Impact of chronic exercise on counteracting chronic stress-induced functional and morphological pancreatic changes in male albino rats

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Abstract

Chronic stress has been linked to many diseases resulted from dysfunction of both the nervous system and peripheral organ systems. Yet, the effects of chronic stress on the pancreas have received relatively little attention. This work aims to investigate the influence of chronic stress exposure on both the endocrine and exocrine pancreatic function and morphology and its possible mechanism of action, and also to evaluate the impact of chronic exercise with moderate intensity on ameliorating the stress-induced pancreatic changes. Forty adult male albino rats were used and divided into four groups: control group, exercised group (3 weeks of swimming exercise), stressed group (3 weeks of immobilization stress), and stressed group practicing exercise (3 weeks of exercise, concomitant with 21 daily sessions of stress). On the final day of the experiment, all rats were sacrificed. Biochemical, immunohistochemical, and histological studies were conducted. The results showed that chronic immobilization stress produced hyperglycemia, hyperinsulinemia, and increased homeostatic model assessment of insulin resistance index (HOMA-IR) with increasing exocrine pancreatic injury markers by increasing oxidative and inflammatory status of the pancreatic tissue. Histological study showed the injurious effect of stress on the morphology of pancreatic tissue. Physical exercise protected the pancreas from the negative effects of stress through its anti-inflammatory and anti-oxidative effects, evidenced by increasing pancreatic interleukin 10 and total antioxidant capacity and decreasing pancreatic tumor necrosis factor-alpha, and malondialdehyde with ameliorating most of the histological changes induced by stress exposure. Physical exercise effectively counteracts chronic stress-induced pancreatic changes through different mechanisms.

Keywords Pancreas · Exercise, stress · Inflammation · Oxidative stress

Introduction

Stress is defined as the response of the brain and the body to stimuli arising from the internal and environment signals (Goldstein and McEwen 2002). Normally, the body responds to the stressful stimuli in a physiological way. The hypothalamic–pituitary–adrenal (HPA) axis and the sympathetic

nervous system (SNS) are woken up to release chemical mediators in order to protect the body from stress. In contrast, prolonged traumatic or psychological stress can lead to cellular and systemic equilibria disruption, leading to dysfunction of both the nervous and peripheral organ systems with consequent development of both psychiatric and physical disorders, which is called stress-related disease (Duric et al. 2016).

The traditional mechanisms linking stress and disease have focused on activation of the HPA axis and the SNS. Moreover, alterations in HPA axis and SNS have indirect effects on target systems (Rohleder 2014). Lately, inflammation is proposed strongly to link stress to stress-related diseases, and many studies reported that excessive inflammation directly contributes to the pathophysiology of stress-related diseases (Liu et al. 2017). Binker and Cosen-Binker (2014) reported that chronic stress appeared as a risk factor to develop pancreatitis by sensitizing the exocrine pancreas through tumor necrosis factor-alpha (TNF- α).

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One of the factors that protect against stress, the so-called stress buffering, is physical activity. Exercise is commonly assumed to be a behavioral approach for relieving stress, and the direct relationship between exercise and health is well established for many pathological disorders as coronary heart disease, metabolic syndrome, bone mineral density, gastrointestinal disorders, and life satisfaction or depression (Gerber and Puhse 2009; Seo et al. 2014; ter Steege and Kolkman 2012), and also there is evidence that physical activity may modify the inflammatory process among the tissue (Geffken et al. 2001).

The positive effect of physical exercise on insulin sensitivity is well established. Due to the relationship between exercise and the improvement in glycemic homeostasis, some studies set a relationship between exercise and insulin secretion by pancreas (Almeida et al. 2012).

Previous studies reported that the exocrine pancreatic dysfunction could be induced by various physical conditions such as diabetes, obesity, and aging. Moreover, dietary components and food consumption had been reported to affect the pancreatic enzyme synthesis and secretion. However, little is known about the effects of physical exercise on the exocrine pancreatic function (Shiroya and Minato 2015).

The current work was designed to study two main points: first to investigate the influence of chronic immobilization stress exposure on both the endocrine and exocrine pancreatic function and morphology and its possible mechanisms of action, and second to shed more light on the impact of moderate-intensity physical activity represented by chronic swimming exercise on counteracting the stress-induced pancreatic changes.

Materials and methods

Ethical approval

Minia University Faculty of Medicine, Research Ethics Committee “FMREC” approved this research proposal regarding source of the animals, health status, inclusion criteria, exclusion criteria, caging, comfort, and the detailed experimental design and procedures. It was conducted in agreement with the NIH Guide for Care and Use of Laboratory Animals (Health and Services 1992).

Experimental groups and animals

Forty adult male healthy Sprague–Dawley albino rats (8–10 weeks) weighing between 200 and 250 g were used throughout the present study. Rats were purchased from the National Research Center, Cairo, Egypt. Rats were housed in hygienic plastic cages in a clean, well-ventilated room and

were given free access to food (a standard diet of commercial rat chow) and water with normal light and dark cycles. Rats were left to acclimatize to the environment for 2 weeks prior to inclusion in the experiment. The rats were divided randomly into four different groups of 10 animals each: control group (sedentary and housed without disturbances), exercised group (3 weeks of swimming exercise), stressed group (3 weeks of immobilization stress), and stressed group practicing exercise (3 weeks of exercise, concomitant with 21 daily sessions of stress). This study was conducted in Minia University, Faculty of Medicine.

Models

Swimming exercise was done in a cylindrical tank of 100 cm diameter and 50 cm depth, containing water at 30–32 °C. The rats in groups promote more forceful exercise than rats allowed to swim alone, so individual swimming was preferred so as not to produce a stress effect by the forceful exercise. Rats were allowed to acclimate to the shallow water previous to the experiment in order to reduce stress. Swimming was accomplished between 8:00 and 9:00 A.M. in order to exclude diurnal effect. The swimming period was initially by 15 min/day and was then increased gradually until reaching 60 min/day. After a week of this training period, the rats were allowed to swim for 60 min/day, five times a week, for 3 weeks (Aydin et al. 2009). Swimming exercise displays advantages over other models of exercise and is commonly used as a proper model of physical exercise, as it belongs to the natural behavior of a rat, which under appropriate experimental circumstances mainly involves physical exercise with minimal emotional arousal, and it can be considered as moderate-intensity exercise (Lima et al. 2013).

Immobilization stress rats were immobilized for 60 min/day for 21 days. Rats were immobilized individually in 15–19-cm long and 5-cm-wide well-ventilated plastic rodent restrainers that allowed for a close fit to rats. Animals were unable to barrel-roll or turn. Immobilization stress was considered an easy and convenient way to induce both psychological stress (escape reaction) and physical stress (muscle work), resulting in restricted mobility and aggression (Dhanalakshmi et al. 2007). It was supposed to be the most severe type of stress in rodent and has a comparative effect in humans (Gilgun-Sherki et al. 2002). The duration of immobilization stress has been displayed to be sufficient to produce manifest elevations in corticosterone, which is consistent with a physiologic stress response (Bielajew et al. 2002). In this study, measurement of serum corticosterone levels confirmed stress exposure.

Stressed group practicing exercise was allowed to swim for 3 weeks, concomitant with 21 daily sessions of stress.

Animal sacrifice, sample collection, and parameters measured

On the last day of the experiment, all rats were sacrificed by decapitation, but without anesthesia after 10 h of fasting. Rats exposed to immobilization stress were sacrificed 5 min following the end of the stressor. Blood samples were collected immediately in 10-ml Eppendorf tubes, left to clot, and then delivered into centrifuge tubes to be centrifuged at 3000 rpm for 20 min; serum samples were separated in 2-ml Eppendorf tubes to be used immediately as fresh samples (preferred) or to be stored on -20°C until used. Serum samples were used to determine serum corticosterone using enzyme-linked immunosorbent assay kit (ELISA) from Enzo Life Sciences, Inc., USA; serum amylase and serum lipase by enzymatic calorimetry from Bio-Assay, USA; serum insulin level using ELISA kits from RayBio®, USA; and serum glucose by enzymatic calorimetry from Biodiagnostic, Giza, Egypt.

The pancreas of the rats were rapidly dissected out and were divided; some specimens were stored at -80°C for tissue assay, and some specimens were fixed for paraffin embedding.

Pancreas specimens were weighed and then homogenized separately in potassium phosphate buffer 10 mM; pH 7.4. The ratio of tissue weight to homogenization buffer was 1:10. The homogenates were centrifuged at 5000 rpm for 10 min at 4°C . The resulting supernatant was used for determination of tumor necrosis factor- α (TNF- α) by ELISA kit from MyBioSource, USA; interleukin-10 assay (IL-10) by ELISA kit from MyBioSource, USA; malondialdehyde (MDA) using colorimetric assay kit from MyBioSource, USA; and total antioxidant capacities (TAC) using colorimetric assay kit from MyBioSource, USA.

Homeostatic model assessment of insulin resistance (HOMA-IR) index

In order to determine HOMA-IR index, the values of fasting plasma glucose and insulin levels were used; the formula is $\text{HOMA-IR} = (c_i \times c_g)/22.5$, where c_i is fasting insulin level ($\mu\text{U/ml}$) and c_g is fasting glucose level (mmol/l) (Fukushima et al. 1999).

Histological and immunohistochemical study

Small specimens were taken from the pancreatic tail tissue, rapidly fixed at 10% buffered formalin, and then subjected to study by light microscopy.

Tissues were proceeded to:

- 1- Hematoxylin and eosin (H&E) (Suvana et al. 2018)
- 2- Masson trichrome (Suvana et al. 2013)

- 3- Immunohistochemical staining for estimation of anti-insulin antibody using the avidin–biotin peroxidase system was done. Rabbit mono-clonal anti-insulin antibodies [Abcam, USA (ab181547)] was used at a dilution of 1:500 followed by counterstaining with hematoxylin according to the manufacturer's guidelines (Côté 1993).

Positive tissue control: Slides of control rat pancreatic tissue.

Negative control: Slides of control pancreatic tissue were subjected to the same previous steps, but skipping the step of using the primary antibody.

Image capture

The sections were examined using a light microscope (Olympus, Japan). Photomicrographs were digitally captured using a high-resolution color digital camera (Olympus, Japan) adapted to the microscope, connected to a computer.

Measuring the mean area fraction of anti-insulin immunoreactivity

Image J 22 software (open-source Java image processing program) was used. Area fraction was measured in a standard measuring frame per eight photomicrographs in each group using a magnification $\times 400$ by light microscope connected to the monitor screen. The areas containing positive immunoreaction tissues proceeded for measuring (Ibrahim and Khaled 2014).

Morphometric analysis

Quantitative data were collected for three parameters:

1. *Pancreatic histological scoring* was performed on the H&E-stained slides from each rat, using power $\times 400$. Scoring was carried out of 0–4 of the following parameters: degeneration, lymphocytic infiltration, and hemorrhage by counting the mean number of affected foci, as follows: 0 = absent, 1 = mild, 2 = moderate, 3 = severe, and 4 = overwhelming (Binker et al. 2010b). The degrees of all previous parameters were measuring semi-quantitatively in 10 random fields within each slide (three sections per animal).
2. *Mean diameter of islets of Langerhans.* Measurement of the average islet core diameter and excluded the extension areas were done. This was carried out using power $\times 400$ from 10 successive non-overlapping fields.
3. *The mean number of anti-insulin β cells in the islet.* The mean numbers of immunoreactive β cells were measured semi-quantitatively. Measurements were performed in 10 random fields from each rat using 400 power fields (Tulic et al. 2009). Counting the nuclei of all positive cells inside one islet in the field was done. A total number of 10 islets for each rat were counted.

Table 1 Serum corticosterone level in the different studied groups

Parameters	Control group	Exercised group	Stressed group	Stressed group practicing exercise
Serum corticosterone (pg/ml)	234 ± 19	272 ± 29	515 ± 42 ^{ac}	308 ± 35 ^{ab}

Results represent the mean ± SE ($n = 8$). ^a Significant difference from control group, ^b significant difference from stressed group, and ^c significant difference from exercised group, $P < 0.05$

Data handling and statistical analysis

Data analysis was performed using SPSS version 20 (SPSS Inc., Chicago, Illinois, USA). Data were expressed as mean ± standard error (mean ± SE). Significant differences between groups were carried out by one-way ANOVA. P value ≤ 0.05 was considered statistically significant.

Results

Biochemical analysis

Serum corticosterone

As shown in Table 1, there was no significant change in the serum corticosterone level in the exercised group if compared to the control group, while in the stressed group there was a significant increase in its level if compared to the control group. Exercise in the stressed group practicing exercise reversed the effect of stress and produced a highly significant decrease in its serum level if compared to the stressed group ($P < 0.05$).

Endocrine pancreatic parameters

In Table 2, the exercised group showed a significant decrease in fasting serum glucose and insulin levels if compared to the control group, with decrease in insulin resistance as indicated by the significant decrease in HOMA-IR level. Chronic stress exposure produced a reversal effect since there was a significant increase in fasting serum glucose, fasting serum insulin, and HOMA-IR levels in the stressed group compared to the control group. Chronic exercise in

the stressed group practicing exercise antagonized the effect of stress on these parameters and produced a significant decrease in their levels if compared to the stressed group ($P < 0.05$).

Serum exocrine pancreatic injury markers

In exercise group, there was no significant change in the exocrine pancreatic injury markers, serum amylase and serum lipase, in the exercised group compared to the control group, while in the stressed group there was a significant increase in their levels if compared to the control group. Chronic exercise in the stressed group practicing exercise reversed the effect of stress and produced a significant decrease in their serum levels if compared to the stressed group ($P < 0.05$) (Table 3).

Pancreatic tissue oxidative and inflammatory parameters

In Table 4, the exercised group showed a significant decrease in the pancreatic tissue MDA and the proinflammatory cytokine, TNF- α , with significant increase in the pancreatic tissue TAC and the anti-inflammatory cytokine, IL-10, compared to the control group ($P < 0.05$).

Stressed group showed a reversal effect with significant increase in the pancreatic tissue MDA and TNF- α , while it caused a significant decrease in the pancreatic tissue TAC and IL-10 if compared to the control group ($P < 0.05$).

Chronic exercise in the stressed group practicing exercise reversed the effect of stress and produced a significant decrease in the pancreatic tissue MDA and TNF- α , with a significant increase in the pancreatic tissue TAC and IL-10 if compared to the stressed group ($P < 0.05$).

Table 2 Endocrine pancreatic markers in the different studied groups

Parameters	Control group	Exercised group	Stressed group	Stressed group practicing exercise
Fasting serum glucose (mmol/L)	4.2 ± 0.5	3.3 ± 0.2 ^a	9.4 ± 1.1 ^{ac}	5.6 ± 0.7 ^{abc}
Fasting serum insulin (μ IU/ml)	30.6 ± 2.5	25.5 ± 1.9 ^a	70.3 ± 4.1 ^{ac}	37.8 ± 2.6 ^{abc}
HOMA-IR	5.7 ± 0.6	3.7 ± 0.2 ^a	29.4 ± 1.6 ^{ac}	9.4 ± 0.9 ^{abc}

Results represent the mean ± SE ($n = 8$). HOMA-IR: homeostatic model assessment of insulin resistance index. ^a Significant difference from control group, ^b significant difference from stressed-group, and ^c significant difference from exercised group, $P < 0.05$

Table 3 Exocrine pancreatic injury markers in the different studied groups

Parameters	Control group	Exercised group	Stressed group	Stressed group practicing exercise
Serum amylase level (U/L)	14.2 ± 1.3	15.4 ± 2.1	39.8 ± 3.2 ^{ac}	22.7 ± 1.9 ^{abc}
Serum lipase level (U/L)	485.9 ± 19.7	510.4 ± 30.5	1460.5 ± 34.2 ^{ac}	780.3 ± 25.8 ^{abc}

Results represent the mean ± SE ($n = 8$). ^a Significant difference from control group, ^b significant difference from stressed group, and ^c significant difference from exercised group, $P < 0.05$

Histological results

H&E results

Pancreatic sections of the control and exercised groups exhibited the same morphological picture; they showed normal pancreatic tissue architecture. The pancreatic tissue formed of lobules separated by thin interlobular septa containing blood vessels and ducts. The pancreatic lobules consisted of both endocrine and exocrine portions. Regarding the endocrine portion, it was formed by islets of Langerhans; pale areas between the exocrine acini. The islet's cells were polygonal in shape; they had central rounded vesicular nuclei. Most of the peripherally situated cells had darkly stained nuclei. The pancreatic acini consisted of pyramidal shape cells with basal basophilia and apical acidophilia (Figs. 1 and 2).

Regarding the stressed group, immobilization stress resulted in variable morphological changes in the pancreatic tissue. One of the most characteristic findings of this group was the distorted lobular architecture. Wide inter-lobular septa occupied by invasion of massive connective tissue adipose tissue were noticed. Inter-lobular septa showed dilated arteries and veins surrounded by pale edematous areas. Dilated ducts lined with flattened epithelium with retained secretion were clearly noticed. Moreover, most Langerhans cells had small dark nuclei. Others appeared with distorted shapes and showed less cellularity if compared with the two previous groups. Regarding the acini, most acini showed different focal morphological changes; some acinar cells appeared with no acidophilia, others appeared with no basophilia while others did not show neither acidophilic nor basophilic portions. In most sections, the acinar cells appeared with pyknotic nuclei

along with numerous cytoplasmic vacuolations. Inflammatory cell infiltrations were frequently seen (Fig. 3).

In the stressed group practicing exercise, it was noticed that exercise ameliorated most of the previous morphological changes occurred in the stressed group. More or less normal pancreatic lobules were noticed in this group. Less apparent widening of interlobular septa and less notable dilated ducts and blood vessels were observed. The islets showed apparent more cellularity if compared with the stressed group. But some dilated congested blood capillaries were seen between Langerhans cells. Most Langerhans cells appeared with vesicular nuclei. Regarding the acini, it approached the corresponding healthy pancreatic slides. Almost all acini retained their acidophilic and basophilic portions and appeared with vesicular nuclei (Fig. 4).

Masson's trichrome stain

Control and exercise groups stained by Masson's trichrome showed delicate amounts of collagen fibers surrounding interlobular ducts, arteries, and veins. While in the stressed group, massive collagen fibers were seen surrounding all previously mentioned structures. Furthermore, interlobular diffuse collagen invasion was noticed. In stressed group practicing exercise, little amount of collagen fiber deposition around the previous mentioned areas was seen (Fig. 5).

Immunohistochemical staining for anti-insulin

The negative control slides showed no immune reactions while the positive control slides showed β cells with brown cytoplasmic staining (Fig. 6).

Table 4 Oxidative and inflammatory status of the pancreatic tissue in the different studied groups

Parameters	Control group	Exercised group	Stressed group	Stressed group practicing exercise
Pancreatic tissue TNF- α (pg/ml)	18.3 ± 1.9	12.8 ± 2.1 ^a	38.9 ± 2.3 ^{ac}	22.6 ± 1.5 ^{abc}
Pancreatic tissue IL-10 (pg/ml)	17.8 ± 1.5	22.5 ± 1.1 ^a	9.3 ± 0.7 ^{ac}	20.4 ± 2.1 ^{ab}
Pancreatic tissue TAC (μ M/ml)	86.5 ± 3.1	98.4 ± 4.2 ^a	49.5 ± 2.4 ^{ac}	76.3 ± 3.2 ^{abc}
Pancreatic tissue MDA (μ M/ml)	42.2 ± 2.2	35.4 ± 1.7 ^a	75.9 ± 3.7 ^{ac}	56.1 ± 3.1 ^{abc}

Results represent the mean ± SE ($n = 8$). TNF- α , tumor necrosis factor- α ; IL-10, interleukin-10; TAC, total antioxidant capacities; MDA, malondialdehyde. ^a Significant difference from control group, ^b significant difference from stressed group, and ^c significant difference from exercised group, $P < 0.05$

Fig. 1 Photomicrographs of rat pancreatic tissue of the control group showing (a1, a2) pancreatic lobules (P) that consist of the exocrine portion (E); composed of closely packed secretory acini and the pale-stained area; endocrine portion (islets of Langerhans (gl)). The interlobular CT septum (curved line) separates these lobules (L) and contains blood vessels (BV) and duct (D). (b) Islets of Langerhans consist of endocrine cells arranged into cords that have both pale (black arrowhead) and dark (yellow arrowhead) cells. Blood sinusoids (arrow) are seen between these cells. (c) Pancreatic acini formed of pyramidal-shaped acini with basal vesicular nuclei with prominent nucleoli (black arrow). The acinar cells display intense basal basophilia (yellow arrow) and apical acidophilia (star). H&E, scale bar: a1, a2 $\times 200 \mu\text{m}$; b, c $\times 50 \mu\text{m}$

In control and exercised groups, the β cells displayed positive cytoplasmic expression occupying most islet cells with negative reaction in the acini, while in stressed group sections,

few positive immunostained cells appeared in apparently shrunk islets. Other few cells showed faint immunoreaction, while other β cells displayed a completely negative reaction.

Fig. 2 Photomicrographs of rat pancreatic tissue of the exercise group showing (a) pancreatic lobules (P) that consist of the exocrine portion (E); composed of closely packed secretory acini and the pale-stained area; endocrine portion (islets of Langerhans (gl)). The interlobular CT septum separates these lobules (L) and contains blood vessels (BV) and duct (D). (b) Islets of Langerhans consist of endocrine cells arranged into cords that have both pale (dark arrowhead) and dark (yellow arrowhead) cells. Blood sinusoids (arrow) are seen between these cells. (c) Secretory acini formed of pyramidal-shaped acini with basal vesicular nuclei with prominent nucleoli (black arrow). The acinar cells display intense basal basophilia (yellow arrow) and apical acidophilia (star). H&E, scale bar: a $\times 200 \mu\text{m}$; b, c $\times 50 \mu\text{m}$

Fig. 3 Photomicrographs of rat pancreatic tissue of the stressed group showing (a) distorted lobular architecture (D), wide inter-lobular septa (curved line), and the invasion of massive CT adipose tissue (ad). (b) Dilated artery (A) and vein (V) surrounded by edematous area (star). (c) Most Langerhans cells become dark and small (circle). (d) Islet of Langerhans (gl) appears with less cellularity. (e) Dilated duct lined with flattened epithelium (arrow) with retained secretion (D) is seen. (f) Some

acini appear with no acidophilic portion (yellow star); others appear with no basophilic nor acidophilic portion (blue star). Notice the pyknotic nucleoli (arrows). (g) Acinar vacuolations (V) and inter-acinar inflammatory cells (circle). Some acini lost their basophilic part (arrow). Notice the severe dilated intra-lobular duct (D). H&E, scale bar: a, b $\times 200 \mu\text{m}$; c, d, e, f, g $\times 50 \mu\text{m}$

Regarding the stressed group practicing exercise, more positive reaction in apparently more numerous β cells was clear seen while other cells still showed negative reaction (Fig. 6).

Morphometric analysis

The mean areas containing degeneration, inflammatory cell infiltration, and hemorrhage in all studied groups

Stressed group showed a highly significant increase in all these parameters while stressed group practicing exercise showed a significant decrease in all previously mentioned parameters if compared with the stressed group (Table 5).

The mean area fraction of anti-insulin immunopositive cells

No significant difference between control and exercised groups was detected ($P > 0.05$). There was a highly significant decrease in the mean area fraction of anti-insulin expression in the stressed group if compared to the control and exercised

groups ($P < 0.0001$). Meanwhile, stressed group practicing exercise exhibited a high significant increase in this parameter if compared with stressed group (all $P < 0.001$) (Table 6).

The mean diameter of islets (μm) and the mean number of insulin positive β cells per islet

No significant difference between control and exercised groups was detected ($P \geq 0.05$), but a highly significance decrease in the two parameters was estimated in stressed group if compared to the previous two groups (all $P < 0.001$). Comparing stressed group practicing exercise to stressed group, there was a highly significant increase in these parameters (all $P < 0.001$) (Table 6).

Discussion

Stress activates the hypothalamic pituitary adrenocortical (HPA) with elevating the levels of glucocorticoids (GCs)

Fig. 4 Photomicrograph of rat pancreatic tissue of the stressed group practicing exercise showing (a) more or less normal pancreatic lobules (P). Less notable dilated artery (A) and vein (V) are observed. Notice the less apparent widening of interlobular septae (curved line). (b) Islet of Langerhans (gl) showing apparent increase in islet cellularity. Notice the less numerous inflammatory cells (circle). Dilated congested blood capillaries (arrows) are seen between Langerhans cells. Most Langerhans cells appear with vesicular nuclei. (c) Less dilated intra-lobular duct (D). Almost all acini retain their apical acidophila and basal basophilia. Most acinar cells appear with vesicular nuclei (arrows). H&E, scale bar: a $\times$ 200 μ m; b, c $\times$ 50 μ m

(cortisol in humans; corticosterone in rodents) which are part of the humoral adaptive response to the stressor (Sapolsky et al. 2000). This explains the significant

increase in the serum corticosterone level observed in the present study with stress exposure and verifies the efficacy of the stress model.

Fig. 5 Photomicrographs of rat pancreatic tissue. (a, b) Control and exercise groups, respectively, showing scant collagen fibers (arrows) surrounding duct (D), artery (A), and vein (V). (c1, c2) Stressed group. (c1) Massive collagen deposition around the dilated interlobular artery (A) and vein (V) is noticed. (c2) Notice the interlobular diffuse collagen

invasion (star) and the intense collagen (arrow) surrounding interlobular duct (D). (d) Stressed group practicing exercise showing less apparent collagen fiber deposition around duct (D), artery (A), and vein (V). Masson trichrome, scale bar $\times$ 200 μ m

Fig. 6 Photomicrographs of rat pancreatic tissue immunostained for anti-insulin. (a) Negative control for anti-insulin. (b, c) Group I and group II respectively displaying positive cytoplasmic immunostained β -cells staining (white arrow) occupying most of the islet's cells (white arrows) with negative reaction in the acini (stars). (d) Stressed group showing few positive immunostained β cells in apparently shrunken islet (white

arrow). Few cells show weak immunoreaction (black arrow), while other cells display a negative reaction (yellow arrow). (e) Stressed group practicing exercise showing positive reaction in apparent more numerous β cells (black arrow). Other β cells show negative reaction (yellow arrow). Immunohistochemistry, counterstained with H, scale bar $\times 50 \mu\text{m}$

Previous studies reported that stressors could impair neuroendocrine, reproductive, and metabolic functions (Rosmond 2005). In the current study, chronic stress affected the functional and morphological structure of the endocrine and exocrine pancreas; it was reported that the inter-relationship of the endocrine–exocrine parts of the pancreas is a complex one because of the close functional and anatomical links (Soliman 2012).

Regarding the endocrine pancreatic function, serum biochemical analysis revealed that chronic stress produced a significant increase in the serum insulin and glucose levels, with increasing insulin resistance reflected by increasing HOMA-IR index. These results come in line with previous studies (Eguchi et al. 2011; Rafacho et al. 2010; Zardooz et al. 2012) that reported that stress affects the endocrine pancreas and can lead to the production of diabetes mellitus by different mechanisms.

Previous studies reported higher β -cell glucose transporter 2 (GLUT2) and insulin receptor substrate 2 (IRS-2) protein expression in animals that were treated with a synthetic glucocorticoid (Rafacho et al. 2008), leading to hyperinsulinemia that resulted from increasing the glucose-stimulated insulin secretion (GSIS). However, some other *in vivo* studies propose that the increase in GSIS is a direct result of increased insulin resistance from peripheral tissues which drive insulin secretion (Rafacho et al. 2011). Glucocorticoids were reported to have an insulin-resistant effect in most tissues, including skeletal muscle, adipose, and brain, through impairments in the translocation of the glucose transporter 4 (GLUT4) protein (Piroli et al. 2007) in addition to up-regulating the key hepatic gluconeogenic enzymes, such as phosphoenolpyruvate carboxykinase and glucose-6-phosphatase (G6P), both *in vitro* and *in vivo* (Van Raalte et al. 2009).

Table 5 Effect of stress on the pancreatic morphology in the studied groups

	Control group	Exercised group	Stressed group	Stressed group practicing exercise
Degeneration and necrosis	0.6250 ± 0.2631	0.7500 ± 0.2500	5.000 ± 0.6268^{ac}	3.250 ± 0.3660^{abc}
Mononuclear cellular infiltration)	0.3750 ± 0.1830	0.3750 ± 0.1830	20.625 ± 1.711^{ac}	4.125 ± 0.7181^{abc}
Hemorrhage	0.000 ± 0.000	0.000 ± 0.000	4.375 ± 0.6529^{ac}	2.000 ± 0.3273^{abc}

Results represent the mean $\pm$ SE ($n = 8$). ^a Significant difference from control group, ^b significant difference from stressed group, and ^c significant difference from exercised group, $P < 0.05$

Table 6 Effect of stress on the mean area fraction of anti-insulin, the mean diameter of islets (m), and insulin-positive β cells per islet (β cells/islet) in the studied groups

	Control group	Exercised group	Stressed group	Stressed group practicing exercise
Mean area fraction of anti-insulin	5.200 $\pm$ 0.7297	45.671 $\pm$ 0.8403 ^b	11.473 $\pm$ 0.7232 ^{ac}	35.100 $\pm$ 0.6586 ^{abc}
Mean diameter of islets (m)	137.38 $\pm$ 0.9051	140.88 $\pm$ 1.777	60.000 $\pm$ 1.909 ^{ac}	107.88 $\pm$ 6.672 ^{abc}
Insulin-positive β cells per islet (β cells/islet)	8.250 $\pm$ 0.7500	86.125 $\pm$ 0.9149	26.625 $\pm$ 2.138 ^{ac}	47.375 $\pm$ 1.438 ^{abc}

Results represent the mean $\pm$ SE ($n = 8$). ^aSignificant difference from control group, ^bsignificant difference from stressed group, and ^csignificant difference from exercised group, $P < 0.05$

The glucocorticoid-induced peripheral insulin resistance affects the β cell and leads to an increase in insulin biosynthesis for compensatory adaptations (Rafacho et al. 2009).

The two pancreatic enzymes, amylase and lipase, were considered consistent biomarkers of laboratory diagnosis for the exocrine pancreatic injury in both humans and animals; their serum concentrations rise within hours of the pancreatic injury (Carroll et al. 2007; Smith et al. 2005). In the present study, the stressed group showed a significant increase in serum amylase and lipase levels if compared to the control group indicating the marked stress-induced injury in the exocrine pancreatic tissue. Muniraj et al. (2015) reported that the destruction of the pancreatic acinar cells resulted in increased serum pancreatic enzyme levels as in pancreatitis and pancreatic cancer.

In an attempt to explain the mechanism beyond the stress-induced pancreatic function and structure abnormalities, some oxidative stress and inflammatory markers were measured in the pancreatic tissue.

Regarding the oxidative status of the pancreatic tissue with chronic stress exposure, the results of the present study showed a significant increase in the level of pancreatic tissue MDA with significant decrease in TAC. TAC, which considers the cumulative effect of all antioxidants, and MDA can be used as general biomarkers for biological oxidative stress (Kadiiska et al. 2005). This finding is consistent with the finding of some previous studies (Şahin and Gümüslü 2004) who stated that stress could disrupt the balance of the oxidant/antioxidant system and cause oxidative damage to several tissues and affecting tissue function by altering the enzymatic and non-enzymatic antioxidant status. Binker et al. (2010a) reported that the stress-induced pancreatic injury in the pancreatic islet and acinar cells might be attributed to the generation of reactive oxygen species (ROS) due to oxidative stress.

Concerning the inflammatory status of the pancreatic tissue with chronic stress exposure, the results of the present study showed a significant increase in the level of pancreatic tissue pro-inflammatory cytokine TNF- α with a significant decrease in the anti-inflammatory cytokine IL-10. These findings come in line with the results of Miller et al. (2009) that reported that

severe stress overactivates the immune system, leading to the imbalance of inflammation and anti-inflammation. Reports from different studies have confirmed pro-inflammation induced by stress, including C-reactive protein (CRP), IL-6, TNF- α , IL-1 β , and the transcription factor of “nuclear factor kappa B (NF- κ B)” in many cell types (Gu et al. 2009; Mazzon and Cuzzocrea 2008; Munhoz et al. 2004; Pavlovic et al. 2008), including pancreatic acinar cells (Kim et al. 2008; Liu et al. 2009). Moreover, stress-induced oxidative stress and generation of ROS, proved in the present study and reported in previous studies, might also be implicated in the increase of all inflammatory parameters (Soliman 2012).

In the present study, the results of the biochemical analysis correlated with many cellular and morphological changes observed upon stress exposure in the endocrine–exocrine pancreatic portion. Histologically, in some sections of the stressed group, the general shape of the islets of Langerhans was dramatically changed. Afifi (2012) reported that high serum glucose level resulted in stimulation of fibronectin and collagen I and III secretion by endothelial cells, leading to enhanced fibrosis and loss of islet shape. Moreover, the stressed group showed marked reduction in Langerhans cellularity with less numerous insulin immunoreactivity, and this was further confirmed by the statistical analysis.

For estimation of the effect of the stress in increasing extracellular matrix formation, Masson's trichrome stain was carried out. In stressed group, intense collagen depositions were detected. It was reported that insulinopenia might cause such damaging hazards on pancreas (Ricciolo et al. 2004). Apte et al. (2009) localized the pancreatic stellate cell (PSCs) in the islets, upon stimulation, in response to ROS; these cells started to divide, change their morphology into myofibroblast-like cells, and began to release extracellular matrix components.

The hazardous effects of stress on the β cells were supported by the immunohistochemical results; the rats exposed to stress revealed less numerous insulin immunoreactivity, and this was further confirmed by the statistical analysis.

In the stressed group, the edematous areas surrounding the dilated blood vessels were clearly noticed. This edema could be explained as the damaging effect of oxidation on capillary

endothelial membranes that resulted in enhanced microvascular permeability and edema (Shidham et al. 2001). However, other researchers (Takács et al. 2002) stated that endogenous nitric oxide (produced in response to high ROS) led to an increase in vascular permeability and protein extravasations.

Inter-lobular massive adipocytes were detected also in the stressed group. The same results were reported in a previous study and were recorded as pancreatitis (Bello et al. 2017).

Histologically, the focal loss of acidophilic part seen in the stressed group was an indicator for the decrease of pancreatic zymogen, and it was due to increased intracellular calcium as a result of high ROS, which in turn resulted in the disturbance of the apical zymogen granule exocytosis and calcium-induced gene transcription, which in turn inhibited the pancreatic secretory function (Brown et al. 1997).

Furthermore, the stressed group showed inter-lobular inflammatory cell infiltration beside apoptotic cells. Fonseca et al. (2010) stated that ROS release from exposure to stress could be implicated in the enhancement release of almost all inflammatory mediators in pancreatic tissue. Also, ROS might directly damage DNA with subsequent enhanced apoptotic process (Binker et al. 2010a). Furthermore, stress-induced increase in TNF- α was strongly suggested to be the cause of apoptosis in pancreatic cells (Binker et al. 2010a).

In studying the effect of chronic swimming exercise, it was found that contrary to the effects of acute swim stress on rats (Contarteze et al. 2008), the long-term chronic swimming exercise elicited no stress response in rats as reflected in the present study by corticosterone levels in the exercised group; however, it significantly decreased the serum corticosterone level in the stressed group practicing exercise if compared to the stressed group.

In the stressed group, there was a significance decrease in Langerhans' islet diameter, indicating their severe distortion. Also, there was a significant decrease in immunopositive cells for anti-insulin, and it referred to the marked damage in these cells. Also, the surface area fraction of β cells was decreased in this group if compared with the control one. The affection of β cell was matching with the significance increase in serum glucose level.

Regarding the effect of chronic exercise training on the endocrine pancreatic function, the exercised group showed a significant decrease in the glucose level, insulin level, and HOMA-IR index if compared to the control group. In the exercise stressed group, exercise counteracts the stress-induced increase in insulin level, glucose level, and HOMA-IR. These results were explained by Teixeira de Lemos et al. (2011) who demonstrated that chronic exercise produced a significant decrease in plasma insulin level owing to improved peripheral insulin resistance rather than changes in the β cells. Not as much insulin was needed in the exercised groups because contraction of the muscle during exercise increased the

transport of glucose into the muscle without insulin and increased GLUT4 to the plasma membrane to increase the effectiveness of insulin which also decreases plasma glucose level (Rawal et al. 2013).

Regarding the two pancreatic enzymes, amylase and lipase, exercise training alone failed to produce any significant changes in their levels. However, in the stressed group practicing exercise, the level of these enzymes was significantly decreased if compared to the stressed group, which indicated that exercise reduced the stress-induced exocrine pancreatic injury.

Although many studies verified the positive impact of exercise on stress system dysregulation and comorbidities (Booth et al. 2012; Koo and Kim 2018; Tsatsoulis and Fountoulakis 2006), the exact mechanisms for exercise to counteract stress have not been established. In the present study, histological and biochemical analyses of the pancreas were done in an attempt to understand the possible mechanisms beyond exercise-induced pancreatic effects with and without chronic stress.

Biochemical analysis of the pancreatic tissue revealed that in the exercised and exercise stressed groups, there was a significant reduction in the level of MDA and TNF- α with a significant increase in the level of TAC and IL-10 if compared to the control and stressed groups, respectively, revealing the anti-oxidant and anti-inflammatory effect of chronic exercise.

These results come in line with previous studies which reported that regular exercise attenuated the oxidative damage in the brain (Marosi et al. 2012), liver (Viboolvorakul et al. 2009), kidney (Navarro et al. 2004), skeletal muscle (Leeuwenburgh et al. 1994), blood (Franzoni et al. 2005), and heart (Starnes et al. 2003). It was reported that an active lifestyle and moderate exercise have been demonstrated to be useful in the prevention of oxidative stress since chronic exercise promoted the increase in antioxidant defenses, which removed free radicals and stabilized the reactive species production (Bouazid et al. 2018).

The antioxidant defense tools of the body consist of non-enzymatic antioxidants (coenzyme Q10, glutathione, uric acid, lipoic acid, bilirubin, etc.) and antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase, etc.). The exercise-induced ROS generation resulted in increased activity of enzymatic antioxidants, which then led to an increased resistance to the oxidative challenges with limiting free-radical production and oxidative damage (Simioni et al. 2018). The differential oxidative stress and antioxidant defense responses in the pancreatic tissue after exercise were also reported in Raza et al.'s (2016) study.

Regarding the inflammatory response in the exercised and exercise stressed group, the results of the present study agree with several interventional studies which reported that exercise reduced inflammatory markers, particularly CRP, TNF- α , interleukin-8, interleukin-18, and soluble IL-6 receptor

(Majka et al. 2009; Timmerman et al. 2008; Yu et al. 2009), while it increased the levels of anti-inflammatory factors such as interleukin-10, interleukin-12, interleukin-4, and transforming growth factor beta 1 (Lavie et al. 2009; Sloan et al. 2007; Stefanov et al. 2013; Yeh et al. 2009).

Histologically, the pancreatic tissue in the stressed group practicing exercise restored nearly its normal general architecture if compared with the stressed group. This improvement is in accordance with Puterman et al. (2014) who suggested that physical activity was a good modifiable behavior that could limit the hazardous health effects of ongoing stress.

The pancreatic lobules were separated by thin interlobular septa with invisible adipose tissue invasion if compared with the stressed group. Heiskanen et al. (2018) reported that short-term training efficiently decreased ectopic fat along the pancreatic tissue, and exercise training could therefore decrease the risk of type 2 diabetes. In this study, most islets restored their normal shape and appeared with more cellularity. Also, the acini more or less appeared normal. Most pancreatic acini restored its basophilic and acidophilic portions. It was in line with Amaral et al. (2015) who noticed that practiced walking (type of exercise) showed normalization of the endocrine part of the pancreas of animals with metabolic syndrome (Amaral et al. 2015). They also noticed acinar hypertrophy and increased zymogen granules in rats subjected to chronic physical exercise. In this current study, exercise markedly attenuated the pancreatic apoptotic cells noticed in the stressed group. It was in the same line with Ghorbanzadeh et al. (2017) who suggested that voluntary exercise has synergistic anti-apoptotic effects on pancreas tissue of type 2 diabetic rats. Protective effects of this intervention were probably carried out through the reduction of glucose and hemoglobin A1C blood levels of rats suffering from diabetes (Ghorbanzadeh et al. 2017).

There was a significant decrease in inflammatory cell infiltration in stressed group practicing exercise if compared with the stressed group. It was in agreement with Allen et al. (2015) who stated that chronic exercise exerts anti-inflammatory effects via several distinct mechanisms. These include actions dependent and independent of changes in adipose tissue mass (Allen et al. 2015).

Conclusion

Physical exercise is a proper lifestyle that can be considered as one of the most valuable non-pharmacological approaches for counteracting the stress-induced pancreatic functional and morphological negative changes through its anti-inflammatory, anti-oxidative, and anti-apoptotic effects. These health-promoting effects of exercise can be followed routinely in humans to counteract the stress-induced negative effects on different body systems and organs especially the pancreas.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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