A Comparison between the Impact of Two Different Exercise Protocols on Advanced Glycation End Products in Type 2 Diabetic Rats

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Abstract: AIM: This study was designed to compare the impact of two different exercise protocols on the serum level of advanced glycation end products (AGEs) in type 2 diabetic rats. **METHODS:** This study was performed on 40 male albino rats, weighed 200-250 g. At the start of the study, 10 male rats were separated and used as a control group (group I). Induction of diabetes in another 30 rats was done by a single intravenous injection of 45 mg/kg streptozotocin (STZ). The diabetic rats were randomly divided into two groups: group II (sedentary group) (n = 10) and group III (exercised group) (n = 20). Then, the exercised group was further subdivided into two subgroups: IIIa (were subjected to chronic regular moderate exercise protocol for 8 weeks and IIIb faced another protocol in the form of irregular strenuous exercise for the same period. **RESULTS:** The data in group II (sedentary group) as compared to group I (control group), showed that the injection of streptozotocin resulted in significant increase in the fasting blood glucose, serum AGEs and insulin levels and HOMA-IR. Also, there was significant decrease in the extracellular superoxide dismutase (SOD) enzyme serum level. Meanwhile, in subgroup IIIa (as compared to group II), regular moderate exercise protocol in diabetic rats produced significant reduction in the fasting blood glucose, serum AGEs and insulin levels and HOMA-IR. At the same time, there was significant increase in serum level of superoxide dismutase. Lastly, in subgroup IIIb (as compared to group II), the irregular intense exercise protocol in diabetic rats caused significant reduction in fasting blood glucose but there was insignificant decrease in serum AGEs and insulin levels and HOMA-IR. This was associated with insignificant increase in the superoxide dismutase serum level. **CONCLUSION:** In type 2 diabetes, regular moderate exercise protocol is more valuable program to reduce serum level of advanced glycation end products than irregular severe one. This regular moderate protocol will be very helpful in the prevention of development of diabetic complications.
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Key Words: Diabetes, AGEs, regular moderate exercise, irregular intense exercise.

1. Introduction

Diabetes mellitus has emerged as one of the main alarms to human health in the 21st century. Pronounced changes in the human environment, behavior and lifestyle have accompanied globalization, which resulted in growing rates of diabetes [(1)]. In the year 2010, 284 million people were recorded as suffering from this disease, and it is suggested that this figure will increase to 439 million in the year 2030 [(2)].

Diabetes mellitus is a chronic debilitating disease characterized by hyperglycemia, which is the main cause of the increased risk of developing complications such as kidney failure, adult blindness, non-traumatic amputations, cardiovascular disease, and nerve damage according to its duration and extent [(3)].

Glycation is a reaction that takes place when simple sugar molecules, such as fructose or glucose, becomes attached to proteins or lipids without the moderation of an enzyme (Maillard reaction). This results in the formation of heterogeneous class of compounds known as advanced glycation end products (AGEs). The formation of AGEs is an important biochemical abnormality that accompanies diabetes mellitus, normal aging and, likely, inflammation in general [(4,5)].

The blood level of AGEs appears to be increased in diabetes because of hyperglycemia, oxidative stress and high quantities of free fatty acids (FFA) [(6)].

The formation of AGEs can be retarded or attenuated, but not completely abolished, through an efficient glycemic control [(7)]. The regular practice of physical activity could be one interesting approach to controlling glycemia through the reduction of peripheral resistance to insulin, attenuating the formation of AGEs, as well as associated oxidative stress [(8)].

In cases of type 2 diabetes, the oxidative stress results in formation of ROS that disrupt transmission

pathways between the insulin receptors and the glucose transport system, which leads to an onset of insulin resistance [9]. These ROS participate in the formation of AGEs, and also mediate its effects on target tissues. The connection between clinical complications of diabetes and oxidative stress arises from the formation of high doses of AGEs in this metabolic disorder [10,11]. Therefore, the association between oxidative stress and AGEs may explain, at least in part, the close relationship between hyperglycemia and tissue damage.

Nowadays, regular physical activity can prevent or manage a wide range of health problems and concerns, including stroke, metabolic syndrome, obesity, depression, arthritis and certain types of cancer. Regular activity also promises mental-health benefits, like relieving stress and anxiety. In fact, life style modification especially exercise becomes a very important method for the prevention, treatment of diabetes and in the prevention of the development of its complications [12,13].

Experimental protocols that use animal subjects are therefore developed when it would not be appropriate to use human subjects for studies of exercise's impact.

The regular practice of moderate intensity physical exercise (training) showed capacity to reduce body weight, improve insulin sensitivity, increase circulating levels of high density lipoprotein (HDL), decrease triglyceride levels and normalize blood pressure [14]. An immediate effect of exercise is the increased maximal oxygen consumption and metabolic activity. This condition leads to an imbalance between free radicals and antioxidants, as the increased consumption of oxygen for respiration generates increased amounts of ROS [15].

The enhancement of the antioxidant enzymatic capacity, the improvement of vasodilatation capacity of blood vessels [16] and the favorable modifications of lipoprotein blood profile and the increment of LDL oxidation resistance [17] are favorable adaptations produced by regular physical exercise in diabetic patients. On the other hand, when a subject is unaccustomed to exercise or when it is practiced too intensively, it can induce considerable oxidative stress and damage to the exercised muscles [18,19].

Our body runs out of antioxidants during extreme exercise because of free radicals which overwhelm our cells and consequently oxidative stress rises far beyond healthy levels. Over time, this damage increases our risk of heart disease, cancer, and early death [20].

Extracellular superoxide dismutase (SOD) enzyme promotes the dismutation of the superoxide radical to form hydrogen peroxide (H_2O_2) and oxygen. This antioxidant enzyme is able to combine with ROS, generating less reactive species [21, 22].

Recently, **Oliveira *et al.*,** compared the effects of 12 weeks training with 3 different types of exercise (aerobic training, strength training and combined training) on type 2 diabetes mellitus (T2DM) male and female human subjects, demonstrating that the aerobic training program provided important upregulation in antioxidant enzymes and increased NO bioavailability, which may help in minimizing oxidative stress and the development of the chronic complications of diabetes [23]. However, it is now clear that physical exercise, especially too intense or sporadic could cause damage to muscle cells or inflammatory reactions within them; some of this damage is due to the formation of ROS [24, 25, 26].

As the formation of AGEs and its accumulation in the tissues has a key role in the development of diabetic complications, it was very essential to study the effect of exercise in different forms on the serum AGEs level in diabetic patients. Unfortunately, there has been little research about this subject in spite of its great importance. Hence, our idea arises to study this aspect to clarify the vital role of selecting specific exercise protocol in treatment of diabetes and prevention of the development of its complications.

2. Material and Methods

2.1 Animals

This study was carried out on 40 male albino rats, weighed 200-250 grams. The animals were obtained from animal house of the Faculty of Science, Tanta University. The handling of the animals was carried out in accordance with the ethical guidelines for investigations and approved by the local ethical committee for the care and use of laboratory animals. The rats were handled daily, housed in isolated animal cages, and kept under a 12-hour light-dark cycle at room temperature and humidity 70–75%. They had free access to water all over the period of the work. Before the start of the work, all animals underwent 2 weeks acclimatization period.

2.2 Experimental design

The animals were kept on standard commercial rat show and tape water *ad libitum* for the whole period of the experiment. At the start of our study 10 male rats were separated and used as a control group (group I).

Another 50 rats were prepared to develop diabetes mellitus by a single intravenous injection of 45 mg/kg streptozotocin (STZ) (Sigma, Chemical Co., St. Louis, MO, USA) dissolved in sodium citrate buffer (0.1 mol/liter, pH adjusted to 4.5) at a concentration of 20 mg/ml immediately before use. After 3 days, fasting blood samples were collected from tail vein, and analyzed for blood glucose by using a glucometer (Aquo-Check, Roche). Animals

showing fasting blood glucose higher than 200 mg/dl were considered as diabetic and used for the study.

Thirty diabetic rats were chosen and randomly divided into two groups: group II (sedentary group) (n = 10) and group III (exercised group) (n = 20). Then, the exercised group was further subdivided into two subgroups: IIIa and IIIb. Lastly, The diabetic rats were subjected to exercise protocol (1) and (2) for subgroups IIIa and IIIb respectively for 8 weeks.

Exercise protocol (1) for subgroup IIIa

Swimming was done in a cylindrical tank, 120 cm in diameter and 80 cm in height, with 40 cm warm water (30 - 32 ^{o}C). The animals were placed in the tank every day at the same hour (10.00 - 11.00 am) and the training was monitored by the same person. The rats initially swam (10 min/day) five days/week which allowed the animals to adapt to water environment without determining physical condition through swimming practice with progressive increase of 10 minutes/day up to the maximal time of 60 minutes. The 60 minutes/day time schedule was kept until the end of the experimental period. Swimming program was performed according to **Volpato *et al.*,** [(27)].

Exercise protocol (2) for subgroup IIIb

Swimming was done in the previously described tank but a load equals to 5% of the body weight of the rat was attached to its tails [(28)]. In addition, the animals were placed in the swimming tank two days/week at different times each day. Also, the rats were kept in the swimming tank until exhaustion which was identified by the beginning of the rat to sink.

The sedentary rats were allowed to swim for just 30 seconds each day. After each exercise session, the animals were gently dried with a cloth towel.

2-3 Blood sampling

To minimize the acute effects of the exercise, exercised animals were sacrificed 48 hrs after the end of the last training session. At the end of the experimental period, the rats were fasted per night and at the next morning they were anaesthetized by intra-peritoneal injection of pentobarbital sodium (50 mg/Kg body weight) [(29)]. Then, they were sacrificed and blood samples were collected and centrifuged at 3000 rpm for 10 minutes and the separated serum was then pipetted into clean storage tubes to be tested.

2-4 Biochemical assays

Serum AGEs was measured by using Rat Pentosidine Enzyme-linked immunosorbent assay (ELISA) Kit supplied by Elabscience Biotechnology Co., Ltd, Beijing, China. We chose pentosidine as a biomarker for AGEs because it is a well characterized and easily detected member of this large class of compounds. Fasting blood glucose was measured by using Roche Hitachi 912 Chemistry Analyzer according to the manufacturer's instructions. Insulin level was determined by radioimmunoassay (RIA) using Immulite device (RIA-Immulite, IML2000, IML 2500 insulin) provided by Siemens Medical Solutions Diagnostics. Insulin resistance was calculated using the formula of the Homeostasis Model Assessment (HOMA-IR)=fasting insulin (uIU/ml) x fasting glucose (mg/dl)/405. Extracellular Superoxide dismutase enzyme was determined by using Rat Enzyme-linked immunosorbent assay (ELISA) Kit for Superoxide Dismutase , Soluble (SOD) supplied by Uscn Life Science Inc., USA.

2-5 Statistical analysis

Data were processed using the Statistical Package for Social Sciences® (SPSS) program v. 20 (Chicago, IL, USA). Descriptive statistics were used, as means (M) and standard deviations (SD), frequency distribution, and comparisons. One way ANOVA test was used to compare between groups, followed by post hoc test (least significant difference) for inter-group comparisons. We considered differences to be statistically significant if *P* values were < 0.05.

3. Results

By checking the results of group II (sedentary group) as compared to group I (control group), the injection of streptozotocin resulted in increase in the fasting blood glucose from 82.90±6.12 to 228.5±11.07mg/dl, serum AGEs (pentosidine) from 35.75±1.72 to 49.08±2.53 ng/ml, insulin level from 6.67±1.33 to 13.70±3.12 uIU/ml and HOMA-IR from 1.38±0.39 to 7.79±2.12. Also, there was decrease in the superoxide dismutase serum level from 8.57±2.21to 5.80±1.06 ng/ml. All the previously mentioned changes were statistically significant as compared to group I (p_1< 0.001) [Table1, Figs.1-5].

Meanwhile, in subgroup IIIa (as compared to group II), regular moderate exercise protocol in diabetic rats produced reduction in the fasting blood glucose to 129.4±6.19 mg/dl, serum AGEs (pentosidine) to 38.93±2.03 ng/ml, insulin level to 8.25±2.02 uIU/ml and HOMA-IR was decreased to 2.65±0.78. In the same time there was increase in the superoxide dismutase serum level to 7.84±1.84 ng/ml. Also, all the changes in group III were statistically significant as match up to group II (p_2< 0.001) [Table1, Figs.1-5].

Lastly, by screening the results of subgroup IIIb (as compared to group II), we noticed that the intense exercise protocol in diabetic rats caused significant reduction in fasting blood glucose to 213.8±9.99 mg/dl but there was insignificant decrease in serum AGEs level to 47.10±3.06 ng/ml (p_3=0.072), insulin level to 12.28±2.80 uIU/ml (p_3=0.198), and HOMA-

IR to 6.54±1.80 (p_3=0.063), This was associated with insignificant increase in the superoxide dismutase serum level to 5.99±0.93 ng/ml (p_3=0.452) [Table1, Figs.1-5].

Table (1): Effect of diabetes and the two exercise protocols on fasting blood glucose, AGEs (pentosidine), SOD, insulin and HOMA-IR.

	Group I (control)	Group II (sedentary diabetic)	Subroup IIIa (DM + moderate exercise)	Subgroup IIIb (DM + intense exercise)
FBG mg/dl	82.90±6.12	228.5±11.07*	129.4±6.19*	213.8±9.99*
		$p_1 < 0.001$	$p_2 < 0.001$	p_3=0.001
Insulin uIU/ml	6.67±1.33	13.70±3.12*	8.25±2.02*	12.28±2.80
		$p_1 < 0.001$	$p_2 < 0.001$	p_3=0.198
HOMA-IR	1.38±0.39	7.79±2.12*	2.65±0.78*	6.54±1.80
		$p_1 < 0.001$	$p_2 < 0.001$	p_3= 0.063
AGEs ng/ml	35.75±1.72	49.08±2.53*	38.93±2.03*	47.10±3.06
		$p_1 < 0.001$	$p_2 < 0.001$	p_3= 0.072
SOD ng/ml	8.57±2.21	5.80±1.06*	7.84±1.84*	5.99±0.93
		$p_1 < 0.001$	$p_2 < 0.002$	p_3= 0.452

The data are expressed as mean± SD. (*) means significant when $p < 0.05$
P1 means group II versus group 1
P2 means group III versus group I1
P3 means group IV versus group 1I
DM = Diabetes Mellitus; Ex.=Exercise; FBG= Fasting blood glucose; AGEs= Advanced glycation end products ; SOD= Superoxide dismutase

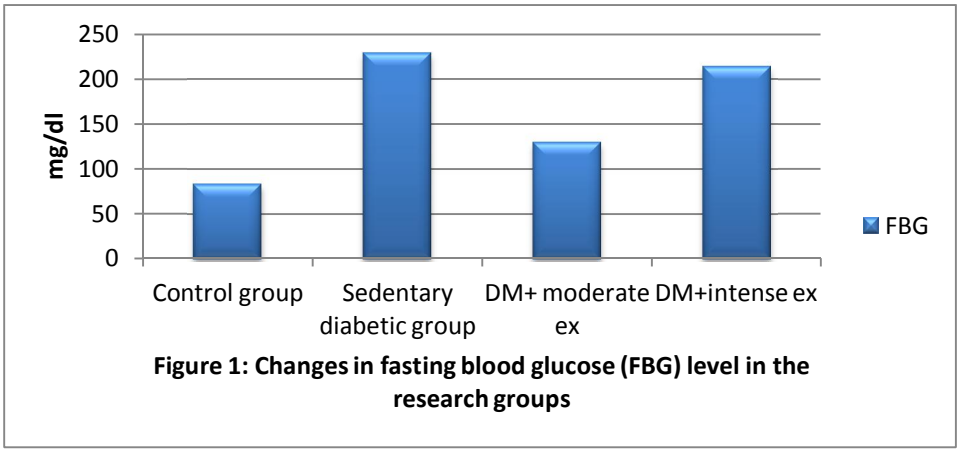

Figure 1: Changes in fasting blood glucose (FBG) level in the research groups

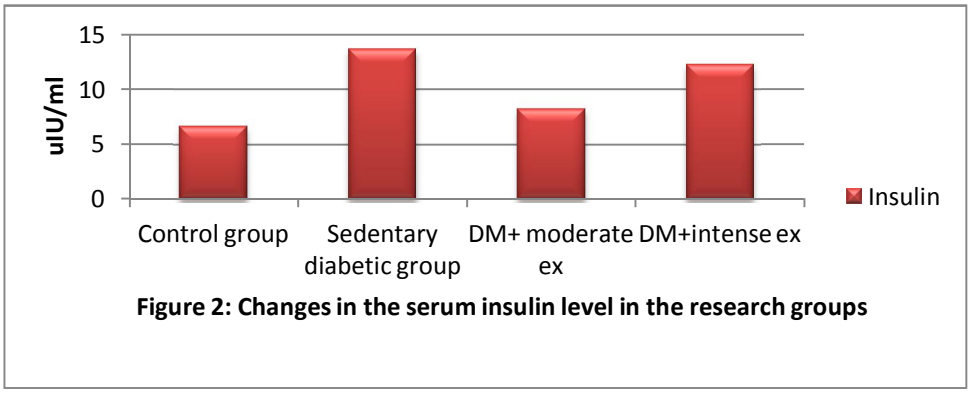

Figure 2: Changes in the serum insulin level in the research groups

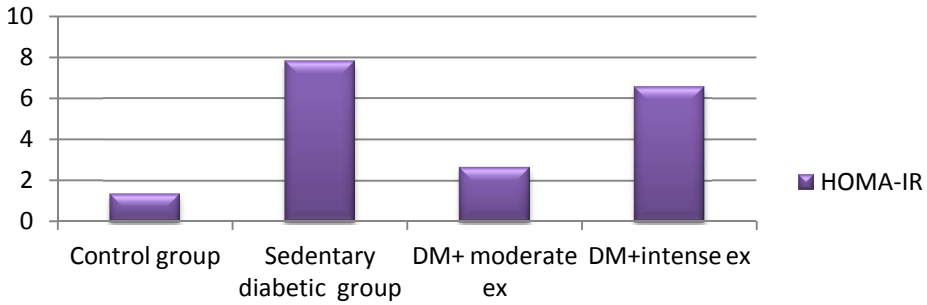

Figure 3: Changes in HOMA-IR in the research groups

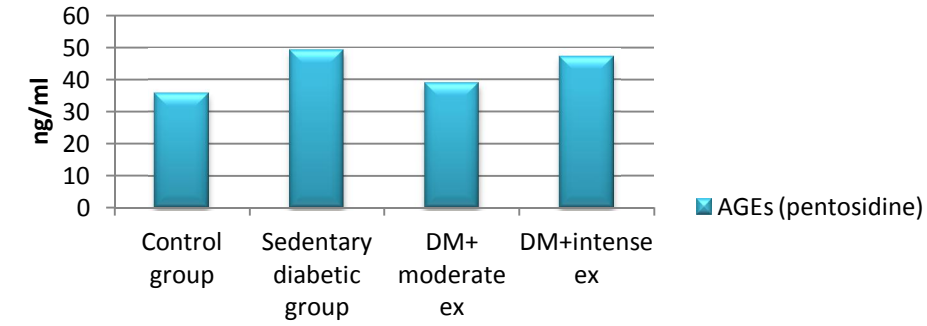

Figure 4: Changes in serum advanced glycation end products (AGEs) level in the research groups

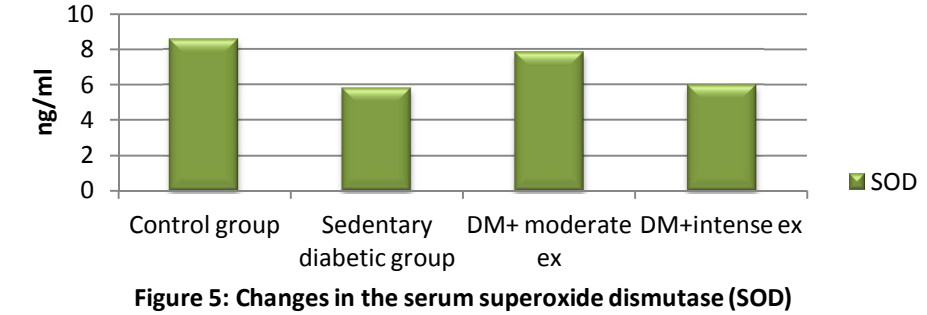

Figure 5: Changes in the serum superoxide dismutase (SOD) enzyme level in the research groups

4. Discussion

Diabetes is a disease characterized by hyperglycemia, a relative or absolute lack of insulin action, and the development of diabetes specific pathology. The final mechanism by which diabetes damages susceptible tissues is by increasing the formation of AGEs and expression of its receptors and its activating ligands [(6)].

Type 2 diabetes (T2D) usually develops in adulthood and is related to obesity, lack of physical activity and unhealthy diets. Exercise is a multifactorial activity that affects virtually every organ and tissue in the body. Exercise programs have been adopted as a key procedure for diabetic patients [(19)] . The problem always lies in selecting a suitable protocol of the activity and the ability of the patient to obey it carefully.

In the present study, we investigated the impact of two different types of exercise protocols, chronic regular moderate and irregular intense, on the serum level of AGEs.

The results of this research showed that chronic regular moderate exercise resulted in significant reduction in the blood glucose, serum AGEs and insulin levels as well as insulin resistance associated with significant increase in the level of extracellular superoxide dismutase (SOD) enzyme.

On the other hand, irregular intense exercise produced less significant decrease in the blood glucose and insignificant decrease in serum AGEs and insulin levels as well as insulin resistance associated with insignificant increase in the level of extracellular SOD enzyme. Comparing the results of the two different exercise protocols showed that moderate exercise caused more significant effect on blood glucose level ($p < 0.001$) than intense one ($p < 0.003$).

The effect of exercise on the serum level of AGEs can be explained by the ability of exercise to improve the hyperglycemia and to reduce its associated oxidative stress. The regular practice of physical activity could be one interesting approach to controlling glycemia through the reduction of peripheral resistance to insulin, attenuating the formation of AGEs, as well as associated oxidative stress [30].

During physical exercise, glucose uptake by the working muscles rises 7 to 20 times over the basal level, depending on the intensity of the work performed. However, Intense exercise provokes the release of insulin-counter regulatory hormones such as glucagon and catecholamines, which ultimately cause a reduction in the insulin action [31]. This explains the more superiority of regular moderate exercise over sever intermittent one in controlling hyperglycemia as shown in our results. Furthermore, moderate regular exercise has been indicated as to have an "insulin-like" activity because of the increase of muscle's capacity to capture circulating glucose, due to stimulation of GLUT-4 increment and decreased intramuscular fat reserves [32]. Additionally, it causes stimulation of insulin action on cells of the organs involved in the exercise [33].

Maeda *et al.,* reported that moderate regular exercise increases basal production of nitric oxide [34]. This will increase glucose transport as NO is a critical mediator of insulin and has a role in the signal transduction mechanism [35].

However, it was proven that extenuating exercise only lowers the level of glucose, lipids and insulin whilst aggravating the inflammatory profile and oxidative stress [36].

The effect of acute and sever exercise on glycemia is likely due to the ability of skeletal muscle contractile activity to activate glucose transport, as this pathway appears to be normal in animal models of insulin resistance [33] and in T2DM subjects [37]. This can explain the significant decrease in blood glucose shown in our results.

Inagaki *et al.,* reported that the blood level of AGEs appears to be increased in diabetic patients, not only because of hyperglycemia and oxidative stress, but also because of high quantities of free fatty acids which are responsible for the development of insulin resistance [38].

Regular exercise improves dyslipidaemia in rats, namely by reducing the total cholesterol and triglycerides [39]. Furthermore, it stimulates lipolytic activity and promotes the use of free fatty acids (FFA) as an energy source through increased activity of lipoprotein and hepatic lipases [40]. As the serum level of FFA decreases, the insulin resistance will decrease and consequently AGEs formation will also decrease. This could explain the reduction in AGEs observed in subgroup IIIa in the current study.

In addition, **Turcotte and Fisher,** showed that the increased capacity of the muscle to oxidize fat in response to exercise is a major mechanism by which exercise training improves insulin sensitivity and consequently control glucose and AGEs levels [41] as revealed in our results.

Also, regular exercise is able to activate an alternative pathway: the adenosine monophosphate-activated protein kinase (AMPK) [42]. This enzyme acts on the liver, muscle and adipocytes by increasing fatty acid oxidation, decreasing cholesterol synthesis, and lipolysis. It also plays an important role in decreasing the glucose levels, being able to stimulate GLUT-4 increment and even modulating insulin secretion from pancreatic islets [43].

In previous studies, the acute, irregular or sever bouts of exercise presented a significant glucose-lowering effect in diabetic human and rats, accompanied by a reduction on insulinemia and a decrease in insulin resistance, evaluated by HOMA-IR [36, 44, 45]. These results are deviated from that of the current study because of the difference in used protocols.

It is to be noted that exercise is associated with increased formation of free radicals, mainly due to increased O_2 consumption by active tissues. Several studies have shown that the amount of free radicals in biological tissues is increased especially after strenuous exercise, which coincides with the presence of tissue damage [20,36,46]. On contrast, many studies suggested that, chronic regular exercise of moderate intensity (training) positively alters the oxidative homeostasis of cells and tissues, by decreasing the

basal levels of oxidative damage and increasing resistance to oxidative stress [14, 47, 48]. The previously mentioned data were in accordance with the current study results.

It was proved that AGEs formation may contribute to the antioxidant diminution observed in uncompensated diabetes, where the loss of glucose homeostasis, with the consequent glycation of antioxidant enzymes such as catalase, glutathione peroxidase, glutathione reductase and reduced glutathione, may attenuate their activity [8].

However, it was verified by **Teixeira *et al.,*** that extenuating exercise only lowers the level of glucose, lipids and insulin whilst aggravating the inflammatory profile and oxidative stress. On the other hand, chronic (habitual) exercise had beneficial effects on all the previously mentioned biomarkers and profiles with consequential change in serum level of AGEs [36].

Alessio *et al.,* reported that considerable oxidative stress and muscles damage is a clear result in persons unaccustomed to exercise or performing too intensive physical activity [18].

Another recent study by **Patil *et al.,*** mentioned that heavy and sustained exercise training generates large quantities of free-radicals that likely outstrip the buffering capacity of the system, leaving these individuals susceptible to oxidative stress [49]. The problem arises when total concentration of ROS formation is higher than the cellular defense systems capacity to neutralize and eliminate them as occurred in long-term exhausting exercise.

Meanwhile, regular exercise causes adaptations in the antioxidant capacity, protecting cells against the harmful effects of oxidative stress, thus preventing cellular damage [50,51]. These adaptations may be mediated, at least in part, by a hyper-regulation of basal nitric oxide (NO) production [52]. Increased nitric oxide level stimulates the activity of SOD enzyme [53] and this will improve the oxidative stress in type 2 DM and lowers the serum level of AGEs as shown in our results.

It was proved that AGEs augmented hyperglycemia-associated depletion in endothelial nitric oxide production and block nitric oxide activity and consequently caused the production of ROS [54]. The resulting oxidative stress will augment the production of AGEs and aggravates the condition. Regular exercise cut this vicious circuit via increasing nitric oxide (NO) bioavailability, ROS detoxification and decreasing ROS generation [19].

The regular exercise was able to prevent serum oxidative stress, viewed by the reduction of lipid peroxidation, evaluated by the increment of SOD activity, as shown in the current results, thus reinforcing the antioxidant action of training [50, 51].

Our results showed that intermittent intense exercise insignificantly correct the already elevated SOD levels found in the sedentary diabetic rats. This may be due to the increased production of large quantities of free radicals by strenuous exercise that exceed the antioxidant defenses of the body with persistence of the oxidative stress [49]. Also, long-term intense exercise produced a decreased in the levels of antioxidants and an increase in ROS, resulting in a reduction in NO bioavailability [55] and consequently SOD level.

5. Conclusion and Recommendations:

We can conclude that, in type 2 diabetes, chronic regular moderate training produces beneficial effects on diabetic metabolism and AGEs formation than irregular exhausting one. This regular moderate exercise protocol will be very helpful in the prevention of development of diabetic complications. Exercise is considered the best preventive and treatment option for diabetes, but unfortunately, many people with diabetes do not or cannot exercise regularly.

We recommend a judicious choice of exercise protocol for diabetic patients by physicians, namely, considering the type, duration and intensity, which seem to differently affect diabetes pathophysiology. For future research, the effects of the new different exercise protocols for maintaining the optimum health and avoiding complications in diabetics remains to be explored.

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