Effects of Chronic Exercise and/or Food Restriction on Growth Factors in Diabetic Male Rats

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Abstract
Physical training has a significant effect on GH/IGF-1 axis in diabetic rats that may improve metabolic disturbances caused by diabetes on glucose homeostasis. Also, nutrition is one of the major factors regulating this axis. The aim of the present study was to compare the effects of food restriction and/or aerobic exercise on the growth hormone (GH)/insulin-like growth factor-1 (IGF-1) axis in diabetic rats. In this work six groups, each was formed of ten rats, 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: Serum glucose, insulin, free fatty acids and leptin as well as hepatic glycogen stores and Insulin-Like Growth Factor-1 (IGF-1) mRNA gene expression in liver were estimated. Diabetes increased serum glucose, free fatty acids and leptin and decreased insulin, liver glycogen stores and IGF-1 mRNA gene expression in the liver. Physical training significantly decreased serum glucose and leptin, significantly increased serum insulin, liver IGF-1 mRNA gene expression and glycogen stores, and insignificantly increased serum GH in diabetic rats. Diet restriction significantly decreased serum glucose, insulin, free fatty acids and leptin and insignificantly increased serum GH in diabetic rats. Combining physical training with diet restriction significantly decreased serum glucose, insulin, free fatty acids and leptin and insignificantly increased liver IGF-1 mRNA gene expression and serum GH level in diabetic rats.

In conclusion, physical training counteracts the inhibitory effects of diabetes on IGF-1 levels and in turn improves glucose homeostasis; while diet restriction caused an nearly no change in IGF-1 gene expression in liver of diabetic rats and an insignificant increase in serum GH levels. The combination of physical training and diet restriction didn’t affect the levels of IGF-1 gene expression in diabetic rats nor the serum GH levels significantly, but had a significant improvement in glucose homeostasis.

Key Words: IGF-1 – Growth hormone – Diabetes – Physical training – Diet restriction.

Introduction
CLINICAL studies demonstrated that poorly controlled diabetes mellitus is associated with growth retardation and reduced serum insulin-like growth factor 1 (IGF-1) [1,2]. IGF-1 is a polypeptide that has 48% amino acid sequence identity with proinsulin and induces changes in protein and carbohydrate metabolism, its gene is expressed in many tissues but the liver and bone are the primary sources of circulating IGF-1 [3].

The effect of exercise on growth factors may be different in local tissues than in the circulation [4]. Growth hormone (GH) for example is produced only in the pituitary and is known to stimulate hepatic IGF-1. This process is responsible for most of the IGF-1 found in the circulation. Moreover, exercise training can stimulate local muscle production of IGF-1 even in the absence of GH [5]. GH/IGF-1 pathway plays an important role in the maintenance of skeletal muscle mass and function in adults [6,7]; IGF-1 also plays a central role in exercise-associated muscle hypertrophy [8].

High levels of activity and fitness had been shown to increase the secretion of both GH and IGF-1 [9,10]. Regular exercise improves metabolic control in diabetic individuals and is an important component of treatment in diabetes mellitus [11].

Nutrition is one of the main regulators of circulating IGF-1. In humans serum IGF-1 concentrations are markedly lowered by energy deprivation. Energy is critical in the regulation of serum IGF-1 concentrations. Indeed after fasting optimal intake of both energy and protein is necessary for the rapid restoration of circulating IGF-1. Food restriction induces glucosidic profile modification that induces modification of hormonal regulation of growth factors or leptin [12].

Energy balance during periods of exercise training may influence circulating IGF-1 and related growth mediators [13].
The aim of this study is to compare the effects of food restriction-low carbohydrate diet and/or aerobic exercise on the GH/IGF-1 axis in diabetic rats.

Material and Methods

The present study was carried out in the Physiology Department, Faculty of Medicine, Cairo University. Sixty male albino rats with body weights 150-200 grams were included in this study. The animals were purchased 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 10 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: 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. \[14\]

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) \[15\].

Training:
Training included daily swimming 30 minutes/day in 2 sessions each formed of 15 minutes separated by 10 minutes rest, 5 days/week, for 12 weeks \[16\].

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, insulin, GH, IGF-1, leptin and free fatty acids (FFA). Then, animals were sacrificed and livers were rapidly excised for further detection of liver glycogen stores.

Measurement of fasting blood glucose level:
Serum glucose was measured using oxidase-peroxidase method \[17\].

Measurement of serum insulin:
Serum insulin levels were analyzed using enzyme-linked immunosorbent assay ELISA (Dako, Carpinteria, CA) according to the manufacturer’s instructions \[18\].

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

Measurement of FFA:
FFA was assessed in serum sample by colorimetric method using kit supplied by Zen-Bio, Inc. – Biotechn. North Carolina USA, according to manufacture instruction \[19\].

Measurement of GH and leptin:
GH was measured by ELISA kit supplied by (SPI BIO, France) according to manufacture instruction; also leptin level was assessed by Rat Leptin ELISA Kit supplied by (Ray Biotech.Inc. USA) \[20\].

Detection of IGF-1 gene expression by polymerase chain reaction (PCR):
Total RNA was extracted from liver tissue homogenate using RNAeasy purification reagent (Qiagen, Valencia, CA) according to manufacturers Single stranded cDNA was synthesized from the total RNA as follows. In brief, 1 µg RNA was pre-incubated with 1 µL oligo (dT) 15 primer, and diethylpyrocarbonate (DEPC)-treated water was added to a total volume of 9.5 µL at 70ºC for 10min, and then rapidly chilled on ice. To the annealed primer/template, 4 µL 5 x RT (reverse transcriptase) buffer, 0.5 µL dNTP (10mmol/L each), 25U ribonuclease inhibitor (Promega, Madisson, USA), 200U Moloney murine leukemia virus reverse transcriptase and DEPC-treated water were added to a final volume of 20 µL. The reaction was incubated at 42ºC for 1h and terminated by placing it on ice after deactivation at 70ºC for 10min. The resultant cDNA was used as a template for subsequent polymerase chain reaction (PCR). The PCR mixture contained 5 µL 10 x Taq buffer (Promega, Madisson, USA), 4 µL dNTP (10mmol/L each), 2 µL gene-specific primers, 2.5U Taq DNA polymerase and 2 µL cDNA in a total volume of 50 µL. Thirty cycles of PCR amplification were performed with an initial incubation at 94ºC for 3min and a final extension at 72ºC for 7min. Each cycle consisted of denaturation at 94ºC for 30s, annealing at 53ºC for 30s, and extension at 72ºC for 30s.

Presence of RNA in all samples was assessed by
analysis of the ‘house-keeping’ gene β-actin. The primer sequence was shown in Table (1). PCR products were electrophoresed on 2% agarose stained with ethidium bromide and visualized by ultraviolet transilluminator. Semiquantitation was performed using gel documentation system (BioDO, Analyser, Biometra, Göttingen, Germany). According to the amplification procedure, relative expression of each studied gene (R) was calculated according to the following formula: Densitometrical units of each studied gene/densitometrical units of β-actin.

Table (1): The oligonucleotide primers sequence.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sequence</th>
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<tbody>
<tr>
<td>IGF-1 Forward</td>
<td>5' - AGCCAAGGGGCAGAGTTTAT-3'</td>
</tr>
<tr>
<td>Reverse</td>
<td>5' - CGCCTTCACGCAAACATATA-3'</td>
</tr>
<tr>
<td>β-actin Forward</td>
<td>5' - TCA CCC TGA AGT ACC CCA TGG AG-3'</td>
</tr>
<tr>
<td>Reverse</td>
<td>5' - TTG GCC TTG GGG TTC AGG GGG-3'</td>
</tr>
</tbody>
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Statistical analysis:

The data were statistically analyzed using the statistical package SPSS (version 12). Values were expressed as mean ± standard deviation (SD). The groups were compared with one-way ANOVA followed by a post-hoc analysis using the Bonferroni test for multiple comparisons. The level of statistical significance was set at \( p \leq 0.05 \).

Results

The effect of food restriction-low carbohydrate diet and/or aerobic exercise on the GH/IGF-1 axis was investigated in diabetic rats. Results were shown in Table (2) & Fig. (1).

There was no significant change \( (p>0.05) \) in the levels of glucose and insulin between group 1 (Sedentary fed ad lib) and group 2 (Trained fed ad lib). 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 in glucose levels in group 6 compared to group 4.

While there was a significant decrease \( (p<0.05) \) in insulin value in group 3 compared to group 1, group 2, group 4 and group 6, no significant difference was observed on comparing group 3 to group 5.

No significant differences in glucose levels between groups 4 and 5. Moreover, no significant change in insulin levels between groups 4, 5 and 6.

Exercise in normal rats did not significantly affect glucose and insulin levels compared to normal sedentary rats, while it reduced glucose levels significantly in diabetic trained rats (group 5) compared to diabetic sedentary fed ad. libitum (group 3).

The diet restriction and exercise decreased the glucose level and elevated the insulin level in the diabetic rats but did not reach the normal control levels.

The results showed that diabetes induced reduction of liver glycogen compared to non-diabetic rats. Diet restriction alone resulted in insignificant decrease in liver glycogen, while exercise alone resulted in marked significant increase in hepatic glycogen. Combination of diet restriction and exercise showed a significant elevation of glycogen compared to diabetic fed ad lib group. However, the glycogen level was not restored to its normal control content in the liver with diet restriction, exercise or combination of both.

The results showed that exercise in normal rats did not affect FFA level significantly compared to sedentary rats. Also, the results demonstrated that diet restriction is more effective in decreasing FFA than exercise in diabetes. Diet restriction alone or in combination with exercise resulted in marked reduction in FFA level.

Exercise and/or diet restriction could decrease the levels of leptin in diabetic rats but not to the extent of normal control values.

Diabetes significantly decreased GH level compared to normal control values. Diet restriction, exercise or combination of both could elevate the GH levels slightly above the corresponding value of diabetic rats, however those elevations were insignificant to the normal control values (Table 2).

Diabetes markedly decreased the IGF-1 gene expression compared to normal control rats. Exercise training in diabetic rats resulted in significant increase in IGF-1 gene expression. Diet restriction alone or combined with exercise resulted in insignificant change in liver IGF-1 gene expression level compared to diabetic rats (Table 2 & Fig. 1).
Exercise training in diabetic rats leads to significant increase in IGF-1 gene expression, while diet restriction alone or combined with exercise results in insignificant change in IGF-1 gene expression level compared to diabetic rats.

Diabetes type 1 is induced using streptozotocin (STZ) (60mg/kg), this results in significant increase in glucose levels in all groups treated with STZ as compared with non diabetic groups: Group 1 (Sedentary fed ad lib) and group 2 (Trained fed ad lib).

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 [15] 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 [21].

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 [22].

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 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 [23]. The possible mechanism by which FFA exert this effect is by increasing levels of pancreatic islet carnitine palmitoyl transferase-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 β pancreatic islets respond less and glucose-induced insulin secretion is abolished [24].

In the present study glycogen contents in the livers were measured and the results shows that diabetes markedly impairs the ability of the liver to produce glycogen as demonstrated in the significant decrease in hepatic glycogen after STZ administration. Physical training increases hepatic glycogen in trained diabetic groups but does not restore it to the normal control values.

Diabetes decreases the activity of hepatic glycogen synthase leading to decreased liver glycogen [15]. It was reported that diabetes impairs the hepatic glycogen synthesis whether in the fed ad libitum state or diet restricted state [25]. These results are in agreement with other study which reported that endurance exercise induces chronic adaptations in liver, enabling diabetic rats to restore their hepatic stores of glycogen [26].

The mechanism underlying the effect of exercise on liver glycogen is evidenced in a study by Remedio et al. [27] who reported that effect of exercise on liver glycogen is through better absorption of blood glucose and its conversion into liver glycogen (glycogenesis) or even through decrease glycogenolysis. It was reported that the difference in liver glycogen between trained and untrained diabetic rats is due to that intermittent exercise is a muscle recruitment function that spares liver glycogen and that exercise induces hormonal and metabolic changes which during rest causes improved glucose homeostasis in diabetic rats and it’s associated with GH/IGF-1 pathway [28].

The present study shows significant changes in the serum levels of FFA in STZ-induced diabetic rats as compared to non diabetic rats. Exercise alone does not significantly affect FFA level in diabetic or non diabetic rats. Diet restriction, alone or in combination with exercise, results in marked reduction in FFA level in diabetic rats. Diabetes inhibits tissue lipase that leads to increased serum FFA that increases the insulin resistance. Diet restriction has decreased levels of FFA in diabetic rats whether trained or not [29]. In contrast to the present study, it was reported that exercise elevates the levels of FFA after one bout of exercise in fasting rats with or without glucose infusion [30].

The results of the present study are in accordance with results of another study that levels of serum leptin is increased in chronic diabetic rats in which fat-specific insulin receptors were knocked-out and this may be related to the decrease in plasma adiponectin, indicating the complex interplay between glucose metabolism and adipokines [31].

Contradictory results were reported by German et al. [32] that after 2 weeks of inducing diabetes by the use of streptozotocin, depletion of body fat stores resulted in markedly reduced plasma leptin levels, however that contradiction may be explained by the difference in the duration of their experiment (2 weeks) and that of the present work (12 weeks). The author also reported that leptin deficiency may induce insulin resistance and this may have a role in the development of diabetes.

Insulin appears to increase leptin secretion, in turn, leptin increases peripheral insulin sensitivity while decreasing insulin secretion from pancreatic beta cells. Leptin increases skeletal muscle glucose uptake and oxidation, and suppresses hepatic glucose output. Effects of leptin on lipid metabolism might reduce lipotoxicity and therefore contribute to the improvement of hepatic, skeletal and whole body insulin sensitivity and so, it improves hyperglycemia, insulin resistance, hyperinsulinemia, dyslipidemia and hepatic steatosis in lipoatrophic diabetes and this may indicate adaptive responses of leptin in diabetes [33].

In the present study, exercise induces a significant decrease on leptin levels in diabetic rats which is in accordance with the results obtained by Ozcelik et al. [34] who investigated the effect of exercise on obese diabetic patients and concluded that leptin levels decrease in response to 12 weeks of training and this decline was so closely associated to the decrease in body fat mass. The effect of diet restriction on leptin levels in this study is in consistence with other human as well as animal studies [15,35], that a decrease in plasma leptin concentrations in fasting humans or animals may
be a potent stimulus of the food intake by an action on appetite, an important homeostatic response to maintain the body energy stores.

In the present study, STZ-induced diabetes results in significant decrease in GH levels as compared to non diabetic rats. Exercise dose not affect GH level in normal rats. In diabetic rats, diet restriction, exercise or combination of them elevates the GH slightly above the value of diabetic rats but this elevation is statistically insignificant compared to the diabetic or even the normal control values.

The results of the present study are consistent with that of Gahete et al. [36], who reported significant decrease in GH plasma levels in diabetic rats compared to normal control groups with reduction in pituitary GH peptide content and increase in the pituitary GH Releasing Hormone-Receptor and decrease in GH mRNA levels in pituitary cells. The inhibitory effect of diabetes on GH secretion may be due to factors associated with catabolic state [37].

A possible mechanism by which fasting increases GH secretion is that fasting increases IGFBP-1 and 2 leading to a marked drop in the IGF-1 levels that removes the inhibitory feedback effect exerted by IGF-1 on GH secretion [38]. Another study, postulated another mechanism to explain the fasting associated increase of GH, assumes that gastric “ghrelin” secretion which is increased by fasting also contributes to that rise of GH and that ghrelin is the specific endogenous ligand for GH secretagogue-receptor (GHS-R) [39].

In the current study, exercise training has insignificantly elevated levels of serum GH; this is in agreement with a study done by Takashi [40] who reported that GH levels were increased in response to exercise in obese subjects with type-2 diabetes. GH and the growth hormone-releasing hormone (GHRH) were increased in response to strenuous exercise in healthy subjects performing exercise on cycle ergometer for 6 weeks [41]. The stimulatory effect of exercise on serum GH levels can be explained by the inhibitory effect of exercise on hypothalamic somatostatinergic tone mediated by activation of the central cholinergic system, GHRH increases during exercise possibly due to another ligand that has not been fully understood [42].

In the present study, STZ-induced diabetes results in significant decrease in IGF-1 gene expression in the liver as compared to non diabetic rats. Exercise alone significantly increases the IGF-1 mRNA gene expression in the livers of both normal non-diabetic and diabetic rats. Diet restriction alone or combined with exercise insignificantly changes the IGF-1 gene expression in the livers of diabetic rats.

The results of the present work are in agreement with previous study [21] which showed that diabetes is associated with reduced IGF-1 blood concentrations. Han et al. [43] also reported that diabetes reduces the expression of hepatic IGF-mRNA as well as serum IGF-1 levels; this effect may be related to a reduced hepatic responsiveness to GH. In another study it was demonstrated that circulating levels of IGF-1 are decreased in diabetic rats, this was attributed to the increase in IGFBP-1, so proportionally more IGF-1 will be bound to it. IGFBP-1 can cross capillary walls contrary to IGFBP-3 that is actually decreased in diabetic rats [44].

The interrelationships between IGF-1, IGFBP-1 and insulin are complex. IGF-1 generation and activity are insulin dependent thus in conditions of insulin deficiency, IGF-1 levels are depressed whereas levels of its inhibitor IGFBP-1 is elevated, in return IGF-1 may have an important role in the promotion or maintenance of insulin secretion and Beta cell mass [45]. Experimental studies have demonstrated that the IGF-1 receptor may be important in promoting pancreatic beta cell development and survival and these effects may be mediated through the insulin receptor substrate-2 signaling pathway such knockout animals are born with insufficient beta cell to maintain glucose homeostasis and they can’t produce new or sustained survival of existing beta cells [46].

In the present study diet restriction caused an insignificant change in the levels of IGF-1 gene expression in the liver, this is consistent with Karrie et al. [47] who reported that there was no significant difference in IGF-1 levels in mice fed on low carbohydrate diet and mice fed on balanced diet. However IGF-1 levels are much reduced in mice fed on low carbohydrate diet and mice fed on balanced diet. IGF-1 levels are much reduced in mice fed on low carbohydrate diet and mice fed on balanced diet.

In humans, serum IGF-I concentrations are markedly lowered by energy deprivation. Energy is critical in the regulation of serum IGF-I concentrations. Indeed, after fasting, optimal intake of both energy and protein is necessary for the rapid restoration of circulating IGF-I. Food restriction induces glucosidic profile modifications that induce modification of hormonal regulation of growth factors or leptin. However, that in adult human energy may be somewhat more important than protein in this regard. While the lowest protein
intake is able to increase IGF-I in the presence of adequate energy, there is a threshold energy requirement below which optimal protein intake fails to raise IGF-I after fasting. When energy intake is severely reduced, the carbohydrate content of the diet is a major determinant of responsiveness of IGF-I to GH. The essential amino acid content of the diet is also critical for the optimal restoration of IGF-I after fasting, when protein intake is reduced [12].

It is reported that long term physical training restored hepatic IGF-1 levels in diabetic rats and could contribute to the restoration of this hormone in these animals, serum IGF-1 levels show a significant correlation with hepatic IGF-1 [48]. On the other hand, Nindl et al. [49], proposed that longer periods of training result in increased concentrations of IGF-I. Such findings have led to the hypotheses that the IGF-I system may exhibit a two-phased response (an initial decrease, followed by an increased after 5 weeks).

The results of another study demonstrated that, the IGF-1 response to physical training depends on the intensity of training: Short term physical training (5 weeks) reduces circulating IGF-1 levels whereas prolonged training (>5-6 weeks) increases circulating IGF-1 levels. It has been shown that in normal and diabetic rats, short term physical training (four weeks) decreases IGF 1 concentration, whereas six weeks of physical training doesn’t change the blood IGF-1 in control rats but restores this parameter in diabetic rats [50].

Similarly, diabetes also reduces IGF-1 concentrations but these are restored by chronic exercise. The precise source of blood IGF-1 in trained diabetic animals and humans remains uncertain but probably involves the liver since there is a marked reduction (80%) in the serum IGF-1 concentrations of mice with IGF-1 deficient livers [51]. So, diabetes causes a reduction of IGF-1 gene expression in liver and so, IGF-1 circulating levels, but physical training improves this condition. Physical training exerts this effect by increasing the sensitivity and responsiveness to insulin. Several of the beneficial effects of exercise result from interactions between specific hormones and growth factors such as IGF-1 [50].

A study done by Ryan et al. [52] who reported that exercise with calorie restriction improves insulin sensitivity and glycogen synthase activity in obese post-menopausal women with impaired glucose tolerance. It was also reported that combining food restriction with exercise training increases insulin sensitivity and this in turn causes a decrease in blood glucose levels and decreased plasma triglycerides, cholesterol and free fatty acids [53]. Haus et al. [54], had proposed that changing lifestyle (exercise training and calorie restriction) is very efficient in decreasing plasma free fatty acids and so, increasing insulin sensitivity.

The results of the present work support the expectation that physical training counteracts the inhibitory effects of diabetes on IGF-1 levels and in turn improves glucose homeostasis; this can be explained by the effect of exercise on increasing sensitivity to insulin and initiating the transcription at promoter 1 of the IGF-1 gene, while diet restriction caused an insignificant change in IGF-1 gene expression in liver of diabetic rats and an insignificant increase in serum GH levels.

The combination of physical training and diet restriction didn’t affect the levels of IGF-1 gene expression or the serum GH levels significantly in diabetic rats, but had a significant improvement in glucose homeostasis; however the precise mechanism by which this combination affects GH/IGF-1 axis needs further exploration.

References

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