Effects of L-arginine versus exercise on acinar and beta-cells of pancreas in high-fat diet-fed albino rats: a histological and immunohistochemical study

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Abstract

Fatty acids and glucose are the main nutrients in energy metabolism and of particular interest in metabolic diseases as they may cause obesity. This work aimed to study the histological effect of L-arginine versus exercise on acinar and beta-cells of pancreas in high-fat diet-fed albino rats. Forty adult male albino rats were divided into group I and group II. Group I served as the control group. Rats of group II were further classified into high fat chow (HFC), HFC+ exercises (EXC) and HFC + L-arginine (Arg) subgroups. After 12 weeks, rats were anesthetized and the pancreas was dissected out. Specimens were processed and examined by light and electron microscopy. HFC subgroup showed increased serum glucose, insulin and triglycerides. There was highly significant increase in mean body weights, pancreatic weights, and mean area % of insulin immunoexpression. Acinar and beta-cells showed irregular and dilated rER, damaged mitochondria, cytoplasmic vacuoles and few zymogen granules. Also, pancreatic stellate cells and neutrophils could be seen. Exercise improved many histological changes due to HFC, whereas the effect of L-arginine was more pronounced. In conclusion, L-arginine can be used as a safe and effective way to protect pancreas from fatty changes.

Keywords: Beta-cells, acinar cells, exercise, L-arginine, obesity, pancreas.

Özet

Albino sıçanlarda L-arjinin ve egzersizin yüksek yağ içerikli beslenmede pankreasın asinar ve beta hücrelerine etkisi: Histolojik ve immünohistokimyasal bir çalışma

Yağ asitleri ve glikoz enerji metabolizmasının temel bileşenleridir ve obeziteye sebep olmalarından dolayı metabolik hastalıklarda dikkati çeker. Bu çalışma yağlı diyetli beslenmiş albino farelerinde L-arjinin ve egzersizin pankreastaki asinar ve beta hücrelerine etkilerini araştırmayı amaçlamıştır. 40 erişkin erkek albino fare Grup I ve Grup II’ye ayrılmıştır. Grup I kontrol grubu olarak kabul edilmiştir. Grup II fareleri kendileri aralarında yüksek yağlı besin (HFC), yüksek yağlı besin ve egzersiz (EXC), yüksek yağlı besin ve L-arjinin olmak üzere alt gruplara (Arg) ayrılmıştır. 12 hafta sonra fareler anestezili tabi tutulmuş ve pankreasları çıkarılmıştır. Örnekler ışık ve elektron mikroskoplarda incelenmiştir. HFC grubunda serum glukoz, insulin ve trigliseriderin arttığı gözlemehmiştir. Ortalama vücut ağırlıklarında, pankreas ağırlığı ve adaçık ve insulin immünokşpresyonu gösteren yüzde alanların ortalamasında önemli bir artış tespit edilmiştir. Asinar ve beta hücreleri düzensiz ve genişlemiş rER, hasarlı mitokondri, sitoplazmik kofullar ve birkaç...
zimojen granülleri göstermiştir. Bununla birlikte, pankreatik stellat hücreleri ve nötrofiller görünmüştür. Egzersiz, HFC sebebiyle oluşan birçok histolojik değişikliği düzelmiştir, fakat L-arjinin etkileri daha fazla göz çarpmaktadır. Sonuç olarak, L-arjinin pankreasi yağ asitlerinin yol açtığı değişimlerden korumak üzere güvenli ve etkili bir şekilde kullanılabılır.

Anahtar kelimeler: Beta hücreleri, asinar hücreleri, egzersiz, L-arjinin, obezite, pankreas.

INTRODUCTION
Fats and glucose are the main nutrients in energy metabolism in most organisms. They are of particular interest in metabolic diseases such as diabetes and obesity. The specific pathogenesis of these metabolic diseases remains unclear. The deleterious effects of elevated fatty acid levels on the pancreatic acinar and beta (β) cell viability and function has been elevated recently (Yan et al., 2012).

Obesity resulting from an energy imbalance can be attributed to the current lifestyle which includes little physical activity and excessive intake of foods rich in saturated fats. Consumption of high-fat and physical inactivity are potential triggers of type 2 diabetes and insulin resistance (de Ferranti and Mozaffarian, 2008). The coincidence of obesity, insulin resistance, hypertension and hyperlipidemia are commonly referred to as the ‘metabolic syndrome’. Obesity approximately affects 20–40% of the population in industrialized cities, and its prevalence is expected to rise further in the next decades (Laaksonen et al., 2004).

Fat accumulation in the liver, muscle and pancreas causes an increase of cellular free fatty acids, decrease in β-oxidation and accumulation of triglycerides (Carr et al., 2004; Pessayre, 2007). Consequently, elevated insulin levels, effects on pancreas, development of acute pancreatitis and pancreatic cancer may be expected (Akiyama et al., 1996; Mathur et al., 2007; Brown et al., 2009).

Exercise has been prescribed as part of the treatment of obesity and type 2 diabetes (Ropelle et al., 2006; Vieira et al., 2009). Recent studies reported that exercise increases insulin sensitivity under normal conditions and improve impaired insulin action in insulin-resistant cases (Perrini et al., 2004). Moreover, it improves glucose homeostasis by enhancing glucose uptake in skeletal muscle and adipose tissues (Holloszy, 2005; Park et al., 2007).

L-arginine, an essential amino acid for adults, is a precursor for the synthesis of biologically important molecules, including nitric oxide (NO), polyamines, creatine, proline, and glutamate (Wu and Morris, 1998). Available evidence shows that physiological levels of arginine and NO promote fat oxidation and decrease fat synthesis in tissues (Jobgen et al., 2006). Recent studies showed that dietary L-arginine supplementation reduces adiposity in genetically obese rats. It is responsible for altering the balance of energy intake and expenditure in favor of fat loss or reduced growth of white adipose tissue (Mc Knight et al., 2010).

As obesity becomes a major public health crisis worldwide that annoys developed countries, as well as developing ones, therefore, this study aimed to discuss the effect of L-arginine versus exercise on pancreatic acinar and beta-cells in high-fat diet fed albino rats using histological, immunohistochemical and histomorphometrical methods.

Materials and Methods
Animals
Forty adult male albino rats; six months old, weighing 200± 3 gm, were used in the present study. The animals were kept in the animal house in the Bilharzial and Medical Research Center, Faculty of Medicine, Ain Shams University. Animals were housed in separate cages with mesh wire cover and allowed one week for acclimatization at room temperature with 12hrs light and dark cycle before beginning the experiment. Rat’s chow diets were purchased from Meladco for Animal Food, El-Obour, Al sharkia, Egypt. Chow pellets and tap water were provided ad libitum. All procedures were done according to ethical guidelines for animal use in research. Body weight of rats was recorded early in the morning at the end of the experiment.
Experimental design

All rats were divided randomly into two main groups: group I (the control group), comprising 10 rats and group II (the experimental group) comprising 30 rats. Rats of group I were given a standard chow (SC) and water ad libitum for 12 weeks. Standard chow contained 76% calories from carbohydrates, 10% from fat, and 14% from protein. Rats of group II were randomly classified into three subgroups: high fat chow (HFC) (subgroup IIa), high fat chow + exercise (EXC) (subgroup IIb) and high fat chow+ L-arginine (Arg) (subgroup IIc). Each subgroup had ten rats. Exercise and L-arginine were applied concomitantly with HFC for 12 weeks. HFC contains 26% calories from carbohydrates, 60% from fat, and 14% from protein. Fresh chow was provided daily and any remaining chow from the previous day was discarded. The mineral and vitamin content of the two diets were identical (Table 1). The diet was designed and prepared at the National Research Institute for Nutrition, Giza, Egypt, according to the National Nutrition Database.

### Table 1. Composition and energy content of the standard chow (SC) and high-fat chow (HFC).

<table>
<thead>
<tr>
<th>Content (g/kg)</th>
<th>SC</th>
<th>HFC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>160</td>
<td>190</td>
</tr>
<tr>
<td>Cornstarch</td>
<td>620.7</td>
<td>250.7</td>
</tr>
<tr>
<td>Sucrose</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Lard</td>
<td>-</td>
<td>320</td>
</tr>
<tr>
<td>Fiber</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Vitamin mixture</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Mineral mixture</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>L – cystin</td>
<td>1.8</td>
<td>1.8</td>
</tr>
<tr>
<td>Choline</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Antioxidant</td>
<td>0.008</td>
<td>0.008</td>
</tr>
<tr>
<td>Total grams</td>
<td>1,000</td>
<td>1,000</td>
</tr>
<tr>
<td>Energy content (kcal/kg)</td>
<td>3,573</td>
<td>5,404</td>
</tr>
<tr>
<td>Carbohydrates (%)</td>
<td>76</td>
<td>26</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Lipids (%)</td>
<td>10</td>
<td>60</td>
</tr>
</tbody>
</table>

Exercise protocol

Rats were submitted for one week of adaptation to swimming, followed by eleven weeks of swimming exercise training protocol. The rats were adapted to swimming for 20 min on the first day; 30 min on the second day, until they reached 60 minutes daily (1 week) while swimming exercise training protocol was applied as follows: 2 x 30 minutes with 5 minutes of interval/ day, five days per week, for eleven weeks. The rats swam in groups of five each. Adaptation and exercise training was conducted in 80 × 60 × 50 cm plastic swimming pool that were filled to a depth of 30 cm with water at temperature controlled at 30 ± 2°C (Farias et al., 2012).

L-arginine

N^G^-Nitro-L-arginine (Product No. 483125 ) package of white to off-white colored powder of 100mg/ package (Merk KGaA, Darmstadt, Frankfurter, Germany), was used at 88.5 mg/kg/d and introduced to rats in drinking water as 1.51% L-arginine-HCL for twelve weeks (Jobgen et al., 2006; Mc Knight et al., 2010).

Biochemical analysis

All blood samples were obtained from rat’s tail vein at the end of experiment and collected in glass tubes containing 0.05 ml 15% fluid EDTA. Samples were placed on ice and centrifuged within 10 min. Plasma was then stored at −80°C until assayed. Plasma triglyceride was measured by spectrophotometric method (Beckman Analyzer; Beckman, Fullerton, CA). Immunoreactive insulin was measured by solid-phase radioimmunoassay (Coat-A-Count Insulin; DPC, Los Angeles, CA). Fasting blood glucose level was measured using Accu–Check glucometer (Roche Diagnostics, Mannheim, Germany) (Everard et al., 2012).

Tissue sampling

At the end of the experiment, the rats were fasted overnight. They were anesthetized by intraperitoneal injection with sodium thiopental (40 mg/kg of body weight) (Sigma Chemical Co., ST Louis, MO, USA) (Toblli et al., 2012) and blood was collected from the tail vein for biochemical analysis. The abdomen was rapidly opened.
and the pancreas was dissected out, freed from fat and lymph nodes and weighed before preparing it for both light and electron microscope study.

**Light microscopic studies**

Specimens from the pancreas were fixed in 10% buffered formalin overnight at room temperature. Tissue samples were dehydrated in alcohol, cleared in xylol, and embedded in paraffin. Tissue sections (5 μm thickness) were stained with H&E stain (Bancroft and Gamble, 2008) and immunohistochemical stains for insulin immunoeexpression (Kostromina *et al.*, 2010).

**Staining procedures for immunohistochemistry**

Paraffin sections on positive slides were immunostained using an avidin–biotin technique. Slides were deparaffinized, rehydrated, rinsed in tap water, and embedded in 3% H2O2 for 10 min to block endogenous peroxidase. Sections were immersed in antigen retrieval solution (10 mmol/l sodium citrate buffer, pH 6) and subjected to heat-induced antigen retrieval for 20 min in a microwave. The slides were incubated for 45 min at room temperature with primary antibody after its dilution with phosphate buffer solution (PBS) at concentration 1:150. Drops of streptavidin peroxidase were added to the slide, and then washed with PBS for 5 min. Diaminobenzidine was added to slides as a chromogen, after which the slides were washed with distilled water. Finally, the slides were stained with Harris hematoxylin, dehydrated, and cleared in xylene. Positive reaction is strong brown cytoplasmic staining of beta-cells in the pancreas leaving the rest of the pancreatic tissue negative (Zhang *et al.*, 2012). Guinea pig polyclonal insulin antibody was obtained from Abcam Biochemical, USA (Cat. no ab7842).

**Electron microscopic studies**

Specimens for electron microscopy were immediately fixed in 2.5% phosphate-buffered glutaraldehyde (pH 7.4). Thereafter, they were post-fixed in 1% osmium tetroxide in the same buffer at 4°C, dehydrated, and embedded in epoxy resin. Semi-thin sections (0.5 μm) were cut with a diamond knife and stained with 1% toluidine blue, examined and photographed by light microscopy. Ultrathin sections (70-90 nm) were stained with uranyl acetate and lead citrate (Glauert and Lewis, 1998) and examined and photographed using a JEOL JEM 1010 electron microscope (Jeol Ltd, Tokyo, Japan) in the Electron Microscope Research Laboratory of the Histology and Cell Biology Department, Faculty of Medicine, Zagazig University, Egypt.

**Morphometric studies**

Leica Qwin 500 (Imaging System, Cambridge, UK) was used for measuring the area of the pancreatic islets/μm² from H&E stained sections and area % of insulin immunoeexpression from immunostained sections. Five non-overlapping high-power fields (×400) were taken from each block for all rats. Morphometric measurements were taken within frame area 2934288.00 μm². Morphometry was carried out at the Image Analysis Unit, Anatomy Department, Faculty of Medicine, Taibah University, Al madinah Al monawarrah, KSA.

**Statistical analysis**

Statistical analysis was performed using SPSS software (SPSS 13.0, Chicago, Illinois, USA). One-way analysis of variance and post-hoc least significant difference (LSD) were performed for intergroup comparisons. All data are expressed as mean ± SEM. P ≤ 0.05 and P ≤ 0.001 were considered significant and highly significant, respectively.

**Results**

**Biochemical results**

HFC subgroup showed high significant increase (p <0.001) in blood levels for fasting glucose, insulin and triglyceride when compared with control group. HFC + EXC subgroup showed significant increase (p <0.05) in fasting blood glucose level and highly significant increase (p <0.001) in insulin and triglyceride levels compared to the control group. HFC + L-arginine subgroup showed non-significant increase (p >0.05) in all measured biochemical parameters except for Insulin level in blood which was significantly different (p<0.05) from the control group (Table 2).
Histological results

Light microscopic examination

H&E stained sections of pancreas from control group showed multiple rounded or oval acini contained cells that had basal basophilic and apical acidophilic cytoplasm. Islet of Langerhans had large pale cells with pale nuclei in the center. Also small dark cells with small nuclei in the periphery were seen (Fig. 1a). Subgroup IIa had large-sized islet of Langerhans with increased cell population and wide intercellular spaces compared to control group. Many cells had small darkly stained nuclei with acidophilic cytoplasm in its center. Thick wall of the interlobular duct, segmented islets by fibrous tissue, multiple congested blood vessels and extravasated RBC could be seen (Fig. 1b,c). Subgroup II-b had islet of Langerhans contained wide intercellular spaces, few centrally located cells had small darkly stained nuclei and few extravasated RBCs. Islet of Langerhans was smaller in size compared to subgroup II-a (Fig. 1d). Subgroup II-c had rounded and small islet of Langerhans with apparently normal shaped central cells and narrow intercellular spaces compared to subgroups IIa and IIb. Many congested blood vessels in the islet and thin wall of intralobular duct were observed (Fig. 1e,f). Insulin immunoperoxidase stained sections showed strong positive immune reaction in cytoplasm of beta-cells of islets of Langerhans of all groups (Fig. 2a,b,c, d). Large sized islets with multiple cell populations were seen in subgroups II-b and II-c (Fig. 2b, c) compared to remaining groups.

Toluidine blue stained sections of control group showed multiple acini formed of multiple cells that had rounded nuclei, prominent nucleoli and basophilic zymogen granules. Centroacinar cells had pale cytoplasm. Islets of Langerhans had rounded shape and contained many cells with sharply demarcated nuclei and prominent nucleoli (Fig. 3a). Subgroup IIa showed acinar cells had few zymogen granules and large sized islets contained many cells with dark and small sized nuclei and vacuolated cytoplasm in its center (Fig. 3b). Subgroup II-b showed cytoplasm of all pancreatic acinar cells was occupied by small-sized vacuoles and many zymogen granules. Islet of Langerhans had cells contained sharply demarcated nuclei and prominent nucleoli (Fig. 3c). Subgroup II-c showed multiple zymogen granules and few vacuoles in the acini. Rounded islets of Langerhans contained multiple cells with rounded nuclei, prominent nucleoli and pale cytoplasm could be seen (Fig. 3d).

Electron microscopic examination

Control group showed that pancreatic acinar cell had euchromatic nucleus, prominent nucleolus, multiple rounded electron dense zymogen granules, regularly arranged rER, oval shape mitochondria with parallel cristae and lysosomes. Centroacinar cells occupied the central part of the acini had euchromatic nucleus and electron lucent cytoplasm. Beta-cells of islet of Langerhans were formed of sharply demarcated nucleus, prominent nucleolus and its cytoplasm contained electron dense granules surrounded by electron lucent halo (Fig. 4-a, b, c , d).

Subgroup II-a showed that some pancreatic acinar cells had small heterochromatic nucleus, irregularly arranged and dilated rER, damaged cristae of mitochondria, multiple large electron lucent cytoplasmic vacuoles and absence of zymogen granules were noticed. Pancreatic stellate cell had multiple cytoplasmic processes and elongated nucleus. Neutrophile with segmented nuclei and chromatin band connecting nuclei could be seen. Cytoplasm of beta-cells of Langerhans contained few electron dense granules surrounded by electron lucent halo, when compared to control group, multiple cytoplasmic vacuoles, dilated rER and some mitochondria with damaged cristae as compared to control group (Fig. 5 a, b, c, d, e).

Subgroup II-b showed that pancreatic acinar cells had sharply demarcated euchromatic nuclei and prominent nucleoli, regularly arranged and dilatated rER, oval shaped mitochondria with parallel cristae and some cytoplasmic vacuoles, Golgi apparatus, primary and secondary lysosomes contained vesicles and zymogen granules. Beta-cells had sharply demarcated euchromatic nuclei, many electron dense granules surrounded by electron lucent halo, dilated Golgi apparatus, rER, some mitochondria with damaged cristae, and
multiple cytoplasmic vacuoles (Fig. 6 a, b, c, d, e).

Subgroup IIc showed that pancreatic acinar cells had euchromatic nuclei, prominent nucleoli, oval shaped mitochondria with parallel cristae, many zymogen granules and lysosomes. Beta-cells had sharply demarcated euchromatic nuclei, prominent nucleoli, many electron dense granules surrounded by electron lucent halo, regularly arranged rER, and mitochondria with parallel cristae. Cytoplasmatic vacuoles were absent from acinar and beta-cells (Fig.7a, b, c).

**Morphometric results**

Mean body weight of rats (/gm) for group I and subgroups IIa, IIb and IIc were 247.2 ± 4.1, 297.9 ± 11.4, 274.0 ± 7.4 and 254.6 ± 4.8 consecutively. In comparison with control group, there was high significant differences (p< 0.001) represented by 20.5 % higher weight gain in subgroup IIa while subgroups IIb and IIc showed significant (p< 0.05) and no significant (p>0.05) increase in weight gain represented by 10.8% and 7.4%, respectively (Fig. 8-a).

Mean pancreatic weight (/gm) for group I and subgroups IIa, IIb and IIc were 1.25± 0.02, 2.23 ± 0.1, 1.79 ± 0.14 and 1.36 ± 0.03 consecutively. In comparison with control group, there was high significant increase (p< 0.001) represented by 78% and 43.2% increase in weight of pancreas in subgroups IIa and IIb respectively, while subgroup IIc showed no significant increase (p>0.05) represented by 8.8% (Fig.8-b).

Mean area (/ μm²) of islet of Langerhans for group I and subgroups IIa, IIb and IIc were 269953.2± 45.06, 819697.8 ± 17209.9, 443025.7± 14835.6and 356653.2± 18252.07 consecutively. In comparison with control group, there was highly significant increase (p < 0.001) in area of islets of Langerhans represented by two folds increase in subgroup IIa, while subgroups IIb and IIc showed only significant increase (p < 0.05) represented by 64.1 % and 32%, respectively (Fig. 8-c).

Mean area % of insulin immunoexpression for group I and subgroups IIa, IIb and IIc were 6.4± 0.15, 38.4 ± 0.25, 11.2± 2.3 and 7.04± 0.27 consecutively. In comparison to control group, there was highly significant and only a significant increase (p< 0.001) and (p< 0.05) in the mean area % of insulin represented by 5 folds and 75.0% increase in subgroups IIa and IIb respectively, while subgroup IIc showed no significant increase (p>0.05) represented by 10% (Fig.8-d).

**Discussion**

Obesity is a major health crisis worldwide and new treatments are needed to fight this epidemic. Fat-enriched diets had been used as a model for obesity, dyslipidemia and insulin intolerance in rodents as it achieved metabolic disorders closely resemble that of human (Woods et al., 2003). Accordingly, researchers had focused on the regulation of lipid metabolism and energy partitioning to prevent and treat disorders of obesity.

In the present study, the mean body weight of rats showed higher weight gain in subgroup IIa, while subgroups IIb and IIc showed significant and non-significant increase compared to control group. Also the mean pancreatic weight was highly significantly increased in subgroups IIa and IIb compared to subgroup IIc. Fraulob et al., (2010) stated that HFD significantly increased body weight and visceral fat. Jobgen et al., 2009 reported that HFD enhanced adipogenesis and lipogenesis, whereas L- arginine reduced white fat gain in retroperitoneal, subcutaneous, epididymal and mesenteric pads and increased brown fat masses in diet induced obesity. Brown adipose tissue is rich in mitochondria where fatty acid oxidation results in heat production due to uncoupling protein -1 (Cannon and Nedergaard, 2004). Recent studies showed that regular aerobic exercise reduces the amount of visceral fat mass and body weight through its appetite-suppressive action resulted from increasing the sensitivity to leptin and insulin signaling in the hypothalamus (Kaastra et al., 2006; Flores et al., 2006).

In the current research, subgroup IIa showed highly significant increase in blood levels for fasting glucose, insulin and triglyceride, whereas subgroup IIb showed significant increase in fasting blood glucose level and high significant increase in insulin and triglyceride levels.
Histological changes of pancreas in high-fat diet-fed albino rats

Table 2. Mean ±SEM of glucose (mM), insulin (pM) and triglyceride (mM) levels in the blood (p-values were compared to the control group).

<table>
<thead>
<tr>
<th></th>
<th>Fasting blood glucose level (Mean ±SEM)</th>
<th>p</th>
<th>Insulin level in blood (Mean ±SEM)</th>
<th>p</th>
<th>Triglyceride level in blood (Mean ±SEM)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.0 ± 0.4</td>
<td></td>
<td>94.5 ± 0.53</td>
<td>0.94 ± 0.005</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HFC</td>
<td>9.5 ± 0.5</td>
<td>0.001</td>
<td>118.6 ± 0.66</td>
<td>0.001</td>
<td>1.70 ± 0.09</td>
<td>0.001</td>
</tr>
<tr>
<td>EXC + HFC</td>
<td>7.15 ± 0.5</td>
<td>0.04</td>
<td>107.6 ± 0.71</td>
<td>0.001</td>
<td>1.5 ± 0.06</td>
<td>0.001</td>
</tr>
<tr>
<td>L-arginine+HFC</td>
<td>5.5 ± 0.053</td>
<td>0.60</td>
<td>95.05 ± 0.74</td>
<td>0.055</td>
<td>1.00 ± 0.02</td>
<td>0.36</td>
</tr>
</tbody>
</table>

![Photomicrographs of pancreas sections showing:](image)

**Figure 1.** Photomicrographs of pancreas sections showing: **a)** Control group has multiple rounded or oval acini (A) contain cells have basal basophilic and apical acidophilic cytoplasm. Islet of Langerhans (I) has large pale cells with pale nuclei (thin arrows) in the center and small dark cells with small nuclei (thick arrows) in the periphery. **b&c)** Subgroup II-a has large-sized islet of Langerhans (I) with increased cell population and wide intercellular spaces compared to control group. Many cells have small darkly stained nuclei with acidophilic cytoplasm (arrows) in its center. Thick wall of the intralobular duct (D), segmented islets by fibrous tissue (I), multiple congested blood vessels (black arrows) and extravasated RBC (white arrow) were seen. **d)** Subgroup II-b has islet of Langerhans (I) contains wide intercellular spaces, few centrally located cells have small darkly stained nuclei (thin arrows) and few extravasated RBCs (thick arrows). Note: Islet of Langerhans has smaller size compared to subgroup II-a. **e&f)** Subgroup II-c has rounded and small islet of Langerhans (I) with apparently normal shaped central cells and narrow intercellular spaces compared to subgroups IIa and IIb, many congested blood vessels (arrows) in the islet and thin wall of intralobular duct (D). (H&E; a, b, c, d, e & f x 400).
Figure 2. Photomicrographs of pancreas sections showing strong positive insulin immunoreaction (arrow) in beta-cells for all groups. Large sized islets with increased cell populations are seen in (Figs 2-b & c) compared to remaining groups. a) Control group, b) Subgroup II-a, c) Subgroup II-b, d) Subgroup II-c. (Insulin immunoperoxidase reaction; a, b, c &d x 400).

Figure 3. Photomicrographs of pancreas sections showing: a) Control group shows multiple acini (white arrows) formed of multiple cells have rounded nucleus, prominent nucleolus and basophilic zymogen granules. Centroacinar cells have pale cytoplasm (red arrow). Islets of Langerhans have rounded shape and contain many cells with sharply demarcated nuclei (N) and prominent nucleoli. b) Subgroup II-a shows acinar cells have few zymogen granules (white arrows) and large sized islets containing many cells with dark and small sized nuclei and vacuolated cytoplasm (black arrows) in its center. c) Subgroup II-b shows cytoplasm of all pancreatic acinar cells (white arrows) are occupied by small-sized vacuoles and many zymogen granules. Islet of Langerhans has cells containing sharply demarcated nucleus (N) and prominent nucleolus. d) Subgroup II-c shows multiple zymogen granules (white arrows) and few vacuoles in acini. Rounded islets of Langerhans containing multiple cells with rounded nuclei (N), prominent nucleoli and pale cytoplasm can be seen. (Toluidine blue; a, b, c &d x 1000).
Histological changes of pancreas in high-fat diet-fed albino rats

Figure 4. Electronmicrographs of control group from pancreas sections showing: a) Euchromatic nucleus (N) and prominent nucleolus of pancreatic acinar cell and multiple rounded electron dense zymogen granules (yellow arrows). b) Higher magnification of (Fig. 4-a) showing regularly arranged rER (yellow arrow) around the nucleus of pancreatic acinar cell, oval shaped mitochondria (M) with parallel cristae, zymogen granules (z) and lysosomes (L). c) Centroacinar cells (yellow arrows) occupying central part of the acini have euchromatic nucleus and electron lucent cytoplasm. d) B-cell of islet of Langerhans is formed of sharply demarcated nucleus (N), prominent nucleolus (n) and cytoplasm contains electron dense granules surrounded by electron lucent halo (yellow arrows). (TEM; a x 4000, b x 10000, c X 12000 & d x10000).
Figure 5. Electronmicrographs of subgroup II-a from pancreas sections: a) Some pancreatic acinar cells have small heterochromatic nucleus (N) Note; absence of zymogen granules. b) Pancreatic acinar cell has irregularly arranged rER (arrow), damaged cristae of mitochondria (M), multiple large electron lucent cytoplasmic vacuoles (V) and no zymogen granules. c) Pancreatic stellate cell (S) has elongated nucleus. Dilated rER of pancreatic acinar cells can be seen (yellow arrow). d) Neutrophile with segmented nuclei (N) and chromatin band (yellow arrow) connecting nuclei can be seen between pancreatic acinar cells. e) Cytoplasm of beta-cell of Langerhans contains few electron dense granules surrounded by electron lucent halo (yellow arrows), when compared to control group, and multiple cytoplasmic vacuoles (V). f) Higher magnification shows dilated rER (yellow arrow) and mitochondria with damaged cristae (M). (TEM; a x 4000, b x 6000, c X 12000,  d x8000, e x 8000 & f x 15000).
Figure 6. Electron micrographs of subgroup II-b from pancreas sections showing: a) Pancreatic acinar cells have sharply demarcated euchromatic nuclei (N) and prominent nucleoli (n). b) Higher magnification reveals pancreatic acinar cell has dilated rER (yellow arrow) oval shaped mitochondria (M) with parallel cristae and some cytoplasmic vacuoles (V) can be seen. c) Pancreatic acinar cell has regularly arranged rER (red arrow), Golgi apparatuses (green arrow), primary lysosomes (black arrow) and secondary lysosomes (yellow arrow) containing zymogen granules (Z). d) Beta-cell has sharply demarcated euchromatic nuclei (N) and electron dense granules surrounded by electron lucent halo (yellow arrows). e) Higher magnification reveals dilated rER (yellow arrow), mitochondria with damaged cristae (M), and cytoplasmic vacuoles (V). (TEM; a x 5000, b x 10000, c x 15000, d x6000 & e x 12000).
Figure 7. Electronmicrographs of subgroup II-c from pancreas sections showing: a) Pancreatic acinar cells have euchromatic nuclei (N) and prominent nucleoli (n), oval shaped mitochondria (M) with parallel cristae, many zymogen granules (Z), and lysosomes (L). No cytoplasmic vacuoles can be seen. b) Beta-cell has sharply demarcated euchromatic nuclei (N), prominent nucleoli (n) and many electron dense granules surrounded by electron lucent halo (yellow arrows). c) Cytoplasm of beta-cell contains regularly arranged rER (yellow arrow) and mitochondria with parallel cristae (M). No cytoplasmic vacuoles are seen in beta-cell. (TEM; a x 5000, b x 4000 & c x 10000).

Figure 8. Histograms showing; a) Mean body weight (gm), b) Mean pancreatic weight (gm), c) Mean area for islets of Langerhans (um²), and d) Mean area % of insulin immunoeexpression for all groups. P-values in each histogram were compared to control group.
In contrast, subgroup IIc showed non-significant increase in all measured biochemical parameters. HFD is associated with increased circulating free fatty acids, glucose, and insulin production, insulin resistance and triglycerides (Gallou-Kabani et al., 2007; Karasawa et al., 2009). Schadeva et al., (2009) added that the increased peripheral tissues resistances to insulin as well as production of high amounts of glucose by the liver were beyond the elevated blood glucose level. In contrast, exercise reduces visceral adiposity, serum triglycerides, and increases peripheral tissue sensitivity to insulin (Charbonneau et al., 2005). During exercise and after glycanogen depletion, fatty acid becomes the major fuel for the metabolism in muscles (Gauthier et al., 2004; Slentz et al., 2009). Tan et al., (2012) noticed that oral administration of L-arginine increased insulin sensitivity, reduced plasma and hepatic glucose and diminished white-fat mass through enhancement of AMP activated protein kinases (AMPK) expression resulting in reduction in triglycerides.

During this work, subgroup IIa showed large sized islet of Langerhans represented statistically by highly significant increase in its mean area % compared to control group. On the contrary, there was noticed reduction in the previously mentioned parameters from subgroups IIb to IIc. Previous studies reported that islets of Langerhans showed more than 120% increase in its area in HFD compared to only 40% increase in Exercised-HFD (Marques et al., 2010). Previous data were explained by islet hyperplasia, hypertrophy and neogenesis from precursor cells (duct cells) as a compensatory mechanism to increased demands for insulin resulted from tissue resistance (Jetton et al., 2005; Cole et al., 2012). Islet hyperplasia and hypertrophy are mainly due to beta-cell proliferation (Schadeva et al., 2009). However, in exercise and L-arginine treated rats the increased insulin sensitivity and decreased insulin resistance can explain reduction in islet's area % (Park et al., 2007; Jobgen et al., 2009; Pi et al., 2012).

In the current work, the mean area % of insulin immunoeXpression showed highly significant, significant and non-significant increases in its expression in subgroups IIa, IIb and IIc consecutively. These results were in agreement with data obtained by Marques et al. (2010), who found that the number of insulin-positive cells was significantly increased up to 60% in rodents fed HFD to compensate with the increased tissue resistance to insulin while it was reduced due to improvement of the previous cause in exercised group when examined in isolated islets (Huang et al., 2011). In the same field, L-arginine protects beta-cell against chemical and immune insults in isolated islets due to simple membrane depolarization, promotion of glutathione synthesis and antioxidant defense mechanism (Krause et al., 2011).

Light microscope-examined sections revealed wide intercellular spaces in the islets and appearance of fibrous tissue as well as thick wall of intralobular ducts in subgroup IIa, while ultrathin sections showed many cells that had dark and small sized nuclei in islets (indicative of degeneration) and vacuolated cytoplasm. These results were in agreement with previous studies reported an increased number of cells with pyknotic nuclei, vacuolation and extensive edematous swelling in many acinar cells as well as appearance of cytoplasmic vacuoles as an indicative criterion for early pancratitis (Parimal et al., 2000). Inflammation, fat deposition in acinar and islet cells as well as cell atrophy and fibrosis affecting the lobules and periacinar areas of the pancreas was observed in high-fat diet fed group (Yan et al., 2012). The increase in collagen synthesis, during inflammation of pancreas, resulted from activation of pancreatic stellate cells in response to FFA and lipid peroxidation (Zhang et al., 2008).

In this study, congestion of blood vessels with extravasated RBCs was seen in HFD subgroup. This result was explained by development of endothelial dysfunction together with inflammation (Panchal et al., 2011). Non-alcoholic fatty pancreatic disease, pancreatic microcirculatory disturbances and oxidative stress production are all the causes behind congestion of blood vessels, extravasation of RBCs and escape.
of tissue fluids, causing widening of intercellular spaces (Yan et al., 2006).

In the current work, exercise treated subgroup showed few extravasated RBCs, whereas in L-arginine treated subgroup there were many congested blood vessels but without extravasations of RBCs. This result can be explained by the antioxidant role of L-arginine on the rats treated with alloxan, recovery of endothelium-dependent relaxation in patients with type 2 diabetes (Vasilijevic et al., 2007) and improvement of endothelial cell function (Lucotti et al., 2006). L-arginine stimulates NO production by endothelial cells (Fu et al., 2005) that contributes to the increase in blood flow causing congestion of blood vessels (Park et al., 2007; Praphatsorn et al., 2010; Tan et al., 2012).

The current work revealed neutrophile infiltration, dilatation of rER, damaged mitochondria, cytoplasmic vacuoles, decrease of secretory granules in both acinar and beta-cells and the appearance of pancreatic stellate cell (PSC) in subgroup IIa. These results were in accordance with those of Adeghate et al. (2006), who stated that endoplasmic reticulum expansion may be the early sign of beta-cell damage. In addition, intracellular vacuoles resulted from lipid accumulation as a consequence of continuous oversupply of FFAs (caused by enhanced lipolysis and adipocyte dysfunction) causes impairment of FFA oxidation in the mitochondria (Morino et al., 2006) and increased endoplasmic reticulum stress (Özcan et al., 2004).

In agreement with the previous studies done by Zhang et al., (2008) and Yan et al., (2012) who detected inflammation and activation of PSC in high fat fed rats. A possible reason for observed inflammation may be due to sluggish metabolism in high-fat diet-fed animals. Many studies reported that administration of L-arginine (Lucotti et al., 2006) and exercise (Teixeira de Lemos et al., 2009) attenuate production of inflammatory cytokines. Oxidative stress and excess FFAs following HFD stimulate PSC to proliferate and induce repair mechanisms through production of abundant extracellular matrix, which is the most characteristic finding in pancreatic fibrosis (Siech et al., 2009). Amelioration of fibrosis by inhibiting oxidative stress in pancreas (Gómez et al., 2004; Miyauchi et al., 2007; Tasci et al., 2007) via exercise and L-arginine may be the hope in near future.

In conclusion, exercise decreased the deleterious histological effects of HFC but supplementation of L- arginine gave more improvement to all measured fields in this study. Further studies directed on the histological effect of combination of both exercise and L- arginine may be required in the future.

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Conflicts of interest

The authors declare no conflict of interest.

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