Effect of Endurance Exercise and/or Diet Restriction on Mitochondrial Bioenergetics Function in Skeletal Muscle of Diabetic Male Albino Rats

NASHWA ELTABLAWY, M.D. and EMAN F. KAHELEEL, M.D.
The Department of Physiology, Faculty of Medicine, Cairo University

Abstract

Reduced mitochondrial capacity in skeletal muscle occurs in type 1 diabetic patient and in those at increased risk for this disorder, but the extent to which mitochondrial dysfunction in type 1 diabetic patients is remediable by physical activity and/or diet restriction intervention is uncertain. The aims of our study were to assess the effect of diet restriction and or endurance training on: 1- Expression of Peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1 α) the major transcriptional coactivator regulating the expression of the oxidative phosphorylation (OXPHOS) gene, estrogen related receptor alpha (ERR α) and mitofusion2, 2- Expression of genes of mitochondrial enzymes involved in mitochondrial oxidative metabolism, 3- Lipid peroxidation as measured by (malondialdehyde) MDA and 4- Glucose metabolism.

Material and Methods: In this study six groups, 8 rats for each, were included: A sedentary fed ad libitum group, a trained fed ad libitum group, a diabetic sedentary fed ad libitum group, a diabetic trained group, a diabetic diet restricted group, and a diabetic trained-diet restricted group. For each group the following parameters were measured: 1- Serum glucose, serum insulin, 2- Glycogen, MDA level in gastrocnemius muscle and 3- Gene expression by real time PCR for PGC-1 α, ERR α, Mitofusin2, GLUT4, carnitine palmitoyltransferase-1 (CPT-1) and citrate synthase.

Results: Our results showed that streptozotocin (STZ) induced diabetic rats had significant increase in glucose level and significant decrease in insulin level. Endurance training or diet restriction caused significant decrease in glucose level, while combination of diet restriction and endurance exercise synergistically caused significant decrease in comparison to diet restriction alone but still significantly higher than the normal level in the control rats. There was significant increase in MDA content of gastrocnemius muscles of STZ-induced diabetic rats which significantly decreased by diet restriction, more decreased by endurance exercise, combination of diet restriction and endurance exercise synergically caused more decrease to the normal level as normal control rats. Glycogen content of gastrocnemius muscle of STZ-induced diabetic rats was decreased significantly which it was increased significantly by endurance exercise when compared to diabetic rats. Also our results showed significant decrease in mRNA levels of genes related to mitochondrial biogenesis such as endurance exercise training, promotes phenotypic adaptations in skeletal muscle toward a more oxidative phenotype. Specifically endurance exercise training leads to fiber type transformation, mitochondrial biogenesis, angiogenesis, and other adaptive changes in skeletal muscles along with improved insulin sensitivity and metabolic flexibility in both rodents and humans [1-5].

Conclusion: Diet restriction and/or endurance training in diabetic rats induces mitochondrial bioenergetics in skeletal muscle, and enhances mitochondrial function and improve glucose metabolism by skeletal muscle possibly due to increase PGC-1 α and ERR α. Therefore represent a key strategy in the prevention and treatment of type 1 diabetes and in those at increased risk for this disorder.

Key Words: Type 1 diabetic – Exercise – Diet restriction – PGC-1 α – ERR α – Mitofusin2.

Introduction

It is well known that increased contractile activity, such as endurance exercise training, promotes phenotypic adaptations in skeletal muscle toward a more oxidative phenotype. Specifically endurance exercise training leads to fiber type transformation, mitochondrial biogenesis, angiogenesis, and other adaptive changes in skeletal muscles along with improved insulin sensitivity and metabolic flexibility in both rodents and humans [1-5].

One of the most adaptations to endurance exercise training is an increase in skeletal muscle mitochondrial size and density [6]. A training-induced increase in mitochondrial content is associated with reduced risk for several chronic diseases, likely due, in part, to an enhanced ability to oxidize carbohydrate and lipid [7]. In contrast, decreased mitochondrial volume and a reduced capacity for substrate oxidation have been linked
to obesity [8], insulin resistance [9], aging and Type 2 diabetes [10]. Increasing skeletal muscle mitochondrial content through exercise training may, therefore represent a key strategy in the prevention and treatment of chronic diseases [11-13]. Elucidating the molecular mechanisms that promote exercise-induced mitochondrial bioenergetics is thus an important area of scientific investigation with significant therapeutic application.

Peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1 α) transcriptional coactivator identified through its interaction with PPARγ in brown fat cells, was found to be greatly unregulated in brown fat and skeletal muscle in response to cold exposure [14]. Also it was found that PGC-1 α mRNA and protein expressions are very responsive to endurance exercise [15,16]. It was reported that PGC-1 α is activated in old animals under caloric restriction (CR) [17]. The expression of PGC-1 α is decreased in skeletal muscle of type 2 diabetic patients [18] and with physical inactivity [19]. PGC-1 α is now known to be involved in the regulation of thermogenesis, energy metabolism, and other biological processes of various organ systems [20-22]. PGC-1 α interacts with and coactivates a growing list of transcription factors including estrogen-related receptor- α [23], thyroid receptor [14], nuclear respiratory factor-1 (NRF-1) [24], NRF-2 [25], and myocyte enhancer factor-2 (MEF2) [26]. As a consequence, PGC-1 α is involved in the coordinated regulation of nuclear and mitochondrial-encoded genes required for contractile and metabolic adaptations in skeletal muscle [27,28]. PGC-1 α upregulation (20-27%) in insulin-resistant muscles (obese Zucker rats) improved insulin-stimulated glucose transport, reduced intramuscular lipids (triaclylglycerol, diacylglycerol, ceramide), increased mitochondrial fatty acid oxidation (subsarcolemmal mitochondria only, not intermyofibrillar mitochondria), and increased expression of GLUT4 [29]. A strong positive correlation ( r=0.74; p<0.05) between changes in PGC-1 α and cytochrome c oxidase (COX) activity was used as an index of mitochondrial adaptations [30] there is now ample evidence that a massive PGC-1 α overexpression (600-2,000%) have unexpected pathophysiological consequences [31].

Leick et al., [32] reported that PGC-1 α is not mandatory for exercise- and training-induced adaptive gene responses as reduced basal level mitochondrial respiratory function in a mouse model of global gene disruption of the PGC-1 gene (PGC-1-KO) was corrected by endurance exercise training and increases in hexokinase II, aminolevulinate synthase 1, and cytochrome oxidase (COX) protein expression in response to endurance exercise.

The estrogen-related receptor α (ERRα) is one of the first orphan nuclear receptors identified [33]. Both ERRα and PGC-1 α are up regulated in human skeletal muscle following endurance exercise [34,35]. ERRα, in combination with its transcriptional co-activator PGC-1 α, is known to regulate genes involved in mitochondrial energy-producing pathways in cardiac and skeletal muscle [34,36]. ERRα activates genes involved in multiple key energy production pathways, including cellular fatty acid uptake, fatty acid oxidation [37], and mitochondrial electron transport/oxidative phosphorylation [38].

Mitofusin2 (Mfn2) is a mitochondrial membrane protein that participates in mitochondrial fusion and regulates mitochondrial metabolism in mammalian cells [39,40]. Soriano et al., [41] demonstrate a stimulatory effect of PGC-1 α on Mfn2 mRNA and protein expression in muscle cells. PGC-1 α also stimulated the activity of the Mfn2 promoter, ERRα also activated the transcriptional activity of the Mfn2 promoter, and the effects were synergic with those of PGC-1 α. Mfn2 loss of function reduced the stimulatory effect of PGC-1 α on mitochondrial membrane potential. The previous results indicate the existence of a regulatory pathway involving PGC-1 α, ERRα, and Mfn2. Alterations in this regulatory pathway may participate in the pathophysiology of insulin-resistant conditions and type 2 diabetes (40,42).

Our study was performed in order to investigate the possible role of exercise and or food restriction on mitochondrial function and glucose metabolism in skeletal muscle of STZ-induced diabetic rats.

Material and Methods

The present study was carried out in the Physiology Department, Faculty of Medicine, Cairo University from March 2013 – July 2013. 48 male albino rats with body weights 150-200 grams were included in this study. The animals were and placed in the animal house of the faculty. They were housed in wire mesh cages at room temperature with 12:12 dark-light cycles.

Animals were randomly divided into 6 groups of 8 rats each: Group 1: Sedentary fed ad libitum group, Group 2: Trained fed ad libitum group, Group 3: Diabetic fed ad libitum group, Group 4: Diabetic diet restricted group, Group 5: Diabetic trained and fed ad libitum group and Group 6: Diabetic trained and diet restricted group.
Experimental diabetes: Was induced in fasting rats by single intraperitoneal injection of freshly prepared Streptozotocin (STZ) (60mg/Kg body weight; Sigma Aldrich Co., Germany) dissolved in citrate buffer [43].

Diet restriction:
All groups were allowed to fed ad libitum for one week then food was restricted to the particular studied groups (Groups 4,6) by about 50% (low carbohydrate diet) [44].

Training:
The exercise protocol consisted of swimming exercise (1 hr/day, 5 days/week) [45] for 12 weeks in a swimming tank filled with water at a temperature of 37°C. Daily swimming divided into 2 sessions each formed of 30 minutes separated by 1 hour rest. At the completion of each period of swimming exercise the rats were removed from the water, carefully dried and returned to their cages. The exercised rats underwent a swimming programme consisting of gradually increasing periods of swimming in the first 4 days the duration of exercise was gradual increased from an initial period of 15min to the maximum permissible period of 30min.

At the end of the experimental protocol, blood samples from all overnight-fasting rats were collected, by introducing fine capillary tube at the inner canthus of the eye into the venous plexus, to assess serum levels of glucose and insulin. Then, animals were sacrificed and gastrocnemius muscle were rapidly excised for further detection of GLUT4, glycogen, MDA, ERRα, CPT-1, PGC-1α, citrate synthase and Mitofusin2.

Measurements of fasting blood glucose level:
Serum glucose was measured using oxidase-peroxidase method [46].

Measurement of serum insulin:
Serum insulin levels were analyzed using enzyme-linked immunosorbent assay ELISA (Dako, Carpinteria, CA) according to the manufacturer’s instructions [47].

Assessment of glycogen level:
Glycogen was measured in muscle sample by Colorimetric method using Glycogen Assay Kit (abnova USA) according to manufacture instruction.

Measurement of Malondialdehyde (MDA):
To measure the MDA concentration, 100mg of muscle tissue in 1mL PBS, pH 7.0 was homogenized with micropestle in microtube. 20% TCA was added to muscle homogenate to precipitate the protein, and centrifuged. Supernatants were collected and thiobarbituric acid (TBA) solution was added to the supernatants. After boiling for 10 minutes in water bath, the absorbance was measured. Concentration of MDA in supernatants of muscle homogenate was calculated using the standard curve [48].

Detection of gene expression by real time PCR:
Mitofusin2, citrate synthase, carnitine palmitoyltransferase-1 (CPT-1), ERR alpha, GLUT4 and PGC-1 alpha gene expression was measured by real time PCR briefly, Real-time quantitative PCR.

Total RNA was isolated from muscle tissue homogenate using RN easy Purification Reagent (Qiagen, Valencia, CA) according to manufacturers instruction. The RNA sample was dissolved in RNase-free water and quantified spectrophotometrically. The integrity of the RNA was studied by gel electrophoresis on a 1% agarose gel, containing ethidium bromide. First-strand cDNA synthesis was performed with the Super Script Choice System (Life Technologies, Breda, the Netherlands) by mixing 2 µg total RNA with 0.5 µg oligo (dT) primer in a total volume of 12 µL. After the mixture was heated at 70°C for 10min, a solution containing 50mmol/L Tris˙HCl (pH 8.3), 75mmol/L KCl, 3mmol/L MgCl2, 10mmol/L DTT, 0.5mmol/L dNTPs, 0.5 µLRNase inhibitor, and 200U Superscript Reverse Transcriptase was added, resulting in a total volume of 20.5 µL; this mixture was incubated at 42°C for 1h; total volume was adjusted to 100 µL with RNase-free water and stored at 80°C until further use.

For real-time quantitative PCR, 5 µL first-strand cDNA was used in a total volume of 25 µL containing 12.5 µL SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA) and 200ng of each primer.

Primers, which shown in Table (1), PCR reactions, consisting of 95 ˚C for 10min (1 cycle), 94C for 15s, and 60°C for 1min (40 cycles), were performed on an ABI Prism 7900HT Fast Real Time PCR system (Applied Biosystems).

Data were analyzed with the ABI Prism 7500 sequence detection system software and quantified using the v1 ·7 Sequence Detection Software from PE Biosystems (Foster City, CA). Relative expression of studied genes was calculated using the comparative Ct method. All values were normalized to the beta actin genes [49].
Fasting serum glucose and insulin:

Significant increase (p<0.05) in glucose level in Group 3 (Diabetic fed ad lib) compared to Group 1 (Sedentary fed ad lib), Group 2 (Trained fed ad lib), Group 4 (Diabetic diet restricted), Group 5 (Diabetic trained) and Group 6 (Diabetic trained and food restricted) was seen.

There was a significant decrease (p<0.05) in insulin value in diabetic rats (Group 3) when compared to normal rats (Group 1, Group 2).

Statistical method:

The data was coded and entered using the statistical package SPSS version 15. The data was summarized using descriptive statistics: Mean, standard deviation, median, minimal and maximum values for quantitative variables. Statistical differences between groups were tested using ANOVA (analysis of variance) with post Hoc-Bonferroni test for quantitative normally distributed variables while Nonparametric Mann Whitney test and kruskal-Wallis test were used for quantitative variables which aren’t normally distributed. p-values less than or equal to 0.05 were considered statistically significant.

Table (1): Primer sequences used for RT-PCR.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
</tr>
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<tbody>
<tr>
<td>Mitofusin</td>
<td>F: 5'-ACGGTGGAGGTCAATGCAG-3'</td>
</tr>
<tr>
<td>Citrate synthase</td>
<td>F: 5'-GCGCTGAAAAGCCTTCTCTC-3'</td>
</tr>
<tr>
<td>CPT-1</td>
<td>Forward 5'<em>-CCGAGCTCAGTGGAGCCTCA-3</em></td>
</tr>
<tr>
<td>ERR alpha</td>
<td>Forward 5'<em>-GCC GAA GCT GCA TGG ACA CT-3</em></td>
</tr>
<tr>
<td>GLUT4</td>
<td>Forward 5'<em>-AAAGAAGCAGGACACTAAACC-3</em></td>
</tr>
<tr>
<td>PGC-1 alpha</td>
<td>Forward 5'<em>-TGGCCAGATCTTCTGAAC-3</em></td>
</tr>
<tr>
<td>Beta actin</td>
<td>Forward 5'_-TGCT GGC ACC CCTTCT ACA ATG3</td>
</tr>
</tbody>
</table>

Results

Fasting serum glucose and insulin:

Significant increase (p<0.05) in glucose level in Group 3 (Diabetic fed ad lib) compared to Group 1 (Sedentary fed ad lib), Group 2 (Trained fed ad lib), Group 4 (Diabetic diet restricted), Group 5 (Diabetic trained) and Group 6 (Diabetic trained and food restricted) was seen.

Exercise in normal rats did not significantly affect glucose and insulin levels compared to normal sedentary rats (p>0.05), while it reduced glucose levels significantly in diabetic trained rats (Group 5) compared to diabetic sedentary fed ad. libitum (Group 3). While exercise had no significant effect on insulin level as there was no significant difference between (Group 3) and (Group 4).

The diet restriction and diet restriction & exercise (Groups 4,6) decreased the glucose level and the insulin level in the diabetic rats compared to diabetic sedentary fed ad. Libitum (Group 3) (Figs. 1,2).
PGC-1 α, ERR α and mitofusin2:

There was significant decrease ($p<0.05$) in mRNA of PGC-1, ERR alpha and mitofusin2 in diabetic rats (Group 3) when compared to normal rats (Groups 1, 2). Their values are not affected by diet restriction (Group 4) as there were no significant change when compared by diabetic rats ($p>0.05$). Exercise in diabetic rats (Group 5) caused significant increase in comparing PGC-1 α, ERR alpha and mitofusin2 with their values in diabetic rats (Group 3). Also exercise in diabetic rats (Group 5) returned ERR alpha and mitofusin2 to normal values as there was no significant change when compared to normal control rats (Groups 1, 2). While PGC-1 α was not returned to normal value by exercise as there was significant decrease when compared to normal control rats (Groups 1, 2). Diet restriction and exercise (Group 6) showed significant increase ($p<0.05$) in PGC-1 mRNA when compared to diet restriction alone (Group 4) (Figs. 3-5).
GLUT4:

GLUT4 level was not changed in normal rats by exercise as there was no significant difference between Group 1 (sedentary fed ad libitum) and Group 2 (trained fed ad libitum), while diabetes caused significant decrease ($p<0.05$) in Group 3 (diabetic fed ad libitum) when compared to the control (Group 1). Diet restriction had no effect on GLUT4 as there was no significant difference ($p>0.05$) between Group 4 (diabetic diet restricted) and Group 3 (diabetic rats). But exercise and exercise and diet restriction in diabetic rats (Groups 5,6) caused significant increase ($p<0.05$) in GLUT4 when compared to diabetic (Group 3) (Fig. 6).

Exercise and diet restriction restore GLUT4 level within normal as there was no significant difference in GLUT4 level between (Group 6) and normal rats (Groups 1,2).

Glycogen:

There was no significant difference in glycogen in skeletal muscle of normal rats ($p>0.05$) between trained group (Group 2) and Group 1 (sedentary fed ad libitum). There were significant decrease ($p<0.05$) in all diabetic Groups 3,4,5 and 6 when compared to (Groups 1,2).

Exercise caused significant increase ($p<0.05$) in glycogen (Group 5) when compared to its value in diabetic rats (Group 3) and diet restriction (Group 4).

Diet restriction (Group 4) had no significant change ($p>0.05$) in glycogen level when compared to values of diabetic rats (Group 3). Also diet restriction with exercise (Group 6) showed no significant change in glycogen when compared to its value caused by exercise alone (Group 5) (Fig. 7).

MDA:

Diabetes in group 3 caused significant increase ($p<0.05$) in MDA when compared to normal rats (Groups 1,2). Exercise or diet restriction in diabetic rats (Groups 4,5) caused significant decrease in MDA when compared to its value in diabetic rats (Group 3) but still significantly higher than MDA value of normal control rats. Exercise and diet restriction in diabetic rats (Group 6) caused significant decrease in MDA that reach its value as normal control rats as there is no significant change between group 6 and 1,2 ($p>0.05$) (Fig. 8).

CPT-1:

There was significant decrease ($p<0.05$) in CPT-1 mRNA in diabetic rats (Group 3) when compared to its value in normal rats (Groups 1,2). Diet restriction in diabetic rats (Group 4) had no significant effect on CPT-1 when compared to diabetic rats,
while exercise in diabetic rats (Group 5) caused significant increase ($p<0.05$) in its value when compared to diabetic rats. Also exercise return its level as normal control as there is no significant difference ($p>0.05$) in CPT-1 values between diabetic rats with exercise and normal control rats (Fig. 9).

**Citrate synthase:**

There was significant decrease ($p<0.05$) in citrate synthase in diabetic rats (Group 3) when compared to its value in normal rats (Groups 1,2). Diet restriction or exercise in diabetic rats (Groups 4,5) had no significant difference ($p>0.05$) effect on Citrate synthase when compared to its value in diabetic rats. Combination between diet restriction and exercise in diabetic rats (Group 6) caused insignificant ($p>0.05$) increase in citrate synthase when compared to its value in diabetic rats (Group 3) (Fig. 10).

**Discussion**

This study was done to investigate the possible role of exercise and or diet restriction in diabetic rats on mitochondrial function.

In the present study there was significant increase in glucose levels in all groups treated with STZ as compared with non-diabetic groups. Diet restriction or exercise alone results in significant reduction in the glucose level of diabetic rats, moreover combination of both have synergistic effect but do not restore normal control value in non diabetic rats.

These results are in agreement with previous study conducted by Filaire and his colleagues [44] who reported that diabetic rats showed hyperglycemia but training and food restriction significantly reduced blood glucose concentrations, despite; exercise training doesn’t restore normal blood glucose levels [50].

Several mechanisms may act locally to improve glucose uptake and disposal after exercise. Those include increased muscle blood flow, increased insulin binding to its receptor (IR), increased IR turnover and increases glucose transport by stimulating GLUT4 translocation to the muscle cell surface [51].

In the present study, STZ induced diabetes results in a significant decrease in insulin level as compared to non diabetic rats. The chronic exercise alone did not affect insulin level in both diabetic and normal rats, while food restriction alone [52] or in combination with exercise significantly decreases insulin level.

It is possible that food restriction exerts its effects on insulin secretion by alteration in different steps in the mechanism of insulin gene expression, biosynthesis or secretion due to the elevated levels of serum FFA accompanying food restriction [53]. The possible mechanism by which FFA exert this effect is by increasing levels of carnitine palmitoyl transferase-1 (CPT-1) activity in pancreatic islets that leads to decreased Fatty acyl-CoA which is an important coupling factor in the secretion of insulin by stimulating protein kinase C isoforms, activation of ATP-sensitive K+ channels and acetylating proteins to target them to appropriate membrane sites, so 0 pancreatic islets respond less and glucose-induced insulin secretion is abolished [54].

The mRNA expression level of PGC-1 α is lower in the skeletal muscles of diabetic patients [55]. Reductions in PGC-1 α have also been ob-
served when fasting insulin concentrations are increased and with an increased body mass index in diabetes-prone humans [56]. Thus, there appears to be a potential role for PGC-1α in the etiology of insulin resistance in human skeletal muscle. This linkage is strengthened by the fact that PGC-1α is also down regulated in selected animal models of insulin resistance and type 2 diabetes [57,58].

The previous studies are in agreement with the present study which showed that Real-time RT-PCR analysis of gene expression of PGC-1α in the gastrocnemius muscle of diabetic rats was significantly decreased compared to normal rats. Exercise did not affect PGC-1α mRNA of in normal rats, Its level was significantly elevated in diabetic rats by exercise, not by diet restriction compared to diabetic rats fed at libitum but this elevation did not reach to its value in normal rats. Many studies showed that PGC-1α mRNA and protein expressions are very responsive to endurance exercise [59-64]. Also our results showed that exercise with diet restriction significantly increase PGC-1α mRNA than diet alone [65].

The possible mechanisms that might regulate this response is that exercise caused activation of AMP-activated protein kinase (AMPK), calcium/calmodulin-dependent protein kinase (CaMK) II and p38 mitogen-activated protein kinase (MAPK) which are upstream modulators of PGC-1α expression in skeletal muscle [59,66,67]. And transcription factors that are coactivated by PGC-1α, such as myocyte enhancer factor 2 (MEF2) [68], leads to a second phase of adaptation characterized by an increased expression of PGC-1α and higher protein content, which could serve to sustain the increase in mitochondrial content.

In the present study exercise in normal rats caused insignificant increase in ERRα mRNA when compared to normal sedentary rats. STZ induced diabetes caused significant decrease in ERR-2α mRNA in gastrocnemius muscle when compared to normal trained rats. Its level was elevated by exercise, not by diet restriction when compared to diabetic rats and this elevation reached to its level in normal trained rats.

The results of the present study are consistent with that of Cartoni et al., [64] who reported that exercise leads to increases in the gene expression of PGC-1α and its coactivators of mitochondrial biogenesis, ERRα and NRF-2 as well as that of the mitochondrial fusion proteins Mfn1 and Mfn2.

ERRα serves as a critical nodal point in the regulatory circuitry downstream of PGC-1α to direct the transcription of genes involved in mitochondrial energy-producing pathways in cardiac and skeletal muscle. ERRα null mice show defects in lipid metabolism and decreased expression of genes coding for fatty acid oxidation enzymes and oxidative phosphorylation components [38]. Herzo et al., [69] suggest that enhancing ERRα activity could have beneficial effects on glucose metabolism in diabetic subjects by two distinct mechanisms: Increasing mitochondrial oxidative capacity in peripheral tissues and liver, and suppressing hepatic glucose production.

Bach et al., [39,40] reported that Mfn2 expression is down regulated in skeletal muscle in animal or human obesity and in type 2 diabetic patients which is in accordance with the results in the present study which showed that STZ induced diabetes caused significant decrease in Mfn2 mRNA in gastrocnemius muscle of rat.

Our results showed that exercise in normal rats caused insignificant increase in Mfn2 mRNA when compared to normal sedentary rats. But exercise in diabetic rats increased and restore Mfn2 mRNA level to its level in normal rats. In contrast to our results Alvarez et al., [70] found that exercise caused no increase in Mfn2 in young type 2 diabetic subjects. While Little [71] et al., reported that exercise increases Mitofusin2 (71%) protein content in patients with type 2 diabetes.

The GLUT4 protein is recruited from intracellular sites by insulin and exercise by different signaling pathways. It translocates to the cell membrane and transverse tubules, where it mediates the transport of glucose into the muscle cells [72,73]. The increase in GLUT4 expression is mediated by the transcription factors myocyte enhancer factor 2 (MEF2A) and D and a GLUT4 enhancer factor (GEF) [74]. PGC-1α coactivates MEF2A and also increases MEF2A protein expression by activating NRF-1 [75], and possibly, other transcription factors that regulate MEF2A expression. Furthermore As a consequence of this adaptive increase in GLUT4, muscle glycogen storage following glycogen depleting exercise occurs more rapidly and to a greater extent in the trained than in the untrained state [76,77].

Physical training increases muscle GLUT4 protein and mRNA in patients with NIDDM [78,79]. In the present study we found that GLUT4 mRNA in the skeletal muscle of STZ-induced diabetic rats was decreased [78], but exercise training reversed diabetes-induced decrease of GLUT4 mRNA. Other studies on the skeletal muscles of rats [80,81] found
that exercise training increased GLUT4 protein expression in insulin deficiency and insulin resistance.

In the present study exercise had no effect on glycogen of the muscle in normal rats, while diabetic rats had decreased glycogen content of gastrocnemius muscle which increased by exercise but did not reach its value in normal rats. Exercise training-induced increases in muscle glycogen content could be regulated by multiple mechanisms, including enhanced insulin sensitivity, glycogen synthase expression, allosteric activation of glycogen synthase, and protein phosphatase 1 (PP1) activity [82].

PGC-1 alpha was shown to increase muscle glycogen stores via several mechanisms including stimulation of glucose import, suppression of glycolytic flux, and by down-regulation of the expression of glycogen phosphorylase and its activating kinase, phosphorylase kinase alpha. These findings identify PGC-1 alpha as a critical regulator of skeletal muscle fuel stores. Conversely, PGC-1 alpha-deficient animals exhibited reduced rates of muscle glycogen repletion post-exercise [28].

In the present study exercise in normal rats had no effect on CPT-1mRNA, while Hildebrandt et al. [83] found that CPT-1mRNA is significantly increased in gastrocnemius muscle of normal rats. Our results showed significant decrease in CPT-1mRNA in STZ-induced diabetic rats and exercise but not diet restriction caused significant increase in CPT-1mRNA to the normal level. In contrast to other studies results Huang et al., [84] found that exercise or diet restriction for 8 weeks having no effect on CPT-1mRNA in skeletal muscle of in KKAy obese/diabetic mice. While exercise increased CPT-1mRNA and protein in skeletal-muscle obese mice [85] and human [86]. It was also reported that exercise increased CPT-1mRNA in skeletal muscle of healthy subjects under the effect of striated muscle activator of Rho signalling (STARS) which is a transcriptional target of PGC-1 α and ERRα [37]. Meredith et al., [87] demonstrated that PGC-1 stimulates CPT-1 β gene expression in rat neonatal myocytes through interaction with myocyte enhancer factor 2 (MEF2).

Lipid peroxidation of cellular structures, a consequence of increased oxygen free radicals, is thought to play an important role in atherosclerosis and microvascular complications of diabetes mellitus [88]. Reactive oxygen species (ROS) in turn, can profoundly modify, and even damage, mitochondrial function [89]. PGC-1 α expression is induced by ROS, and PGC-1 α has been shown to limit the accumulation of ROS and be protective against oxidative damage [90].

In our study, MDA (marker of lipid peroxidation) content of skeletal muscle was significantly increased in diabetic group. Exercise decreased the elevated MDA. Our result is consistent with the other studies results [91-93] who indicated an increase in lipid peroxidation in diabetes mellitus and decreased by exercise.

It was reported that caloric restriction resulted in significantly reduced malondialdehyde in hepatocytes of streptozotocin-induced diabetic rats [94]. Also our study showed that diet restriction decreased significantly the elevated MDA content of skeletal muscle.

Our results showed significant decreased in citrate synthase (CS) mRNA in diabetic rats which did not changed by diet restriction but increased insignificantly by exercise and diet restriction. Lehti et al., [95] found that Citrate synthase activity was lower in the skeletal muscles of diabetic mice compared with healthy mice (p<0.05). Trained healthy and trained diabetic mice both had higher citrate synthase activity than their respective untrained controls [96]. It was reported that overexpression of PGC-1 in skeletal muscle of rats increased the activity of citrate synthase [97].

We concluded that diet restriction and/or endurance training in diabetic rats induces mitochondrial bioenergetics in skeletal muscle, and enhances mitochondrial function and improve glucose uptake by skeletal muscle possibly due to increase PGC-1 α and ERRα. Therefore represent a key strategy in the prevention and treatment of type 1 diabetes and in those at increased risk for this disorder.

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