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The effect of 6 weeks resistance training on serum levels of IL-18 and TNF-α in type I diabetic male rats

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ABSTRACT

Diabetes mellitus (DM) and chronic inflammation are strongly related to increased cardiovascular risk. This study aims to investigate the influence of physical training on the inflammatory markers of diabetic rats. Adult male Wistar rats were distributed into Sedentary Control (SC), Trained Control (TC), Sedentary Diabetic (SD) and Trained Diabetic (TD) groups were used. Diabetes was induced by Stroptozotosin (55 mg/bw-i.v.). Training protocol consisted of resistance training(elevate upward weights), at 32 +/- 1 degrees C, one hour/day, five days/week, supporting an overload equivalent to 5% of the body weight, during four weeks. At the end of the experiment the rats were sacrificed by decapitation and blood samples were collected for glucose, IL-18 and TNF-α determination. The results were analyzed by one way at a significance level of 5%. Diabetes reduced blood insulin, HDL and increased blood glucose, IL-18, TNF-α .TG and LDL count. Physical training restored glycemia and inflammatory markers count in diabetic rats. In summary, physical training was able to improve metabolic and immunological aspects in the experimental diabetic rats.

Keywords: Physical training, inflammatory markers, diabetes mellitus.

INTRODUCTION

Many chronic diseases are now in pandemic proportions and increasingly a major cause of morbidity and mortality worldwide. Diabetes mellitus plays a starring role in this problem (1,2) with diabetic complications being a very important public health issue.

Recently, the effect of physical activity on immune function has been studied intenselyin diabetics patient. This is an important area of study because exercise may modulate the immune system's ability to monitor and protect the individual from disease and to repair damage. In most of these studies, aerobic exercise (AEX) and aerobic conditioning (ACO) have been the independent variables. Consequently, the functional immune response to ACO seems relatively clear. However, the immune response to resistance exercise (REX) is not as clear because few studies have been published. The immune response to REX may be different than that to AEX because of the different physiological demands of these 2 types of exercise. Resistance conditioning (RCO) does not have a significant effect on heart rate, blood pressure, cardiac output, stroke volume, vascular resistance, or the arteriovenous oxygen saturation difference during submaximal treadmill exercise (3,4).

During acute exercise muscles release IL-6, and levels can increase significantly (5). inflammatory cytokines as well as plasma concentrations TNF-α, IL-1, IL-1ra, IL-10, IL-18 and sTNF-r can increase to various magnitudes during a bout of exercise (6,7,8).

The pro-inflammatory markers TNF-α and IL-1β do not seem to increase in short periods of moderate intense exercise,although conflicting results have been documented (7,9).

The extent to which these changes occur in patients with a chronic inflammatory disease is important to address to ensure that Exercise is performed in a safe manner where inflammation is not being further amplified.

In an almost paradoxical way, however, participation in regular exercise (i.e., training) can reduce basal or resting levels of many inflammatory markers (6).

Type 1 pro-inflammatory cytokines such as interferon(IFN)- γ ,interleukin(IL)-1β,IL-12, IL-18 and tumour necrosis factor (TNF)-α realise by macrophage and T lymphocytes in the vicinity of pancreatic beta cells have repeatly been implicated in the pathogenesis of Type I(insulin-dependent)diabetes mellitus (10). IL-18 is another type 1 cytokine primatily produced by macrophages and closely related to the IL-1 family of cytokines. It has been shown to play a pivotal part in generation of type 1 cytokine responses through its ability with IL-12 to up-regulated IFN- γ production from T cells and natural killer cells .An up-regulated production of IL-18 could therefore be an important pathogenic event in the dysregulated production of IFN-γ and other type 1 cytokines thought to predispose to immuoinflammatory diseases such as type I diabetes. TNF-α is a pleiotropic inflammatory cytokine that is mainly produced by monocytes, macrophages, and T cells. In addition, and similar to other inflammatory cytokines, the expression and synthesis of TNF-α is not limited to hematopoietic cells. Furthermore, recent studies show that TNF-α can be stored within cells in a proactive form, and the TNF-α–converting enzyme can rapidly increase levels of the active cytokine (11).

In addition, relevant TNF-α effects have been reported, such as induction of apoptosis and necrotic cell death (12,13).

Cytokines are a group of pharmacologically active, low molecular weight polypeptides that possess autocrine, paracrine, and juxtacrine effects with characteristic features (16). These molecules cluster into several classes (i.e., interleukins, tumor necrosis factors, interferons, colony-stimulating factors, transforming growth factors and chemokines), which are relevant humoral mediators in a highly complex, coordinated network regulating inflammatory immune

responses with the participation of different cytokine-associated signaling pathways. In addition, they exert important pleiotropic actions as cardinal effectors of injury. Cytokines are produced by a wide variety of cells in the body, playing an important role in many physiological responses that have a therapeutic potential.

At the present time it is recognized that chronic low-grade inflammation and activation of the innate immune system are closely involved in the pathogenesis of diabetes mellitus.[19–20] Increasing evidence suggests that individuals who progress to diabetes mellitus display features of inflammation years before the disease onset (7,14). Moreover, population-based studies suggest that inflammatory parameters, including inflammatory cytokines, are strong predictors of the development of diabetes (8,10). The main cytokines involved in the pathogenesis of diabetes are IL-1, TNF-α, and IL-6.

However, there are few data about of the impact resistance exercise on inflammatory markers function and inflammation in type 1 diabetes, especially in type 1 diabetic patients.

The aim of this study was thus to investigate whether a 6-weeks supervised resistance exercise program could suppress the inflammatory cascade and trigger anti inflammatory mediators in male rats with type 1 DM.

MATERIALS AND METHODS

Male Wistar rats were used in the experiments (180-210 g; 40-day-old). They were kept at 25°C with a 12/12 light/dark cycle, and fed with Purina rat food and water *ad libitum*. All experiments with the animals were performed in accordance with the specific Brazilian resolutions of the Bioethics of Experiments with animals (law N° 6.638 of May 8th 1979; Decree N° 24.645 of July 10, 1934, Brazilian College of Animal Experimentation).

Diabetes induction

Diabetes was induced by an intravenous injection (55 mg/kg b.w.) of Stroptozotosin. After two days, blood samples were obtained with animals in the fed state to determine the plasma glucose concentration. Rats which were not diabetic (<14,7 mmol/L) or too severely diabetic (>35,5 mmol/L) were eliminated from the study.

Training protocol

For the study, the rats were randomly distributed in four groups (n = 8 per group), Sedentary Control (SC), Trained Control (TC), Sedentary Diabetic (SD) and Trained Diabetic (TD). The training included daily swimming with load of 5% of the body weight, one hour/day, five days/week, for four weeks.

Cytokines detections

Serum TNF-α and IL-18 levels were measured by enzyme-linked immunosorbent assay (ELISA) technique (enzyme-amplified sensitivity immunoassay (ELISA) kits (from Bender Med Systems and Invitrogen Life Science Co,.USA). These assays detected only Rat cytokines and the minimum detectable concentrations in our laboratory were 11 pg/Ml for TNF-α and 15.6 pg⁄mL for IL-18.

Glucose was measured from whole venous blood with a glucose monitor (Glucometer; Bayer Diagnostics, New York, NY).

Statistical Analysis

The Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA), version 16, was used for statistical analysis. All data are presented as mean ± SEM. Before statistical analysis, all variables were checked for normality and homogeneity of variance by using the Kolmogorov-Smirnoff and Levene tests, respectively. The data obtained were tested by ANOVA followed by Tukey's post-hoc multiple comparison test. P<0.05 was considered statistically significant.

RESULTS

After the experimental period stroptozotosin-induced diabetes decreased serum insulin and liver glycogen.

The mean plasma level of IL-18 in Trained Diabetic and trained control rats were 30/79±6/60 and 30/79± 8/70 pg/ml after exercise respectively. Therefore, the calculated free IL-18 was 39/68±15/64 pg/ml in control group, increased to 46/24±5/92 (p = 0.05). Table 1 and 3. The mean plasma level of TNF-α in Trained Diabetic and trained control rats were 30/38±7/98 and 40/38±5/14 pg/ml after exercise respectively. Therefore, the calculated free TNF-α was 27/65±6/11 pg/ml in control group, increased to 46/28±9/64(p = 0.05). Table 2 and 4.

Table 1: Comparison of IL-18 levels among groups obtained from ANOVA

IL-18	ANOVA				
	Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Between Groups	1356.725	3	452.242	4.532	.010
Within Groups	2794.013	28	99.786		
Total	4150.738	31			

Table 2: Comparison of TNF- α levels among groups obtained from ANOVA

TNF-α	ANOVA				
	Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Between Groups	1633.246	3	544.415	10.007	.000
Within Groups	1414.547	26	54.406		
Total	3047.793	29			

Table 3: Multiple comparison of IL-18 obtained data by TUKEY test.

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
CON Healthy	Training Healthy	8.88375	6.32824	.660	-11.0114	28.7789
	CON Diabetic	-6.56625	5.91350	.832	-25.9268	12.7943
	Training Diabetic	8.88375	6.00253	.614	-10.5590	28.3265
Training Healthy	CON Healthy	-8.88375	6.32824	.660	-28.7789	11.0114
	CON Diabetic	-15.45000*	3.72327	.007	-26.9185	-3.9815
	Training Diabetic	.00000	3.86311	1.000	-11.7956	11.7956
CON Diabetic	CON Healthy	6.56625	5.91350	.832	-12.7943	25.9268
	Training Healthy	15.45000*	3.72327	.007	3.9815	26.9185
	Training Diabetic	15.45000*	3.13791	.001	5.9509	24.9491
Training Diabetic	CON Healthy	-8.88375	6.00253	.614	-28.3265	10.5590
	Training Healthy	.00000	3.86311	1.000	-11.7956	11.7956
	CON Diabetic	-15.45000*	3.13791	.001	-24.9491	-5.9509

Asterisks in the superscript indicate significant difference at the level of 0.05.

Table 4: Multiple comparison of TNF- α obtained data by TUKEY test

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
CON Healthy	Training Healthy	-12.73107*	3.81745	.013	-23.2036	-2.2586
	CON Diabetic	-18.63143*	3.94265	.000	-29.4474	-7.8155
	Training Diabetic	-2.73607	3.81745	.890	-13.2086	7.7364
Training Healthy	CON Healthy	12.73107*	3.81745	.013	2.2586	23.2036
	CON Diabetic	-5.90036	3.81745	.426	-16.3729	4.5721
	Training Diabetic	9.99500	3.68801	.054	-.1224	20.1124
CON Diabetic	CON Healthy	18.63143*	3.94265	.000	7.8155	29.4474
	Training Healthy	5.90036	3.81745	.426	-4.5721	16.3729
	Training Diabetic	15.89536*	3.81745	.002	5.4229	26.3679
Training Diabetic	CON Healthy	2.73607	3.81745	.890	-7.7364	13.2086
	Training Healthy	-9.99500	3.68801	.054	-20.1124	.1224
	CON Diabetic	-15.89536*	3.81745	.002	-26.3679	-5.4229

Asterisks in the superscript indicate significant difference at the level of 0.05.

DISCUSSION

The major forms of diabetes can be categorized as type 1 or type 2 (14). In type 1 diabetes, which accounts for 5–10% of cases, the cause is an absolute deficiency of insulin secretion resulting from autoimmune destruction of the insulin-producing cells in the pancreas. Type 2 diabetes (90–95% of cases) results from a combination of the inability of muscle cells to respond to insulin properly (insulin resistance) and inadequate compensatory insulin secretion. Less common forms include gestational diabetes mellitus (GDM), which is associated with a 40–60% chance of developing type 2 diabetes in the next 5–10 years (15). Diabetes can also result from genetic defects in insulin action, pancreatic disease, surgery, infections, and drugs or chemicals (14,15).

Genetic and environmental factors are strongly implicated in the development of type 2 diabetes. The exact genetic defects are complex and not clearly defined [14] , but risk increases with age, obesity, and physical inactivity.

The goal of treatment in type 1 diabetes is to achieve and maintain optimal BG, lipid, and blood pressure (BP) levels to prevent or delay chronic complications of diabetes. Many people with type 1 diabetes can achieve BG control by following a nutritious meal plan and exercise program, losing excess weight, implementing necessary self-care behaviors, and taking oral medications, although others may need supplemental insulin. Diet and PA are central to the management and prevention of type 1 diabetes because they help treat the associated glucose, lipid, BP control abnormalities, as well as aid in weight loss and maintenance. When medications are used to control type I diabetes, they should augment lifestyle improvements, not replace them.

Conraads et al. have showed that combined endurance/resistance training in patients with chronic heart failure has an anti-inflammatory effect through significantly reduction of plasma TNF-α receptor. The patients had a four month exercise program (three times/week), each session was consisted of 30 min resistance and 20 min endurance training 17.

Recently found that person with type 1 diabetes have to the increased serum IL-18 in the early stages of the disease (16). This mechanism results in an individual patient will develop a chronic inflammation. Such a mechanism would have a direct role in pancreatic β-cell destruction (17). Several studies on human and models showed that deficiency in IL-18 decreases T helper activities and levels of IL-18 in the sub-clinical stages of disease is associated with increase in

antibody against beta cells and thereby the role of mentioned cytokines is known in the pathogenesis of these patients (14). TNF-α levels increases after a period of moderate intensity resistance training. Inflammatory cytokine response is different to infection and sports. TNF-α increased with exercise training suggests that the inflammatory effects of exercise on the body is created and its levels after exercise starts to decline and not remain such as persistent infection in the body.

In general, research shows that physical activity is inversely associated with levels of TNF-α and IL-18. Regular physical activity has beneficial effects on all organs and tissues and the effects on reducing inflammation, inflammatory factors and inflammatory markers. Inflammatory cytokines that reduce the body adapt to exercise in order to create an inflammatory environment. So do at least one hour per week of physical activity have beneficial effects.

In the present study, we found the beneficial effect of resistance training on the muscle strength and balance of Diabetic patients. This change will improve their fitness and quality of life. Beside reduction in the inflammatory cytokines, reduction of clinical disability of Diabetic subjects, demonstrate the performance of RESISTANCE training as an exercise program.

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