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# INFLUENCE OF MODERATE PHYSICAL TRAINING ON THE GH/IGF-1 AXIS IN DIABETIC RATS

**R.J. Gomes[1], F.H. Caetano[2], M.A.R. Mello[1], E. Luciano[1]**
*Depts. of [1]Physical Education, [2]Biology, State University of São Paulo (UNESP), Rio Claro-SP, Brazil*

**Abstract.** The influence of moderate physical training on serum growth hormone (GH), insulin-like growth factor –1 (IGF-1) and binding protein ( IGFBP-3) in experimental diabetic rats was investigated. Male Wistar rats were divided into 4 groups, sedentary control (SC), trained control (TC), sedentary diabetic (SD) and trained diabetic (TD). Experimental diabetes was induced of Alloxan (35mg/b.w.) The training program consisted by swimming 5 days/week, 1 h/day, supporting a load of 2.5% b.w., during 6 weeks. Then, the rats were sacrificed and blood was collected for determinations of serum glucose, insulin, GH, IGF-1 and IGFBP-3. Samples of liver were used to evaluate glycogen, protein and DNA contents. The results were analyzed by ANOVA, and Bonferroni test and the significance level was set at 2.5%. Diabetes decreased serum GH, IGF-1, IGFBP-3 and liver glycogen stores in SD group. Physical training promoted increase in serum IGF-1 in both TC and TD groups (SC=82±15; TC=103±13; SD=77±16; TD=112± 29 ng/ml) and liver glycogen store in TD group when compared to SD (SC=5.2±1.2; TC= 6.2±1; SD=2±0.5; TD=5±1.8 mg/100mg). Therefore, physical training contributes to the increase in liver glycogen content and to rise of insulin-like growth factor level in diabetic rats. It was concluded that moderate physical training promotes important adaptations related to GH-IGF-1 axis in diabetic organisms.

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*Key words: Diabetes Mellitus* - Growth hormone - Insulin-like growth factor (IGF-1) - Binding protein (IGFBP-3) - Physical training

Reprint request to: Dr. Ricardo José Gomes, Dept. of Physical Education, State University of São Paulo (UNESP), Rio Claro-SP, Brazil, Avenida 24A nº1515, Bela Vista, Rio Claro – SP, Brazil. Caixa Postal 199; CEP 13506-900; Fax 35340009, E-mail: ricarjg@ibestmail.com.br

## Introduction

*Diabetes Mellitus* is a chronic disease, characterized by extensive biochemical alterations, which occur in consequence of an absolute, or relative insulin deficiency. Diabetes leads to a variety of complications that include nephropathy, retinopathy, and cerebrovascular and cardiovascular disease [19]. Poorly controlled *Diabetes Mellitus* is associated often with impaired growth in animals and sometimes in humans [32]. These metabolic alterations may modify growth hormone (GH) and insulin-like growth factor (IGF-1) synthesis and releasing [31]. GH acts on hepatocytes IGF-1 production. IGF-1 is the major component of the GH-IGF-1 axis, a system of growth mediators, receptors, proteases, and binding proteins that control somatic and tissue growth [16,17]. The IGF-1 family includes three known ligands (IGF-1, IGF-2, and insulin), six binding proteins (IGFBP-1 through-6), and cell surface receptors that mediate the actions of the ligands (IGF-1 receptor, insulin receptor and the IGF-2 mannose-6-phosphate receptor) [6]. IGF-1 or Somatomedin-C is a polypeptide that has 48% amino acid sequence identity with proinsulin and induces changes in protein and carbohydrate metabolism. The IGF-1 gene is expressed in many tissues but liver and bone are the primary sources of circulanting IGF-1. More than 80% of circulanting IGF-1 is carried in a trimeric complex composed of IGFBP-3, the largest molecular weight IGFBP, and a liver – derived glycoprotein known as the acid-labile subunit (ALS) [27].

The effect of exercise on growth factors may be different in tissues from that in the circulation. 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 blood [17]. However, exercise training can stimulate local muscle production of IGF-1 even in the absence of GH [33]. The growth hormone/insulin-like growth factor (GH/IGF-1) pathway plays an important role in the maintenance of skeletal muscle mass and function in adults [5,18]. IGF-1 also plays a central role in exercise-associated muscle hypertrophy [1,29]. Physical activity influences tissue growth, but the mechanisms linking exercise with muscle hypertrophy, increased capillarization and mitochondrial capacity, and stronger bones are not completely understood [26]. High levels of activity and fitness have been shown to increase both the secretion of the growth hormone/insulin-like growth factor 1 [9,17,23].

Regular exercise improves metabolic control in diabetic individuals and is an important component of treatment in diabetes *mellitus* [19]. However less is known about the role of moderate exercise on GH-IGF-1 axis in diabetic organisms. Thus, the

propose of this study was to assess the effects of moderate physical training on serum GH, IGF-1 and IGFBP-3 in diabetic Wistar rats.

## Materials and Methods

*Population:* Forty male Wistar rats were used for the study. They were separated at the postnatal day 48 (150-200g) and were allowed standard food and water *ad libitum.* For the study, the rats were randomly distribuited in four groups (n=10 per group), Sedentary Control (SC), Trained Control (TC), Sedentary Diabetic (SD) and Trained Diabetic (DT). Diabetes was induced by an i.v. injection of Alloxan Sigma (35 mg/kg b.w.). After 5 days, blood samples were obtained from animals in the fed state to determine the plasma glucose concentration. Rats that were not diabetic (<250 mg/dl) or too severely diabetic (>600 mg/dl) were eliminated from the study.

*Protocol:* The training included daily swimming with load of 2.5% b.w., 1 h/day, 5 d./wk, for 6 weeks. At the end of the training period, hematocrit was measured at rest, to ensure that measurements of metabolite and hormone concentrations were not influenced by changes in plasma volume. A capillary tube sample of blood was spun at 3,000 rpm for 3 min., and the hematocrit was determined. Then, the rats were sacrificed 48 h after their last exercise bout and the samples blood collected for glucose, insulin, GH, IGF-1 and IGFBP-3 determinations.

*Analytical methods*: Glucose. Serum glucose concentration was measured by a colorimetric method [15].

Insulin. Serum insulin concentration was determined by radioimmunoassay (RIA, Kit Coat-A-Count, USA).

Growth Hormone. Serum GH concentration was measured by radioimmunoassay (RIA) with the use of the commercially available kit (Coat-A-Count, USA).

Insulin-like growth factor (IGF-1). Serum IGF-1 concentration was measured by Immunoradiometric assay (IRMA) using commercially available kit (DSL-5600, Diagnostic System Laboratories Inc. Kits, Webster, TX).

Binding protein (IGFBP-3). Serum IGFBP-3 concentration was measured by Immunoradiometric assay (IRMA) using commercially available kit (DSL-6600, Diagnostic System Laboratories Inc. Kits, Webster, TX).

Samples of liver were used to evaluate glycogen, protein and DNA contents. Glycogen content was obtained following the method described by Dubois *et al.* [8]. Hepatic

total proteins were obtained following the method described by Lowry *et al.* [20]. Hepatic DNA was obtained following the method described by Giles and Mayers [14]. Statistical analysis. Analysis of variance (ANOVA) one-way was performed on these data, and the Bonfferroni test was the n used for comparisons. $P<0.05$ was considered statistically significant. All results are expressed as mean ± SD.

## Results

Table 1 provides the physical and physiological characteristics of the rats used in the study. Sedentary diabetic rats weighed less than nondiabetic rats ($p<0.05$). Diabetes resulted in an increase in glycaemia and in a decrease in insulinaemia in both Sedentary Diabetic and Trained Diabetic groups ($p<0.05$). Hematocrit was similar among all groups (Table 1).

**Table 1**
Body mass (g), blood glucose (mmol/L), insulin (pmol/L) and hematocrit (%) after 6 weeks of training. Values are means ± SD; n = 10 per group. SC (Sedentary Control); TC (Trained Control); SD (Sedentary Diabetic); TD (Trained Diabetic)

| | Body mass (g) | | Glucose (mmol/L) | | Insulin (pmol/L) | | Hematocrit (%) | |
|---|---|---|---|---|---|---|---|---|
| Group | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| SC | 379 | 55 | 6.5 | 0.7 | 109 | 14 | 43 | 1.5 |
| TC | 343 | 43 | 6.7 | 0.8 | 116 | 15 | 44 | 1.5 |
| SD | 277$^{a,b}$ | 49 | 24.3$^{a,b}$ | 5.5 | 86$^{a,b}$ | 14 | 45 | 1.5 |
| TD | 327 | 54 | 22.8$^{a,b}$ | 5 | 89$^{a,b}$ | 13 | 43 | 1.2 |

ANOVA; $P<0.05$; a≠SC; b≠TC; c≠SD; d≠TD

Serum GH concentration (Fig. 1) was lower in diabetic rats compared with nondiabetic rats ($p<0.05$). Moreover, diabetes reduced serum IGFBP-3 levels in Sedentary Diabetic group when compared with both Sedentary Control and Trained Control ($p<0.05$). However, in the Trained Diabetic group, serum IGFBP-3 did not change ($p<0.05$). Physical training increased serum IGF-1 in Trained Control group when compared to Sedentary Diabetic group. Exercise also increased serum IGF-1 in Trained Diabetic group when compared with both Sedentary Control and Sedentary Diabetic groups.

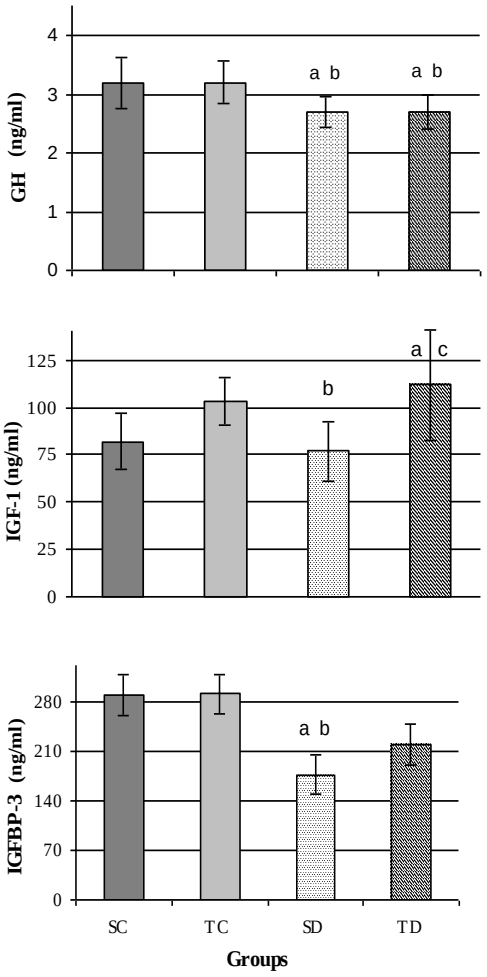


**Fig. 1**
Serum growth hormone (GH; ng/ml), Insulin-like growth factor (IGF-1; ng/ml) and it binding protein 3 (IGFBP-3; ng/ml) concentrations after 6 weeks of training. Values are means ± SD; n = 10 per group. SC (Sedentary Control); TC (Trained Control); SD

(Sedentary Diabetic); TD (Trained Diabetic). ANOVA; P<0.05; a≠SC; b≠TC; c≠SD; d≠TD

Hepatic protein, DNA concentration and the ratio protein/DNA were similar among all groups (Table 2). However, hepatic glycogen concentration was lower in Sedentary Diabetic group (p<0.05). Besides, exercise improved hepatic glycogen concentration in Trained Diabetic group, when compared with Sedentary Diabetic group (Table 2).

**Table 2**
Hepatic protein (mg/100mg), hepatic DNA (mg/100mg), protein/DNA ratio and hepatic glycogen (mg/100mg) after 6 weeks of training. Values are means±SD; n=10 per group. SC (Sedentary Control); TC (Trained Control); SD (Sedentary Diabetic); TD (Trained Diabetic)

| Group | Hepatic ptotein (mg%) | | Hepatic DNA (mg%) | | Protein/DNA ratio | | Hepatic glvocgen (mg%) | |
|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| SC | 2.5 | 0.38 | 0.18 | 0.02 | 14 | 3 | 5 | 1 |
| TC | 2.8 | 0.62 | 0.15 | 0.04 | 19 | 6 | 6 | 1 |
| SD | 2.9 | 0.77 | 0.18 | 0.04 | 16 | 4 | 2a,b,d | 0.5 |
| TD | 2.9 | 0.95 | 0.18 | 0.03 | 16 | 4 | 5 | 2 |

ANOVA; P<0.05; a≠SC; b≠TC; c≠SD; d≠TD.

## Discussion

Aerobic exercise is advocated for glycaemic control in diabetic patiens [11,19]. Many of the health-promoting effects of exercise result from the interaction of specific hormones and growth factors as insulin - like growth factor-1 (IGF-1) [10]. This study showed that Alloxan injection resulted in a decrease in plasma insulin in both sedentary diabetic and trained diabetic groups (p<0.05). Training did not attenuate hyperglycaemia in diabetic group, however, sedentary diabetic rats weighed significantly less than trained diabetic animals.

Serum growth hormone (GH) was reduced in diabetic rats and physical training did not increase this hormone concentration. We can explain this result by high glucose levels, because it is known that high glucose or somatostatin levels inhibit growth hormone secretion [4,7,31]. Our finding is consistent with data of Luciano and Mello [21], who showed a similar reduction in circulating GH levels in sedetary and trained diabetic rats. GH is anabolic hormone that induces a positive nitrogen balance and protein synthesis in muscle. Many of the effects of GH on growth and metabolism are mediated by control of the synthesis of other growth factors. Of these, the insulin-like growth factors (IGFs), are considered to be the most important [6,16].

Our results show that circulating IGF-1 was increased both in trained control and trained diabetic rats despite of low GH levels in diabetic group ($p<0.05$). Previous studies also showed that exercise induces an increase in serum IGF-1 in humans [2,30]. In diabetic rats, exercise improves serum IGF-1 levels and IGF-1 peptide content in gastrocnemius muscle [13]. Whether alternative tissue sources of IGF-1 or IGFBPs contribute to the circulating levels is not known, but even if they do, locally produced growth factors will be diluted and might undergo proteolysis or another degradation in the transit from tissue to the circulating blood [9].

Most circulating IGF-1 is bound by a complex of IGFBP-3 and the "acid-labile subunit" which appears to be not bioavailable to most tissues [17]. Moreover, increasing attention has been paid to the role of IGFBPs in regulating IGF-1 bioactivity. Approximately 80% of the IGFs in plasma are associated with IGFBP-3 in a large ternary complex that restricts their movement across the capillary endothelium, thereby retaining IGF-1 in the circulation [3,29]. Our study showed that IGFBP-3 is reduced in diabetic rats and indicate that a poor glycaemic control is associated with lower IGFBP-3 levels. These data are consistent with the large majority of the literature. Fedele *et al.* [13], reported that the plasma concentration of IGFBP-1 is increased, whereas the concentration of IGFBP-3 is decreased, in diabetic rats compared with nondiabetic animals. In the present study the effect of the exercise on plasma IGFBP-3 concentrations in the nondiabetic rats was insignificant. Another study showed that the plasma concentrations of IGFBP-3 were not significantly altered by exercise compared with the sedentary condition [28]. However, this study showed that circulating IGFBP-3 was increased in trained diabetic rats. IGFBP-3 is considered to be an integrated index of GH action, exercise and training have been reported to increase IGFBP-3, but there is not general agreement about this [23,25].

This study showed also that the moderate exercise no alters hepatic protein, DNA concentrations and the ratio protein/DNA. Farrel *et al.* [12], reported that severely diabetic rats are not able to increase rates of protein synthesis after moderate-intensity resistance exercise, suggesting that there is a critical concentration of circulanting insulin below which appropriate anabolic responses are inhibited. Our study shows that diabetes also had reduced resting hepatic glycogen stores, but the physical training was able to increase this substrate content. Therefore, the results reflect the chronic adaptations induced by physical training. Luciano and Mello [21], similarly showed a reduction in muscle and hepatic glycogen concentrations in experimental diabetic rats and the chronical exercise restored this substrate in muscle of trained group. The underlying mechanisms responsible for the improvements in muscle glucose metabolism are not fully understood, but evidence has demonstred a correlation between PI3-kinase activity and glycogen metabolism [11,19,22].

In conclusion, our study showed that diabetes induced by alloxan reduces plasma insulin, GH and IGFBP-3 concentrations in Wistar rats. Physical training did not reduce serum glucose in diabetic group, but increased hepatic glycogen stores and had a moderate effect on serum IGFBP-3 concentrations. Moreover, plasma IGF-1 was increased by physical training in control group and in diabetic group despite of lower GH concentrations. From these incises it therefore seems that physical training has some “reverse” effect to the deviations elicited by diabetics although training did not significantly affect blood glucose or plasma insulin. Thus, moderate physical training promotes important adaptations related to GH-IGF-1 axis in diabetic organisms, but the mechanisms distinguishing the endocrine vs. the paracrine/autocrine actions of IGF-1 and their binding proteins in diabetic organisms remain to be elucidated.

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