Effect of High Fat Diet on the structure of Pancreas rat and the ameliorative effect of Vitamin D

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Abstract
Background: Diet can cause changes in the pancreas' structure, which have been linked to a variety of metabolic dysfunctions. Many of the metabolic issues associated with high-fat diets (HFD).
Objectives: The study was designed to investigate the effect of HFD on the pancreas and possible impact of Vitamin D (Vit D).
Materials and methods: Four groups of albino rats were included in this study. Negative control group (Group I) received standard chow (SC, 10% fat). Positive control group (Group II) received Vit D3 at a daily oral dose of 10 µg/kg. HFD-treated group (Group III) received high-fat chow. The HFD+ Vit D treated group (Group Ⅳ) received high-fat chow and Vitamin D3. The rats were sacrificed, and the pancreas was removed and prepared for light, ultrastructural, and immunohistochemical examination.
Results: HFD causes an increase in the mean body and pancreas weights, many vacuolations in the islet of Langerhans and pancreatic acini, dilated congested blood vessels, inflammatory cellular infiltration, fatty infiltration, acidophilic exudate, damaged acinar cells and ß cells of islets of Langerhans as well as strong immuno-expression of caspase-3 and TNF-α. Vit D can partially attenuate these findings.
Conclusion: HFD results in significant histopathological changes in the pancreas although Vit D could attenuate these toxic effects through modulation of pancreatic caspase-3 and TNF- α immuno-expression.
Keywords: Pancreas; High fat diet; Vitamin D; Caspase-3; TNF-α.
DOI: 10.21608/svuijm.2023.228257.1644
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Received: 1 August, 2023.
Revised: 19 August, 2023.
Accepted: 20 August, 2023.
Published: 25 Septembre, 2023
Cite this article as: Omnia I. Ismail (2023). Effect of High Fat Diet on the structure of Pancreas rat and the ameliorative effect of Vitamin D. *SVU-International Journal of Medical Sciences*. Vol.6, Issue 2, pp: 736-752.

Introduction

Food with little nutritional value and a high calorie, fat (HFD), and sugar content is being consumed at an increasing rate. Overall physical health and life expectancy have significantly declined as a result of decreased physical activity. Many of the metabolic issues associated with obesity are the result of both previous mentioned causes. Obesity is a major global health issue, and its effects have not yet been fully understood **(Asghar et al.,2021).**

The complex pathophysiological condition of obesity is defined by an excessive deposition of adipose tissue **(Sumińska et al., 2022).** Up until 2015, obesity and/or being overweight afflicted more than one-third of the world's population (**Hruby & Hu, 2015).**

Obesity impairs several organs' and tissues' metabolic processes, increasing the risk of numerous illnesses like cardiovascular conditions, diabetes mellitus (DM), osteopenia and various cancers **(Burki, 2021; Wang et al., 2021 and Zhang., 2022).**

Due to increased consumption of a high-fat diet, saturated fatty acid levels increased. Since a result, the organs' overall structure and physiology are affected as the extra fat tends to concentrate inside of and around the organs. Due to adipocyte hypertrophy and hyperplasia, fatty diets also have a significant impact on the dysfunctional lipid metabolism that leads to an inflammatory environment **(Osborn and Olefsky, 2012).**

In this regard, the immune response's cellular mediators are drawn to the adipose tissue, where they produce pro-inflammatory cytokines to trigger a series of inflammatory reactions **(Qatanani and Lazar, 2007)**.

By boosting blood flow to metabolically active tissues, several hormones, in particular insulin and catecholamines, cooperate to control blood flow into adipocytes (**Laclaustra et al., 2007).**

Pancreas is an exocrine and endocrine organ. Acinar cells, which secrete pancreatic fluids that contain different digestive enzymes and additionally the endocrine cells that release pancreatic hormones perform these functions **(Theodory et al., 2022).**

Consequently, compared to all other tissues, pancreatic cells are exposed to oxidative stress at higher levels and more liable to damage **(Singh et al., 2022).**

Vitamin D (Vit D) involves several metabolites with several functions other than the calcium homeostasis and bone mineralization **(Herrmann, 2023).** The biologically active metabolite 1,25-dihydroxyvitamin D (also known as 25(OH)2D) which is obtained from the diet or from the supplements and formed in the kidneys after being hydroxylated in the liver to 25-hydroxyvitamin D **(Płudowski et al., 2023).** It has been demonstrated that Vit D deficiency is associated with pancreatitis, inflammatory bowel disease, DM (both type 1 and 2), metabolic syndromes, cardiovascular disease, hypertension, multiple sclerosis, psoriasis, and different kinds of cancer **(Holick, 2017 and Cai et al., 2022).**

Additionally, due to Vit D's biochemical activity in the endocrine system, besides its possible anti-inflammatory and immune-modulating capabilities, there is rising interest in Vitamin D's possible impact in the prevention and management of DM and obesity. Moreover, pancreatic cancer, and other pancreatic diseases appear to be influenced by Vit D via its immune-modulatory action **(Sadek and Shaheen, 2013).** Thus, we hypothesized that Vit D may protect against HFD-induced pancreatic damage.

Notwithstanding, there are still ma ny aspects that are not fully understood. The fact that an increase in dietary fat intake is the cause of a rise in adiposity is supported by much research on people conducted in many nations. Animals as

well as humans get obese when they consume a diet high in fat. It remains unclear whether the anti-diabetic effects of Vit D might be associated with prevention of HFD-induced pancreatic damage. In the present study, we will focus on the effect of HFD and co-treatment of Vit D on the cytoarchitecture of pancreas.

Materials and Methods

Chemicals: Dry Vitamin D3 was purchased from Sigma Pharmaceutical Company, Egypt. The item included 104,473.8 IU (2.6 mg) of Vitamin D3 per gramme of powder.

Ethical statement: The current work was approved by Ethical Committee, Faculty of Medicine, Assiut University (Approval number: 17300854). The National Institutes of Health's guide for the care and use of laboratory animals is followed in all investigations (NIH Publications No. 8023, revised 1978) **(Care, 1985).** According to the ARRIVE Guidelines for reporting in vivo research, the current study was conducted **(McGrath et al., 2010).** Throughout the study period, every attempt was taken to minimize suffering of the rats.

Animals: Forty adult male albino rats weighing between 200-220 g were obtained from the Animal House, Faculty of Medicine, Assiut University. The rats were kept in a controlled environment with a 12 hours light/12 hours dark cycle and temperatures between 22º and 24 Cº. Rats were divided equally into 4 groups in random way. Negative control group (Group I) received standard chow (SC, 10% fat) for 8 weeks.

Positive control group (Group II) received Vit D3 at a daily oral dose10 µg/kg for 8 weeks. According to the Institute of Medicine (USA), a chosen current vi D3 dose is equivalent to the tolerated maximum daily limit of 4000 IU (100 g)/day in a 60 kg adult **(Prietl et al., 2013)**. Daily, fresh aqueous Vit D3 dispersions in distilled water were made in amber-coloured glass tanks at a concentration of 2.6 µg Vit D3 (1 mg powder)/ml.

HFD treated group (Group III) received high-fat chow (HFC, 60% fat) for 8 weeks **(Gomaa and Abd El-Aziz, 2017).** Department of Animal Nutrition and Clinical Nutrition, Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt created both types of diets (basic and HFD).

The HFD+ Vit D treated group (Group Ⅳ) received high-fat chow and Vitamin D3 at the same dose for 8 weeks.

The experiment conducted through 8 weeks then the rats were weighted and scarified under urethane anaesthesia (1.2 g/kg body weight I.P.). The pancreas was extracted, weighed, and rinsed with normal saline. Ten percent formalin was used to preserve pancreatic tails.

For light microscope : To examine general pancreatic anatomy, 4-6 µm pancreatic sections were cut and stained with Haematoxylin and Eosin (H&E) after they had been embedded in paraffin blocks **(Bancroft and Gamble, 2008).**

Transmission electron microscopy (TEM): Following immediate scarification of the animals, small pieces of the pancreatic tails were removed, fixed in 5% cold glutaraldehyde for 24 hours. Washed in three to four changes of cacodylate buffer (pH 7.2) was done for twenty minutes in each change. After that, postfixing in cold osmium tetraoxide for 2 hours was performed. Epon 812 and gelatin capsules were used for the embedding process to polymerize. The embedded samples were stored in an incubator at 35°C for one day, 45°C for one day, then 60°C for the final three days. The ultramicrotome was used to create semithin sections (0.5–1 µm) then stained via Toluidine blue and finally were inspected under a light microscope. On copper grids, ultrathin sections (50–80 nm) from selected regions of the trimmed blocks were created. Uranyl acetate and lead citrate were utilized to contrast the ultrathin sections for 10 and 5 minutes,

respectively. Examination was done and the findings were photographed by a transmission electron microscope (TEM, JEOL 100 CX, Japan) at the Electron Microscopy Unit, Assiut University, Assiut, Egypt **(Hyman& Jarvis, 2011).**

Immunohistochemistry (IHC): The coronal sections with a thickness of 3 microns were prepared for IHC methods. Caspase-3 and tumor necrosis factor alpha (TNF-α) were tagged using a modified avidin-biotin peroxidase method in order to display apoptosis and inflammatory indicators receptively. It was done to deparaffinize and hydrate the sections.

Sections were treated with citrate buffer for about 10 minutes to make the antigen visible. For 30 minutes, the slices were exposed to 0.3 percent H2O2 to inhibit endogenous peroxidase activity. The next step involved blocking using horse serum. Following that, different slides were incubated with the two primary antibodies; anti-caspase-3 solution (dilution 1:150, Catalog# RB-1197-B Thermo Fisher Scientific, CA, USA) and anti-TNF-α solution (dilution 1:200; Catalog# Z02918, GenScript, New Jersey, USA) for 20 hours at 4°C. After washing, a second incubation with biotinylated secondary antibodies was carried out. Then, the avidin-biotin complex was incubated. Following exposure to 0.05 percent diaminobenzidine, the sections were ultimately stained with haematoxylin, dried, and mounted **(Elbandrawy et al.,2022).**

Morphometric and statistical analysis: For morphometric investigations of pancreatic islet morphology, three successive H&E-stained sections of the pancreas were examined at a magnification ×100 to estimate the cross-sectional areas of 10 non-overlapping islets as described by **(Ayuob and ElBeshbeishy, 2016).** Caspase-3 and TNF-α area percent in the immune-stained sections of all groups were calculated through the usage of the cell counter plugin in Image J software (version 1.52, Public Domain). The current data were presented as mean ± standard deviation (SD). To compare the animal groups, an ANOVA test was employed. When the p-value was less than 0.05, the difference between the groups was considered significant.

Results

Effect of HFD and Concomitant administration of Vit D on the body and Pancreas weights

HFD treated rats showed significantly higher body and pancreas weights compared to the control rats. The HFD plus Vit D treated group had lower body and pancreas weights compared to the HFD treated group (**Table. 1**).

Table 1. Comparison of the body weight (g), and the pancreas weight (mg) in all studied groups.

Parameters / Groups	The body weight (g)	The pancreas weight (mg)
Group I	244.1± 2.9	132.3± 3.3
Group II	240.5± 4.6	130.1± 1.8
Group III	259.3±5.3 [a, b]	158.5± 4.3[a, b]
Group IV	252.6±8.4 [a, b]	150.4± 4.1 [a, b,c]
P-value	< 0.0001*	< 0.0001*

Data are represented as Mean ± SD.* means statistically significant difference.
a statistically significant as compared with the group I, P < 0.05. b statistically significant as compared with the group II, P < 0.05. c statistically significant as compared with the group III, P < 0.05.

Effect of HFD and Concomitant administration of Vit D on Pancreas architecture

Examining the pancreatic slices from the positive and negative control groups revealed that their histological structures were nearly the same normal structure. As a result, both control groups were represented by the findings mentioned under "Control Group".

Evaluation of H&E-stained pancreatic sections of the control rats displayed normal histological findings; the islets of Langerhans appeared as isolated endocrine cell clusters within the pancreatic lobules surrounding by the pancreatic acini and unstained connective tissue septa separated the pancreatic lobules (**Fig.1a**). The HFD treated group showed many vacuolations in the islets of Langerhans and the pancreatic acini, acidophilic exudate, dilated congested blood vessels, inflammatory cellular infiltration, and fatty infiltration (**Figs.1b, c, d**). The HFD+ Vit D treated group showed mild improvement but the vacuolations in the islets of Langerhans and the pancreatic acini as well as the presence of acidophilic exudate were still seen (**Fig. 1 e**).

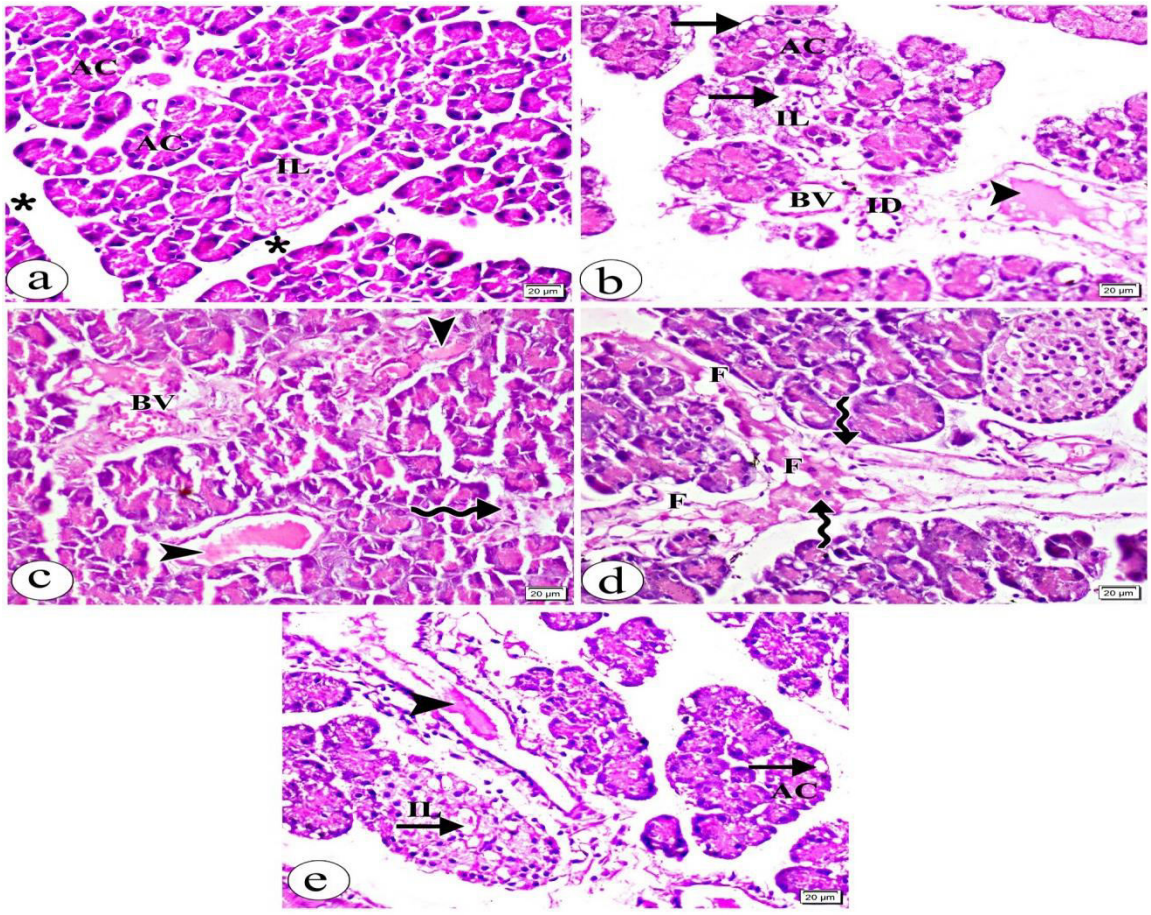

Fig.1. The photomicrographs of the pancreatic tissue sections in (a) The control group showing an islet of Langerhans (IL) surrounded by pancreatic acini (AC). Note unstained connective tissue septa (asterisk) separated pancreatic lobules and intralobular duct (ID). **(b) The HFD treated group** showing many vacuolations (arrow) in the islet of Langerhans (IL) and pancreatic acini (AC). Acidophilic exudate is observed (arrowhead). Note intralobular duct (ID) and blood vessel (BV). **(c) The HFD treated group** showing dilated congested blood vessels (BV), inflammatory cellular infiltration (curved arrow) and acidophilic exudate (arrowhead)**. (d) The HFD treated group** showing inflammatory cellular infiltration (curved arrow) and fatty infiltration (F). **(e) The HFD+ Vit D treated group** showing vacuolations (arrow) in the islet of Langerhans (IL) and pancreatic acini (AC). Note the presence of acidophilic exudate (arrowhead). **(H&E, x 400, Scale bar = 20 µm).**

Regarding the ultrastructure of the pyramidal acinar cells in the control group displayed rounded basal nuclei with prominent nucleoli, many cisternae of rough endoplasmic reticulum (rER), and many mitochondria. In the apical cytoplasm, many electron-dense secretory granules were present (**Fig.2 a**). In the HFD treated group, the pyramidal acinar cells had shrunken deeply stained basal nuclei, dilated rER, destructed mitochondria and many vacuoles. In the apical cytoplasm, few electron-dense secretory granules with different sizes were noticed (**Fig.2 b**). The HFD+ Vit D treated group revealed mild improvement of the pancreatic structure. The pyramidal acinar cells had basal nuclei with prominent nucleolus, destructed mitochondria and some rER still appeared dilated. In the apical cytoplasm, many electron-dense secretory granules were noticed (**Fig.2 c**).

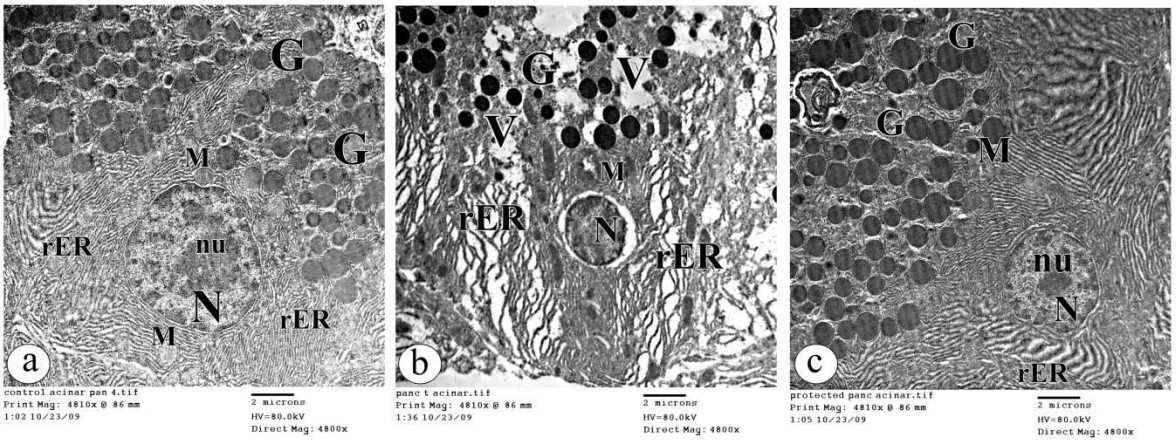

Fig. 2. The electron micrographs of the pyramidal acinar cells in (a) The control group showing rounded basal nucleus (N) with prominent nucleolus (nu), many cisternae of rough endoplasmic reticulum (rER), and many mitochondria (M). In the apical cytoplasm, many electron-dense secretory granules are present (G). **(b) The HFD treated group** showing shrunken deeply stained basal nucleus (N), dilated rough endoplasmic reticulum (rER), destructed mitochondria (M) and vacuoles (V). In the apical cytoplasm, few electron-dense secretory granules (G) with different sizes are noticed. **(c) The HFD + Vit D treated group** showing basal nucleus (N) with prominent nucleolus (nu), destructed mitochondria (M) and some rough endoplasmic reticulum (rER) still appear dilated. In the apical cytoplasm, many electron-dense secretory granules (G) are noticed. **(TEM, X 4800, Scale bar = 2 µm).**

The ß cells of islets of Langerhans in the control group appeared with rounded basal nuclei, prominent nucleoli, many cisternae of rER, and mitochondria. In the apical cytoplasm, several secretory granules having wide halos and dense cores were present (**Fig.3a**).

On the contrary, the HFD treated group had the ß cells of islets of Langerhans with shrunken nuclei, dilated rER, huge vacuoles, and few secretory granules. Some cells had pyknotic dense nuclei and destructed rER (**Figs.3 b & c**). In HFD+ Vit D treated group, the ß cells of islets of Langerhans showed dense nuclei, many secretory granules, few vacuoles, and rarified cytoplasm (**Fig.3 d**).

Effect of HFD and Concomitant administration of Vit D on the immuno-expression for apoptosis and inflammation in the Pancreatic tissue

Examination of the IHC-stained sections in the control group revealed mild Caspase (**Fig.4a**) and mild TNF-α (**Fig. 5 a**) immuno-expression. On the other hand, strong Caspase (**Fig.4b**) and strong TNF-α (**Fig 5 b**) immuno-expression following HFD administration were observed. The HFD+ Vit D treated group showed moderate Caspase (**Fig.4c**) and moderate TNF-α (**Fig 5 c**) immuno-expression.

Comparing the groups' mean values for the area percent of caspase 3 and TNF-α expression revealed statistically significant variations (**Table. 2**).

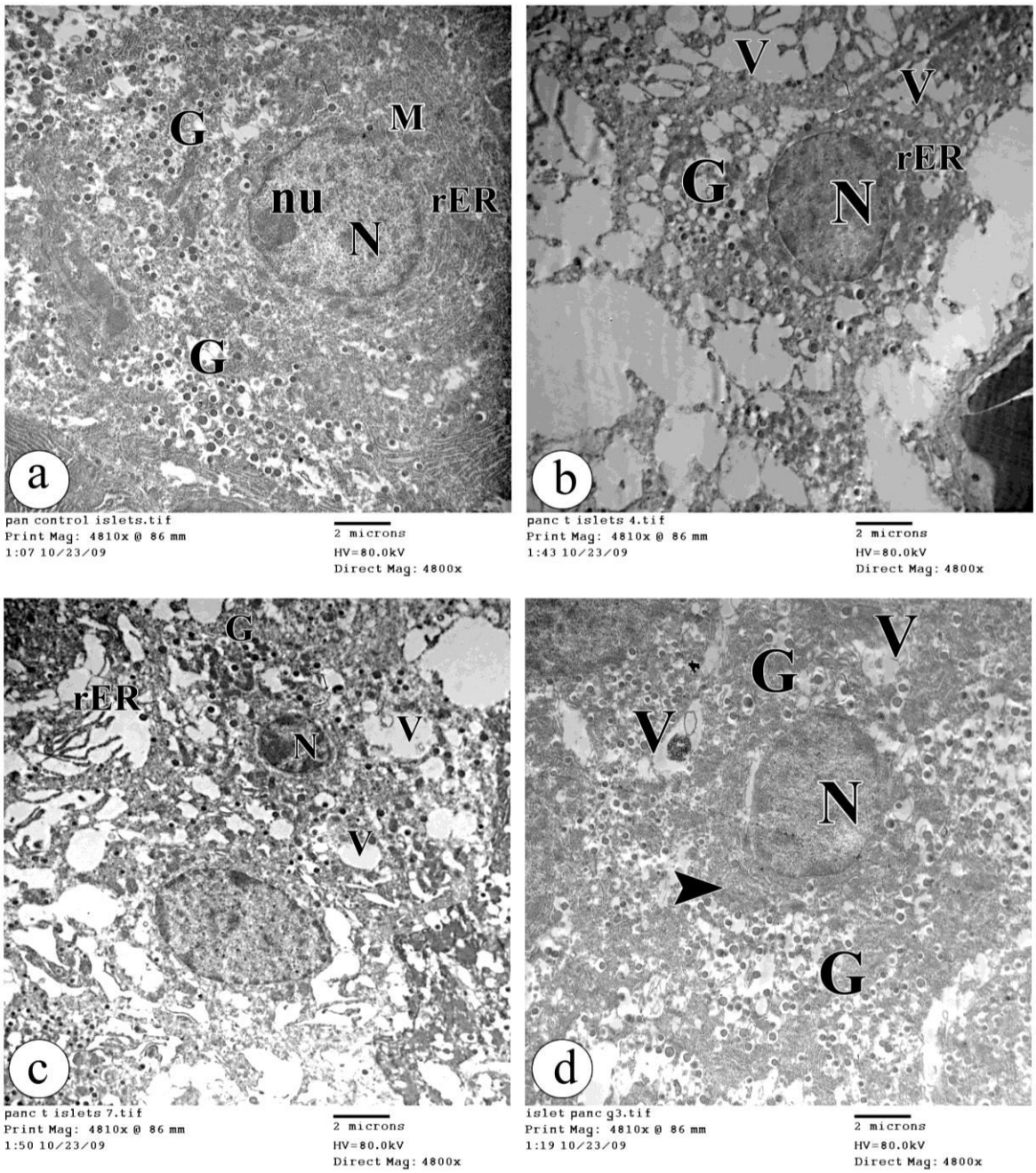

Fig. 3. The electron micrographs of the ß cells of islets of Langerhans in (a) The control group showing a rounded basal nucleus (N) with prominent nucleolus (nu), many cisternae of rough endoplasmic reticulum (rER), and mitochondria (M). In the apical cytoplasm, several secretory granules (G) having wide halos and dense cores are present. **(b) The HFD treated group** showing a shrunken nucleus (N), dilated rough endoplasmic reticulum (rER), huge vacuoles (V) and few secretory granules (G). **(c) The HFD treated group** showing a pyknotic dense nucleus (N), destructed rough endoplasmic reticulum (rER), huge vacuoles (V) and few secretory granules (G). **(d) The HFD+ Vit D treated group** showing a dense nucleus (N), many secretory granules (G) and few vacuoles (V). Rarified cytoplasm (arrowhead) is observed. **(TEM, X 4800, Scale bar = 2 µm)**

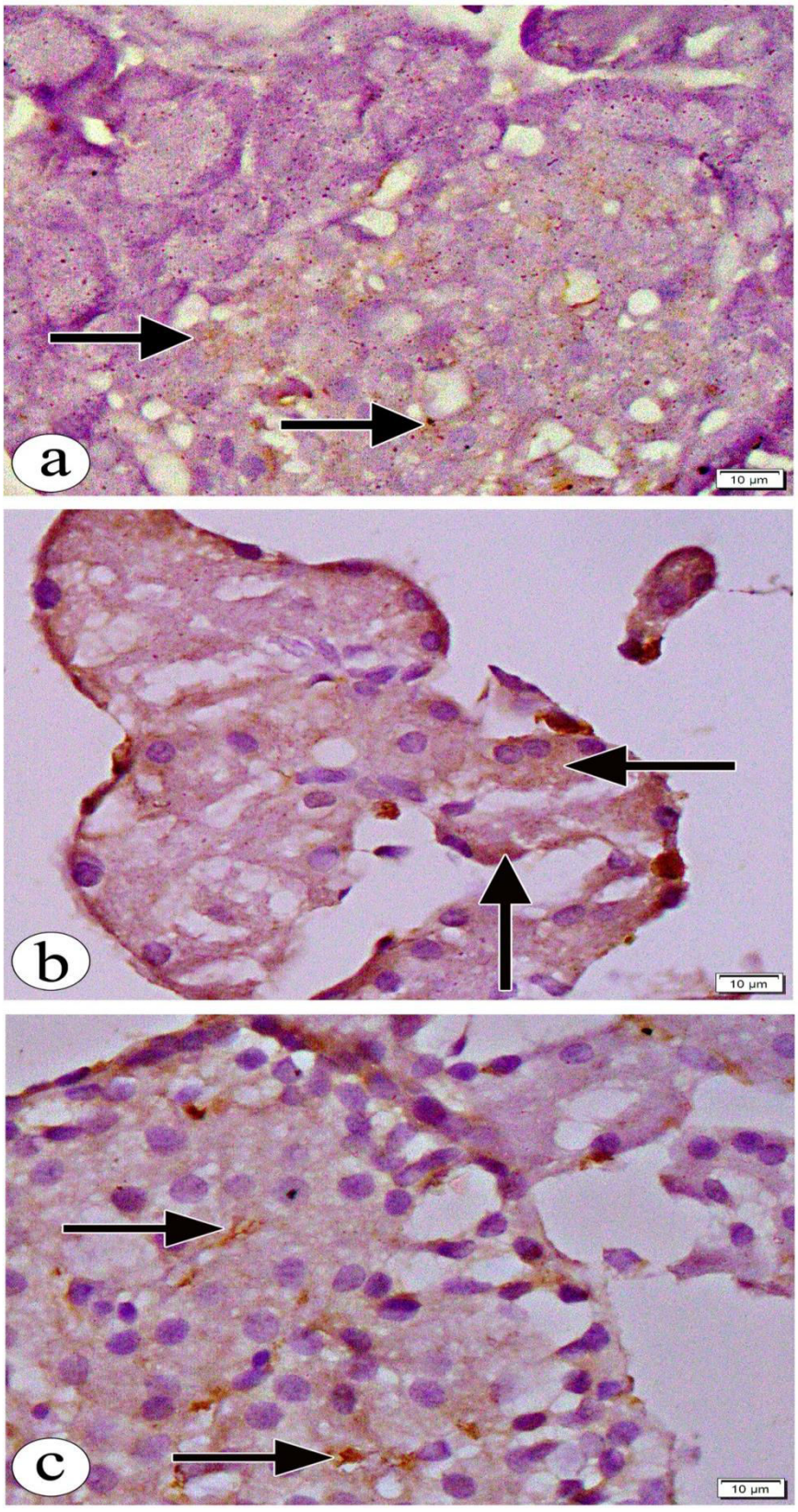

Fig. 4. The photomicrographs of the pancreatic tissue sections in (a) The control group showing a mild Caspase immuno-expression (arrow). **(b) The HFD treated group** showing a strong Caspase immuno-expression (arrow). **(c) The HFD+ Vit D treated group** showing a moderate Caspase immuno-expression (arrow). **(Caspase x 1000, Scale bar = 10 µm)**

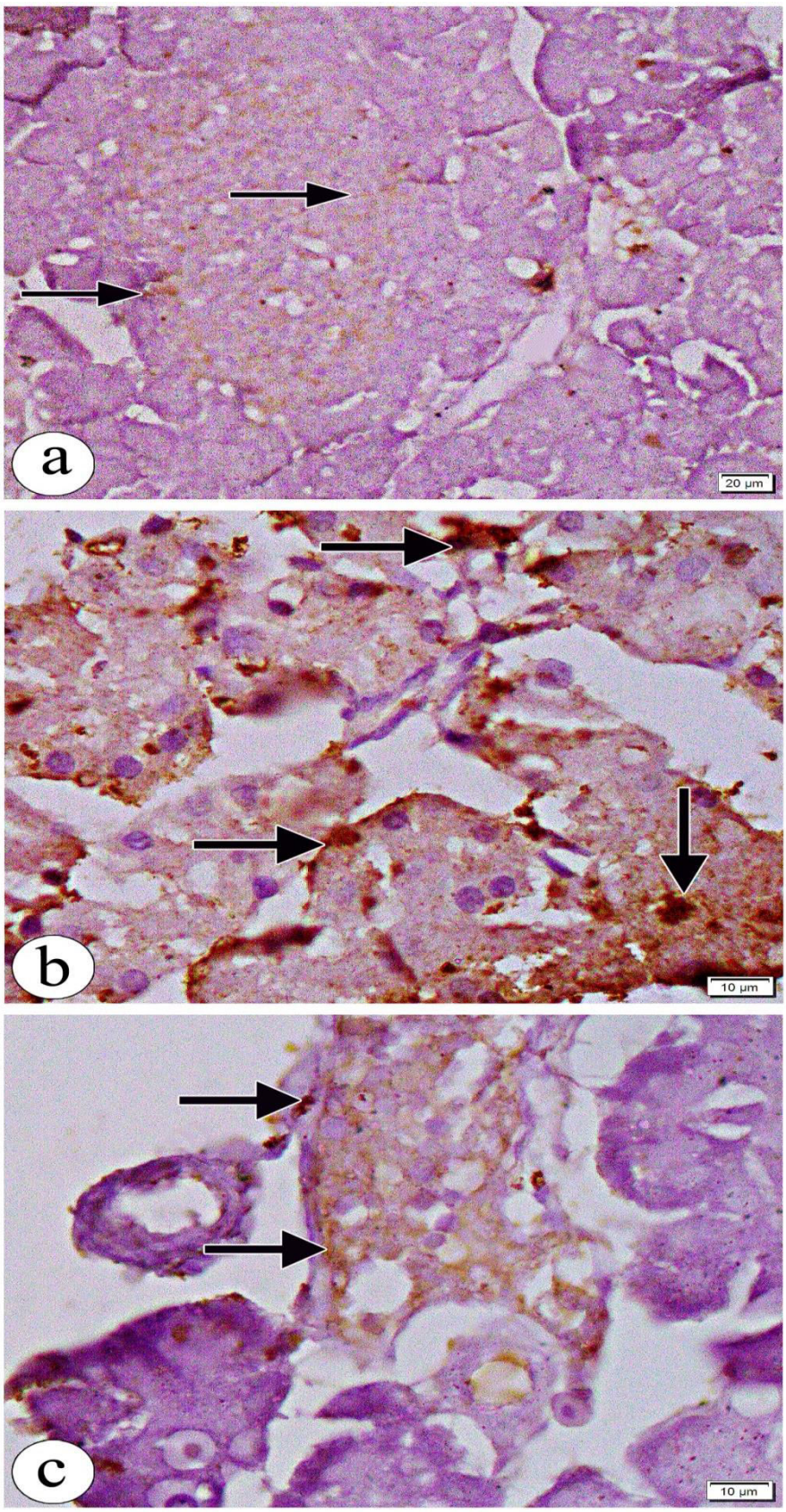

Fig. 5. The photomicrographs of the pancreatic tissue sections in (a) The control group showing a mild TNF-α immuno-expression (arrow). **(b) The HFD treated group** showing a strong TNF-α immuno-expression (arrow). **(c)The HFD+ Vit D treated group** showing a moderate TNF-α immuno-expression (arrow). **(TNF-α x 1000, Scale bar = 10 µm)**

Effect of HFD and Concomitant administration of Vit D on the cross-sectional area of the islets of Langerhans

The morphometric and statistical study demonstrated that the mean cross-sectional area of the islets of Langerhans insignificant decreased in HFD treated rats compared to the control rats. In the HFD+ Vit. D treated group, the mean cross-sectional area of the islets increased compared to HFD treated rats but also there was no statistical significance difference (**Table. 2**).

Table 2. Comparison of the area percent (%) of Caspase expression, the area % of TNF-α expression and the cross-sectional area of the islets (μm^2) in all studied groups.

Parameters / Groups	The area % of Caspase expression	The area % of TNF-α expression	The cross-sectional area of the islets(μm^2)
Group I	4.69 ± 0.63	4.06± 0.77	18013.69± 408696
Group II	4.41± 0.87	3.95± 0.49	20270.84± 948278
Group III	11.37± 0.92 $^{a, b}$	19.34± 0.81 $^{a, b}$	13407.93± 732141
Group IV	6.96±0.93 $^{a, b,c}$	7.98± 0.95 $^{a, b,c}$	14148.12± 121106
P-value	< 0.0001*	< 0.0001*	0.41 ^{ns}

Data are represented as Mean ± SD.* means statistically significant difference ns means no significance difference. a statistically significant as compared with the group I, P < 0.05. b statistically significant as compared with the group II, P < 0.05. c statistically significant as compared with the group III, P < 0.05

Discussion

Obesity is associated with many important complications such as DM, metabolic syndrome **(Roy et al., 2022)**, coronary heart disease, diseases of the gallbladder, liver, and spleen **(Pi-Sunyer, 2002).**

Non-communicable diseases (NCDs), such as DM, cardiovascular diseases, and cancer account for more than 70% of preventable deaths globally and remain one of the major challenges of this century. A significant risk factor for NCDs is obesity (**Kassa and Grace, 2019).**

Commonly, mice on a HFD are employed as models for obesity **(Zhang et al., 2022).** Additionally, it was documented that HFD led to lower cognitive capacity in both humans and animals **(Martinellirt et al., 2022),** ovarian follicular development damage **(Paula et al., 2022),** the breakdown of the intestinal barrier and enhanced cell proliferation that occurs early during the development of colorectal cancer **(Yang et al., 2022).**

In the current study, we aimed to evaluate the effects of HFD and co-treatment Vit D on the cytoarchitecture of pancreas. Our findings showed that HFD for 8 weeks led to an increase in the mean body and pancreas weight, many vacuolations in islet of Langerhans and pancreatic acini, dilated congested blood vessels, inflammatory cellular infiltration, fatty infiltration and acidophilic exudate, damaged acinar cells and ß cells of islets of Langerhans, nuclear pyknosis, depletion of secretory granules, loss of cytoplasmic organelles as well as strong immuno-expression of caspase-3 and TNF-α. Vit D can partially attenuate these findings.

In this work, it was observed an increase in the body weight and pancreas weight. This supports the results of **Roy et al. (2022) and Ge et al. (2023).** This increase in the body weight might be linked to increased circulating free fatty

acids, serum leptin and increased adipocyte size in both subcutaneous and visceral adipose tissue **(Torre-Villalvazo et al., 2018).**

The present increase in the weight of pancreas in HFD treated rats might be linked to increased levels of circulating free fatty acids encouraging lipid build-up in some organs like the liver, skeletal muscle, and pancreatic islets **(Unger, 2003).**

Our findings were in accordance with the results of **Chung et al. (2018)** who found that both the endocrine and exocrine systems were damaged in both diffuse and focal locations in rats received HFD. In addition, several rats' pancreatic islets were completely absent, and some islets had fewer cells than normal. Similarly, a previous work detected a noticeable decrease in islet cell mass as well as islet and acinar lesions **(Rasheed et al., 2022).** Also, our study confirmed the result of **Wang et al. (2023)** who found that HFD result in histopathological alterations in the pancreas, and increased levels of TNF-α.

The present findings via electron microscopic examination support the previous work which concluded that nuclear pyknosis and vacuolization, together with fatty infiltration and structural changes in the islets of Langerhans, are the signs of HFD's harmful impacts on pancreatic β-cells **(Barrientos et al., 2021).** The degenerative changes observed in β-cells under the electron microscopic examination support the results of **Chansela et al. (2022)** who found that the insulin-positive area in medium and large islets was dramatically reduced after exposure to HFD in comparison to the control group.

It was suggested rER stress plays a critical role in pancreatic damage with HFD. The rER is the organelle in charge of secretory and membrane-associated protein synthesis, folding, and trafficking. The rER is directly engaged in lipid droplet production and lipid homeostasis maintenance **(Stevenson et al., 2016).** The rER stress is linked to lipolysis and adiponectin hyposecretion. Professional secretory cells with many ER, such as the hepatocytes, and pancreatic beta-cells, have a high capacity for protein synthesis **(Marciniak et al., 2022).**

This work found with HFD, inflammatory cellular and fatty infiltrations invaded the pancreatic lobules and deformed many acini as well as a significant increase in TNF-α expression. We suggested HFD induced inflammation and fat necrosis that might have a role in pancreatic islets damage and induction of DM. This is in the same line with a previous work reported that due to severe immune cell infiltration and noticeable acinar cell necrosis, HFD increased the severity of acute pancreatitis **(Hong at al., 2020).** Moreover, excessive pancreatic lipid accumulation in Human is defined as non-alcoholic fatty pancreas disease that might progress to non-alcoholic steato-pancreatitis, and this process can be managed with weight loss as well as prevention of HFD consumption **(Chen et al., 2022).**

On the other hand, no inflammatory cellular infiltration, and a significant decrease in TNF-α expression found with Vit D treatment. Therefore, it was suggested Vit D supplementation can suppress inflammatory status induced by HFD in the pancreatic tissue. This agrees with the previous researchers found that both diabetic rats and rats having pancreatitis treated with Vit D showed a decrease in cortisol, proinflammatory cytokines (tumour necrosis factor-α, interleukin (IL)-2, IL-6), C-reactive protein levels and lipid peroxidation enzymes like malondialdehyde in the pancreatic tissue. This carried out via expression of vitamin D receptor and downregulation of toll-like receptor **(Abou Saleh et al., 2020; Cai et al., 2022 and Fathi et al., 2022).**

TNF-α is one of the inflammatory indicators and proinflammatory cytokine

generated primarily by the immune cells (**El-Masry et al., 2020).** Furthermore, TNF-α led to upregulation of other cytokines, generation of free radical species, and cellular death **(El-Ashmawy et al., 2018).** It was suggested that HFD induced generation of free radical species and inflammation.

The current data were in accordance with early findings of **Elbandrawy et al. (2022)** who reported that TNF- and IL-6 levels have been shown to rise in obese rodents and those that exhibit insulin resistance. By preventing insulin-induced tyrosine phosphorylation on the insulin receptor substrate-1 -chain and insulin receptor, TNF- α is a significant contributor to DM type II and insulin resistance. Moreover, the inflammatory activity can contribute to the incidence of insulin resistance, which exacerbates DM, and consequently it has a negative impact on the activity of pancreatic beta cells **(Elbandrawy et al., 2022).**

The IHC study additionally demonstrated HFD elicited caspase-3 positive expression and apoptosis. This is supported by an early study concluded that HFD induced oxidative stress through caspase-3 signal leading to the activation of cell death mechanism and appearance of insulin resistance (**Chansela et al., 2022).** It is generally recognised that apoptosis is a form of programmed cell death that contributes to organ dysfunction syndromes and Caspase-3 protein is essential to the apoptosis process **(Ismail et al., 2022**). Moreover, observed damaged mitochondria and rER in the current work may be linked to cytochrome C liberation, oxidative phosphorylation, and caspase-9 activation which in turn trigger the apoptosis.

Our morphometric findings were in accordance with a previous study showed that HFD led to a decrease in the number of pancreatic islets and the number of β cells per mm^2, as well as loss of the large islets **(Rodriguez-Hernandez et al., 2023).**On the other hand, other authors mentioned that obesity is consistently accompanied by an increase in the number of islets, their hyperplasia, hypertrophy, and an associated rise in insulin levels **(Butler et al., 2003)**

Collectively, the current pancreatic damage in HFD rats might be related to variable reasons. First of all, reactive oxygen species (ROS) production by hypertrophied adipocytes is increased in the obese state that led to dysregulated cytokine and adipokine secretion. ROS have been viewed as a catalyst for necroptosis **(Hong et al., 2020).** Leptin, interleukin 6, and tumour necrosis factor are among the adipokines and cytokines whose release is increased. In contrast, adiponectin, an insulin-sensitizing adipokine, is secreted at lower levels **(Devericks et al., 2022).** Second, increased levels of free fatty acids in the bloodstream induced pancreatic islets' lipotoxicity which in turn led to tissue damage **(Torre-Villalvazo et al., 2018).** Also, elevated plasma fatty acids can influence glucose metabolism and insulin levels by elevation of insulin secretion much more under basal conditions **(Barrientos et al., 2021).** Third, because the adipose tissue sequesters the fat-soluble Vitamin D3, obesity has been associated with Vitamin D3 deficiency **(Patriota et al., 2022).** Moreover, previous authors claimed that the degree of insulin resistance and the level of β-cell dysfunction are correlated with the pancreatic fat deposition. Additionally, increased pancreatic fat concentration may be a precursor to β-cell dysfunction and later development of metabolic syndrome **(Chansela et al., 2022).**

In this study, Vit D can decrease body and pancreas weights, improve the ultrastructure of the pancreatic tissue, as well as reduce inflammation and apoptosis conditions. Our results are consistent with former study revealed that Vit D can normalise cell function, enhance islet morphology and attenuate signs of

apoptosis in the pancreas of Zucker diabetic fatty rats via nuclear factor-κB and divalent metal transporter 1 (NF-κB-DMT1) signalling **(Zhao et al., 2022).**

In addition, Vit D may contribute to the prevention of type 1 DM because of its immune-modulating properties as well as through reduction the expression of CatG and prevention CD4+ T cell activation. Vit D may have an impact on the cell function, insulin sensitivity, and systemic inflammation that are all hallmarks of type 2 diabetes **(Zhang et al., 2021).**

Moreover, the findings from observational studies connected Vit D deficiency to a rise in the prevalence of both type 1 and type 2 DM. Also, prospective studies on the incidence of cancer pancreas typically suggest higher 25-hydroxy Vit D concentrations and inverse associations between UVB dose or exposure and incidence and/or mortality rate of pancreatic cancer **(Wimalawansa, 2018).**

Regarding the ultrastructural findings, an observed improvement in the structure of cells was detected in HFD +Vit D treated rats. This might be explained by the fact that Vit D has an ability to improve pancreatic β-cell function and thereby significantly increased levels of insulin as well as enhanced glucose tolerance in diabetic mice according to **Lai et al. (2022).**

In addition, **Fathi et al. (2022)** documented that the activity of the antioxidant system in particular superoxide dismutase, glutathione, catalase, and total antioxidant capacity have all been shown to be elevated in significant manner with Vit D supplementation.

Conclusion

Taken together, the results of the present study revealed that HFD results in significant histopathological changes in the pancreas although Vit D could attenuate the toxic effects of HFD through modulation of pancreatic caspase-3 and TNF- α immuno-expression. The study adds to our understanding of the pancreas's histopathological response to HFD and offers further proof of Vit D's therapeutic benefits in this condition.

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