Original Article

Effects of Junk Food and Carbonated Soft Drinks on Body Mass Index and Histological Structure of Anterior Pituitary Gland in Young Male Albino Rats

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ABSTRACT

Introduction: Obesity and stunted growth are major health problems that face the population nowadays. Regular intake of junk foods and soft drinks has been associated with obesity and other health conditions. The hypothalamus-pituitary axis serves as the master pathway governing the growth and development. Growth hormone, secreted by pituitary gland, is the one regulating these processes.

Aim of Work: The aim of this study is to throw a light on the effect of junk food and soft drinks on the body mass index (BMI) and structure of pituitary gland.

Materials and Methods: Three-week-old male albino rats (n=40) were divided into; group 1 (control), group 2 (junk food), group 3 (carbonated soft drink) & Group 4 (Junk food and carbonated soft drink). Initial and final weight, length and BMI were measured and calculated for each rat then all rats were sacrificed on the 28th day. Microscopic studies of anterior pituitary gland and morphometric studies for PAS-orange & anti-growth hormone stained sections were done.

Results: Significant increase in weight gain, decrease in length gain and increase in final BMI in all experimental groups compared to control were found. In all experimental groups, H&E stained sections of pituitary gland revealed histological changes of acidophils. PAS-orange stain revealed reduction of orange stained cells. The anti-growth hormone immunostain showed less growth hormone expressive cells. Electron microscope revealed distorted nuclei and reduced secretory granules, vacuolated cytoplasm and swollen mitochondria especially in groups (3&4).

Conclusion: Junk food and carbonated soft drinks consumption led to increase in body mass index and resulted in microscopic changes into light and electron microscopic examination of the anterior pituitary gland.

Received: 24 August 2022, **Accepted:** 19 October 2022

Key Words: Body mass index, junk food, obesity, pituitary gland, soft drinks.

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ISSN: 1110-0559, Vol. 46, No. 4

INTRODUCTION

Obesity and stunted growth are two major health problems which face both the developed and developing countries in the last 30 years. Many serious health problems have arisen by changes in the food habits as the use of processed junk foods[1]. Many of these modern and pre-packaged foods stimulate preferences for sugars, salts, and fats causing excessive food intake[2].

Over the last few years, Studies reported that overweight and obesity prevalence is becoming high and constitutes an emerging concern among pre-school and primary school children. Also, stunting is a significant health problem affecting 1 in 5 children under 5 years of age in low-income and middle income countries[3,4]. Studies have demonstrated that obesity and stunting in children are associated with a decrease intake of nutrient rich foods and an increase consumption of junk foods[5,6].

Fast food meals are frequently accompanied by soft drinks. Most of world's favorite soft drinks comprise of kola which is a known source of caffeine and other components as: phosphoric acid, sugar and chemicals that are used for preservation, coloring and flavor[7]. The intake of soft drinks has increased in the past two decades because of their sweet taste and excessive marketing, in many cases the consumption is without knowledge of the possible hazardous effects to the health if consumed daily[8].

Multiple health hazards have been related to regular intake of soft drinks as liver diseases, tooth decay and type 2 diabetes. Also, the use of sugary sweetened beverages has been found to increase the rate of obesity and insulin resistance[9].

Growth and metabolism are complex physiological processes governed by the hypothalamus-pituitary axis. The release of hypothalamic neuropeptides and control of the pituitary function leads to secretion of many hormones. Growth hormone is an important one of them. Childhood obesity is associated with several metabolic and growth findings as impaired spontaneous growth hormone secretion and decreased growth hormone response to stimulation test[10,11].

 DOI: 10.21608/ejh.2022.157962.1759

According to World Health Organization (WHO), body mass index (BMI) is an index used to measure body fat based on body height and weight for individual. According to the values of BMI, people are divided into four categories such as underweight (< 18.5), normal (18.5 - 24.9), overweight (25.0 - 29.9) and obesity (> 30.0)[12,13].

The exact causes of the relations between junk food and soft drink with obesity and stunted growth were poorly studied. Thus this study investigated their effects on BMI and structure of anterior pituitary gland.

MATERIALS AND METHODS

Animals

Forty young male albino rats aged 3 weeks old were collected immediately after weaning. They were obtained from the animal house - Faculty of Medicine, Tanta University. The animals were kept in clean properly ventilated separated cages. Care of animals and the experimental procedures were carried out according to the rules and regulations laid down by the ethical committee of animal's experimentation of Tanta University. All experimental protocols were approved by ethical committee Tanta University. Approval code: 33235/07/19.

Experimental design

At the beginning of the study, the following measures were estimated for each rat:

a. Initial weight

b. Initial length (nose-to-anus length)[14]

c. Initial body mass index (BMI) was calculated according to the formula {weight (g) / length² (cm^2)}[15]

Then the rats were divided randomly into 4 groups as follows:

Group 1 (Control group): Consisted of 10 rats which received free access to standard diet and water. The diet was composed of [60% carbohydrates(3.6 gm sugar,4.2gm wheat not crushed and 4.2 gm coarse crushed corn), 14% fat (2.8ml sunflower oil) and 26% protein (5.2gm coarse crushed soybeans),] combined in a food processor[16].

Group 2 (Junk food group): Consisted of 10 rats which received free access to junk food diet and water. The junk-food was a mash of: original potato chips, Oreo cookies, Cheetos, Nesquik powdered chocolate flavoring and cheddar cheese combined in a food processor. The diet contains (51% carbohydrates, 25% fat, 11% proteins & 13% fibers and other constitutes) and was adjusted to produce 4.5 Kcal/ gm[17,18,19].

Group 3 (Carbonated soft drink group): Consisted of 10 rats which received free access to standard diet (as in the control group) and alternating carbonated soft drink and water every 12 hours. The carbonated soft drink contains the following constituents {carbonated water, sugar or fructose syrup, caramel color, phosphoric acid, caffeine, emulsifier (gum Arabic) & natural flavor}. Each 100 ml of soft drink contains 41Kcal, 11gm carbohydrates, 11gm sugar &8mg sodium[20].

Group 4 (Junk food and carbonated soft drink group): Consisted of 10 rats which received junk food diet as in group 2 and alternating soft drink and water every 12 hours as in group 3.

The duration of the study was 4 weeks for all groups.

Physical measurements

Length and weight of each rat were measured again at the end of the experiment just before sacrification so that, length gain, weight gain and final body mass index were calculated. Mean and standard deviation were estimated.

Histological methods

In the 28th day of the experiment, the control and experimental rats were anesthetized with ether, sacrificed and subjected for dissection. The pituitary glands were extracted immediately and divided into two halves; the right half was fixed in 10% formol saline for light microscopic examination[21,22]. The left half of the pituitary gland was divided into small pieces and fixed in 3 % glutaraldehyde in 0.1 phosphate buffer solution for transmission electron microscopic examination[23].

Light Microscopic study

a. Haematoxylin and Eosin (H&E) stain used for the study of general histological features of the pituitary gland in each group.

b. Histochemical stain (PAS Orange G stain): is used for detection of the secretory granules in pituitary cells.

Solutions

Periodic acid-1 % aqueous, Schiff's reagent, Celestine blue, Mayer's hemalum. Orange G 4 gm, Phosphomolybdic acid 10 gm, distilled water 200ml.

The Dye and the acid were dissolved in water by shaking periodically for few days and were left completely undisturbed until the solution clarified. The clear solution was pipetted off and stored in tightly stoppered bottle in a dark site.

Steps of staining: sections were brought to water via xylene and ethanol, then placed into periodic acid for 10 minutes.They were rinsed well with tap water, then with distilled water. They were placed in Schiff's reagent for 10-20 minutes and wash off with distilled water for about 10 minutes. They are counterstained with the celestine blue-hemalum sequence, washed well with tap water for 5 minutes. Then they are placed in orange G for 20 seconds. They were rinsed with tap water until pale orange. Finally, dehydrated with ethanol, cleared with xylene and coverslip using a resinous medium. Erythrocytes were stained yellow, while Pituitary acidophils were stained orange. Basophils were stained purpleand chromophobe cells remained unstained.

c. Immunohistochemical study (Anti-growth hormone antibody) (Rabbit polyclonal antibody, AR707-5R, BioGenex antibodies, Fremont, California,USA) was used for immunohistochemical study[24,25].

Steps of staining: immunohistochemical staining was carried out according to the manufacturer's recommended protocol: Paraffin sections were mounted on coated slides. They were deparaffinized with xylene, rehydrated in descending grades of alcohol, then immersed in 0.3% hydrogen peroxide for 10 minutes to block endogenous peroxidase activity. The sections were incubated for 1 hour with polyclonal rabbit antibodies and incubated. The slides were rinsed in PBS (phosphate buffered saline is a buffer solution consists of sodium chloride, sodium phosphate, potassium chloride and potassium phosphate (PH 7.4) 4 times and then incubated with primary antibody enhancer for 10 minutes at room temperature and rinsed again in PBS. Then sections were incubated with peroxidase-compatible chromogen and washed 4 times in distilled water. Finally, all the sections were counterstained with hematoxylin, dehydrated and coverslip using aqueous mounting media DPX (Dibutylphthalate polystyrene xylene). Brown cytoplasmic staining was scored as a positive reaction.

Electron microscopic examination

The specimens were subjected to the following

The small tissue pieces were fixed as soon as possible in 2.5-3 % phosphate buffered glutaraldehyde solution (pH 7.3) for two hours at 4 degrees centigrade and then washed three times in the same buffer. Then they post fixed in 1% phosphate buffered osmium tetroxide for 1 – 2 hours at room temperature, rinsed three times in phosphate buffer solution for 15 minutes each to minimize the possible reaction between the fixative and the dehydrating agents. Dehydration was done in ascending grades of ethanol (Adwick Co., Egypt). After dehydration specimens were transferred into propylene oxide and epoxy resin. Then embedded in capsules, polymerized and sections of 1 micron thickness were prepared. The area needed for ultrastain examination was cut by a diamond knife to make a ribbon of sections about (40 – 50 nm) in thickness. They were then picked on 200 mesh naked copper grids. The grids were examined and photographed on the JEOL 100 JEM (Tokyo Japan) electron microscopic unit of the Faculty of Medicine, Tanta University.

Morphometric measurements

The following measurements were done:

- Optical density of the orange G stain for acidophils was measured in figures of PAS-Orange G-stained slides (×1000) for all groups by image J program. Five not-overlapped images from a slide of each animal from each group were measured. Mean and standard deviation were calculated.
- Optical density of anti-growth hormone immune positive cells was measured in figures of anti-growth hormone-stained slides (×400) for all groups by image J program. Five not-overlapped images from a slide from each animal from each group were measured. Mean and standard deviation were calculated

Statistical analysis

Statistical analysis of physical results and morphometric measurements was conducted. One way analysis of variance (ANOVA) test was used for comparison of the quantitative data among different groups according to the computer program SPSS software version 20. Scheffe test was used for comparison between the *P values* (probability of difference) of different groups. *P values* < 0.05 were considered statistically significant and if *P values* were <0.001 it is considered highly significant[26].

RESULTS

Results of physical measurements

The study of weight changes showed significant increase in weight gain in all experimental groups in comparison with the control group (Table 1). In the contrary, significant decrease in length gain in all experimental groups in comparison with the control group was found (Table 2). Meanwhile there was significant increase in BMI in all experimental groups in comparison with the control group (Histogram 1).

Histological results

Light microscopic examination

a-Hematoxylin and eosin stain (H&E)

The pars distalis consisted mostly of the acidophils which were deeply stained with central rounded nuclei. Chromophobes had large rounded central nuclei within unstained or faintly stained cytoplasm. Basophils had vesicular eccentric nuclei within basophilic cytoplasm. Sinuosoidal capillaries were seen between cells (Figure 1 a).

Sections of pars distalis of the junk-food group showed loss of the branching pattern of the secretory cells with multiple amalgamations of acidophils and dilated sinusoidal capillaries between cells (Figure 1 b).

In sections of carbonated soft drink group, acidophilas with abnormally eccentric nuclei were seen. Some of them appeared shrunken and smaller in size in comparison to the control group. Some cells showed cytoplasmic vacuoles. Congested capillaries were seen between the cells (Figure 1 c).

Sections obtained from the junk food & carbonated soft drink group showed amalgamation of the acidophils. Most of the acidophils had darkly stained nuclei and vacuolated cytoplasm. The blood sinusoids were markedly dilated and congested (Figure 1 d).

b- PAS-Orange stain

Control group sections of pars distalis showed abundant acidophils with their cytoplasm stained orange, some basophils taking purple coloration and scanty unstained chromophobes. Pars distalis of the junk-food group showed reduction of orange stained cells compared to the control group. The sections of carbonated soft drink group showed more reduction of orange stained cells. Whereas, the sections of the junk food & carbonated soft drink group showed marked reduction of orange stained cells (Figure 2). These results were confirmed by measurement of optical density in PAS-orange stained sections which revealed highly significant decrease in the junk food group, the carbonated soft drink group and the group of both junk food and carbonated soft drink in comparison with the control group (P value <0.001) (Table 3, Histogram 2).

c-Anti-growth hormone immunostaining

Pars distalis of the control group showed strong diffuse growth hormone expression which appeared as dark brown cytoplasmic coloration. The junk food group showed less expressive cells while the carbonated soft drink group and group of junk food and carbonated soft drink showed marked reduction of growth hormone expression and decreased color intensity in comparison to control group (Figure 3). Measurements of optical density confirmed the previous results (Table 3, Histogram 2)

Electron microscopic examination

Ultrastructural examination of rat's anterior pituitary gland of the control group showed different types of cells; somatotroph cells, smaller thyrotrophes, large gonadotrophes and corticotrophes with pale cytoplasm and sparse peripheral granules. Somatotrophe cells showed rounded nuclei with regular boundaries. The cytoplasm showed rough endoplasmic reticulum, many variable sized-secretory granules and elongated mitochondria (Figure 4).

The somatotrophes of the junk food group showed slightly distorted nucleui and secretory granules were accumulated at one side of the cell. Some cells showed depleted areas of the secretory granules, dilated rough endoplasmic reticulum and mitochondria with small vesicles. The somatotrophes of the carbonated soft drink group showed shrunken irregular nuclei with areas of clumbed chromatin, vaculated cytoplasm and swollen mitochondria. There was apparent reduction of secretory granules and dilated rough endoplasmic reticulum. The junk food and carbonated soft drink group showed many vaculations of the cytoplasm in the somatotrophes and decreased secretory granules. Shrunken condensed nuclei with clumbed chromatin, swollen dilated mitochondria with destructed cristae and very dilated rough endoplasmic reticulum were seen (Figure 5).

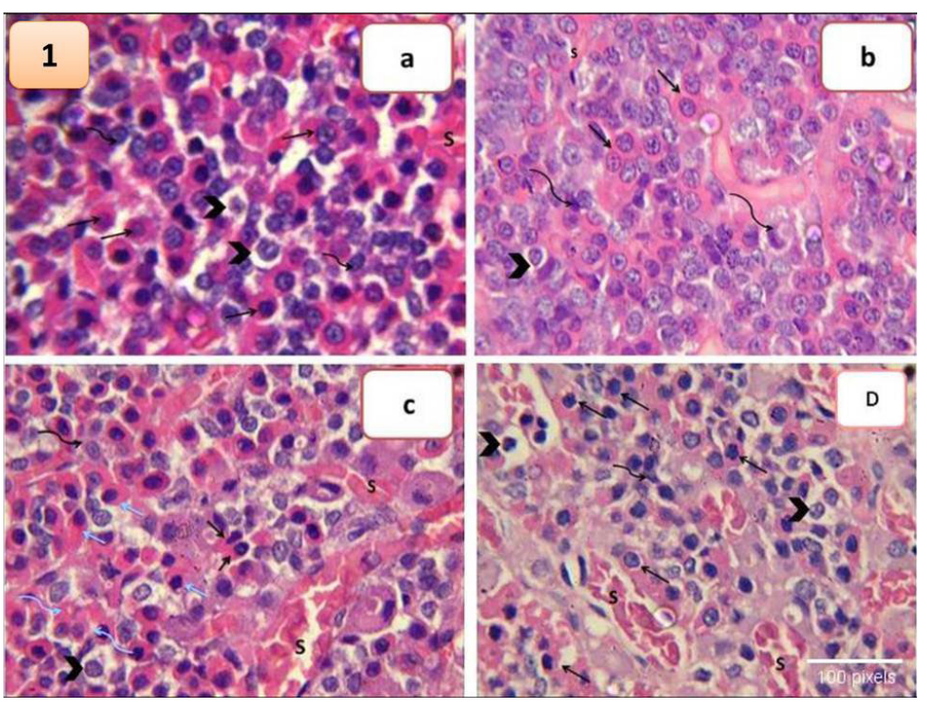


Fig. 1: A photomicrograph of sections of pars distalis in the different groups; (a): the control group showing acidophils (straight arrows), chromophobes (arrow heads) and basophilis (curved arrows). Normal Sinuosoidal capillaries (s) are seen between cell cords. (b): the junk-food group showing loss of the branching pattern of the secretory cells with multiple amalgamations of acidophils (straight arrows). Dilated sinusoidal capillaries (S) appear between cells. Note: basophils (curved arrows) and chromophobe (arrow head).(c): the carbonated soft drink group showing some acidophils with eccentric nuclei (blue straight arrows), shrunken acidophils (black straight arrows) and cells with cytoplasmic vacuoles (blue curved arrows). Basophils (black curved arrow) and chromophobes (arrow head) are also seen. Notice the dilated congested sinusoidal capillaries (S). (d): the junk food and carbonated soft drink group showing most of acidophilic cells with darkly stained nuclei and vacuolated cytoplasm (straight arrows). The blood sinusoids (S) are markedly dilated and congested. Notice: chromophobes (arrow heads) and basophils (curved arrow). (H&E×1000).

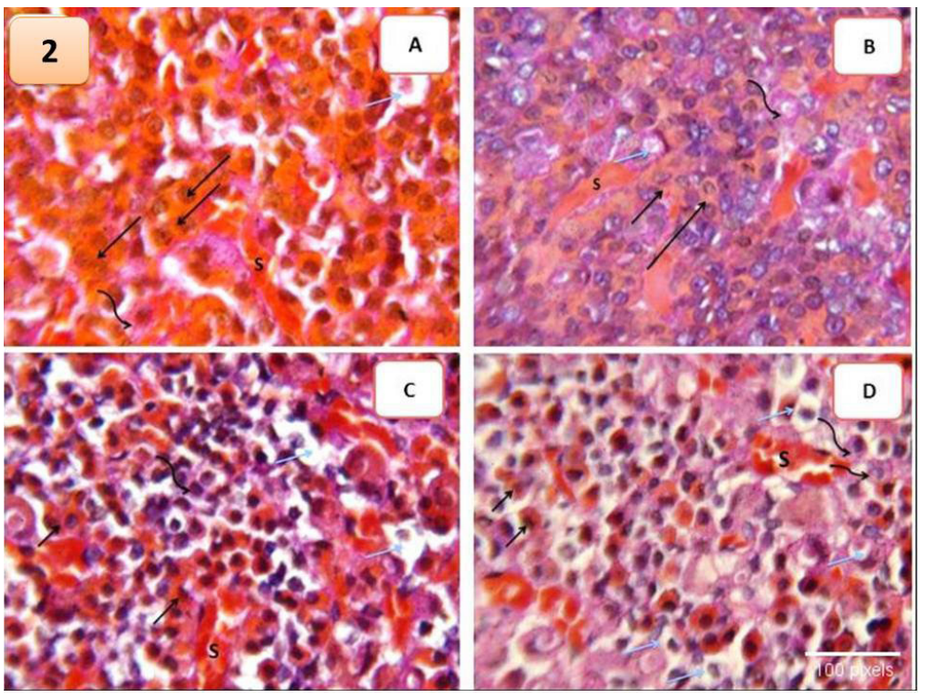


Fig. 2: A photomicrograph of Pas-Orange G stained sections of pars distalis ofdifferent groups; (a): the control group showing abundant acidophils with their cytoplasm stained orange (black straight arrows). Some basophils (curved arrow) taking purple coloration and unstained chromophobes (blue straight arrow) are also seen. There are sinusoids (S) inbetween. (b): the junk-food group showing apparent reduction of orange stained cells (Ac) compared to the control group. (c): the carbonated soft drink group showing apparently marked reduction of orange stained cells (black straight arrows) in comparison to control group. (d): the junk food and carbonated soft drink group showing very little orange stained cells (black straight arrows) in comparison to other groups. Notice unstained chromophobes (blue straight arrows), some basophils (curved arrows) and sinusoids (s) in all groups (Pas-Orange G ×1000).

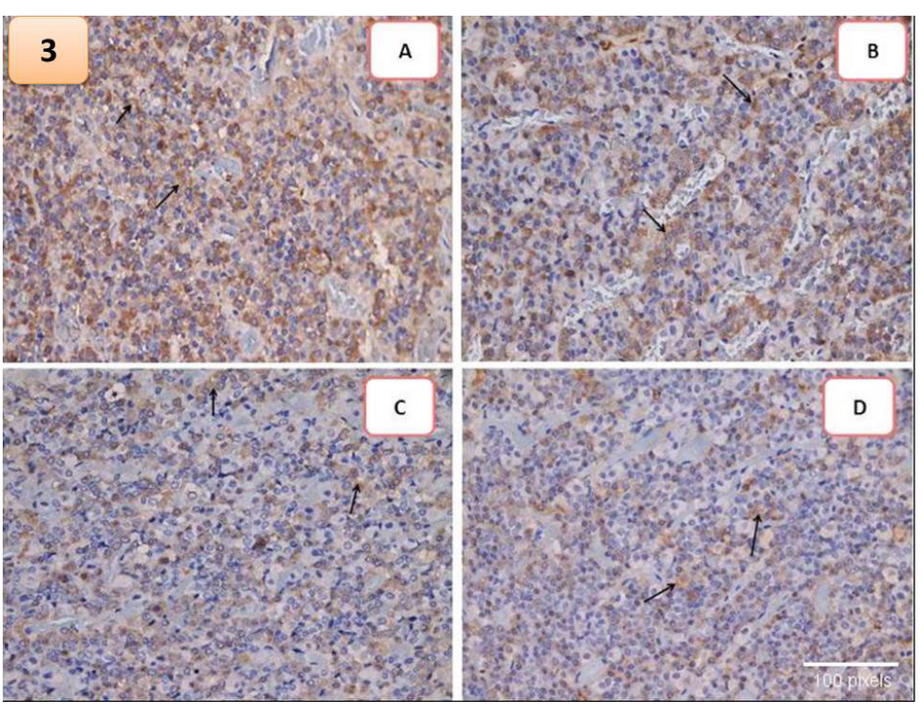


Fig. 3: A photomicrograph of immunostain sections of pars distalis of different groups; (a): the control group showing strong diffuse growth hormone expression which appear as dark brown cytoplasmic coloration (arrows). (b): The junk food group showing less growth hormone expression (arrows) in comparison to the control group. (c): The carbonated soft drink group showing weak growth hormone expression in comparison to control group (arrows). (d): The junk food and carbonated soft drink group showing very weak growth hormone expression (arrows) in comparison to other groups. (Growth hormone immunoreactivity× 400).

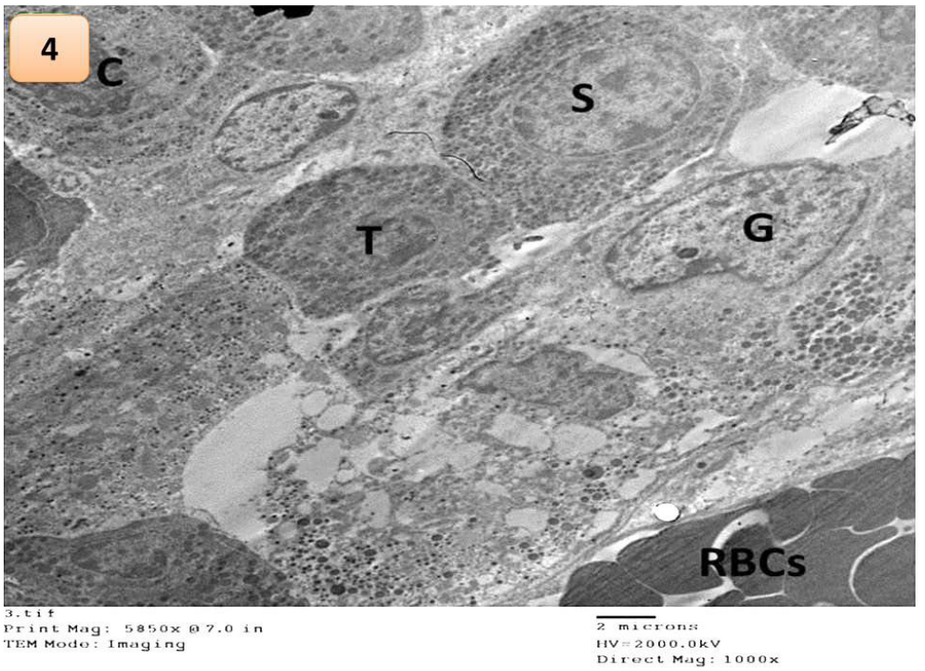


Fig. 4: A transmission electron micrograph of an ultrathin section in the rat anterior pituitary gland of the control group showing different types of cells; somatotroph cell (S) is packed with secretory granules of variable sizes, thyrotroph (T) is smaller with peripherally located less granules, large gonadotroph (G) with less granules of variable sizes and corticotroph (C) with pale cytoplasm and sparse peripheral granules. Notice the capillary sinusoid at the periphery of the figure containing RBCs (Direct magnification x 1000).

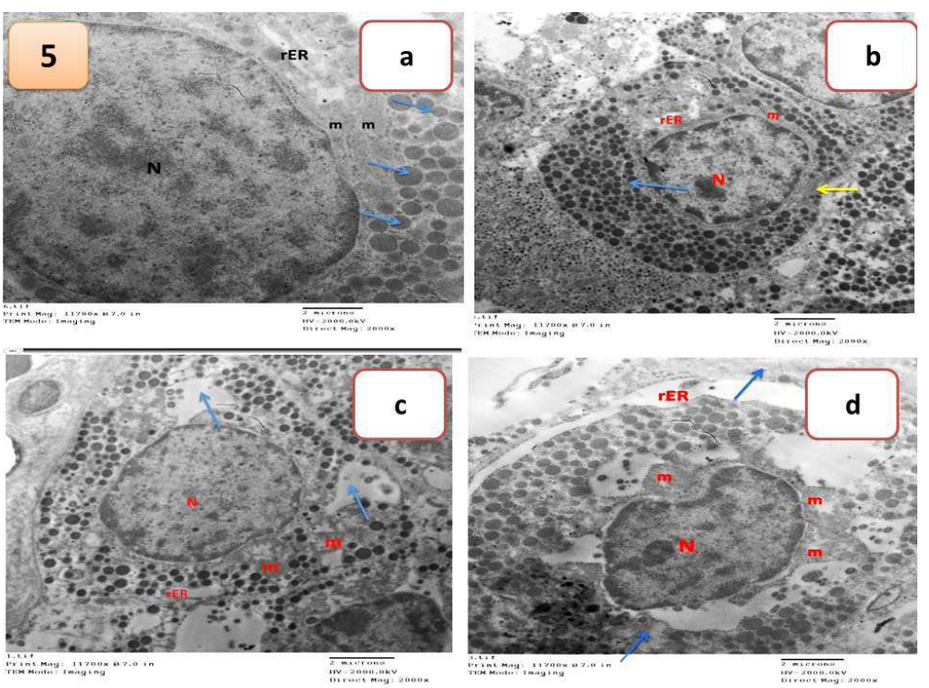


Fig. 5: Transmission electron micrograph of an ultrathin section of somatotrophe cell; (a): of the control group showing the nucleus (N) with dispersed chromatin, intact nucleus envelope and regular boundaries. Elongated mitochondria (m) are seen near the nuclear envelope. Multiple dense granules of variable sizes (blue arrows) and rough endoplasmic reticulum (rER) are also seen. (b): of the junk food group showing a slightly distorted nucleus (N). The secretory granules are accumulated at one side of the cell (blue arrow) with areas depleted of the secretory granules (yellow arrow). Dilated rough endoplasmic reticulum (rER) and elongated mitochondria (m) are seen. (c): of the carbonated soft drink group showing a slightly distorted nucleus (N),vaculated cytoplasm (blue arrows) and swollen vaculated mitochondria (m). Dilated rough endoplasmic reticulum (rER) and apparent reduction of secretory granules are noticed. (d): of the junk food and carbonated soft drink group showing irregular nucleus (N), swollen mitochondria (m) with destructed cristae and very dilated rough endoplasmic reticulum (rER). Notice apparent reduction of secretory granules (blue arrows) (Direct magnification x 2000).

Table 1: Initial, final and weight gain in all groups

Weight changes (grams)		Control	Junk food	Soft drinks	Junk food & soft drinks
Initial	Range	47-53	46-53	47-53	46.5-52.5
	Mean ± SD	49.80± 2.26	49.25± 2.69	49.75± 2.27	49.81± 2.07
Final	Range	162-169	172-179	182-187	192-198
	Mean ± SD	166.10± 2.65	174.44± 2.30	184.11± 1.54	195.56± 1.81
Weight gain	Range	115– 120	123 – 126	129 – 134	138.5- 145.5
	Mean ± SD	117.5± 1.96	121.8± 1.87	126± 2.16	135.5±3.08
	Control & Junk food	Control & Soft drinks		Control and Junk food &soft drinks	
	0.001*	0.000*		0.000*	
	Junk food& Soft drinks	Junk food and Junk food &soft drinks		Soft drinks and Junk food &soft drinks	
	0.001*	0.000*		0.000*	

SD : standard deviation * :highly significant *p value*

Table 2: Initial, final and length gain in all groups

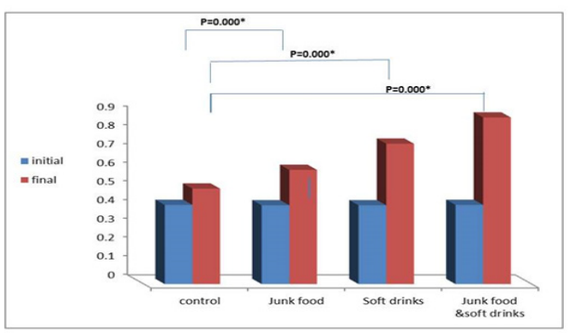
Length (centimeters)		Control	Junk food	Soft drinks	Junk food & soft drinks
Initial	Range	10.5-10.9	10.5-11	10.7-11	10.7-11
	Mean ± SD	10.64± 0.143	10.80±0.173	10.86±0.133	10.86±0.113
Final	Range	18-18.3	16.6-17.1	15.5-15.9	14.8-15.1
	Mean ± SD	18.16±0.11	16.86±0.17	15.77±0.14	15.00±0.1
Weight gain	Range	7.1 – 8.6	6.1 –7.9	4.8 - 6.9	3.9 - 4.9
	Mean ± SD	8.03 ± 0.427	6.68± 0.782	5.62± 0.699	4.32 ±0.382
	Control & Junk food	Control & Soft drinks		Control and Junk food &soft drinks	
	0.001*	0.000*		0.000*	
	Junk food& Soft drinks	Junk food and Junk food &soft drinks		Soft drinks and Junk food &soft drinks	
	0.001*	0.000*		0.001*	

SD : standard deviation * :highly significant *p value*

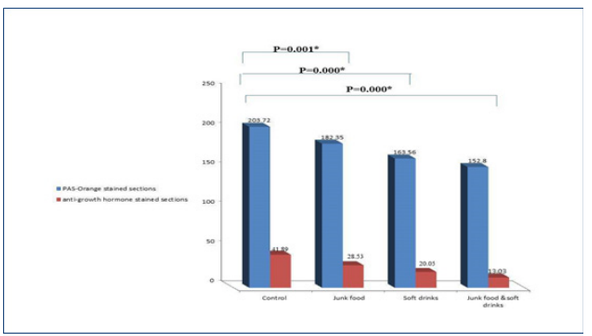
Table 3: Optical density of PAS-orange & anti-growth hormone stained section of all groups

Length (centimeters)		Control	Junk food	Soft drinks	Junk food & soft drinks
Optical density of PAS-orange stain	Range	196.47 – 210.30	178.59 – 184.50	163.01 – 170	150- 164.25
	Mean ± SD	203.72± 5.63	182.35± 2.50	163.56± 2.82	152.80±5.05
Optical density of anti-growth hormone stain	Range	40.11 – 50.88	26.90 – 32.80	19.88 – 23.78	12.89- 16.09
	Mean ± SD	41.89± 3.78	28.53± 3.12	20.05± 1.94	13.03±2.11
	Control & Junk food	Control & Soft drinks		Control and Junk food &soft drinks	
	0.001*	0.000*		0.000*	
	Junk food& Soft drinks	Junk food and Junk food &soft drinks		Soft drinks and Junk food &soft drinks	
	0.001*	0.000*		0.001*	

SD : standard deviation * :highly significant *p value*



Histogram 1: Initial and final body mass index (BMI) in all groups



Histogram 2: Optical density of PAS- orange and anti-growth hormone stained sections of all groups

DISCUSSION

Obesity and its related disorders are increasing in many countries worldwide. This increase over the past few decades is generally attributed to a change in diet habits. Manufactured foods that contain high levels of fat, sugar and salt, but less nutritious in vitamins and essential nutrients; are often qualified as 'junk food'. Moreover, evidences suggest an association between the intake of sugar-sweetened soft drinks and the risk of obesity in children and adults[27,28].

Stunted growth is a reduced growth rate in human development. According to the World Health Organization (WHO), it is defined as the "height for age" value to be less than two standard deviations of the WHO Child Growth Standards median[29]. Studies investigated factors related to stunting among infants and young children found that most children were eating junk foods rather than nutritious diets[6].

Few studies were done demonstrating the effect of junk food and carbonated soft drinks on BMI. Moreover, researches regarding their effect on the structure of pituitary gland were hardly found in the literature. So, this study was designed to throw a light on the possible effect of junk food and carbonated soft drinks on body mass index and histological structure of the anterior pituitary gland in young male albino rats.

Rats were used in this study as animal models for studying obesity and stunted growth based on previous researches which suggested that both humans and rodents tend to gain weight with high-caloric intake [14].

In this experimental work, there was significant increase in weight gain in all experimental groups when compared to control group. These results were in agreement with previous research that stated that cafeteria diet intake for fourteen weeks can cause obesity and increase the white fat mass in Wistar rats[30].

Many studies also reported that there was an increase in body weight after using artificially sweetened soft drinks and investigated that consumption of fructose- sweetened soft drinks led to increase in the body weight and hepatic lipid accumulation in mice[31,32]. Sugar is used as sweeteners in soft drinks, and it had been reported to result in obesity upon consumption[33].

Previous research explained that obesity associated with intake of junk food and soft drinks in preschool and school children may be due to increased calories provided by this type of food and/or drink. Many studies attributed the obesity in children to the decrease in the intake of nutrient rich food and the increase of junk food and soft sweetened drinks ingestion[5].

On the other hand, this study showed significant decrease in length gain in all experimental groups compared with the control. This finding was reported by previous work that stated that total body and femoral lengths were significantly shorter in rats which received ultra-processed food and caloric soft drink than in the control group and explained that stunting growth was caused by these food and drink, not due to caloric deficiency[34].

Likewise, other study reported that linear growth retardation and stunting are manifestations of chronic malnutrition. They added that, this condition can occur due to inadequate intake of food, inappropriate type of diet, or both[35].

On the other hand, this result was on contrary to previous study that reported an increase in length of fructose-fed rats that was greater as compared to controls. The differences may be determined by their breed or the quantity and route of administration of fructose[36].

Obesity can be easily determined from estimating body mass index (BMI) in rats,[14]. Results obtained in this research showed significant increase in final BMI in the experimental groups in comparison to the control group. Similar finding was reported in previous research that groups of laboratory rats consuming water with fructose, aspartame and sucralose from weaning age to adulthood had a gain in body mass higher than that of the control[37].

Also, similar results found increase in percentage of body weight gain, BMI, and abdominal circumference (AC) in comparison to their controls in case of metabolic syndrome rat models including obesity[38,39].

Previous studies stated that fructose over-consumption increases body weight, changes food consumption pattern and lipid profile. They suggested that these changes may be partly responsible for the epidemic increase in obesity and metabolic syndrome in the modern Western world. These findings were reported to occur due to decreased leptin effect and peripheral leptin resistance. Leptin is a known adipocyte-derived hormone that plays a key role in the regulation of body weight through its actions on appetite and metabolism[36,40].

Also, the present study showed increase in the final BMI compared to the initial BMI of the control group. This finding may be explained by the young age of the used rats in this work as previous study suggested that BMI increased with enhancing age in rats up to 90 days of age and remained constant thereafter[14].

The pars distalis of the pituitary gland is the master regulator of the peripheral endocrine function, whereas the somatotrophe cell (one of the acidophils) is the major one which controls mechanisms of growth of the body[41]. Thus, this work focused on study of anterior pituitary gland acidophils by light microscope using H&E and PAS-orange stains. Moreover, this study concentrated on somatotrophes by using immunohistochemical and electron microscopic evaluation.

In this current work, light microscopic study of sections of the junk food group (group 2) showed loss of the branching pattern of the secretory cells with multiple

amalgamations of acidophils and dilated sinusoidal capillaries. No studies have been found in the literature concerning the effect of junk food on the anterior pituitary gland. However, previous work reported histopathological changes in some body organs as vaculations of hepatocytes, pyknosis of some brain neurons and congestion of renal blood vessels after experimental ingestion of junk food for 45 days. They suggested that ingestion of junk food had deleterious effects on these body organs[42].

In this experiment, acidophils of carbonated soft drink group (group 3) showed abnormally eccentric nuclei, some cells appeared shrunken and smaller in size in comparison to the control group. Some cells showed cytoplasmic vacuoles. Congested capillaries were seen. The effect of carbonated soft drinks on the anterior pituitary was hardly found in the previous studies. However, many researches were done to evaluate their use on other organs such as vacuolations between cell fibers of the white matter of the cerebellum with degenerated purkinje cells and also, pathological changes in hepatocytes in rats received carbonated soft drinks[43,44]. Previous work explained that carbonated soft drinks have negative consequences on the overall health. They added that some of the mechanisms of their adverse effects include increase in glutathione 6-dehydrogenase level and gamma-aminobutyric acid (GABA), glutamate and dopamine leading to alteration in brain waves on electroencephalography, stroke and dementia. Also, they increase the oxidative stress by decreasing monoamine oxidative and antioxidants[45].

The junk food and carbonated soft drink group (group 4) showed the highest histological changes which may be due to combined effects of both junk food and carbonated soft drink. Sections of this group showed amalgamation of the acidophils. Most of the acidophils had darkly stained nuclei and vacuolated cytoplasm. The blood sinusoids were markedly dilated and congested.

PAS-orange stain is a special stain where acidophils appear orange G positive and basophils are PAS positive while chromophobes have no stain. The staining characteristics are dependent on the level of secretory activity[41]. The current work revealed highly significant reduction in orange stained acidophils in PAS-orange stained sections in the experimental groups. Likewise, immunohistochemical studies revealed significant decrease in growth hormone expression in the anti-growth hormone stained sections of experimental groups in comparison with the control. Thus, these results may suggest decrease in growth hormone secretory granules of the cytoplasm of somatotrophs and decreased their activity in the experimental groups.

Previous researchers reported that diet-induced obesity is characterized by a reduction in GH release. By using obese mouse models, they proved the reduction in the expression of GH and pituitary receptors important for GH synthesis and release. They suggested that these pituitary changes occurred independently of changes in the expression of hypothalamic regulators of GH secretion (growth hormone releasing hormone and growth hormone inhibiting hormone). Accordingly, they suggest that obesity-associated GH suppression is at least in part due to a primary pituitary defect[46].

The electron microscopic (EM) examination of the pituitary gland in this study confirmed our results regarding PAS-Orange stained sections and anti-growth hormone immunohistochemistry. By EM, somatotrophs of junk food group (group 2) showed reduction of secretory granules. In addition, distorted nucleus, swollen vacuolated mitochondria and dilated congested capillaries were also seen.

Also, somatotrophs of carbonated soft drink group (group 3) showed apparent reduction of secretory granules. In addition, shrunken irregular nuclei with clumped chromatin, vacuolated cytoplasm, swollen vacuolated mitochondria and dilated rough endoplasmic reticulum were also noticed. Accordingly, in the junk food and carbonated soft drink group (group 4) somatotrophes showed extensive changes than groups (2,3).

These results can be explained by previous studies that reported that in cases of growth hormone deficiency, most somatotrophs revealed degenerative changes and reduction in the number of the specific secretory granules. Also others had edematous swollen mitochondria as well as irregular nuclei with more condensed chromatin[47].

Also, the changes found in somatotrophs in this study resembled changes noticed after exposure of some drugs and chemicals. Previous research after Toluene (widely used as organic solvent and in production of many industrial products) exposure to rats resulted in similar changes. The somatotrophs showed cytoplasmic vacuolation, irregular shrunken nuclei with more condensed chromatin as well as swollen mitochondria. These changes were attributed to oxidative stress due to arising of lipid peroxidation with production of reactive oxygen species (ROS) and toxic derivatives which may be also the cause of ultrastructural changes observed in our study[48].

CONCLUSION

The present study proved that both junk food and carbonated soft drinks consumption in rats lead to increase in weight gain and decrease in length gain and accordingly increase in body mass index. They also resulted in structural changes in the anterior pituitary gland especially the growth hormone-releasing acidophils (somatotrophes). More studies are recommended to study the effect of their use over longer periods and to evaluate the growth hormone levels.

CONFLICT OF INTERETS

There are no conflicts of interest.

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الملخص العربى

تأثير الاطعمة الغير مفيدة والمشروبات الغازية على مؤشر كتلة الجسم و التركيب الهستولوجى للغدة النخامية الأمامية فى ذكور الجرذان البيضاء الصغيرة

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الخلفية: تعد السمنة و التقزم مشكلتان صحيتان رئيسيتان تواجهان السكان فى الوقت الحاضر.إن الاستهلاك المستمر للاطعمة الغير مفيدة والمشروبات الغازية يؤدى الى السمنة و مخاطر صحية أخرى و يمثل محور الغدة النخامية المسار الرئيسى للتحكم فى النمو والتطور وينظم هرمون النمو الذى تفرزه الغدة النخامية هذه العمليات.وقد صممت هذه الدراسة لإلقاء الضوء على تأثير الاطعمة الغيرمفيدة و المشروبات الغازية على مؤشر كتلة الجسم وتركيب الغدة النخامية.

المواد والطرق: تم تقسيم أربعين من الجرذان الصغيرة البالغة من العمر ٣ أسابيع إلى ؛ المجموعة ١ (المجموعة الضابطة) ، المجموعة ٢ (الوجبات الغير مفيدة) ، المجموعة ٣ (المشروبات الغازية) ، المجموعة ٤ (الوجبات الغير مفيدة والمشروبات الغازية). تم قياس وحساب الوزن والطول ومؤشر كتلة الجسم في كل مجموعة عند بداية التجربة وعند نهايتها. وفى اليوم الثامن والعشرين تم التضحية بجميع الجرذان. أجريت الدراسات المجهرية الضوئية والإلكترونية للغدة النخامية. وتم إجراء دراسات القياس المورفومتري للأقسام المصبوغة بالحمض الدورى -شيف البرتقالية و الهرمون المضاد للنمو. وتم إجراء التحليل الإحصائي لجميع القياسات المورفومترية.

النتائج: : أظهرت نتائج البحث زيادة ذات دلالة إحصائية في الوزن وانخفاض ذو دلالة إحصائية في زيادة الطول وزيادة ذات دلالة إحصائية في مؤشر كتلة الجسم النهائي في جميع المجموعات التجريبية بالنسبة للمجموعة الضابطة. وأظهرت المقاطع المصبوغة بالهيماتوكسيلين و الايوسين للغدة النخامية تغيرات نسيجية في الخلايا الحمضية في جميع المجموعات التجريبية. أظهرت صبغة الحمض الدورى -شيف البرتقالية نقص فى عدد الخلايا المصبوغة باللون البرتقالى في جميع المجموعات التجريبية. وأظهر هرمون المناعة المضاد للنمو خلايا معبرة لهرمون النمو أقل في جميع المجموعات التجريبية. كشف الفحص المجهري الإلكتروني عن نوى مشوهة وحبيبات إفرازية قليلة. ولوحظ السيتوبلازم المجوف ، والميتوكوندريا المنتفخة والشبكة الإندوبلازمية الخشنة المتوسعة خاصة في المجموعة (٣ و ٤).

الخلاصة: يؤدي تناول الوجبات الغير مفيدة والمشروبات الغازية إلى زيادة مؤشر كتلة الجسم وتغييرات هيكلية في الفص الامامى للغدة النخامية .