Original Article

Effect of Juvenile Obesity on the Islets of Langerhans in Rat and the Possibility of Recovery at Adulthood: A Histological and Immunohistochemical Study

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ABSTRACT

Background: Juvenile obesity is a serious problem that increases the risk of future development of cardiovascular disease and type 2 diabetes at adulthood through programing a metabolic dysfunction during development.

Aim of the Work: to perform a biochemical, histological and immunohistochemical evaluation of the effect of juvenile obesity on the morphology, insulin expression, apoptosis and proliferation of the islets of Langerhans in albino rat and to evaluate the possibility of recovery at adulthood.

Material and Methods: Twenty-one juvenile male albino rats were equally divided into 3 groups; control (fed 11% fat standard diet), high fat diet-induced obesity group (fed 45% fat diet for 8 weeks) and recovery group (fed 45% fat diet for 8 weeks then left for 4 weeks on standard diet). Animals were weighed and fasting blood glucose and serum insulin were quantified. Pancreatic specimens were processed for histological and immunohistochemical staining for detection of insulin, Bcl2 and Ki67.

Results: Juvenile obesity group recorded a significant increase in total body weight and blood glucose coupling with a significant decrease in serum insulin. Histological examination revealed few shrunken islets of Langerhans with cells expressing nuclear alterations and cytoplasmic vacuolation. Peripheral mononuclear infiltration and dilated congested blood capillaries were observed. A significant decrease in the immunohistochemical expression of insulin, Bcl2 and Ki67 was recorded. While a significant improvement of all studied parameters was detected in the recovery group.

Conclusion: Restoration of a normal diet in high fat diet-induced obesity in juvenile rats reinstated the abnormal glucose and insulin levels, ameliorated the altered morphology of islets of Langerhans, and upregulated the suppressed immunoexpression of insulin, Bcl2 and Ki67 in the islets at adulthood.

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Key Words: Bcl2; insulin; islets of langerhans; juvenile obesity; Ki67.

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INTRODUCTION

The overwhelming prevalence of obesity worldwide is one of the greatest challenges for medical research to manage. Obesity is usually associated with metabolic alterations termed as metabolic syndrome[1,2]. Since no age or gender is immune to obesity, it can certainly affect children and adolescents, where juvenile obesity has become a terrifying condition that associates with several diseases[3].

Juvenile obesity epidemic was recorded to be over 124 million children and adolescents, where one in every five children and adolescents was considered overweight or obese[4]. Obese children and adolescents are particularly vulnerable to develop several obesity-related cardiovascular complications such as high blood pressure, high cholesterol, and type 2 diabetes. Previous studies strongly suggested that early life weight gain may impact weight later in life[5,6].

Many studies documented that adult obesity and metabolic dysfunction might have been programmed during early phases of life[7,8]. Adolescence is a crucial station in the way for programming of metabolic dysfunction, thus any changes in the environment during development, its result will appear as a disease during adult life, which proves the importance of adequate nutrition during juvenility[9,10,11].

Juvenile obesity was found to be not only responsible for the development of several complications later in adulthood, yet it was also incriminated in provoking a drop in immunological tolerance, which has a pivotal link with the aggravation of pre-existing illnesses like atopic dermatitis[12].

High fat diet (HFD) is a diet obtaining most of its calories from fat instead of carbohydrate or protein. Juvenile obesity model in rodents using HFD has been successfully used for the study of obesity in young age[13], where metabolic changes during consumption of HFD eventually lead to glucose intolerance and development of type 2 diabetes[14,15]. Moreover, HFD-induced obesity is commonly associated with hypertriglyceridemia, thus increasing the chance of development of secondary hypertriglyceridemic pancreatitis[16].

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Taken together, this study was aimed to perform a biochemical, histological and immunohistochemical assessment of the effect of juvenile obesity on the morphology, insulin expression, apoptosis and proliferation of the islets of Langerhans in rat, and to evaluate the possibility of recovery at adulthood.

MATERIAL AND METHODS

Twenty-one juvenile male Wistar albino rats aged 4 weeks weighing 50-70 grams each were used in this study. The rats were kept in clean properly ventilated cages under standard housing conditions of temperature and humidity. The experiment was approved by the Research Ethics Committee of Tanta Faculty of Medicine, Egypt.

The animals were randomly divided into three equal groups

Group I (Control group): Animals of this group were fed a standard laboratory diet (11% fat) (Table 1) for 8 weeks.

Group II (HFD-induced obesity group): Animals of this group were fed a high fat content diet (45% fat) (Table 1) for 8 weeks to induce obesity[14].

Group III (Recovery group): Animals of this group were fed a high fat content diet (45% fat) for 8 weeks and were then left for another 4 weeks on a standard laboratory diet.

Table 1: Compositions and energy contents of standard diet and 45% fat diet

Calories	Standard lab diet	High fat diet
Calories from protein	24	20
Calories from carbohydrates	65	35
Calories from fat	11	45
Total calories (kcal/kg)	2844	4405

At the end of the experiment, the animals were fasted overnight, weighed and blood samples were collected directly from the heart under light anesthesia. The serum was obtained for storage at -20°C for biochemical assay. Animals were euthanized using pentobarbital (40 mg/kg)[17]. The pancreas was rapidly dissected out to be processed for light microscopy.

Biochemical assay

Fasting blood glucose was assessed using commercial diagnostic kit and expressed in (mg/dl). Fasting serum insulin was analyzed using commercial enzyme immunoassay kit and expressed in (uIU/ml)[14].

Light microscopy examination

Pancreas specimens were immersed in 10% neutral-buffered formalin, washed, dehydrated, cleared and embedded in paraffin. Sections of 5µm thickness were stained with hematoxylin and eosin (H&E)[18].

Immunohistochemical staining

Sections of 5µm thickness were deparaffinized, rehydrated and washed with phosphate buffered saline (PBS) then incubated with 10% normal goat serum in PBS. Sections were incubated in a humid chamber overnight at 4°C with the primary antibodies; rabbit polyclonal antibodies against insulin (as a marker for pancreatic beta cells), Bcl2 (B-cell lymphoma 2; as an antiapoptotic marker) and Ki67 (as a proliferation maker) [ab63820, ab59348 and ab15580 respectively, Abcam, Cambridge, Massachusetts, USA]. The sections were then incubated with biotinylated goat anti-rabbit IgG for 60 min at room temperature then with a streptavidin-biotin-horseradish peroxidase complex for another 60 min. The immunoreactivity was visualized using 3,3′-diaminobenzidine (DAB) hydrogen peroxide chromogen. The sections were counterstained with Mayer's hematoxylin[19].

Morphometric analysis

A Leica light microscope (DM500, Switzerland) coupled to a Leica digital camera (ICC50, Switzerland) was used for image acquisition. The software "ImageJ" (version 1.48v National Institute of Health, Bethesda, Maryland, USA) was used for image analysis. Ten different non-overlapping randomly selected fields from each slide were examined at a magnification of 400 to quantitatively evaluate:

1. The mean number, diameter and area of islets of Langerhans (in H&E-stained sections).
2. The mean color intensity of insulin positive immunoreaction in islet cells (in DAB-stained sections).
3. The mean color intensity of Bcl2 positive immunoreaction in islet cells (in DAB-stained sections).
4. The mean percentage (%) of Ki67 immunopositive islets cells (proliferation index) (in DAB-stained sections).

Statistical analysis

The data were analyzed by using one-way analysis of variance (ANOVA) followed by Tukey's test for comparison between the groups using statistical package for social sciences statistical analysis software (IBM SPSS Statistics for Windows, IBM Corp, Version 22.0. Armonk, NY, USA). Differences were regarded as significant if probability value $p < 0.05$[20].

RESULTS

Mean total body weight

Mean total body weight of rats from group II (83.81±4.21) showed a significant increase compared to the control group (67.43±5.77), whereas, group III (79.66±5.28) revealed a significant decrease compared to group II, yet this represented a non-significant difference from the control group (Table 2).

Biochemical assay

Mean fasting blood glucose in group II (144.07±18.88)

showed a significant increase compared to the control group (86.33±11.09), whereas, group III (94.89±13.94) revealed a significant decrease compared to group II, which represented a non-significant difference from the control group (Table 2).

On the other hand, serum insulin in group II (1.622±0.08) revealed a significant decrease compared to the control group (2.99±0.01), while group III (2.61±0.74) revealed a significant increase compared to group II, which was considered a non-significant difference from the control group (Table 2).

Histological findings

H&E staining

Control group I

H&E stained pancreatic sections from the control group revealed the normal histological architecture of the islets of Langerhans, where they appeared as large defined pale stained areas scattered in between the darkly stained exocrine glandular tissue (Figure 1). The islets were composed of cords of secretory cells separated by blood capillaries, where two types of cells could be recognized; central beta (β) cells occupying most of the islet core with large rounded nuclei, and peripheral alpha (α) cells with smaller nuclei (Figure 2).

HFD-induced obesity group II

Sections from group II revealed few, shrunken and vaguely identified pancreatic islets. The islets showed small-sized cells with pyknotic nuclei, other cells revealed nuclear alterations in the form of large dark nuclei or karyolytic ones. Some cells depicted markedly vacuolated cytoplasm. A mild peripheral mononuclear infiltration and dilated congested blood capillaries were observed (Figures 3, 4).

Recovery group III

Sections from group III showed apparently normal-sized pancreatic islets, yet few small-sized islets were observed. Some cells had moderately vacuolated cytoplasm. Moderately dilated blood capillaries were observed (Figures 5, 6).

Morphometrical analysis of mean number, diameter and area of islets of Langerhans of group II (147.95±11.48, 146.93±9.07, 0.098±0.09 respectively) revealed a significant decrease compared to the control group (253.98±24.69, 178.97±15.06, 0.123±0.06 respectively). Whereas, all three parameters from group III (210.98±16.59, 169.62±11.21, 0.101±0.04 respectively) recorded a significant increase compared to group II, whereas this represented a non-significant difference from the control group (Table 2, Histogram 1).

Immunohistochemical staining for detection of insulin

Immunohistochemically stained sections from the control group revealed a strong positive cytoplasmic immunoreactivity for insulin in the form a brownish coloration in most cells of the pancreatic islets mainly in the central part (beta cells), while cells located in the periphery showed no immunoreactivity (alpha cells) (Figure 7). On the other hand, sections of HFD-induced obesity group II showed a weak positive immunoreactivity in a small number of central cells (beta cells) with no immunoreactivity detected peripherally (alpha cells) (Figure 8). Yet, sections from recovery group III depicted a moderate positive cytoplasmic immunoreactivity in most central cells of the pancreatic islets (beta cells), while peripheral cells expressed no immunoreactivity (alpha cells) (Figure 9).

Morphometrical analysis of the mean color intensity of insulin immunoreaction of group II (29.18±4.48) showed a significant decrease compared to the control group (38.19±2.83), whereas, group III (36.47±5.23) revealed a significant increase compared to group II, however, this represented a non-significant difference from the control group (Table 2, Histogram 1).

Immunohistochemical staining against Bcl2

Immunohistochemically stained sections from the control group revealed a strong cytoplasmic immunoreactivity for Bcl2 in the islet cells (Figure 10). Whereas, HFD-induced obesity group II showed a weak cytoplasmic immunoreactivity in the islet cells (Figure 11). On the other hand, sections from recovery group III depicted a moderate cytoplasmic immunoreactivity in the islet cells (Figure 12).

Morphometrical analysis of the mean color intensity of Bcl2 immunoreaction of group II (11.94±0.47) showed a significant decrease compared to the control group (17.95±1.47), whereas, group III (15.58±2.99) revealed a significant increase compared to group II, whereas, this recorded a non-significant difference from the control group (Table 2, Histogram 1).

Immunohistochemical staining for detection of Ki67

Immunohistochemically stained sections from the control group revealed many islet cells with a positive nuclear immunoreactivity for Ki67 (Figure 13), while sections from HFD-induced obesity group II showed few islet cells with a positive nuclear immunoreactivity (Figure 14). Whereas sections from group III depicted some islet cells with a positive nuclear immunoreactivity (Figure 15).

Morphometrical analysis of the mean percentage of Ki67 immunopositive cells of group II (2.37±0.18) showed a significant decrease compared to the control group (4.06±0.29), whereas, group III (3.61±0.91) revealed a significant increase compared to group II, however, this represented a non-significant difference from the control group (Table 2, Histogram 1).

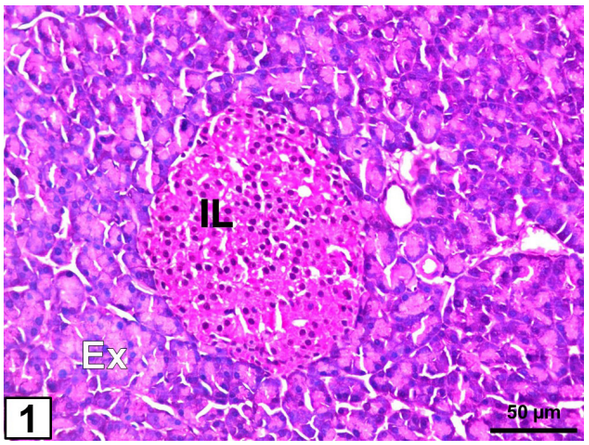


Fig. 1: A photomicrograph of the rat pancreas from the control group I shows the islets of Langerhans (IL) appearing as large defined pale stained areas scattered in between the darkly stained exocrine glandular tissue (EX). (H&E x400, scale bar=50 µm)

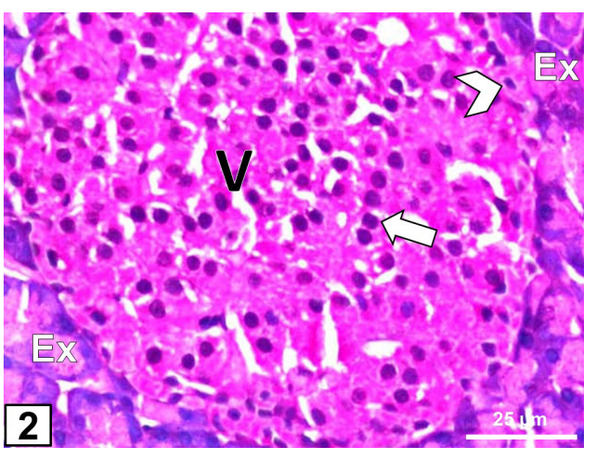


Fig. 2: A photomicrograph of the rat pancreas from the control group I shows the islets composed of cords of secretory cells separated by blood capillaries (V). Two types of cells are recognized; central beta (β) cells with large rounded nuclei (thick arrow) and peripheral alpha (α) cells with smaller nuclei (arrowhead). (H&E x1000, scale bar=25 µm)

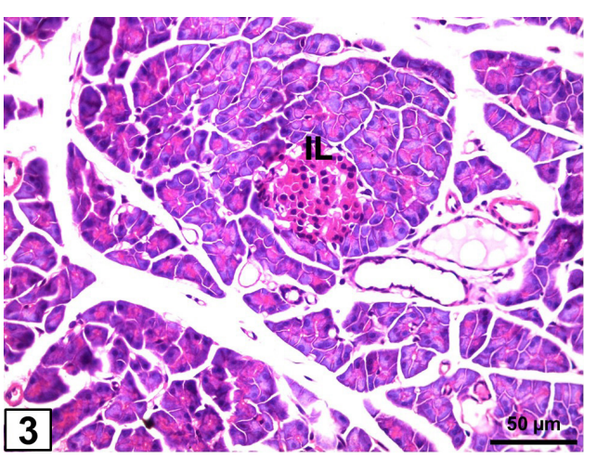


Fig. 3: A photomicrograph of the rat pancreas from the HFD-induced obesity group II shows few, shrunken and vaguely identified pancreatic islets (IL). (H&E x400, scale bar=50 µm)

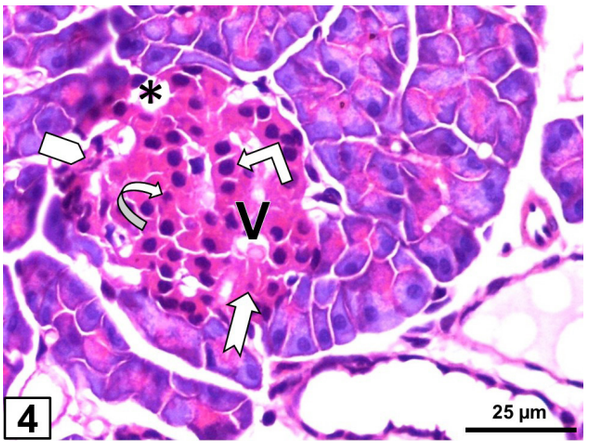


Fig. 4: A photomicrograph of the rat pancreas from the HFD-induced obesity group II shows small-sized cells with pyknotic nuclei (curved arrow), some cells with large dark nuclei (angular arrow), and other cells with karyolytic nuclei (notched arrow). Some cells reveal markedly vacuolated cytoplasm (asterisk). Mild peripheral mononuclear infiltration (bullet arrow) and dilated congested blood capillaries (V) are observed. (H&E x1000, scale bar=25 µm)

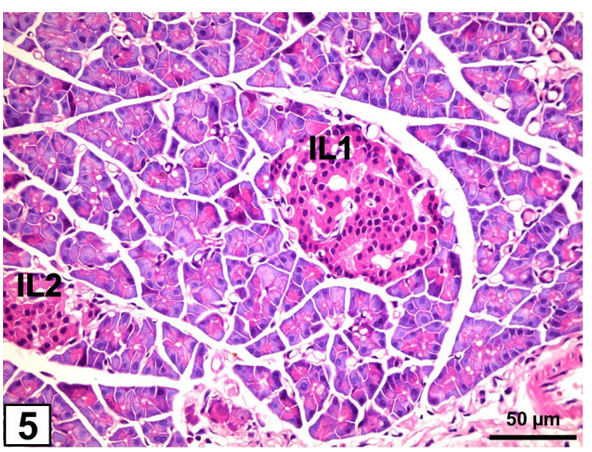


Fig. 5: A photomicrograph of the rat pancreas from the recovery group III shows apparently normal-sized pancreatic islets (IL1), but few small-sized islets (IL2) are observed. (H&E x400, scale bar=50 µm)

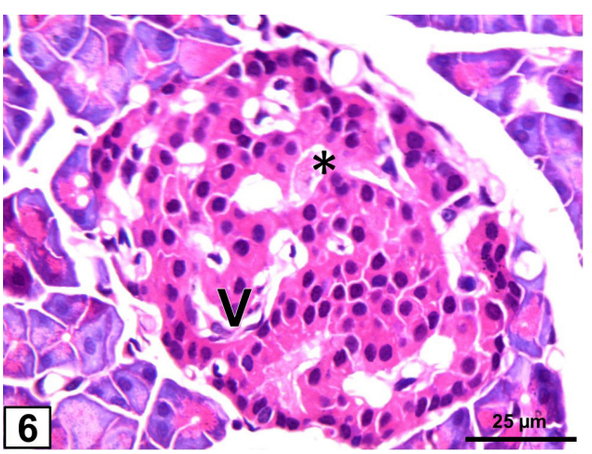


Fig. 6: A photomicrograph of the rat pancreas from the recovery group III shows apparently normal islets where some cells have moderately vacuolated cytoplasm (asterisk). Notice dilated blood capillaries (V) in between the cells. (H&E x1000, scale bar=25 µm)

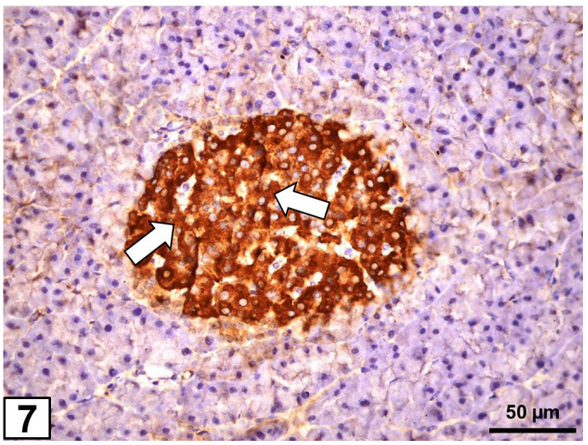


Fig. 7: A photomicrograph of the rat pancreas from the control group I shows a strong cytoplasmic immunoreactivity for insulin in most of the cells of the pancreatic islets mainly in the central part (thick arrows). (Insulin x400, scale bar=50 µm)

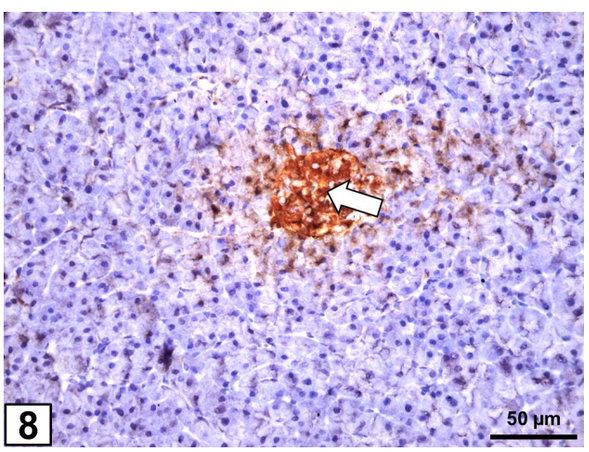


Fig. 8: A photomicrograph of the rat pancreas from the HFD-induced obesity group II shows a weak immunoreactivity for insulin in a small number of central cells (thick arrow). (Insulin x400, scale bar=50 µm)

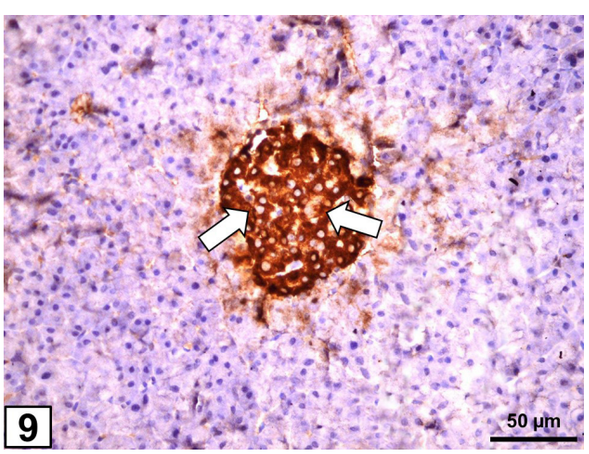


Fig. 9: A photomicrograph of the rat pancreas from the recovery group III shows a moderate cytoplasmic immunoreactivity for insulin in most of the central cells of the pancreatic islets (thick arrows). (Insulin x400, scale bar=50 µm)

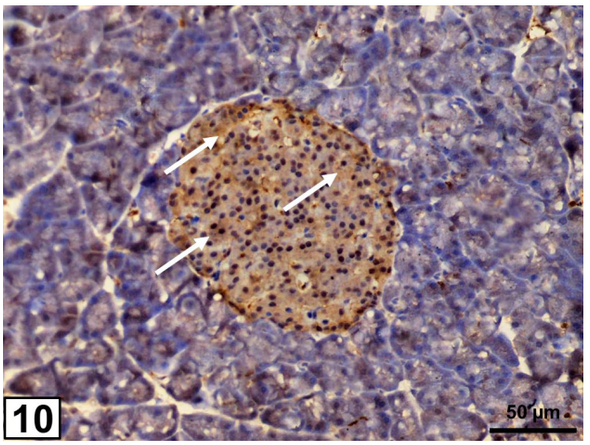


Fig. 10: A photomicrograph of the rat pancreas from the control group I shows a strong cytoplasmic immunoreactivity for Bcl2 in the islet cells (arrows). (Bcl2 x400, scale bar=50 µm)

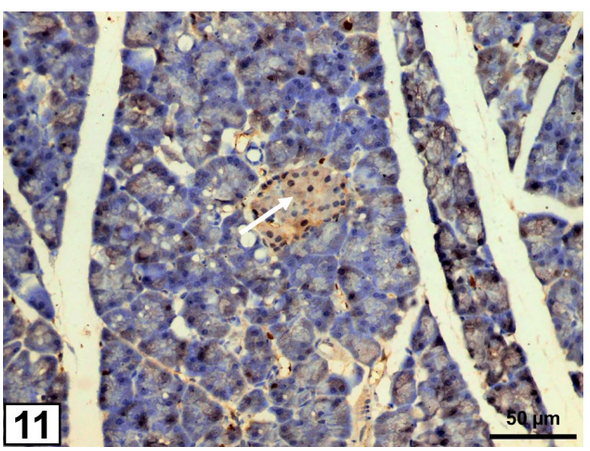


Fig. 11: A photomicrograph of the rat pancreas from HFD-induced obesity group II shows a weak cytoplasmic immunoreactivity for Bcl2 in the islet cells (arrow). (Bcl2 x400, scale bar=50 µm)

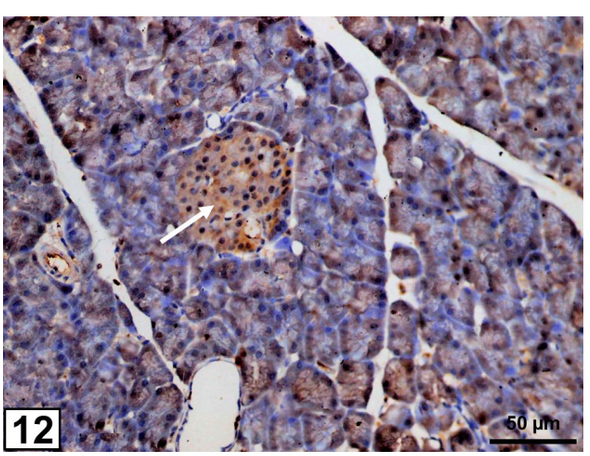


Fig. 12: A photomicrograph of the rat pancreas from recovery group III shows a moderate cytoplasmic immunoreactivity for Bcl2 in the islet cells (arrow). (Bcl2 x400, scale bar=50 µm)

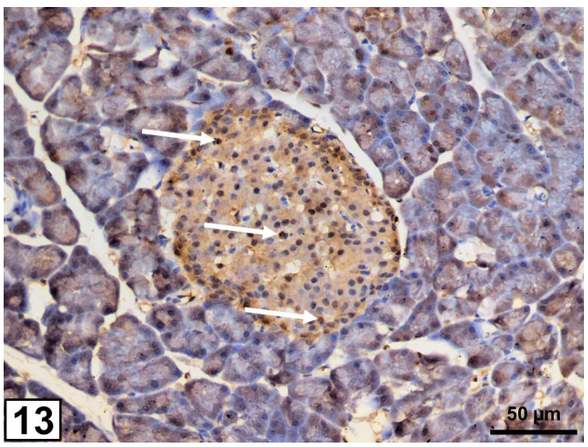


Fig. 13: A photomicrograph of the rat pancreas from the control group I shows many islet cells with a positive nuclear immunoreactivity for Ki67 (arrows). (Ki67 x400, scale bar=50 µm)

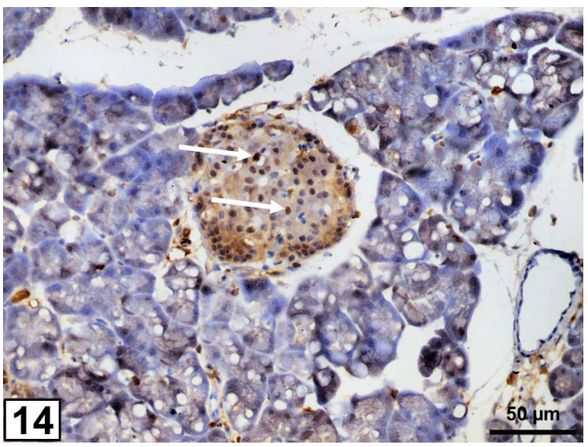


Fig. 14: A photomicrograph of the rat pancreas from HFD-induced group III shows few islet cells with a positive nuclear immunoreactivity for Ki67 (arrows). (Ki67 x400, scale bar=50 µm)

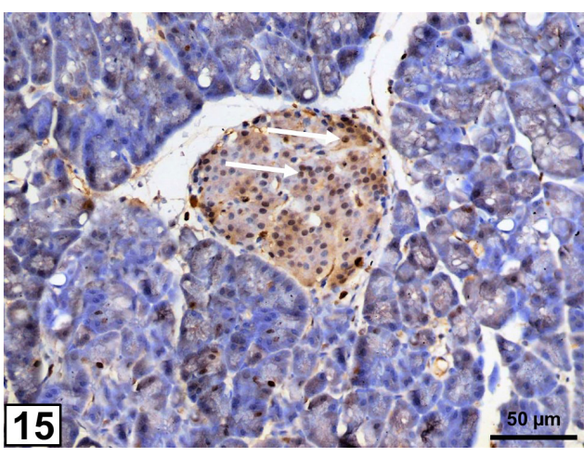
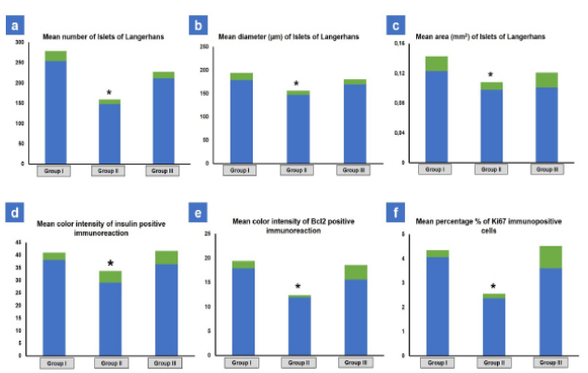


Fig. 15: A photomicrograph of the rat pancreas from recovery group III shows some islet cells with a positive nuclear immunoreactivity for Ki67 (arrows). (Ki67 x400, scale bar=50 µm)



Histogram 1: Morphometrical analysis of a] Mean number of islets of Langerhans, b] Mean diameter (µm) of islets of Langerhans, c] Mean area (mm2) of islets of Langerhans, d] Mean color intensity of insulin positive immunoreaction, e] Mean color intensity of Bcl2 positive immunoreaction, and f] Mean percentage % of Ki67 immunopositive cells. * indicates significance vs control group.

Table 2: Statistical analysis of the different study groups

Parameters		Group I	Group II	Group III
Mean total weight (g)		67.43±5.77	83.81±4.21[a]	79.66±5.28[b]
Mean blood glucose (mg/dl)		86.33±11.09	144.07±18.88[a]	94.89±13.94[b]
Mean serum insulin (uIU/ml)		2.99±0.01	1.62±0.08[a]	2.61±0.74[b]
Islets of Langerhans	mean number	253.98±24.69	147.95±11.48[a]	210.98±16.59[b]
	mean diameter (µm)	178.97±15.06	146.93±9.07[a]	169.62±11.21[b]
	mean area (mm^2)	0.123±0.02	0.098±0.01[a]	0.101±0.02[b]
Mean color intensity of insulin positive immunoreaction		38.19±2.83	29.18±4.48[a]	36.47±5.23[b]
Mean color intensity of Bcl2 positive immunoreaction		17.95±1.47	11.94±0.47[a]	15.58±2.99[b]
Mean percentage % of Ki67 immunopositive cells		4.06±0.29	2.37±0.18[a]	3.61±0.91[b]

Data is expressed as mean ± standard deviation. a $p<0.05$ vs group I, b $p<0.05$ vs group II

DISCUSSION

The overwhelming number of obese children and adolescents represents a major worldwide concern. Since excess food energy is stored as triglycerides (TGs) in adipose tissue, therefore, HFD exceeding the storage capacity of adipose tissue leads to obesity and push TGs to be accumulated in the internal organs causing multiple organ dysfunction[21,22].

In the present study, the mean total weight of rats from HFD-induced obesity group recorded a significant increase compared to the control group. This finding was previously documented in relevant studies, thus supporting the success of the current model of HFD-induced obesity[23,24]. This increase in weight could be explained by the fact that consumption of HFD increases the size and number of adipocytes, affects adipocytes' morphology, hormonal sensitivity, and expression of genes, thus leading to stimulation of adipocyte precursor cells, provoking their differentiation and formation of new tissue[25,26]. Hypertrophy of adipocytes with accumulation of adipose tissue due to increased TGs storage subsequently increase body weight[27]. Nevertheless, Winzell and Ahren[28] suggested that the weight gain was not only related to an increased energy intake but was also attributed to a decreased metabolic rate.

In the current work, a significant increase in the mean fasting blood glucose level in rats from HFD-induced obesity group was recorded compared to the control group. This could be explained by the fact that obesity causes peripheral tissue resistance to the action of insulin with a subsequent rise in blood glucose levels because peripheral tissues no longer take up as much glucose and the liver releases abnormally high amounts of glucose[29]. More importantly, fat accumulation is associated with oxidative stress that promotes insulin resistance by inhibiting phosphorylation of insulin receptor substrate (IRS)-1 with a consequent inhibition of IRS-1-induced phosphatidylinositol 3 -kinase activation, insulin-induced glucose uptake, and translocation of glucose transport protein -4[30].

Additionally, adipocytes normally secrete several adipocytokines which are positively associated with insulin sensitivity and lowering the risk of metabolic syndrome. Yet in obesity, oxidative stress affects adipose tissue secretion, so they secrete low levels and harmful patterns of adipocytokines. Moreover, the secretion of tumor necrosis factor (TNFα) and other pro-inflammatory cytokines increases, thus enhancing insulin resistance through increasing mRNA expression of TNFα in adipose tissue which elevates TNFα level locally and systemically. TNFα in turn decreases the activity of tyrosine kinase enzyme of insulin receptors[31,32]. Nevertheless, accumulating evidences suggested that gut microbiota is an important contributing factor in the development of obesity and insulin resistance. Excess consumption of HFD increases bacterial production of pro-inflammatory lipopolysaccharide and increases gut permeability thus allowing the occurrence of systemic inflammation and obesity-associated metabolic derangements including insulin resistance[33].

In the present work, HFD-induced obesity group revealed few shrunken pancreatic islets with a significant decrease in their mean number, diameter and area compared to the control group. Additionally, multiple nuclear alterations and cytoplasmic vacuolation were associated with some inflammatory signs. These findings were similarly recorded in previous studies[14,16,34]. Many researchers attributed these histological changes to the development of secondary hypertriglyceridemic pancreatitis which could occur even in obese non-diabetic subjects due to the increased levels of free fatty acids (FFAs) resulting in ischemia, acidosis and oxidative stress, thus triggering acute pancreatitis and damaging the islets[16,35]. Whereas other researchers related these histological alterations to the induction of endoplasmic reticulum (ER) stress particularly in obese diabetic subjects, where Yi *et al*.[36] recently reported that the ultrastructure of β-cells of obese mice revealed dilated rough ER with vacuole-like changes and the nuclei had condensed chromatin. They suggested that these changes indicated impairment in the secretory functions of β-cells and confirmed that ER stress enhanced the activating transcription factor 6 signaling pathway, which is a crucial signaling molecule for ERs in β-cells, as one of the potential mechanisms underlying β-cell dysfunction in obesity.

Moreover, in the current study, the significant decrease in the mean number, diameter and area of the islets in HFD-induced obesity group was coupled with a significant decrease in both serum insulin level and insulin immunoexpression in the islets, thus confirming β-cells dysfunction in HFD-induced obesity and came in accordance with previous researchers[14,37]. This immunohistochemical downregulation could be attributed to the β-cells lipotoxicity occurring due to high FFAs level combined with TGs accumulation in the islets of Langerhans. It could be explained by the fact that the primary pathophysiological change in patients with obesity is hypertriglyceridemia, where TGs are then hydrolyzed by the action of lipoprotein lipases into FFAs. Elevated levels of FFAs in blood beyond the capacity of the body lead to deposition of FFAs in non-adipose tissues causing damaging effects to many tissues and cell types[38,39,40] which explains the histological alterations of the islets reported in the current work.

Moreover, Ynsa *et al*.[41] stated that HFD intake resulted in large deposits of fat in the pancreas and twofold decrease of calcium and zinc concentrations in the islets, thus leading to reduction of essential trace element concentrations in pancreas, and in turn affecting its endocrine function. Nevertheless, researchers documented a differential duration-dependent regulation of insulin immunoexpression in the islets; where they reported a significant upregulation upon acute administration of HFD for 3 weeks, whereas a significant downregulation in insulin immunoexpression was recorded upon chronic administration of HFD for 8 weeks[14] as was similarly reported in the current work.

Moreover, the observed nuclear alterations and cytoplasmic vacuolation of the islets' cells in HFD-induced obesity group during the present work were associated

with a significant downregulation of both Bcl2 and Ki67 immunoexpression in the islets as was similarly reported in previous researches[42]. This could be attributed to the effect of FFAs and ER stress during obesity, which result in modulation of the intrinsic pathway of apoptosis controlled by the Bcl2 family of proteins in response to inactivation of Ubiquitin-Proteasome System and hence induces β-cell death[43]. Nevertheless, Golson *et al*.[44] pointed out that normally insulin signaling not only stimulates β-cells proliferation but also inhibits apoptosis through IRS-mediated activation of mitogen-activated protein kinase pathway via the adaptor protein Grb2. Whereas impaired insulin signaling in β-cells in obesity leads to an increased apoptosis and decreased proliferation, as similarly documented in the current study.

On the other hand, in the current study, recovery group revealed an apparently normal morphology together with a non-significant difference in most biochemical, morphometrical, and immunohistochemical expression of insulin, Bcl2 and Ki67 in the islets of Langerhans compared to the control group. These findings were in accordance with many relevant studies that documented the improvement of diet-induced obese rats upon switching back to normal diet[45,46,47]. Additionally, Holmes *et al*.[48] concluded that changing the HFD to a normal diet in diet-induced obese rats improved glucose utilization, where normal diet decreases production of oxidative stress and relieves the ER stress. Many studies concluded that diet-induced obese rats when subjected to caloric restriction, they showed reduced hepatic TGs, hepatic levels of inducible nitric oxide synthase and cyclooxygenase 2 and thus improved levels of lipid peroxidation, reduced the impairment of fasting glucose and reversed the oxidative stress responsible for islets alterations[31,32,49]. Nevertheless, restoration of normal diet was suggested to modulate gut microbiota and reverse the obesity-associated metabolic derangements[33]. Taken together, cutting down the TGs in the recovery group by shifting to the normal standard fat diet was the key event in the reversal of most structural and functional alterations recorded in the HFD-induced obesity group, it could be then assumed that these alterations were mostly initiated through the development of secondary hypertriglyceridemic pancreatitis rather than obesity-induced diabetes type-2. However, it was reported that pancreatitis could eventually lead to the development of pancreatogenic diabetes, referred to as type 3c diabetes, occurring secondary to the endocrine insufficiency[50].

Additionally, Lerea *et al*.[51] suggested that younger obese children were more likely to produce better recovery outcomes than older adolescents and adults when it came to weight loss interventions. They added that management of obesity at younger age could most probably protect the children from the development of obesity at later life stages.

In conclusion, HFD-induced juvenile obesity triggered multiple histological and immunohistochemical alterations in the pancreatic islets through either hypertriglyceridemia pancreatitis or the induction of ER stress, whereas the restoration of a normal diet reinstated the abnormal glucose and insulin levels, ameliorated the altered morphology of islets of Langerhans, and upregulated the suppressed insulin, Bcl2 and Ki67 immunoexpression in the islets at adulthood most likely through lowering the TGs and relieving of the developed ER stress. It is therefore recommended to shift for a normal healthy balanced diet as early as possible for overweight and obese children since recovery could be possible in this early age.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

تأثير سمنة الأطفال على جزر لانجرهانز في الجرذان و إمكانية التعافي عند البلوغ: دراسة هستولوجية وهستوكيميائية مناعية

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مقدمة: السمنة لدى الأطفال مشكلة خطيرة تزيد من خطر التطور المستقبلي لأمراض القلب والأوعية الدموية وداء السكري من النوع ٢ في مرحلة البلوغ من خلال برمجة خلل التمثيل الغذائي أثناء النمو.

الهدف من العمل: إجراء تقييم كيميائي حيوي و هستولوجي و هستوكيميائي مناعي لتأثير سمنة الأطفال على شكل وتعبير الأنسولين و موت الخلايا المبرمج و تكاثرها في جزر لانجرهانز في الجرذان وتقييم إمكانية التعافي في مرحلة البلوغ.

مواد وطرق البحث: تم تقسيم واحد وعشرين من أحداث ذكور الجرذان البيضاء بالتساوي إلى ٣ مجموعات؛ المجموعة الضابطة (غُذيت بنظام غذائي قياسي بنسبة ١١ ٪ من الدهون) ، مجموعة السمنة الناجمة عن نظام غذائي عالي الدهون (غُذيت بنظام غذائي يحتوي ٤٥ ٪ دهون لمدة ٨ أسابيع) ومجموعة التعافي (غُذيت نظام غذائي يحتوي ٤٥ ٪ دهون لمدة ٨ أسابيع ثم تركت لمدة ٤ أسابيع على نظام غذائي قياسي). تم وزن الحيوانات و قياس الجلوكوز والأنسولين في الدم. تمت معالجة عينات البنكرياس من أجل الفحص المجهري الضوئي و الصبغ الهستوكيميائي المناعي للكشف عن الأنسولين و Bcl٢ و Ki٦٧.

النتائج: سجلت مجموعة سمنة الأطفال زيادة كبيرة في الوزن الكلي للجسم و الجلوكوز مع انخفاض ملحوظ في الأنسولين في الدم. كشف الفحص المجهري الضوئي للبنكرياس عن عدد قليل من جزر لانجرهانز المتقلصة ذات خلايا ذات تغيرات نووية و فجوات في السيتوبلازم. و أمكن ملاحظة إرتشاح خلوى على الأطراف و بعض الأوعية الدموية المتسعة المحتقنة. تم تسجيل انخفاض كبير في التعبير الهستوكيميائي المناعي للأنسولين ، Bcl٢ و Ki٦٧. بينما تم تسجيل تحسن كبير لجميع العوامل المدروسة في مجموعة التعافي.

الاستنتاج: استعادة نظام غذائي طبيعي في السمنة التي تسببها الدهون العالية في صغار الجرذان أعاد مستويات الجلوكوز والأنسولين غير الطبيعية ، وحسّن الشكل المتغير لجزر لانجرهانز، وأعاد رفع التعبير الهستوكيميائي المناعي المنخفض للأنسولين و Bcl٢ و Ki٦٧ للجزر عند البلوغ.