INTRODUCTION

Modern lifestyles are frequently distinguished by physical inactivity and copious intake of unhealthy diets. The resultant positive energy balance is a major reason for obesity and ultimately leads to insulin resistance and type 2 diabetes mellitus. White adipose tissue is a metabolically active tissue that acts as endocrine organ that secretes different metabolically active proteins called adipokines which play a major role in the pathogenesis of cardiovascular complication associated with obesity e.g tumor necrosis factor-α (TNF-α), leptin, adiponectin, and resistin (1).

Regular physical exercise (five times/week) can delay or prevent the onset of type 2 diabetes in high-risk individuals with impaired glucose tolerance through increase glucose transport to the muscle fiber (2). At the same time physical activity is accompanied by reduction in the insulin plasma level that results in turning off different enzymes entangled in carbohydrate and lipid metabolism (3). Lifestyle changes, including regular physical activity, have been reported to be more effective in preventing diabetes than drug therapy with metformin (2). This has primarily been attributed to the ability of exercise to improve a variety of metabolic abnormalities and risk factors that are associated with increased atherosclerosis. For example, aerobic exercise has been reported to lower blood pressure, improve dyslipidemia, facilitate weight loss, improve insulin sensitivity, and enhance glucose disposal, thereby reducing the incidence of diabetes (4, 5) Forced swimming allows selecting exercise overloads through the variation from 3% to 6% of body mass of the animal's body and imposes lower mechanical stress due to the water thrust, recruiting different muscle groups and reducing the gravity effects (6).

Adiponectin enhances insulin sensitivity and inhibits many steps in the inflammatory process (7). In the liver, it inhibits both the expression of hepatic gluconeogenic enzymes and the rate of endogenous glucose production. In muscle, it increases glucose transport and enhances fatty acid oxidation, effects that are partly due to the activation of AMP-kinase. In mice, decreased circulating concentrations of adiponectin could be important in producing changes in metabolism consistent with the metabolic syndrome (8).

Leptin and resistin share in the pathogenesis of insulin resistance through decreasing glucose transport in response to insulin and inhibits adipocyte differentiation. In this study HCFD fed rats showed significant elevation in plasma resistin level that illustrate the link between metabolic syndrome and insulin resistance (9).

We hypothesized that exercise training through swimming can improve the effects of HCFD on the glucose homeostasis,
lipid profile, heart histopathology, LVDP as well as the serum adipokines. However, there is no single report in literature investigative the effect of swimming exercise in HCFD fed rats on adiponectin mRNA expression from the cardiac muscle coupled with the LVDP, dp/dt\(_{\text{max}}\) and -dp/dt\(_{\text{max}}\).

MATERIALS AND METHODS

**Animals**

Experiments were performed according to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication No. 85-23, revised 1996). All experimental procedures in this study were approved by the Medical Research Ethics Committee of Mansoura University, Egypt.

Forty male Sprague-Dawley rats weighing 170–200 g, were purchased from the Vaccine and Immunization Authority (Helwan, Cairo, Egypt) and housed (Animal House, Medical Physiology Department, Faculty of Medicine, Mansoura University, Egypt) under controlled conditions (temperature of 23±1°C, and a 12 h/12 h light/dark cycle). The animals were allowed free access to food and tap water.

**Experimental design**

After 1 week of acclimatization to the laboratory environment, the animals were randomly divided into four groups (10 rats each). Animals in group 1 (Con) were used as control group and fed with standard laboratory chow for 15 weeks. Animals in group 2 (Con+Ex) were fed a standard laboratory chow for 15 weeks an obliged to swimming exercise from the 11\(^{th}\) week to the 15\(^{th}\) week. The third group fed a high-cholesterol diet with 10% fructose solution (HCFD) for 15 weeks. Animals of the 4\(^{th}\) group (HCFD+Ex) fed as the third group and perform swimming exercise from the 11\(^{th}\) week to the 15\(^{th}\) week. The high-cholesterol diet, recently described (10), contained 10.1% fat (5% coconut oil and 5.1% linoleic acid), 17% protein, 51.6% carbohydrate and 4% cholesterol. All diets contained 10.1% fat (5% coconut oil and 5.1% linoleic acid), 17% protein, 51.6% carbohydrate and 4% cholesterol. All diets contained a standard mineral and vitamin mixture. Body weight, water and food intake were recorded weekly (11).

**Exercise program**

Swimming was practiced in cylindrical tank (120 cm diameter × 80 cm height) containing temperature-controlled water (30–32°C). Rats were placed in the tank 3 ds/wk, 1 hour each session (9:00–10:00 a.m.). At the end of each exercise session, animals were dried and kept in a warm environment. Sedentary controls were restricted to cage activity. However, on the days of exercise practice, the sedentary animals were removed from their cages and kept for 1 hour in the container (previously cleaned and dried) where the swimming sessions had taken place in order to handle stress. To minimize the acute effect of the exercise, the trained animals were sacrificed 48 hours after the end of the last swimming training session. Food was removed from the animal cages in the night before sacrifice (12).

**Isolated Langendorff-perfused heart**

At the end of study, the rats were anesthetized with an intraperitoneal injection of thiopental sodium (Nesdonal, Specia; Rhône-Poulenc, Paris, France). The hearts were then rapidly excised and perfused according to the Langendorff method at a perfusion pressure of 75 mmHg. The perfusate was a Krebs-Henseleit solution containing NaCl 118 mmol/l, NaHCO\(_3\) 25 mmol/l, KCl 4.75 mmol/l, KH\(2\)PO\(_4\) 1.18 mmol/l, MgSO\(_4\) 1.17 mmol/l, CaCl\(_2\) 1.25 mmol/l, glucose 10 mmol/l (pH 7.4, 37°C), and was bubbled constantly with 95% O\(_2\)/5% CO\(_2\). The left ventricular pressure was measured using a compliant water-filled balloon, connected to a pressure transducer (SensoNor SP 844; Capto, Horten, Norway) via a rigid polyethylene tube introduced into the left ventricle through the mitral valve, and was recorded on a Power Lab acquisition system (AD Instruments Pty Ltd, Castle Hill, Australia). The hearts were paced at 300 beats/minute via electrodes placed on the left atrial wall and connected to a stimulator (6002 model; Harvard Biosciences, Les Ulis, France). The collapsed balloon was filled with saline to obtain a left ventricular end diastolic pressure of 5 mmHg. After 15–20 minutes of equilibration, the left ventricular developed pressure (LVDP), the peak of the positive and negative pressure derivatives (respectively, dp/dt\(_{\text{max}}\) and -dp/dt\(_{\text{max}}\)) were recorded.

**Biochemical measurements**

**Blood samples**

At the end of experimental period, blood samples were collected by cardiac puncture. These blood samples were collected without anticoagulant, left for 10 min, then centrifuged for 10 min at 4000 r/min to obtain serum, which was stored at –20°C until further biochemical analysis for determination of serum insulin, leptin, adiponectin, resistin, triglycerides (TG), total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C) (13), the activities of lactate dehydrogenase (LDH) and creatine kinase isoenzyme (CK-MB) were determined. Serum glucose was determined colorimetrically using a Randox reagent kit (Sigma-Aldrich), according to the method of Trinder (14).

**Calculation of HOMA index**

While the euglycemic hyperinsulinemic clamp is the standard method for measuring insulin resistance, a common method using the homeostasis model HOMA of IR was calculated by using the following equation: HOMA = fasting glucose (mmol L\(^{-1}\)) fasting insulin (mU L\(^{-1}\))/22.5. Typically, a HOMA value >2 is used to identify significant IR (15).

**Liver lipids levels**

Lipids were extracted from tissues using chloroform-methanol (2:1 by volume) by the method of Folch et al. (16). Dried lipid extracts were re-suspended in 1 ml of saline solution (9 g/L NaCl, 1% triton X - 100) and used for the estimation of lipid profile which assayed using reagent kits (Hospitex Diagnostics, Florence, Italy).

**RNA isolation and cDNA synthesis**

After recording the LVDP, hearts were dissected and ventricles were separated. Total RNA was extracted from ventricular homogenate using GS tract\(^{TM}\) RNA isolation kit II (SA-40005, Maxim BioTech, Inc. San Francisco, USA) guanidium thiocynate lysis. The purity and concentration of RNA were quantified by spectrophotometry. Reverse transcription reaction was performed using oligo (dT) primers (USA). The 25 µl cDNA synthesis reaction consisted of 2.5 µl (5×) buffer with MgCl\(_2\), 2.5 µl (2.5 mM) dNTPs (Pharmacia Biotech), 1 µl (10 pmol) Oligo d(T) primer (Pharmacia Biotech), 2.5 µl RNA (2 mg/ml) and 0.5 unit reverse transcriptase enzyme (Qiagen, US). The mixture was Revised 1996). All experimental procedures in this study were approved by the Medical Research Ethics Committee of Mansoura University, Egypt.
incubated at 37°C for 1 hours. PCR amplification was performed in a thermal cycler (Applied Biosystems (ABI), USA) programmed at 42°C for 1 hour, 72°C for 10 min (enzyme inactivation) and the product was stored at 4°C until used.

Real time PCR and quantitative estimation of adiponectin R1 mRNA

For qRT-PCR, a set of primers: forward 5'-GCAGAGATGCACCTCCTGGAA-3', reverse 5'CCCTCTGCCTCTGTCATTTCC-3' (Invitrogen Life Technologies) were designed from the published cDNA sequences of the rat adiponectin gene which amplified a 101 bp product. The reaction was carried out using Rotor-Gene 6000 system (Qiagen, USA) and consisted of 1.25 µl of 2X QuantitechSYBR® Green RT Mix (Fermentas, Germany), 1.0 µl of 25 pm/µl adiponectin primers, 2 µl cDNA (100 ng) and 9.25 µl of RNAse free water. Samples were spun well before loading in the Rotor's wells. The real time PCR program was performed as follows: initial denaturation at 95°C for 10 min.; 40 cycles of 95°C for 15 sec.; annealing at 60°C for 30 sec and extension at 72°C for 30 sec. Data acquisition performed during the extension step. This reaction was performed using Rotor-Gene-6000 system (Qiagen, USA). Negative controls were included in each set of PCR assays where cDNA was substituted with water as a control for contamination from exogenous sources. In addition, RT was omitted in some samples as a negative control for amplification of genomic DNA. Housekeeping gene, GDPH (glyceraldehyde 3-phosphate dehydrogenase) was amplified under similar conditions:

forward: 5'-GAGATACACTTCAATACTTTGACCT-3'
reverse primer: 5'-ATTGATCACTATCTGGGCAA-3';

program was performed as follows: initial denaturation at 95°C for 9 min.; 40 cycles of 95°C for 15 sec.; annealing at 60°C for 90 sec and extension at 72°C for 90 sec. The fold increase in the mRNA expression in all groups was calculated. Comparative quantification analysis using Rotor-Gene-6000 Series Software (Qiagen, USA). PCR products were electrophoresed on 1% agarose gel and visualized by ethidium bromide staining under UV light. The fold increase in the mRNA expression in all groups was calculated. Comparative quantification analysis was done for detection of a probable significance between two different parameters. Results were considered significant if p<0.05.

Statistical analysis

The data were expressed as mean ±standard deviation (S.D.). Data were processed and analyzed using the SPSS version 10.0 (SPSS, Inc., Chicago, Ill., USA). One-way ANOVA was done followed by Tukey's post hoc test. Pearson correlation statistical analysis was done for detection of a probable significance between two different parameters. Results were considered significant if p<0.05.

RESULTS

The changes in body weight, weight gain, food intake, liver weight, heart weight and liver lipids (triglycerides and cholesterol) are shown in Table 1. Feeding rats with HCFD significantly (p<0.05) increased the body weight and weight gain by 38 and 67% respectively. Also, the feeding efficiency increased significantly in the HCFD fed rats. At the same time induction of swimming exercise program significantly (p<0.05) decreased the body weight in the HCF fed rats. Also, swimming significantly reduced the liver weight/body weight ratio in HCF fed rats by 26%. Swimming training significantly (p<0.05) reduced liver triglycerides and cholesterol by 40 and 29% respectively.

From Table 2, the glucose, insulin and HOMA-IR increased significantly (p<0.05) in HCF fed rats as compared with the control group. Also, feeding the rats with HCFD for 15 weeks perturbed the lipid profile by increasing triglycerides, total cholesterol and

<table>
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<tr>
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<th>Con (n=10)</th>
<th>Con + Ex (n=10)</th>
<th>HCFD (n=10)</th>
<th>HCFD Ex (n=10)</th>
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<tr>
<td>Body weight (g)</td>
<td>332±21</td>
<td>317±20</td>
<td>459±38**</td>
<td>391±21**</td>
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<tr>
<td>Weight gain (g/week)</td>
<td>12±6</td>
<td>9±2</td>
<td>20±8**</td>
<td>15±6**</td>
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<tr>
<td>Food intake (g/week)</td>
<td>118.8±7</td>
<td>112.8±5</td>
<td>135±12</td>
<td>105±6**</td>
</tr>
<tr>
<td>Feeding efficiency (%)</td>
<td>12.8±2</td>
<td>11.9±2</td>
<td>25.4±5**</td>
<td>15.8±3**</td>
</tr>
<tr>
<td>Liver weight/body weight (%)</td>
<td>2.59±0.05</td>
<td>2.35±0.04</td>
<td>5.28±0.54**</td>
<td>3.94±1.5**</td>
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<td>Heart weight/body weight (%)</td>
<td>0.28±0.02</td>
<td>0.27±0.008</td>
<td>0.4±0.01**</td>
<td>0.35±0.01**</td>
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<td>Liver triglycerides (mmol/g)</td>
<td>0.26±0.04</td>
<td>0.21±0.05</td>
<td>0.54±0.11**</td>
<td>0.32±0.07**</td>
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<tr>
<td>Liver Cholesterol (mmol/g)</td>
<td>0.27±0.03</td>
<td>0.25±0.04</td>
<td>0.48±0.09**</td>
<td>0.34±0.08**</td>
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a: P<0.05 versus Control; b: P<0.05 versus Con+Ex; c: P<0.05 versus HCF.
LDL-C with significant (p<0.05) decrease in HDL-C. Swimming exercise in the last 4 weeks ameliorate the metabolic disturbances in the glucose homeostasis and lipid profile significantly by decreasing the glucose, insulin, HOMA-IR, triglycerides, total cholesterol as well as LDL-cholesterol with a significant (p<0.05) increase in HDL-cholesterol. Data of the present work showed that feeding rats with HCFD for 15 weeks significantly (p<0.05) increased the cardiac enzymes LDH and CK-MB as compared to the control group. While swimming exercise for the last 4 weeks of the experiment with HCFD significantly (p<0.05) reduced the cardiac enzymes as compared with the HCFD fed rats but still significantly elevated as compared with control rats (Table 2).

Table 3 showed that feeding rats with HCFD significantly (p<0.05) decreased the LVDP, dp/dtmax and –dp/dtmax by 30%, 27% and 40 respectively, versus the control group. On the other hand, swimming exercise at the last 4 weeks with HCFD increased LVDP, dp/dtmax and –dp/dtmax significantly by 27%, 16% and 31% respectively, as compared with HCFD fed rats. Although, the exercise improved the ventricular function LVDP, dp/dtmax and –dp/dtmax remained significantly different from the control group.

Feeding rats with HCFD for 15 weeks increased significantly (p<0.05) the serum adipokines: leptin and resistin and significantly (p<0.05) (Figs. 1A and 1C) and reduced the serum adiponectin as compared with the control group (Fig. 2B). Swimming exercise from the 11th week to the 15th week concomitant with the HCFD significantly (p<0.05) decreased the serum leptin and resistin as compared with the HCFD fed rats while, they still significantly (p<0.05) elevated as compared to the control group. On the other hand serum adiponectin significantly (p<0.05) increased in response to swimming in HCFD+Ex versus the HCFD rats. Additionally, adiponectin still significantly (p<0.05) decreased as compared with the control group.

Table 2. Effect of exercise on glucose, insulin, HOMA-IR, lipid profile and cardiac enzymes in control and HCFD fed rats. Data were expressed as mean ±S.D. of 10 rats.

<table>
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<tr>
<th></th>
<th>Con (n=10)</th>
<th>Con + Ex (n=10)</th>
<th>HCFD (n=10)</th>
<th>HCFD + Ex (n=10)</th>
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<tbody>
<tr>
<td>Glucose (mg/dl)</td>
<td>77.39±8.19</td>
<td>76.41±6.045</td>
<td>139.75±15.28</td>
<td>85.74±12.94</td>
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<tr>
<td>Insulin (µU/ml)</td>
<td>3.157±0.619</td>
<td>2.984±0.527</td>
<td>6.451±1.485</td>
<td>4.512±0.951</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.656±0.0715</td>
<td>0.5942±0.0628</td>
<td>1.215±0.157</td>
<td>0.721±0.0548</td>
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<tr>
<td>Triglycerides (mg/dl)</td>
<td>90.1±5.66</td>
<td>88.2±6.35</td>
<td>158.7±11.89</td>
<td>115.7±15.73</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>85.0±3.51</td>
<td>81.9±4.15</td>
<td>135.7±15.75</td>
<td>99.7±10.61</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>30.6±3.46</td>
<td>25.7±2.48</td>
<td>72.4±4.94</td>
<td>43.4±3.45</td>
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<tr>
<td>HDL-C (rng/ml)</td>
<td>45.4±5.82</td>
<td>48.7±4.45</td>
<td>35.7±2.45</td>
<td>43.4±3.45</td>
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<tr>
<td>LDH (U/L)</td>
<td>1350±273</td>
<td>1289±312</td>
<td>4187±346</td>
<td>2691±218</td>
</tr>
</tbody>
</table>

a: P<0.05 versus Control; b: P<0.05 versus Con+Ex; c: P<0.05 versus HCFD.

Table 3. Effect of exercise on LVDP, dp/dtmax and –dp/dtmax in control and HCFD fed rats. Data were expressed as mean ±S.D. of 10 rats.

<table>
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<tr>
<th></th>
<th>Con (n=10)</th>
<th>Con + Ex (n=10)</th>
<th>HCFD (n=10)</th>
<th>HCFD + Ex (n=10)</th>
</tr>
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<tbody>
<tr>
<td>LVDP (mmHg/g HW)</td>
<td>100.4±2.875</td>
<td>107.2±5.050</td>
<td>69.7±5.078</td>
<td>88.6±2.458</td>
</tr>
<tr>
<td>dp/dt max (mmHg/g HW)</td>
<td>2807.8±94.635</td>
<td>2858±128.478</td>
<td>2024.8±93.073</td>
<td>2360±112.546</td>
</tr>
<tr>
<td>–dp/dt max (mmHg/g HW)</td>
<td>–1833.2±86.511</td>
<td>–1982±79.554</td>
<td>–1090±84.327</td>
<td>–1437.6±103.656</td>
</tr>
</tbody>
</table>

a: P<0.05 versus Control; b: P<0.05 versus Con+Ex; c: P<0.05 versus HCFD.

Fig. 1. Effect of exercise on the serum levels of the leptin, adiponectin and resistin. Data were expressed as mean ±S.D. of 10 rats.

a: P<0.05 versus Control; b: P<0.05 versus Con+Ex; c: P<0.05 versus HCFD.
Fig. 3 showed a significant positive correlation between serum adiponectin level and LVDP for all groups, (r=0.9011) (p<0.001) (n=40).

Histopathological studies of the heart
Hematoxylin and eosin stains

Heart sections of normal control rats showed normal characteristic features of myocardium without cellular infiltration and normal vasculature. Myocardioctye of normal rats had oval-elongate nucleus centrally and homogeneous cytoplasm (Fig. 4A). Rats performed swimming exercise showed also apparently normal myocardial features similar to that of normal control rats. In HCFD fed rats, multi-focal vacuolar degeneration was seen in the myocardial cells (Fig. 4). Moreover, there were congestion of cardiac blood vessel and hyalinosis of its wall, in addition to granularity of the sarcoplasm of focal cardiac myocytes in HCFD fed rats (Figs. 4B and 4C). In contrast, myocardial cell of HCFD fed rat with swimming exercise revealed normal histological structure (Fig. 4D).

Transmission electron microscope

The observed ultrastructure of the control rat hearts (Fig. 5A). Cardiac myocytes contained regular and normal myofibrils, mitochondria, sarcoplasmic reticulum and T tubules and other organelles. There were well-defined myofibrils with a normal striation pattern and numerous interspersed globular mitochondria (Fig. 5A). Myofibrils were highly ordered arrays of dark anisotropic A bands and light anisotropic bands. Light bands were biseected by a thick, dark Z band. The ultrastructure of myocardial alterations in HCFD fed rats included loss and lysis of myofibrils with loss of normal striation pattern in the myofibrils and emptying of the myocyte, vacuolization and mitochondrial swelling, condensed or mitochondrial disruption and cristae destruction (Figs. 5B and 5C). Cardiac myocyte from this group showed degeneration and necrosis. Z-line is irregular, interrupted and thickened in some areas (Fig. 5C). Mega mitochondria were also seen in this group of rats (Fig. 5B). Cardiac myocyte from HCFD + exercise showed mild changes in striation pattern with swollen and condensed mitochondria. Myofibrils loss and interrupted Z-line were mild and improved in comparison to HCFD fed rats (Fig. 5D).
DISCUSSION

In the present study feeding rats with high cholesterol and fructose diet for 15 weeks is known to produced metabolic dysfunction and dyslipidemia. Induction of swimming exercise in the last four weeks in HCFD fed rats was used to investigate the possible protective role of swimming on cardiac function in metabolic syndrome rats. Swimming in the present study markedly attenuated the metabolic disturbances as well as the dyslipidemia produced by the HCFD. Also, the cardiac performance was significantly improved in response to swimming exercise in HCFD fed rats as evidenced by the measurement of the LVDP, dp/dt and –dp/dt.

Regular exercise has long been associated with reduced cardiovascular risk (4). This has primarily been attributed to the ability of exercise to improve a variety of metabolic abnormalities and risk factors that are associated with increased atherosclerosis. Exercise has been reported to decrease blood pressure, improve dyslipidemia, facilitate weight loss, improve insulin sensitivity, and enhance glucose disposal, thereby reducing the incidence of diabetes (5). It has been suggested that many of these improvements may be largely due to exercise-induced reductions in weight and insulin resistance (17). Given the increased understanding of the above-described relationships between obesity, insulin resistance, inflammation, and atherosclerosis, it is important to assess whether many of the physiologic benefits attributed to exercise could, in part, be explained by its effect on serum adiponectin level.

In the current study, swimming of the control rats insignificantly (p>0.05) decreased the lipid profile and increased HOMA-IR. Rats fed with chow diet lack the excess fat stores which are produced by positive energy balance. Also, the LVDP.

Fig. 4. Microscopic examination (Hx & E 40) of the heart (4A): control group, (4B and 4C): HCFD group and (4D): HCFD+ Exercise. Arrow is pointing for lipid droplets.

Fig. 5. Transmission electron microscope of the heart (5A): control group, (5B and 5C): HCFD group and (5D): HCFD+ Exercise. N: nucleus, S: sarcoplasm of a cardiomyocytes; M: mitochondria; V: vacuoles; Arrows, Lipid droplets.
Moreover, liver TG and cholesterol were increased in rats fed on HCFD compared to normal group. These results might be explained by the higher caloric content of HCFD as compared to normal basal diet. The increased fat and carbohydrates content of the diet is responsible for increased weight gain. These results were in agreement with Matos et al. (18) and Rezq and El-Khamisy (19) who demonstrated that high fat cholesterol diet is used to induce hypercholesterolemia and impaired glucose tolerance. In response to swimming exercise in the HCFD rats showed a significant decrease in body weight, liver weight/body weight ratio as well as liver lipids versus the HCFD fed rats (Table 1).

Moreover, the relative liver and heart weight to body weight ratio, showed a significant increase in HCFD fed rats as compared to normal group. These results might be explained by the accumulated fat in the liver and heart cells. Moreover, liver TG and cholesterol were increased in rats fed on HCFD (Table 1).

These results were confirmed by histopathological and transmission electron microscope examination which showed that fatty changes of the sarcoplasm with increased intracellular lipids of focal cardiac myocytes. This was in accord with Rezq and El-Khamisy study (19), who observed intracellular lipid accumulation in cardiomyocytes in response to high cholesterol diet. While swimming in HCFD significantly reduced liver and heart weights to body weight ratio and liver lipids as compared to HCFD fed rats. Also, swimming exercise to HCFD fed rats decreased the feeding efficiency significantly as compared with the HCFD fed rats. Different mechanisms are involved in the appetite suppression during exercise as changes in the leptin sensitivity during exercise (20) because it exerts an anorexigenic effect on the hypothalamic neurons. It decreases food intake and increases energy expenditure by affecting the balance between orexigenic and anorexigenic hypothalamic pathways. Low leptin levels are responsible for the compensatory increase in appetite and body weight and decreased energy expenditure following caloric deprivation. Moreover, lesions in the lateral hypothalamic nuclei resulted in decreased food intake (21). Binding of leptin to its hypothalamic receptors activates a signaling cascade in the arcuate nucleus that results in inhibition of orexigenic pathways as indicated by decreased mRNA expression of neuropeptide Y (NPY) and agouti-related peptide (AgRP), and stimulation of anorexigenic pathways as suggested by increases in the mRNA levels of alpha-melanocyte-stimulating hormone (α-MSH) and cocaine and amphetamine regulated transcript (CART) (22, 23). Also, plasma acylated ghrelin concentration and hunger are suppressed during exercise (24).

Additionally, in response to high caloric intake glucose, insulin, cardiac enzymes (CK-MB and LDH) and HOMA-IR increased significantly (Table 2) with disturbed lipid profile as compared with the control group. In response to swimming for the last 4 weeks, HCFD, the glucose, insulin, HOMA-IR, cardiac enzymes and the lipid profile decreased significantly with increased LDL-cholesterol as compared with the HCFD fed rats and remain significantly increased as compared with the control group. These results are in agreement with other studies performed in the animal model of metabolic syndrome (11).

In the current study, rats fed with HCFD fed rats showed that serum adiponectin level and adiponectin mRNA expression from the cardiac muscle decreased significantly in comparison to the control group (Figs. 1 and 2). Our results were in accord with previous study which found that adiponectin levels were significantly decreased in non alcoholic steatohepatitis (NASH) as compared to not-NASH patients. This difference was found even after the adjustment for potential confounders. Also, they concluded that adiponectin was negatively associated with insulin sensitivity and the grades of steatosis (25). Whereas swimming increased the expression of cardiac adiponectin mRNA and serum adiponectin significantly in HCFD fed rats versus the HCFD fed rats (Figs. 1 and 2). We hypothesize that adiponectin level significantly increased in the exercising group due to reduced white adipose tissue mass. It was thought that adipocytes were the only site of adiponectin synthesis and secretion. However, several studies have now uncovered the potential for adiponectin production by bone-forming cells (26), cardiomyocytes and skeletal muscle cells (27-31). Adiponectin has insulin sensitizing effects on the liver and skeletal muscle (32). It is an anti-inflammatory cytokine that is decreased significantly with central obesity and type 2 diabetes mellitus (7). In the liver, it inhibits both the expression of hepatic gluconeogenic enzymes and the rate of endogenous glucose production. In muscle, it increases glucose transport and enhances fatty acid oxidation, effects that are partly due to the activation of AMP-kinase. Adiponectin may produce a direct role in mediating insulin-stimulated glucose uptake by regulation of both lipids and glucose metabolism. It has a significant role in T2DM and metabolic syndrome, due to its insulin sensitizing, anti-inflammatory and anti-atherogenic properties (33). Adiponectin synthesis and expression from epididymal adipocytes were inhibited by angiotensin II (34) while they could be stimulated by increased NO bioavailability (35). Results of the present study were in agreement with other studies (36-39).

Aerobic exercise through increasing adiponectin level has been reported to lower blood pressure, improves dyslipidemia, insulin sensitivity, glucose disposal and facilitates weight loss thereby reduces the incidence of metabolic syndrome and diabetes (5). So, we can hypothesize that restoration of insulin sensitivity by swimming exercise might be mediated, mainly or at least in part, by mechanisms involving adiponectin that significantly increased in HCFD+Ex group compared with HCFD group. In recent studies performed by Guo et al. (31) who concluded that STZ-induced diabetes up-regulated adiponectin receptors in the heart. Despite an increase in cardiac adiponectin receptor 1 expression, there was an increased cardiac inflammatory response and a decreased GLUT4 expression associated with a reduction in circulating adiponectin and cardiac adiponectin expression. Another study of Shen et al. (39) who reported that obesity and insulin resistance reduced the adiponectin expression. This runs parallel with our results that showed that HCFD fed rats exhibited a reduction in the cardiac adiponectin expression as compared with the control. In response to weight gain and impaired lipogram the ventricular functions were disturbed as denoted by the significant decrease in LVDP, dp/dt max and -dp/dt min in HCFD fed rats versus normal rats. This could be attributed by the fatty infiltration of the cardiac myocytes, vacuolization and mitochondrial swelling, condensed or mitochondrial disruption and cristae destruction (Figs. 5B and 5C). Cardiac myocyte from this group showed degeneration and necrosis. Z-line is irregular, interrupted and thickened in some areas (Fig. 5C). Mega mitochondria were also seen in this group of rats. Hyperglycemia increased the oxidative stress in the cardiac tissue that stimulates apoptosis and cell death that may proceed to cardiomyopathy as noticed by previous researchers (40-42). Also, increased levels of circulating glucose, free fatty acids have
(FFA), and insulin (43, 44); and the combination of heart failure and T2DM induces complex metabolic changes in the myocardium (45). Moreover, systemic insulin resistance with hyperinsulinemia, contributes to the development of diastolic dysfunction in metabolic syndrome (46).

Swimming exercise improved ventricular function as evidenced by increasing the LVDP, dp/dt\text{max}, and –dp/dt\text{max}, significantly in HCFD+Ex group as compared to HCFD fed rats. Possibly the improved function could be due to significant increase in adiponectin that may exert a potent function on the cardiomyocytes. Post analysis showed that there is a direct positive correlation between serum adiponectin and LVDP (r=0.9001) (p<0.001). This was in agreement with Ding et al. (30) who concluded that cardiomyocyte derived adiponectin may serve as a reserved regulator of myocardial energy metabolism when circulating adiponectin becomes scarce in situations such as metabolic syndrome X. The protective effect of adiponectin on the cardiac muscle could be caused by inhibiting inducible NOS and NADPH-oxidase expression and resultant oxidative stress (45). Another possible mechanism could be attributed through AMPK and COX-2-dependent mechanisms (46). Additionally the lipid lowering effect of exercise might reduce the pro-atherogenic effect of hypertriglyceridemia through increased expression of peroxisome proliferator activated receptor (PPAR) which plays a crucial role in adipokines formation and adipogenesis as detected by Krskova et al. (57) who concluded that adiponectin is a protective factor in obesity inflammation and insulin resistance (52), disease severity and mortality (48, 49). Elevated adiponectin levels likely reflects an attempt to mitigate pro-inflammatory or impaired metabolic states and demonstrates a balance between protective and harmful pathways in left ventricular (LV) systolic dysfunction and HF. Adiponectin resistance has been described by a small number of studies examining human tissue (52) and animal models that may be confounded in some chronic human disease states by the counter-regulation of poorly described adiponectin paralogues that may have overlapping functions with adiponectin (53).

Another aim of this study was to investigate the lowering effect of swimming on circulating adipokines: leptin and resistin (Fig. 1) by reducing the adipose tissue mass. The circulating levels of leptin and resistin are concomitant with the degree of obesity and insulin resistance (54, 55). Leptin plasma concentration and mRNA expression in adipose tissue are directly related to obesity severity, as an increased of fat mass is associated with an increase of leptin which makes leptin an indicator of the total fat mass (56). Leptin and resistin are considered as starvation hormones that are decreased with fasting and weight loss. In our study the level of leptin and resistin decreased significantly in response to exercise in HCFD fed rats in agreement with the results obtained by Rajala et al. (57) who reported that resistin impairs insulin action and shares in the pathogenesis of insulin resistance. In the current study the serum level of leptin and resistin decreased significantly with swimming exercise as compared with HCFD fed rats. Decreased leptin and resistin could be attributed to the decrease body weight as well as the adipose tissue content. In agreement with our data the study done by Milan et al. (58) who reviewed that resistin expression decreased significantly in obese rats due to weight loss. Our results were in accord with previous studies (59-64).

Also, the cardiac enzymes increased significantly in rats with HCFD fed rats as compared with control group. These changes were reversed by swimming exercise in the last 4 weeks of the study in HCFD fed rats (Table 3).

Swimming exercise could be an effective therapy to patients with metabolic syndrome and type 2 diabetes mellitus by increasing adiponectin and improving insulin sensitivity. Further research is needed to define the beneficial effects of adiponectin and its receptors on the physiology and ion channels of the cardiac muscle.

In summary, high cholesterol and carbohydrate diet for 15 weeks reduced the expression of adiponectin mRNA in the cardiac muscle and cardiac performance. While, swimming exercise could be considered as efficient treatment of metabolic syndrome as it increased serum adiponectin level and its cardiac expression.

Acknowledgements: The author acknowledges the help of Dr. Asem Shalaby (Pathology Department, College of Medicine, Mansoura University, Egypt) and Dr. Reffat Eid (Pathology Department, College of Medicine, King Khalid University, Saudi Arabia) of their assistance. Also, I introduce my deep thanks to Dr. Amr Abbas (Physiology Department, College of Medicine, Mansoura University, Egypt) and Dr. Ayman El-Sammoudy (Biochemistry Departments, College of Medicine, Mansoura University) for providing the technical help for the completion of the study.

Conflict of interest: None declared.

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Received: December 12, 2012
Accepted: April 15, 2013

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