A short-term training program reduced oxidative damage in elderly diabetic rats

Manuel Rosety-Rodríguez,* Alejandra Camacho,** Miguel Ángel Rosety,* Gabriel Fornieles,* Antonio Jesús Díaz,* Ignacio Rosety,* Natalia García,* Antonio Pardo,* Francisco Javier Ordonez**

*School of Sports Medicine, University of Cadiz. **Hospital SAS Juan Ramón Jiménez, Huelva.

ABSTRACT

Introduction. Recent studies concluded long-term training programs have improved antioxidant system in young and adults diabetic rats. However, to our knowledge, little attention has been paid to elderly individuals. Objective. To assess the influence of a shorter training program in reducing oxidative damage in elderly diabetic rats. Material and methods. Twenty-four male homozygous Zucker diabetic fatty rats (Gmi, fa/fa) aged 18-weeks with an average weight of 370-450 g were purchased. After a 2-week period of environmental adaptation, animals were randomly distributed into exercised group (n = 12) that performed a 6-week swimming training protocol and sedentary group (n = 12). Animals were sacrificed 24-h after the last exercise session under anesthesia. Serum metabolic profile was determined. Lipid oxidation, expressed as malondialdehyde and protein oxidation, expressed as carbonyl groups, were assessed in plasma samples. This protocol was approved by an Institutional Ethics Committee. Results. Exercised rats improved significantly their metabolic profile in comparison to controls. Plasma malondialdehyde (1.58 ± 0.39 vs. 2.06 ± 0.41 nmol/mL; p = 0.016) and carbonyl group levels (1.37 ± 0.33 vs. 1.62 ± 0.58 UA; p = 0.011) were also significantly lowered in exercised rats when compared to sedentary counterparts. Conclusions. A 6-week swimming training program reduced lipid and protein oxidation in elderly fatty diabetic rats. Further studies on this topic are required.

Seis semanas de entrenamiento reducen el daño oxidativo en ratas diabéticas de avanzada edad

RESUMEN

Introducción. El daño oxidativo ha sido propuesto como diana terapéutica en la diabetes tipo 2. Recientes estudios sugieren que el ejercicio físico podría mejorar las defensas antioxidantes. Sin embargo, son programas de larga duración y centrados en animales jóvenes. Objetivo. Estudiar la influencia de un programa de entrenamiento corto (seis semanas) en la reducción del daño oxidativo de ratas diabéticas de edad avanzada. Material y métodos. Se utilizaron 24 ratas diabéticas Zucker (Gmi, fa/fa) de 18 semanas de edad y un peso de 370-450 g. Tras un periodo de adaptación de dos semanas los animales se distribuyeron aleatoriamente en un grupo experimental (n = 12) que realizó un programa de natación de seis semanas, tres sesiones/semana y un grupo control (n = 12). Se evaluó el perfil lipídico y glucémico sérico. Los niveles plasmáticos de malondialdehído se determinaron mediante HPLC y los grupos carbonilos mediante técnicas de Western-Blot. El estudio fue aprobado por un Comité de Ética institucional. Resultados. Los animales que realizaron el ejercicio mejoraron significativamente su perfil metabólico en comparación con el grupo control. También mostraron niveles plasmáticos significativamente menores de MDA (1.58 ± 0.39 vs. 2.06 ± 0.41 nmol/mL; p = 0.016) y grupos carbonilos (1.37 ± 0.33 vs. 1.62 ± 0.58 UA; p = 0.011) que los encontrados en animales sedentarios. Conclusiones. Un programa de entrenamiento de seis semanas redujo significativamente la oxidación de lípidos y proteínas en ratas diabéticas de edad avanzada. Futuros estudios en esta línea son aún necesarios.

Key words: Oxidative stress. Exercise. Type 2 diabetes mellitus. Zucker rat. Aged.
INTRODUCTION

The increasing prevalence of type 2 diabetes mellitus (T2DM) together with the associated morbidity and mortality calls for additional therapeutic strategies. Evidence derived from both epidemiological and mechanistic studies suggests that oxidative stress has an important role in mediating the pathologies of diabetic complications. Accordingly, oxidative stress has been pointed out as a therapeutic target in T2DM.\(^1\)

Fortunately, several papers have reported long-term training programs have improved antioxidant system in young and adults diabetic rats.\(^4,5\) However, to our knowledge, little attention has been paid to elderly individuals. Mainly if we take into account type 2 diabetes prevalence increases directly with age in Latin America Societies.\(^6\) Further, the management of diabetes mellitus in the elderly is a complex process.\(^7\)

In addition, it would be of interest to reduce the length of training programs previously published. Although extrapolation from animal studies to humans requires caution, shorter training programs may contribute to facilitate their follow-up, reducing drop-out rates.\(^8\)

For the reasons already mentioned, the present study was designed to explore whether a short, 6-week, training program was able to reduce oxidative damage in elderly diabetic fatty rats.

MATERIAL AND METHODS

Animals

To get this goal 24 male homozygous Zucker diabetic fatty (ZDF) rats (Gmi,\(fa/\fa\)) aged 18 weeks with an average weight of 370-450 g were purchased. Animals were housed in single cages in an environmentally controlled laboratory (temperature 22 °C) with a 12:12-h light-dark cycle. A standard rodent chow adjusted to their body weight (100 mg/g of weight) was provided. Tap water was given ad libitum.

Swimming training program

After a 2-week period of environmental adaptation, animals (n = 24) were randomly distributed into exercised group (n = 12) that performed a 6-week swimming training protocol and sedentary group (n = 12).

The exercised group swam individually in plastic tanks 50 x 100 x 45 cm, once per day (1-h), 3 days per week (Monday; Wednesday; Friday), during daily dark phase under red light to enable visual observation. During swimming sessions rats wore elastic chest bands to which attachable loads could be added. Rats commenced exercising without any additional load for the first week. However during the second week of treatment rats had 3% body weight added, increasing 1% each week until the end of the study. It should be emphasized loads were adjusted with the body weight every week.

To minimize stress associated to cold or hot water exposure, temperature was monitored and maintained at 32 °C. In order to separate the effects of exercise and the stress associated to the exercise environment, sedentary rats were individually placed in identical swimming tanks but sat in shallow water at the same temperature, duration and frequency than exercised rats. Furthermore, animals were towel dried and left for 1 h in a heated room to minimize the effects of cold exposure.\(^9,10\)

Animals were sacrificed 24-h after the last exercise session under anesthesia using 1 mL of ketamine injection (1 g/10 mL) by cervical dislocation.

Metabolic profile

Blood samples were collected by cardiac puncture and was centrifuged for 15 min at 3,000 rpm. The serum, thus obtained, was used for biochemical analysis.

Plasma glucose and lipid profile (triglyceride and total cholesterol concentrations) were assessed on a BM/Hitachi 902 Automatic Analyzer with the use of standard Roche enzymatic kits (Roche Diagnostics Co, Indianapolis, IN).

Glycosylated hemoglobin (HbA1c) was determined using a specific monoclonal antibody with a turbidimetric readout (DCA 200+ Analyzer, Bayer Diagnostics). Finally, insulin levels were assessed using the ultrasensitive rat insulin enzyme-linked immunosorbent assay kit (Mecodia, Sweden).

Lipid and protein oxidation

Lipid peroxidation, expressed as malondialdehyde (MDA) level, was measured in plasma samples by high performance liquid chromatography.\(^11\) Briefly, a 150 \(\mu\)L aliquot of MDA standard or plasma, in triplicate, was added to polypropylene microcentrifuge tubes kept on ice. The MDA standard was generated from 1,1,3,3-tetraethoxypropane (TEP) by hydrolysis when heated with thiobarbituric acid (TBA) reagent during the assay (1 mole of TEP generates 1
mole of MDA) and a standard curve (0.125-1.0 µM) was obtained by diluting the 20 µM TEP standard (stable at 4°C for up to 1 month) in water. To precipitate proteins and release the MDA bound to the amino groups of proteins and other amino compounds, 75 µL of cold 1 M HClO4 was added to the standard and plasma samples and the tubes were mixed immediately. The tubes were centrifuged at 12,000 g for 3 min at 4 °C and 150 µL of supernatant transferred into 2 mL glass chromatography vials in a rack on ice. 50 µL of 1 M NaOH was added to all vials and vortex immediately. The fluorescent MDA-TBA derivative was prepared by adding 800 µL TBA reagent (0.25% TBA, 1.25 mM diethylenetriaminepentaacetic acid in 2.5 M acetate buffer pH 3.5) and 10 µL of 5% butylatedhydroxytoluene in ethanol. Samples were mixed, placed in a shaking water bath at 95 °C for 60 min and immediately cooled in ice water and kept at 7 °C prior to analysis.

To determine changes in protein carbonylation, we performed an assay for carbonylated proteins provided by Chemicon (Oxyblot oxidized protein detection kit; Millipore, Amsterdam, Netherlands). Briefly, carbonyl groups in the protein side chains are derived to 2,4-dinitrophenylhydrazone (DNP-hydrazone) by reaction with 2,4-dinitrophenylhydrazine. The DNP-derived protein samples are subjected to gel electrophoresis and subsequent western blotting. Densitometry of the 2,4-dinitrophenylhydrazine-derived bands in the gel was performed and normalized to a standard control sample loaded on all gels and expressed as arbitrary units (AU) as previously stated.12

**Ethics and statistical assessment**

It should be emphasized our protocol attended U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training. Further this protocol was approved by an Institutional Ethics Committee.

Results were expressed as mean ± SD and 95% confidence intervals (95%CI). The statistical analysis of the data was performed using Student’s t-test for unpaired data. The significance of the changes observed was ascertained at p < 0.05.

**RESULTS**

Serum metabolic profile in both exercised and unexercised ZDF rats is listed in table 1.

Regarding lipid oxidation, expressed as plasma levels of malondialdehyde (MDA), we found significant differences between exercised and sedentary rats [1.58 ± 0.39 (1.34-1.69) vs. 2.06 ± 0.41 (1.80-2.33) nmol/mL; p < 0.001] suggesting that sedentary animals are more vulnerable to oxidative stress.

Similarly, exercised diabetic fatty rats showed lower levels of carbonylated proteins in comparison to sedentary counterparts [1.37 ± 0.33 (1.28-1.46) vs. 1.62 ± 0.58 (1.49-1.73) UA; p = 0.017].

**DISCUSSION**

The present study has generated some interesting and significant data. But firstly, it should be pointed out that Zucker Diabetic Fatty (ZDF) rat represents a good animal model not only for studying T2DM physiopathology but also for assessing the effects of therapeutic options such as intervention programs based on exercise.13 In fact, the development of diabetes is quite similar to what is seen in obese humans since it results from its hyperphagic eating behavior due to a leptin receptor mutation (fa gene).14 In this respect it should be emphasized sample size was similar to the largest ones reported in previous studies on Zucker diabetic fatty rats.9,13,14

And secondly, we chose swimming because it is widely used to identify biochemical and molecular

Table 1. Comparative analysis of metabolic profile in exercised (n = 12) and sedentary ZDF rats (n = 12) at the end of the study.

<table>
<thead>
<tr>
<th></th>
<th>Exercised</th>
<th>Sedentary</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>433.2 ± 3.1</td>
<td>405.8 ± 3.7</td>
<td>0.17</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>29.4 ± 1.7</td>
<td>33.2 ± 1.9</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Hb1Ac (%)</td>
<td>10.6 ± 0.7</td>
<td>11.1 ± 1.9</td>
<td>0.34</td>
</tr>
<tr>
<td>Insulin (pmol/L)</td>
<td>1206.7 ± 11.9</td>
<td>1342.5 ± 12.4</td>
<td>0.026*</td>
</tr>
<tr>
<td>Total-cholesterol (mmol/L)</td>
<td>4.11 ± 0.6</td>
<td>5.25 ± 0.7</td>
<td>0.035*</td>
</tr>
<tr>
<td>Triglycerols (mmol/L)</td>
<td>3.03 ± 0.4</td>
<td>4.26 ± 0.6</td>
<td>0.011*</td>
</tr>
</tbody>
</table>

Hb1Ac: glycosylated hemoglobin. Results were expressed as mean ± SD. *p value < 0.05.
responses to exercise. Mainly if we take into account it is an uniform type of activity that is less traumatic to animals. Further we did not find significant changes in body weight in agreement with previous studies. Interestingly, a study that employed forced running as the exercise regime showed that body weight increased in exercised animals despite a lack of differences in muscle mass or fat mass. Although we did not measure glycosuria in our study, it has been reported that 25-30% of the caloric intake is excreted in untreated ZDF rats. With respect to biochemical indices, diabetic fatty rats that performed our training protocol improved significantly both glycemic and lipid profiles. Similar results were found after a 12-week training program in diabetic fatty rats. In addition it was reported a 7-week training program improved biochemical abnormalities associated to diabetes. It should be emphasized our protocol induced similar effects lasting just 6 weeks. We have also found hyperinsulinemia was significantly reduced in exercised rats when compared with sedentary counterparts. However, our results suggested that exercised rats were still insulin resistant on a systemic level, as indicated by their elevated plasma insulin concentrations. This hyperinsulinemia could be a reflection of insulin resistance in the liver that was not ameliorated by swimming training protocol. Further long-term studies on this topic are required to clarify this issue.

In a previous study, we found total antioxidant status (TAS) was significantly higher in exercised Zucker diabetic fatty rats in comparison to counterparts that did not perform our 6-week training program. Similarly it has been published a 12-week swimming train-ing increased TAS in ZDF rats. Furthermore, a 6 month swimming protocol improved significantly antioxidant enzyme activities in the soleus muscle of exercised rats. Accordingly, to the best of our knowledge, the present 6-week training protocol is the shortest protocol published in the literature.

It should be also pointed out our 6-week training protocol lasted just 6 weeks in contrast to longer protocols based on exercise that were previously published in the literature. This finding is of particular interest since it may contribute to shorten the length of training protocols applied to patients with T2DM. It may finally lead to reduce drop-out rates in training programs, promoting their applicability to ordinary clinical settings. As an example, in a recent study, 84 diabetic patients aged over 60 years were enrolled to perform a 6-month training program. It should be pointed out ten subjects withdrew from the study.

Finally, it may be concluded a short swimming program improved metabolic profile as well as reduced lipid and protein oxidation in elderly Zucker diabetic fatty (ZDF) rats. Further long-term, well-conducted studies on this topic are required.

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**Correspondence and reprint request:**

Francisco Javier Ordonez
School of Sport Medicine
University of Cadiz
Pza. Fragela s/n
11003, Cadiz, España.
Tel.: +34 956-01-5201
Fax: +34 856-17-2005
Correo electrónico: franciscojavier.ordonez@uca.es

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