

EFFECT OF OBESITY ON GENERAL HEALTH AND ON PAROTID SALIVARY GLANDS OF RATS (HISTOLOGICAL AND ULTRASTRUCTURAL STUDY)

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ABSTRACT

BACKGROUND: Many persistent diseases, including type II diabetes, coronary heart disease, and osteoarthritis, are positively correlated with obesity. Obesity increases fat cell formation, insulin resistance, inflammation, and the production of reactive oxygen species that damage DNA, lipids, proteins, cell growth, and signal transduction. Therefore, destruction in the salivary gland structure results in the impairment of the function of the salivary gland.

OBJECTIVES: The purpose of the study is to explore the effect of a high-fat diet on the parotid salivary gland structure by histological and ultrastructural analysis.

MATERIALS AND METHODS: Twenty-four adult male albino rats were randomly split into two equal groups of 12 rats each. Group I, the control group, were fed normal rat chow. Group II were fed a high-fat diet for 8 weeks. The rats' weights were measured weekly and their general health was observed daily, while a serological analysis was performed at the end of the experimental period. The rats were euthanized, and the parotid salivary glands were dissected out and prepared for light and electron microscopic examination.

RESULTS: Group II displayed a loss in the typical configuration of the parotid salivary gland and the clinical observations showed an increase in body weight, food intake, water intake, hair loss, and a decrease in physical activity, while insulin, cholesterol, glucose, and calcium blood serum levels increased in comparison to the control group.

CONCLUSION: A high-fat diet initiates obesity, insulin resistance, a decrease in general health, and a disruption in the structure of the parotid salivary glands.

KEY WORDS: Obesity, High-fat diet, Parotid salivary gland, Histological results, Ultrastructural results.

RUNNING TITLE: Effect of obesity on parotid salivary glands structure.

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INTRODUCTION

A medical disease known as obesity occurs when excess body fat builds up to the point that it could be harmful to one's health. The main contributing factors to obesity include excessive food intake, a lack of exercise, and genetic predisposition. Certain cases of obesity are primarily brought on by genetics, endocrine issues, drugs, or mental health issues. (1)

Obesity increases the risk of several illnesses, especially osteoarthritis, heart disease, type II diabetes, obstructive sleep apnea, and some types of cancers (1). Moreover, obesity may alter oxygen metabolism, which induces significant oxidative stresses (2). The phenomenon of oxidative stress (OS) is one of the pathogenic mechanisms causing the emergence of insulin resistance (IR), which is when the concentration of steady-state reactive

oxygen species (ROS) is "transiently or chronically increased, resulting in insulin resistance by influencing different points in insulin receptor signal transduction, leading to damaging effects on cellular metabolism and constituents." (1)

All vital cellular components, including DNA, lipids, and proteins, are harmed by the increased formation of ROS, which also disturbs cellular metabolism by altering gene expression, signal transduction, cell development, and apoptosis. The imbalance between ROS and antioxidants causes damage to the salivary glands with the parotid gland being the most adversely affected. (2) Moreover, obesity can result in generalized body inflammations caused by the release of pro-inflammatory cytokines (TNF, IL-6, and IL-1), stimulation of NADPH oxidase, and generation of ROS in adipocytes and

macrophages. Adipocytes also discharge monocyte chemoattractant protein-1 (MCP-1), which draws monocytes to adipose tissue and changes them into tissue-resident inflammatory M1 phenotype macrophages. (2)

A high-fat diet (HFD) can negatively affect general health by initiating obesity and increasing blood cholesterol levels and blood pressure. Moreover, it causes insulin resistance and an increase in blood glucose levels, which may cause type II diabetes. (3) Consuming a large amount of fats also damages the structure of the salivary glands. As the main function of the salivary glands is to produce saliva, which plays an important role in maintaining oral hygiene, the consumption of large amounts of fat can thus decrease oral hygiene. (3)

Finally, salivary gland dysfunction is related to many nutritional disorders such as the onset of type II diabetes. Type II diabetes is initiated by insulin resistance from a high-fat diet, leading to the atrophy of salivary glands. (3)

Thus, the goal of this study is to explore the impact of a high-fat diet on overall health and oral health.

MATERIALS AND METHODS

1. Experimental Animals

The animals were sourced from the Faculty of Medicine's animal house at Alexandria University in Egypt. In this study, 24 mature male albino rats in good health (150-200 g), who were around six months old, were used. They had free use of food and drink, good airing, and a steady light/dark cycle (12/12 h) while being kept under identical regulated laboratory circumstances of a temperature between 22 and 25 °C in specially built cages with wire mesh bottoms. The cages were cleaned twice a day due to the excessive urine associated with diabetes.

1.1 Randomization and study design

This investigation uses carefully supervised experimental animals. The required sample size for statistical tests was calculated by the Department of Biomedical Informatics and Medical Statistics at the Medical Research Institute of Alexandria University in Egypt. A representative sample size was determined using an alpha of 0.05 and 80% study power. The numbers assigned to each rat ranged from 1 to 12. The animals were split into two equal groups using a random number generator (Prism G. version 5.04, GraphPad Software Inc: San Diego, CA, USA) as follows:

Group I: (Control group, n=12) the rats were kept on typical rat food with typical fat levels (up to 20%). (2)

Group II: (High-fat diet group, n=12) rats were fed a diet including 59.8% fat, 20.1% protein, and 20.1% carbohydrates. (2)

Study setting

The present study was completed in the Faculty of Medicine at the University of Alexandria. The histological processes were carried out in the Oral

Biology department at the Faculty of Dentistry, Alexandria University, Egypt.

Percentage of fat in food: Normal rat chow contains fat up to 20% and was increased to 59.8% in each day in the high-fat diet group. (2)

2. Outcome evaluations

2.1-Overall health

Any decrease in food or water intake, changes in physical activity, changes while handling, or even hair loss were all considered clinical signs. (4)

2.2-Body weight

The body weight of each rat was measured weekly throughout the period of the experiment. (4)

2.3-Biochemical analysis

Blood was drawn from the tail veins of the sedated rats. Centrifugation was used to separate the serum (3000 rpm, 15 min) before it was frozen at -80 °C. Standard laboratory procedures were used to evaluate the amount of serum cholesterol level.

Fasting blood insulin levels were determined using rat-specific insulin ELISA (enzyme linked immunoassay) kits. An auto analyzer was used to measure the levels of serum glucose and calcium that were acquired after euthanizing the rats. (2)

2.4-Histological technique

The parotid salivary glands were fixed in 10% neutral-buffered formalin, cleaned, and dried out with increasing concentrations of ethanol, then clarified with xylene and inserted in paraffin wax blocks. A light microscope was used to view sections that were sliced at a 4 m thickness and dyed with hematoxylin and eosin. (2)

2.5- Preparations for ultra-structural specimens

The sample was fixed for 2 hours in 2.5% glutaraldehyde buffered with 0.1 mol/l PBS at pH 7.4. Samples from the parotid salivary glands were postfixed for an hour at 4°C in the same buffer with 1% osmium tetroxide. The samples were prepared and immersed in Epoxy resin in BEEM capsules at 60°C for 24 hours in the Electron Microscope Research unit at the Faculty of Science, Alexandria University. Ultrathin sections were made using Leica ultra-cut UCT (Leica, Cambridge, UK), dyed with uranyl acetate and lead citrate, and analyzed by transmission electron microscopy (Tokyo, Japan) (Alexandria, Egypt).(5)

2.6-Statistical analysis

Results gained from the blood tests, body weight, amount of food and water intake were analyzed using IBM SPSS software package version 24.0. The two groups were compared using the ANOVA test, the data was expressed as a mean, and the significance of the results was judged at the 5% level.(6)

Availability of data and materials

The published publication includes all the materials and datasets that were used in the current investigation.

Ethics approval and consent to participate.

The Animal Care and Use Research Ethics Committee of the Faculty of Dentistry at the University of Alexandria (IORG0008839-0468-06/2023) approved this animal experiment. The handling and use of the animals were done in accordance with all applicable national, institutional, and/or international laws and regulations. The current study's content follows the standards for revealing animal research, known as ARRIVE (Animal Research: Reporting in Vivo Experiments).(4)

RESULTS

1. Clinical assessment

• Hair loss

Throughout the eight weeks, a daily clinical examination of the quantity of hair covering the rats' skin was performed. In contrast to the rats in the control group, the rats in Group II had patches of exposed skin (hair loss) during the last two weeks.

• Behavioral changes

Daily clinical assessments of the rats' behavior were evaluated throughout the experiment period, and the group consuming a high-fat diet showed normal behavior throughout.

• Changes in activity

Changes in activity were recorded three times a day, for an hour each time. By counting the number of movements made, it was discovered that the high-fat diet group's activity decreased in contrast to the control group during the final three weeks of the trial.

• Amount of food intake

As shown in Fig. 1a, the mean amount of food consumed differed significantly between the high-fat diet group and the control group ($p=0.015828637$).

Amount of water intake

A statistically notable variation between the mean water intake of the high-fat diet group and the control group was shown in Fig. 1b ($p=0.024542349$).

2. Changes in body weight

The high-fat diet group's mean weight gain was statistically superior to that of the control group, as shown in Fig. 1c ($p=0.0433$).

3. Serological analysis

a) Calcium serum level

Fig. 2a demonstrated that although it was not statistically significant, the high-fat diet group's mean calcium serum level increased when compared to the control groups. $p=0.765220713$.

b) Cholesterol level

According to Fig. 2b, the high-fat diet group's serum cholesterol level was significantly increased when compared to the control group $p=0.000952729$.

c) Blood insulin level

According to Fig. 2c, the high-fat diet group's blood insulin level was significantly increased in comparison to the control group $p=1.89912E-05$.

d) Blood Glucose level

Fig. 2d demonstrated that there was no statistically significant distinction between the control group and the high-fat diet group in terms of blood glucose level $p=0.09009446$.

4. Light-microscopy outcomes

Control group

The histological pictures served as the basis for the histological analysis (Fig. 3). The parotid salivary gland's many structures were examined, and a dense connective tissue capsule that houses cells, blood vessels, and fat cells could be seen (Fig. 3a). Also, it displayed rounded serous acini with pyramidal cells surrounding them and an intercalated duct with cuboidal cells lining its narrow lumen (Fig. 3b). Secretory striated ducts lined by tall columnar cells with distinct basal striations and closed to normal blood capillary (Fig. 3c). There were typical excretory ducts situated in the interlobular septa, lined with pseudostratified columnar epithelium surrounding a normal lumen (Fig. 3d).

High-fat diet group

Histological analyses were performed using histological pictures (Fig 4). The assessment of the structure of the parotid salivary gland presented an abnormal accumulation of adipocytes in the connective tissue capsule (Fig 4a). It also showed degenerative changes in the acini and vacuolation of the cytoplasm of acinar cells and broadening of the intercalated duct. (Fig 4b). Dilated striated duct with loss of their striations as well as degeneration of the lining epithelium and fibrosis around the striated duct associated with dilated blood capillary. (Fig 4c). In addition, the evaluation showed an enormous widening of the excretory duct lined by degenerated pseudostratified epithelium and surrounded by connective tissue fibrosis with stagnated saliva inside its lumen (Fig 4d).

5. Ultrastructural results

Control

The outcomes of the transmission electron microscope served as the basis for the ultrastructural studies (Fig. 5). The assessment of the various structures and organelles in the parotid salivary gland revealed a significant quantity of well-structured rough endoplasmic reticulum in the cytoplasm of the serous cell with a typical euchromatic nucleus along with a consistent nuclear membrane (Fig. 5a). A typical intercalated duct lined by cuboidal cells with rounded central euchromatic nuclei and several electron rich secretory granules (Fig. 5b). The secretory striated duct's typical histological structure was also observed; it was lined by tall columnar cells with typical euchromatic nuclei and typical basal infoldings with longitudinally orientated mitochondria (Fig. 5c).

High-fat diet group

The results of the transmission electron microscopic analysis were used to serve the ultra-structural studies (Fig. 6). The assessment of the parotid salivary gland presented serous acini with heterochromatic nuclei, some fat droplets, vacuolations appearing in the cytoplasm, and dilated swollen rough endoplasmic reticulum. Multiple immature electron lucent secretory granules and loss of the cell junctions were also visible between the two adjacent cells (Fig. 6 a & b). Degenerated mitochondria and dilated Golgi apparatus were noted (Fig. 6b). Results also showed atypical, intercalated ducts with partially degraded epithelial lining and a wide lumen exhibiting some degraded vacuolization of the cytoplasm (Fig. 6c). In addition, disorganized and degenerated organelles were observed as dilated rough endoplasmic reticulum, Golgi apparatus, and mitochondria (Fig. 6d). The secretory striated duct illustrated severe widening of the lumen and disintegration of the epithelial lining in the form of heterochromatic nuclei, loss of the basal infoldings, degenerated mitochondria and irregular basal lamina (Fig 6 e & f).

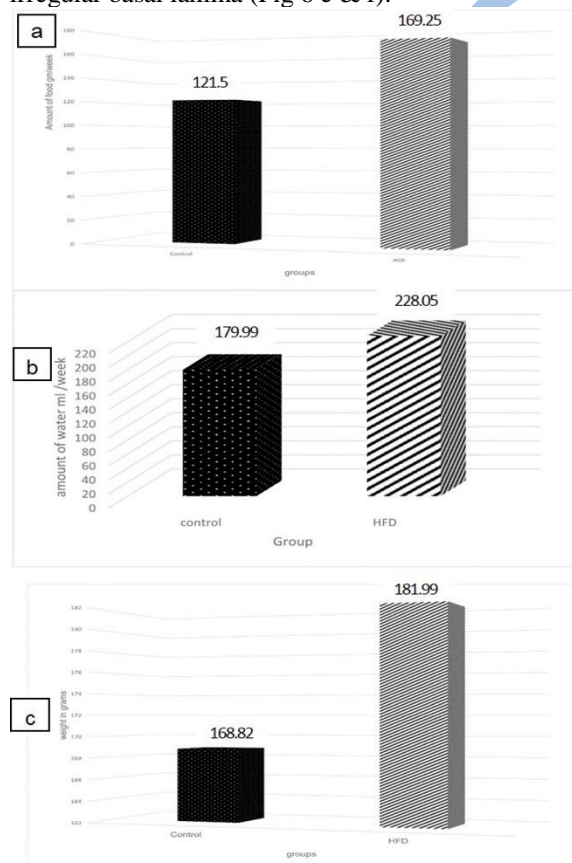


Figure 1: Graphic comparison of the two relevant groups, a high-fat diet and a control diet, demonstrates: (a) Bar graph viewing the average amount of food intake in grams. (b) Bar graph viewing the average of water intake in ml. (c) Bar graph viewing the average of body weight in grams

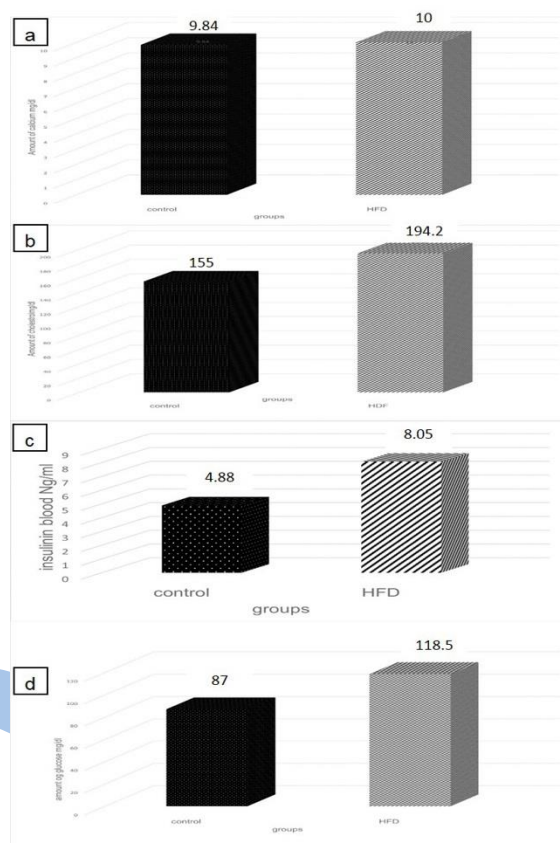


Figure 2: Graphical comparison of the two relevant groups, control and high-fat diet: (a) Bar graph concerning the average of calcium blood level in mg/dl, (b) Bar graph concerning the average of cholesterol in blood in mg/dl, (c) Bar graph concerning the average of insulin in blood Ng/ml, (d) Bar graph concerning the average of glucose in blood mg/dl

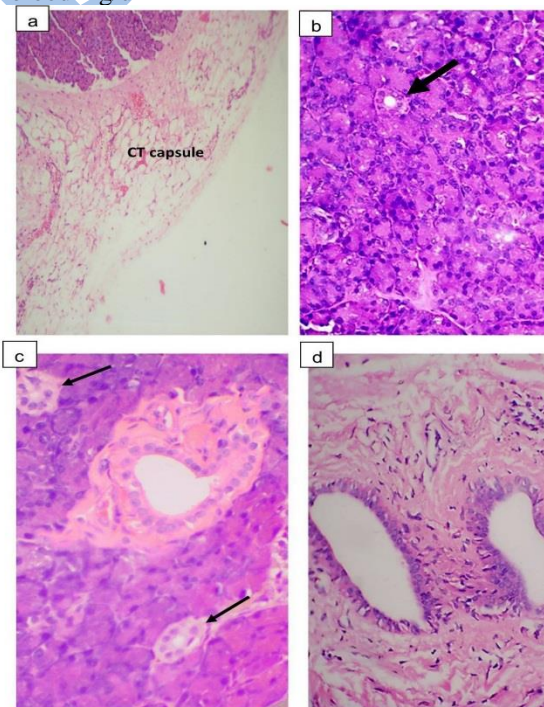


Figure 3: Light micrograph (LM) of the parotid salivary gland of the control group showing: a) A fully formed connective tissue capsule (CT capsule)

containing fat cells, blood vessels, cells, and fibers Original magnification of [H&E: 100] b) Regular serous acini and cuboidal cells lining the intercalated duct's narrow lumen (arrow) c) Normal secretory striated ducts (arrow), closed to blood capillaries, are surrounded by tall columnar cells with distinct basal striations. d) A typical excretory duct with a normal lumen is lined by pseudostratified columnar epithelium. Original magnification: 400 [H&E]

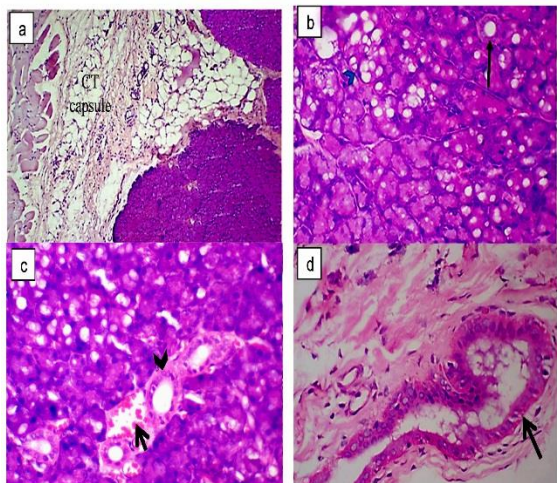


Figure 4: Light micrograph (LM) of the parotid salivary gland of the high-fat diet group showing: a) Extensive fat accumulation in the connective tissue capsule (CT capsule) with numerous inflammatory cells [H&E: original magnification $\times 100$] b) Dramatic vacuolation of the cytoplasm of acinar cells and widening of the intercalated duct (arrow) c) Marked widening in the lumen of the striated duct with loss of their basal striations and degeneration of the lining epithelium (arrowhead), dilation of a blood vessel filled with RBCs (arrow) d) Abnormal widening of the excretory duct (arrow) lined by vacuolated pseudostratified epithelium and encircled by fibrosis of the connective tissue; Note, stagnated saliva can be also seen [H&E: original magnification $\times 400$]

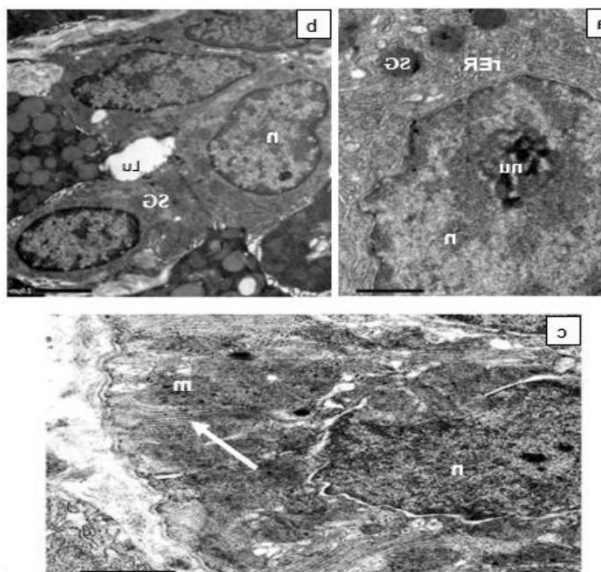


Figure 5: Transmission electron micrograph (T.E.M) of parotid gland of the control group: a) Normal euchromatic nucleus (n) of serous cell with regular nuclear membrane and prominent nucleolus (nu) containing a substantial amounts of well-structured rough endoplasmic reticulum (rER), and a few spherical electron dense secretory granules(SG) (8000). b) A normal intercalated duct with a narrow lumen (Lu) and cuboidal cells with rounded euchromatic nuclei (n) and sparse, electron-dense secretory granules (SG) near the apex $\times 3000$. c) Part of the striated duct showing centrally located euchromatic nucleus (n) with typical normal basal folding (arrow) and longitudinal oriented mitochondria (m) $\times 6000$

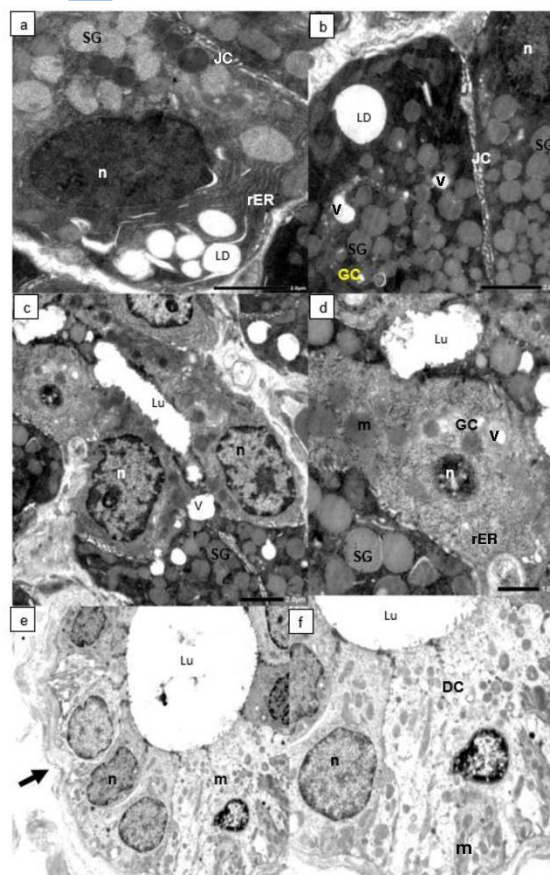


Figure 6: Transmission electron micrograph (T.E.M) of parotid gland of the high-fat diet group illustrating: a) Heterochromatic nucleus(n) and dilated rough endoplasmic reticulum (rER) of a serous cell , there are some lipid droplets (LD). Note multiple immature electro-lucent secretory granules (SG) with slight separation between the two adjacent cells (JC) $\times 4000$. b) Part of serous cell with immature secretory granule (SG) and a lipid droplet (LD) with some vacuolations (v), and dilated Golgi complex (GC)with slight separation between adjacent cells (JC). $\times 4000$. c) Wide lumen (Lu)and a largely deteriorated epithelial lining in an intercalated duct. Deteriorated organelles, apoptotic nucleus (n), and some vacuolization of the cytoplasm (v) $\times 2500$. d) High magnification of the

previous inset of (c) showing apoptotic nucleus (n), dilated rough endoplasmic reticulum (rER) and Golgi complex (GC), and deteriorated mitochondria (m) with vacuolations in the cytoplasm (v) $\times 5000$. e) Secretory striated duct with loss of basal infoldings, irregular disintegrated mitochondria, dissolution in the cytoplasm and some heterochromatic nucleus (n), with irregular basal lamina (arrow) and wide lumen (Lu) (original magnification $\times 1500$). f) View of the preceding inset micrograph at high magnification (Fig. 6e), showing, apoptotic nucleus (n) and dissolved cytoplasm (DC). Note damaged disorganized mitochondria (m) (original magnification $\times 2500$)

DISCUSSION

The Obesity Medicine Association states that "obesity is described as a multifactorial, neurobehavioral disorder that is chronic, progressive, relapsing, and curable, wherein an increase in body fat results in abnormal fat mass and adipose tissue dysfunction, which have a negative impact on metabolic, biomechanical, and psychosocial health effects" (8).

One of the most important causes of obesity is a hypercaloric diet caused by an excess of fats. High-fat diets cause adipocyte accumulation and enlargement. Adipocytes that have reached hypertrophy emit substances including TNF, alpha and insulin-like growth factors, which cause the adipocytes to proliferate (9).

Moreover, the accumulation of adipocyte cells triggers the proinflammatory cytokines, reactive oxidative stresses, and insulin resistance. These previously mentioned factors can all lead to a weakening in general body health, as well as defects in the structure and functions of salivary glands (4-6).

In addition, the production of saliva is the salivary glands' primary function. The salivary glands are crucial for maintaining oral hygiene, but they are also linked to other metabolic disorders, such as the beginning of type II diabetes brought on by insulin resistance brought on by a high-fat diet (10).

In this study, rats were chosen because they are widely available and inexpensive to maintain. Moreover, they have a relatively adequate lifespan and well-characterized salivary glands. Male rats were used to prevent any hormonal changes that might have an impact on the results (11). Rats could become obese and have a rise in the oxidative stressors that cause insulin resistance in just eight weeks (known as the prediabetic stage) (2). The parotid salivary gland in this study was chosen because their antioxidant system is less able to combine ROS than the submandibular glands' antioxidant system, which is why they appear to be more affected by the destruction caused by high fat diet (12).

The present work revealed that the body weights of the animal groups fed a high-fat diet were

significantly greater than that of the control group. This suggests that diets rich in fat are accountable for the rise in adiposity. Failure to adjust the rate of fat oxidation to the excess fat in the diet contributes to adiposity (9).

The current study illustrated that there was a reduction in the activity of the high-fat diet group in the last three weeks. This is regarded as a main sign of metabolic syndrome, which also includes high blood pressure, central obesity, pro-inflammatory and pro-thrombotic states, and oxidative stress (13). Moreover, there was a good amount of hair loss in the high-fat diet group in the last two weeks. A high-fat diet could contribute to an abnormal cycle by introducing inflammatory molecules into the body. These molecules cause oxidative stress that block the regeneration of hair follicles (14).

Furthermore, the high-fat diet groups presented a rise in the amount of water intake, as they reached the prediabetic stage- when the kidneys must work extra hard to filter and absorb the extra glucose this stage causes an increase in water consumption. Also, there was an increase in food intake, which was explained by Luvuno et al (15) that when the body does not absorb enough blood sugar due to insulin resistance, this leads to a decrease in glucose uptake by the cell, thus increasing the feeling of hunger (15).

In other respects, the collection of blood samples from the rats were done before euthanasia to analyze the changes in blood serum, where the high-fat diet group showed a non-significant increase in the serum calcium level. This was in agreement with another study which stated that rats fed a fat-rich diet had larger exposed body surface area as they were obese, thus it makes sense that they would create more vitamin D as it enhances the absorption of calcium from blood (16).

Additionally, in the current study, there was a considerable rise in serum cholesterol levels, which is consistent with recent research showing that rats fed a high-fat diet for 30 days experienced a large rise in blood cholesterol levels (17). Furthermore, in this study, the high-fat diet group had a significant elevation in blood insulin level as the impact of diet high in fat on oxidative stress may be one of the causes that develop IR. This was in agreement with Zalewska A et al (12). Likewise, this study shown an increase in blood glucose levels in group II, although not significantly. This was in coincidence with other researchers who explained that while the amount of increase in blood glucose level does not cause diabetes, it does cause prediabetes, since diets high in fat lead to insulin resistance within 8 weeks (2).

The existing light microscopic results revealed the presence of multiple adipocyte cells in the connective tissue capsule, which is because a high-fat diet leads to adipocyte accumulations (18).

The increase in the intracellular vacuoles inside the serous acini was due to their removal during sample fixation and processing, these vacuoles' appearance may be lipid in nature (2). Another explanation for

the intracellular vacuoles is that oxidative stresses cause the inhibition of insulin signaling. This may lead to insulin resistance, which is the main purpose of acinar cell degeneration (12).

The current histological results revealed marked changes in the ductal system such as dilatation, disintegrated epithelial lining, and retained secretion. These results are similar to the findings of another study which explained that this may be due to glandular malfunction as lipid buildup promotes gland malfunction (5). Moreover, there was a congested blood capillary associated with the striated duct. This might be due to the fact that a high-fat diet leads to inflammatory cell infiltration and congestion of blood vessels, which might be a component of an inflammatory reaction to increase blood flow to fibrotic or degenerating regions (5).

The present findings showed an increase of fibers around the intralobular and interlobular ducts, which is because a high-fat diet led to hypercholesterolemia, which encourages the extracellular matrix's synthesis and breakdown. The activation of fibroblasts to enhance collagen fiber synthesis may be a reaction of the fibroblast stimulating factor. Another theory is that oxidation byproducts like lipid peroxidation compounds can increase collagen production and expression (19).

Since consuming too much fat affects both the lipid profile in plasma and the deposition of fat tissue, the ultrastructural results of this study revealed deteriorated acini and ducts with many vacuoles and fat droplets in its parenchymal cells.

Most of the acinar cells in this investigation exhibited secretory granules that were electron lucent. By lipid peroxidation, the ROS of oxidative stress can change and harm cell membranes made of lipids, inhibiting protein synthesis, and making granules more brittle (5).

Furthermore, the ultrastructural results showed degeneration in most of the cellular organelles, as high-fat diets cause excessive ROS production, which destroys all the important cellular structures, for example DNA found in the nucleus, as well as lipids and proteins which form the outer membrane of all the organelles. In addition, ROS disrupts cellular membranes, causing the striated ducts' basal striation to disappear. These findings all mean the degeneration in both ductal and acinar cells (2).

As a high-fat diet causes cellular apoptosis, there was a presence of a dark apoptotic nucleus in the ductal and acinar cells, with heterochromatin occupying most of the nucleus. This indicates a cell with low metabolic activity. Moreover, the rough endoplasmic reticulum, Golgi apparatus, and multiple vacuolations all showed disorganization and dilation (5). Additionally, both ductal and acinar cells had varying degrees of mitochondrial damage. Alterations to the internal membrane's structure could lead to the destruction of mitochondrial crests due to oxidative stresses, which induce alterations in

the mitochondrial DNA, harm the mitochondrial respiratory chain, and change the permeability of the membrane. As the mitochondria membrane ruptures, it releases apoptotic factors, leading to cellular apoptosis (20).

Moreover, there was a slight separation between the acinar cells. Several ROSs, including hypochlorous acid, nitric oxide, hydrogen peroxide, and peroxynitrite, lead to a disruption in the epithelial tight junctions between the cells (21).

CONCLUSION

According to the study's findings, a high-fat diet may be significantly linked to structural changes in the parotid gland, including severe fibrosis, numerous dilated blood vessels with bleeding, and intracellular vacuolization with lipid droplets. The impact of fat accumulation on glandular function, which may have an impact on oral health, must be investigated further.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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