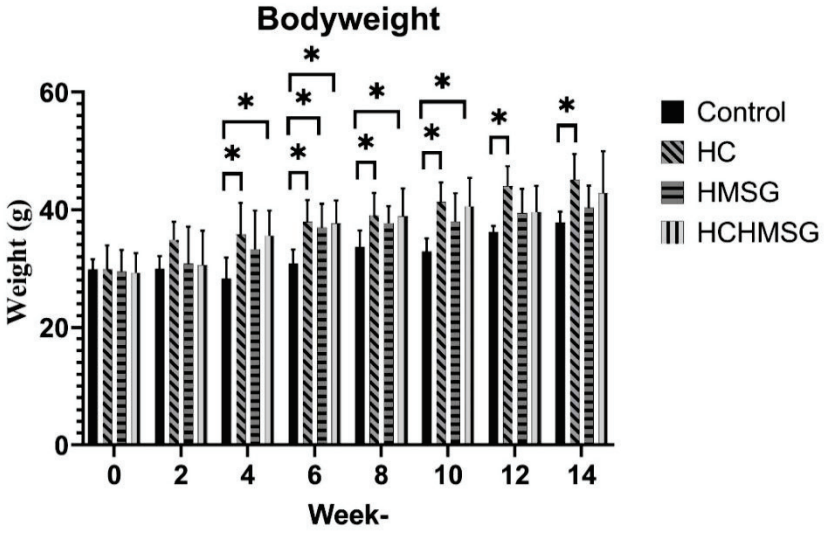

FIGURE 1. Body Weight of the HC, HMSG, and HCHMSG group from week 0 to week 14. The data is stated as the mean ± SD. *$p < 0.05$.

Figure 1 shows the bodyweight of the HC, HMSG, and HCHMSG groups from week 0 to week 14. In the fourth week, there was a significant weight difference between the control, HC, and HCHMSG groups ($p < 0.05$). There was a significant difference between the HCHMSG and control groups until week 10 ($p < 0.05$), while in the 12th to the 14th week, there was no significant weight difference compared to the control group ($p > 0.05$). Meanwhile, the weight difference between the HMSG and the control group is only significant in the sixth week ($p < 0.05$). Another case with the HC group consistently obtained significance from the 4th to the 14th week ($p < 0.05$).

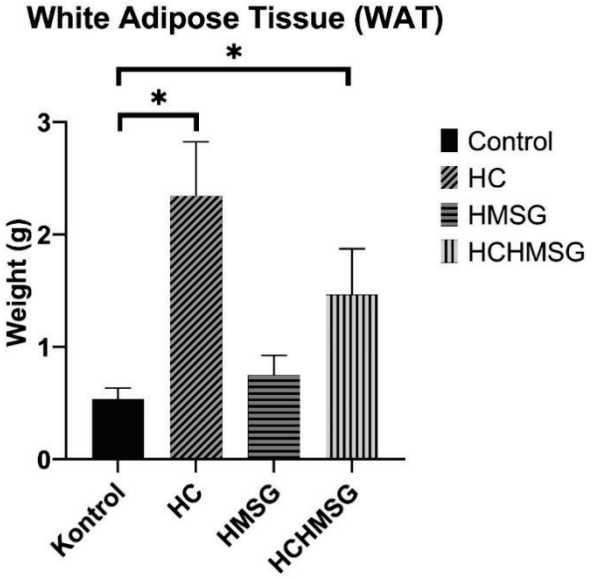

FIGURE 2. Mass of WAT of the HC, HMSG, and HCHMSG group mice at week 14. The data is stated as the mean ± SD. *$p < 0.05$.

Figure 2 shows the WAT mass between the HC, HMSG, and HCHMSG groups. After 14 weeks of the experiment, WAT was collected to determine how the diet affects WAT. There is a significant WAT weight difference in HC and HCHMSG groups compared to the control group ($p < 0.05$) but not in the HMSG group ($p > 0.05$).

DISCUSSION

This study explores the effects of high carbohydrate and high MSG intake estimated as obesogen on WAT and obesity. Obesity occurs due to unbalanced inputs and output. Considering the normal body weight of adult male mice weighing 20-40 grams, then when the weight of mice is above 40 grams, it can be called obesity. [42,43].

In the fourth week, the control group's weight was insignificant compared to the HC and HCHMSG groups (Figure 1). Figure 3 shows that this condition may occur due to the high glycemic index of 71% carbohydrate intake [24,25]. Food intake with a high glycemic index stimulates rapid digestion and absorption [26]. This causes an individual to feel hungry quickly, so the appetite increases and hyperglycemia occurs [3,35,36]. Figure 2 shows that the HC group's WAT exceeds the essential need for mice, thus increasing de novo lipogenesis in the liver, and WAT lipogenesis was not balanced with the energy expenditure [28-30]. This will increase WAT mass due to the high conversion of glucose to TG in fat and increased de novo lipogenesis resulting in free fatty acids. Free fatty acids will increase lipogenesis in WAT so that WAT mass will increase [44-46]. An increase in WAT will result in weight gain [26,40,45,47]—starting from the 4th week of the study until the 14th week (Figure 1).

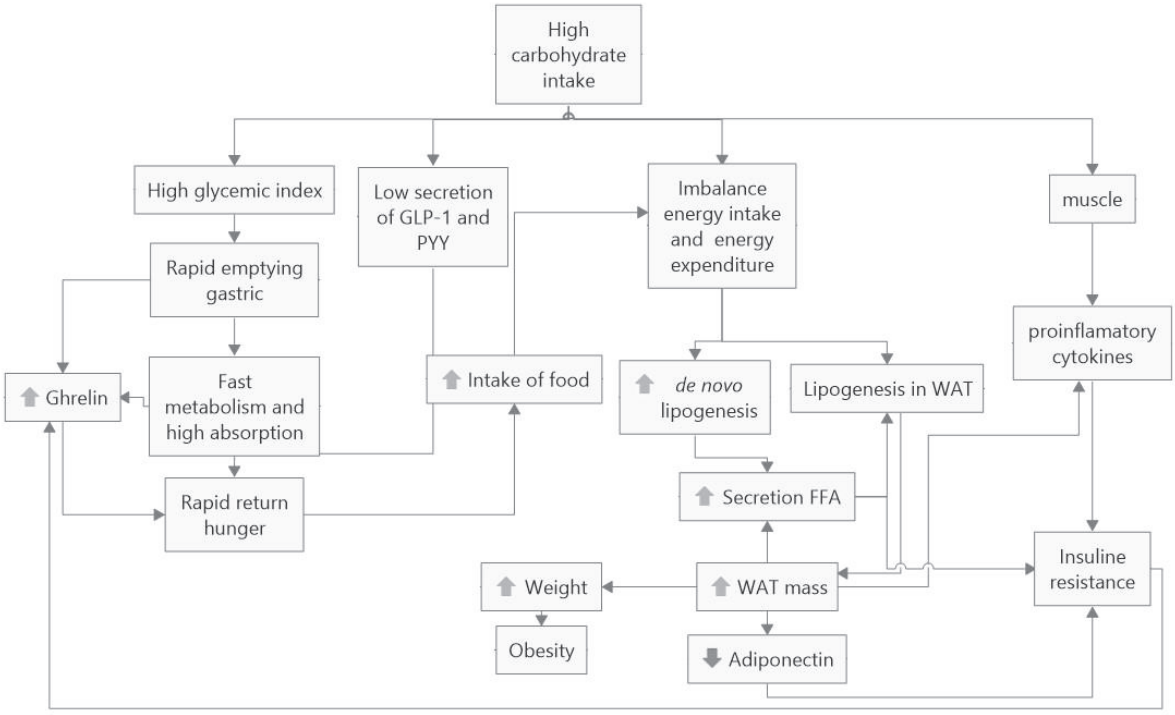

FIGURE 3. Pathophysiology of high-carbohydrate diet-induced obesity. Food with a high glycemic index is digested and absorbed quickly, resulting in hyperglycemia, increased appetite, and frequent hunger. Exceeds of carbohydrate intake increase de novo lipogenesis in the liver and WAT lipogenesis, which will increase WAT mass. An increasing mass of WAT creates insulin resistance, causing ghrelin suppression to decrease and increase ghrelin secretion, further increasing food intake. A slight suppression of the ghrelin hormone and a low increase in satiety hormones (GLP-1 and PYY), thus increasing appetite, further increasing the WAT mass and causing obesity.

Increased WAT mass will decrease adiponectin secretion. Insulin can physiologically inhibit the production of fatty acids in adipose tissue. Increasing insulin production can still compensate for insulin resistance in the early phases. Increased insulin levels will activate lipoprotein lipase on the adipose cell's surface, causing lipolysis to improve insulin resistance further [41,44,48-50]. Adiponectin decreases in skeletal muscles by lowering glucose

uptake by lowering insulin sensitivity, and there is a decrease in beta-oxidation of free fatty acids. The same thing happens with the liver [51-53]. High carbohydrate intake induction increases proinflammatory cytokine levels in the blood [48,54] and skeletal muscles [55]. Increased proinflammatory cytokines play a role in insulin resistance [44,49], inhibiting AKT by inducing serine phosphorylation from IRS-1 (insulin receptor substrate 1) and decreasing insulin sensitivity [46]. Insulin resistance increases appetite because it fails to inhibit the work of ghrelin [56,57].

Compared to high fat or protein intake, high carbohydrate intake showed a slight suppression of the ghrelin hormone and a low increase in satiety hormones (GLP-1 and PYY) in obese individuals. The non-optimal suppression of ghrelin is due to the rapid transfer from the stomach to the duodenum and the rapid absorption of carbohydrates, so ghrelin suppression is only short-lived. At the same time, the low secretion of GLP-1 and PYY still requires further study [58]. Insulin resistance aggravates the condition caused by increased intake, causing ghrelin suppression to decrease and ghrelin secretion to increase, further increasing food intake [56,57].

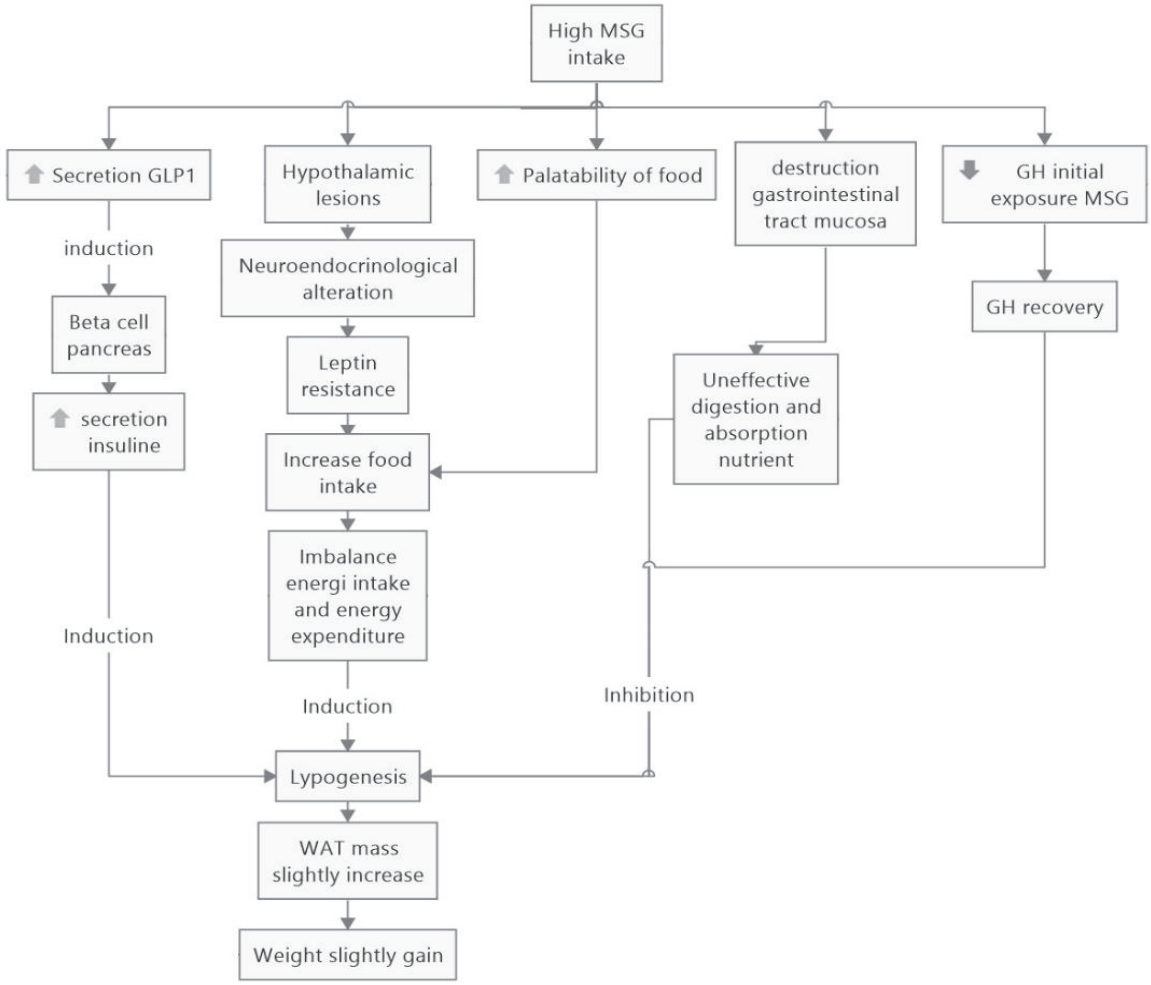

FIGURE 4. Pathophysiology of high-MSG diet-induced obesity. The intake of MSG will increase appetite because MSG increases the palatability of foods. Food consumed in excess will cause excess energy, leading to lipogenesis that will store the extra energy at WAT. WAT will produce leptin to decrease appetite WAT. Still, because MSG is neurotoxic, it will damage the nucleus arcuate and the ventromedial nucleus in the hypothalamus, thus causing the individual leptin resistance. An ever-increasing intake of food that is not offset by expenditure leads to lipogenesis. However, MSG damages the gastrointestinal mucosa, causing digestion and absorption inefficiency. The body cannot convert the food into energy and start to increase growth hormone secretion, even though the secretion of growth hormone decreased at the beginning of induction. Hence, WAT mass slightly increases, and weight is only significant at week 6.

The HMSG group has gained little weight than the control group ($p > 0.05$). Previous research has shown that MSG induction at two months by subcutaneous injection in mice does not decrease Ser9 phosphorylation, so it can still inhibit GSK-3 activation compared to 6 months of induction [59]. In people with DMT2, GSK-3 increased activity was found [60,61]. High GSK-3 activity can lead to increased fat mass, decreased IRS-1 expression, decreased glycogen synthase activity, increased insulin levels, and decreased insulin sensitivity [59-70].

In STC-1 cells, which are model culture cells that secrete GLP-1 induced MSG for 3 hours, there is an increase in GLP-1 excretion. While with 72 hours of MSG induction, there is a decrease in GLP-1 excretion. The 72-hour MSG induction shows similarities to long-term daily consumption [11]. Another study of healthy male volunteers showed similar results in increasing GLP-1 secretion and insulin secretion after one week of MSG-containing intake [71]. This mechanism is possible to happen in the HMSG group. Figure 4 shows an increase in GLP-1 at week 14 due to insufficient induction time. GLP-1 is the main incretin that stimulates insulin secretion from the pancreas, so an increase in GLP-1 can lead to normoglycemia or even hypoglycemia [29-33], and adipose tissue deposition has not occurred massively [34-39]. This mechanism is confirmed by other studies showing that MSG intake increases insulin secretion [72,73]. This explanation explains why the HMSG group is not experiencing DMT2 because insulin can still inactivate GSK and MSG intake acutely causes high GLP-1 secretion to compensate for hyperglycemia conditions.

The addition of MSG to food increases the delicacy, thereby increasing food intake [5,7]. MSG induction damages the arcuate nucleus and the ventromedial nucleus in the hypothalamus, causing leptin resistance and increasing appetite. WAT then produces leptin to control weight and energy balance [5,28,74]. MSG decreases the secretion of growth hormones, aggravating the condition of obesity [75-76]. Pregnant mice and their offspring that were given MSG showed that administering MSG for 30 days would lower growth hormone (GH), but by 90 days, the GH level improved [77]. Growth hormone functions to drain the total and abdominal fat mass [78-79].

The study conducted by El-Aziz et al. (2014) in mice with oral gavage induced with MSG administration for three and six weeks showed experienced weight gain for three weeks, while mice that induced six weeks tended to have stagnant weight and even tended to decrease [80-81].

Several studies have shown different results regarding the effects of MSG induction on body weight. This condition may be due to different induction routes. For example, a study in mice injected with 4g/kg body weight MSG subcutaneously showed an increase in adipose tissue deposition, hyperglycemia, insulin hypersecretion, and pancreatic beta-cell damage [82]. Similar studies showed similar results [83–86]. Other studies have demonstrated stagnant or loss of weight, increased appetite, and decreased anxiety-response in mice with peroral induction [28,87-89].

The mechanism that may occur in the HMSG group is that the high intake of MSG acutely increases the appetite because MSG escalates the palatability of foods. Food consumed in excess will cause extra energy stored at WAT lipogenesis. WAT will produce leptin to lower appetite, but because MSG is neurotoxic, it will damage the nucleus arcuate and the ventromedial nucleus in the hypothalamus, thus causing the individual leptin resistance. An increased intake of food that is not balanced with expenditure leads to increased lipogenesis. However, MSG damages the gastrointestinal mucosa, causing digestion and absorption inefficiency. The food cannot be converted into energy, and increased secretion of growth hormone will happen, even though it will decrease first. Hence, WAT mass only increased slightly, causing a significant weight difference that only happened at week 6.

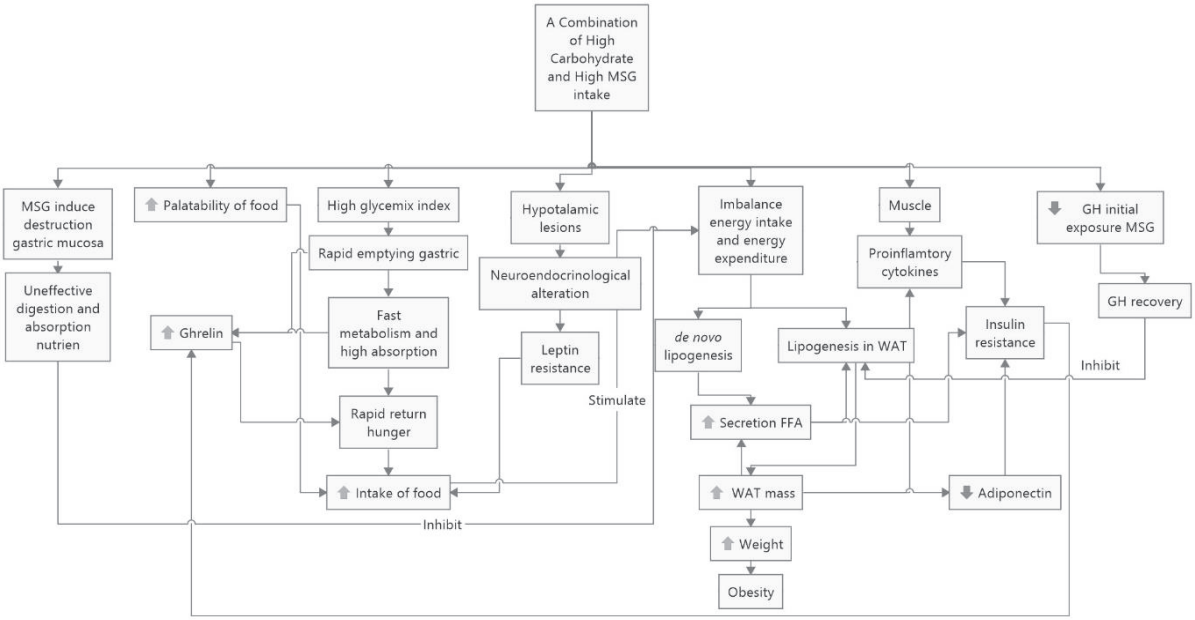

FIGURE 5. Pathophysiology of a combination of high carbohydrate and MSG diet-induced obesity. MSG increases the palatability of these foods, therefore increasing appetite; therefore. Food consumed in excess will cause excess energy, leading to lipogenesis that will store the extra energy at WAT. A high intake of carbohydrates has a high glycemic index; thus, it is digested and absorbed quickly. These phenomenons cause an individual to feel hungry quickly, so the appetite increases. WAT will produce leptin to decrease appetite WAT. Still, because MSG is neurotoxic, it will damage the nucleus arcuate and the ventromedial nucleus in the hypothalamus, thus causing the individual leptin resistance. An increased intake of food that is not balanced with expenditure leads to increase de novo lipogenesis in the liver and WAT, increasing WAT mass. An increasing mass of WAT creates insulin resistance, causing ghrelin suppression to decrease and increase ghrelin secretion, further increasing food intake. A slight suppression of the ghrelin hormone and a low increase in satiety hormones (GLP-1 and PYY), thus increasing appetite, further increasing the WAT mass and leading to obesity. However, MSG damages the gastrointestinal mucosa, causing digestion and absorption inefficiency. The body cannot convert the food into energy and start to increase growth hormone secretion, even though the secretion of growth hormone decreased at the beginning of induction. That is why the weight difference is only significant at the early induction phase.

A significant weight difference was found between the HCHMSG and the control group from week 4 to week 10 ($p < 0.05$). No significant weight difference ($p > 0.05$) was observed from week 12 to week 14. This group intake was predicted to have the most compelling weight gain compared to other groups because the food intake of this group combined two different feed compositions. However, the weight of the HSHMSG group was no higher than the HC group. This research is the first to explore the effectiveness of combining high carbohydrate and MSG intake, thus lacking evidence. So, the researcher hypothesizes about the mechanism of combining high carbohydrate and high MSG intake to the prevalence of obesity.

Figure 5 shows that an early intake of MSG will increase appetite as MSG increases the palatability of foods. Food consumed in excess will cause extra energy that is stored at WAT lipogenesis and increase the mass of WAT. Besides that, a high intake of carbohydrates has a high glycemic index. Food with a high glycemic index is digested and absorbed quickly, resulting in increased appetite and frequent hunger. WAT will produce leptin to decrease appetite WAT. Still, because MSG is neurotoxic, it will damage the nucleus arcuate and the ventromedial nucleus in the hypothalamus, thus causing the individual leptin resistance.

The growth in WAT mass increases the release of free fatty acids. Free fatty acids create insulin resistance, with the result that the liver increases de novo lipogenesis, further increasing WAT mass. In addition, it can also cause an increase in proinflammatory cytokines that stimulates inflammation in fats and muscle, thus decreasing insulin sensitivity. Increased WAT mass will decrease adiponectin secretion. Insulin can physiologically inhibit the production of fatty acids in adipose tissue. Increasing insulin production can still compensate for insulin resistance in the early phases. Increased insulin levels will activate lipoprotein lipase on the adipose cell's surface, causing lipolysis to improve insulin resistance further.

Adiponectin decreases in skeletal muscles by lowering glucose uptake by reducing insulin sensitivity. The same thing also happens in the liver. This feed intake increases proinflammatory cytokines circulating in the blood and skeletal muscles. An increase in proinflammatory cytokines plays a role in insulin resistance, causing ghrelin suppression to decrease and increase ghrelin secretion, further increasing food intake. Compared to high fat or protein intake, high carbohydrate intake showed a slight suppression of the ghrelin hormone and a low increase in satiety hormones (GLP-1 and PYY) in obese individuals, thus increasing appetite, further increasing WAT mass and obesity. However, MSG damages the gastrointestinal mucosa, causing digestion and absorption inefficiency. The food cannot be converted into energy, and increased secretion of growth hormone will happen, even though it will decrease first. Hence, a significant weight difference can be observed from week 4 to week 10. Furthermore, the mass of WAT is not higher than the group that is only given a high intake of carbohydrates only.

CONCLUSION

A high intake of carbohydrates is a potent obesogen as it increases the mass of WAT and leads to the most significant weight gain compared to other types of feed induction. Further research is needed to determine the difference caused by different induction methods between the administration of peroral and injection of MSG to evaluate a high intake of MSG as an obesogen agent. This research demonstrates that the administration of peroral MSG did not cause obesity or show any significant increase in WAT mass. Meanwhile, the combined intake of high carbohydrates and MSG is shown to cause obesity at the end of the study. Although insignificant, the underlying mechanism needs to be investigated further.

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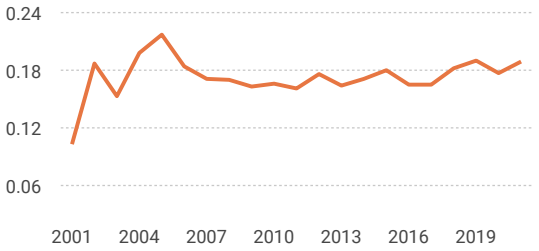

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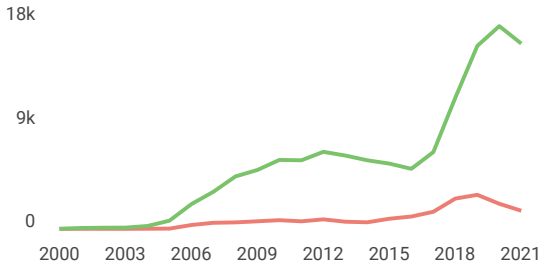

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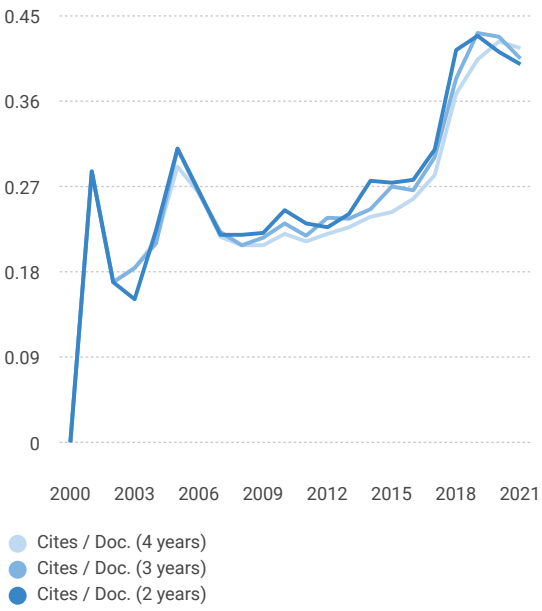

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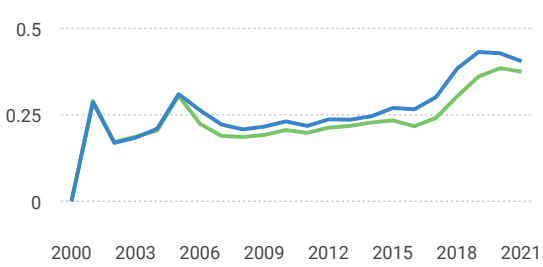

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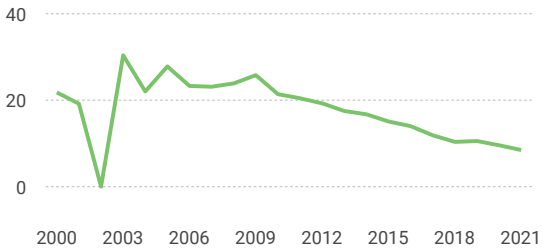

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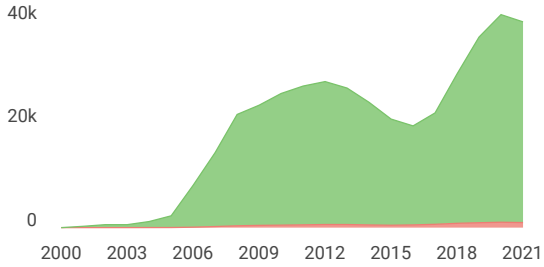

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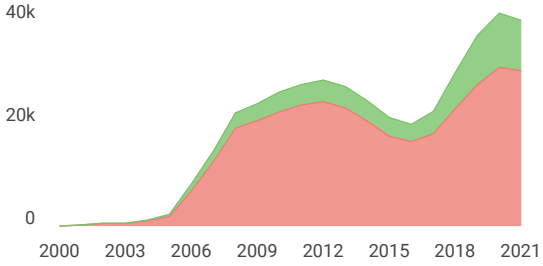

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