ADVANCED GLYCATION END PRODUCTS' RESPONSE TO RESISTANCE TRAINING IN POSTMENOPAUSAL WOMEN WITH TYPE II DIABETES

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Abstract:

The aim of this study was to investigate the role of resistance training (RT) on advanced glycation end products (AGEs) concentrations in postmenopausal women with type II diabetes. Postmenopausal female patients from the Sanandaj Diabetes Association, aged 48-58 years, were randomly assigned to either experimental (n=7) or control (n=8) group. The experimental group has performed a routine program of resistance training for eight weeks, three sessions per week with 70% of one repetition maximum. The control group did not have any type of exercise program during the study. Two-way ANOVA with repeated measures showed that advanced glycation end products' values in the experimental group (compared with the pre-test) was significantly reduced (p=.014). Similarly, AGEs concentrations were significantly lower in the post-test in the experimental group compared with the control group (p=.000). In addition, fasting blood glucose (FBS) levels in the experimental group after 8-week resistance training was associated with a significant reduction (p=.005). The findings suggest that eight weeks of RT reduced blood glucose concentrations, which lead to decreased levels of advanced glycation end products in postmenopausal women with type II diabetes.

Key words: *diabetes mellitus, resistance exercise, glycation*

Introduction

Normally, the primary cause of vascular pathophysiologic changes in diabetic patients is chronic exposure to high levels of glucose in the blood vessels. Although the increase of glucose by different mechanisms affects the cellular features, the most important pathway involved in accelerated atherosclerosis in these patients is an increase in the glycation of proteins, lipids and the formation of advanced glycation end products (AGEs) (King & Banskota, 1994). AGEs are created by non-enzymatic reaction of reducing sugars with free amino groups of proteins, lipids and nucleic acids. Schiff base is the initial product of this reaction. This unstable matter becomes stable Amadori product. Amador's most famous product is called the glycosylated hemoglobin (Talmor, et al., 2008). In fact, AGEs are proteins or lipids that glycate after exposure to sugars; this process is called glycation (Wood & O'Neill, 2012). Stable compounds include carboxy methyl lysyn (CML), pyrraline and pentosidine (Goldin, Beckman, Schmidt, & Creager, 2006).

Patients with diabetes often have high blood concentrations of AGEs (Goh & Cooper, 2008). Glycated hemoglobin, which is the precursor of AGEs, responds to prolonged exercise training (Castaneda, et al., 2002; Dunstan, Daly, & Owen, 2002; Eriksson, et al., 1997). Cross-sectional studies have shown that glycated hemoglobin has a high correlation with weight bearing and high-tension exercises (Eriksson, et al., 1997; Cartee, Young, & Sleeper, 1999). Serum levels of pentosidine as an AGEs index is higher in postmenopausal women due to reduced estrogen levels (Irvine & Taylor, 2009).

Eriksson et al. (1997) showed that three months of resistance training (RT) reduces glycated hemoglobin in type II diabetic patients, while Ishii (1998) showed that four to six weeks of moderate-intensity resistance training (five times a week) does not create changes in glycated hemoglobin. Maiorana (2002) also investigated the effect of two months of RT combined with aerobic training in diabetic women and commented that such a program has no effect on the glucose metabolism and glycosylated hemoglobin.

In addition, higher intensity RT (three sets of eight to ten repetitions with 75 to 85 % of one repetition maximum – 1RM), unlike aerobic exercise, is tolerated by diabetics and has many benefits for them (Saremi, 2011). In a controlled study on older men with type II diabetes, RT led to a 5% increase in insulin action (Saremi, Moslehabadi, & Parastesh, 2011).

The reduction of estrogen biosynthesis in aging has been suggested as a major cause of heart disease in postmenopausal women. A reduced estrogen level due to menopause is a key factor in increased oxidative stress and vascular inflammation in diabetic patients (Nieman, et al., 1998). In addition to diabetes, menopause may also have an influence on glycation end products. In the present study, postmenopausal women were considered because these individuals have lower levels of estrogen and, as previously stated, lower estrogen levels may lead to increased AGEs (Takahashi, Oikawa, & Nagano, 2000).

A few studies have been carried out in relation to the impact of exercise on AGEs (Magalhães, Appell, & Duarte, 2008; Yoshikawa, Miyazaki, & Fujimoto, 2009; Boor, et al., 2009). The results of these studies indicate that AGEs decreases in response to aerobic exercise. However, no report is available on the effect of RT on AGEs. In addition, it has been observed that in pre-diabetics (glucose 100 to 125 mg/dl), RT leads to a decrease in fasting blood glucose levels up to 24 hours after exercise (Black, Swan, & Alvar, 2010). In the present study, diabetes and menopause are simultaneously studied as potential factors contributing to increased glycation end products. According to the aforementioned and due to the popularity of RT (Kraemer, Ratamess, & French, 2002), this raises the question of whether or not eight weeks of moderate intensity RT in postmenopausal women with type II diabetes causes changes in AGEs serum concentrations.

Materials and methods

Subjects and exercise protocol

In an experimental design, both the intervention and control group were studied throughout the pre-test and post-test. During the study of 600 patients who were members of the Sanandaj Diabetes Association (200 of whom were postmenopausal women with type II diabetes), 20 patients were purposefully selected. All subjects were taking diabetes medication (metformin or glibenclamide) three times a day. Subjects had not undertaken a regular exercise program six months prior to the study. Subjects were randomly allocated to either an experimental or a control group, 10 subjects per group. During the 8-week exercise protocol, three patients from the experimental group (due to medical problems or two-time or higher absence from training sessions) and two patients from the control group (due to subjects' lack of willingness to continue working) withdrew from the study and 15 people continued the research to its completion. General characteristics of the subjects are given in Table 1. Health status of the subjects during the study was approved by physicians at the Sanadaj Diabetes Association. One week before the start of the project, subjects were familiarized with the objectives of the executive process and, after explaining the risks, written consent was obtained from all of them. During the familiarization session, the subjects were acquainted with the equipment and resistance training, and underwent a test of one repetition maximum (1RM) for six resistance exercises. One repetition maximum was determined using the equation developed by Brzycki (1993): predicted-1RM = weight lifted / 1.0278 - (0.0278 × number of repetitions). When performing the 1RM test, all subjects were encouraged verbally to exert their maximum effort. After randomly allocating the individuals to the experimental (EG) and control (CG) groups, the intervention group (EG) participated in an 8-week weight training program. Training sessions lasted approximately 60 minutes, and were performed three times a week. They were conducted between 10 a.m. and noon. Resistance exercises included: bench press, leg extensions, hamstring curls, military dumbbell press, standing EZ-curls and lat pull-down. Each exercise was performed in three sets of eight repetitions, with 70% of 1RM. Rest intervals between sets and between exercises were two and three minutes, respectively. In order to control workout intensity, 1RM in every exercise was recorded every two weeks. Prior to each session, a 10-minute warm-up, consisting of stretching exercises (general warm-up) and of two sets of the first exercise performed with 40 to 50% 1RM (special warm-up), was applied and exercise sessions were ended by 5-minute cool-down (only stretching) exercises. During the study period, room temperature was set between 25 to 30 °C. The control group did not do any type of exercise program or similar activities during the study period.

Blood sampling and analysis

The subjects' BMI was calculated by the following formula: body mass $_{(kg)}$ / height $_{(m)}^2$. Blood samples were collected twice: 48 hours prior to the exercise protocol and 36 hours after the completion of the research project. Blood samples (6 ml) were taken from the antecubital vein under normal room temperature after 10-hour overnight fasting and were centrifuged for 15 minutes at 1500g. The serum concentrations of AGEs were determined by AGEs human kit (USCN Life Science Company, China). This assay employs the enzyme-linked immunosorbent method, so an inverse correlation exists between AGE concentration in the sample and the assay signal intensity. In other words, the intensity of color developed is reversely proportional to the concentration of AGE in the sample. The intra-assay coefficient of variation (CV) percentages for AGEs in the experimental and control group were about 23 and 6, respectively. Furthermore, to measure FBS, Iranian Pars Azmun kit and colorimetric method was used. In this assay, hydrogen peroxide reacts with 3, 5-dichloro-2-hydroxybenzenesulfonic acid and 4-aminoantipyrine to produce a pink dye with an absorption at 514 nm.

Statistical methods

With respect to the normal distribution of data (Kolmogorov-Smirnov test), two-way repeated measures ANOVA and Bonferroni *post-hoc* test were used to measure differences within and between the groups. SPSS version 19 was employed to analyze the data and significant level was set at $p<.05$.

Results

Characteristics such as age, body height, body mass and body mass index (BMI) of the subjects are shown in Table 1. These data indicate that the two groups did not differ from each other in terms of body height, age, body mass, and BMI.

The present study findings also showed that AGEs serum in the experimental group was significantly decreased after eight weeks of RT (p=.014). In addition, in the post-test, there was a significant difference between the two groups, thus the mean of the experimental group was lower than the control group (p=.000) (Figure 1).

Moreover, the fasting blood glucose levels (FBS) in the experimental group after eight weeks of resistance training was associated with a significant reduction (p=.005), while no changes were observed in the levels of AGEs and FBS in the control group (Figure 1). Consequently, in the pre--test, there were no significant differences between groups in any of the variables (p=.601 and p=.260 for AGE and FBS, respectively).

Strength changes

As seen in Table 2, resistance exercises (bench press, leg extensions, hamstring curls, military dumbbell press and standing EZ-curls) caused

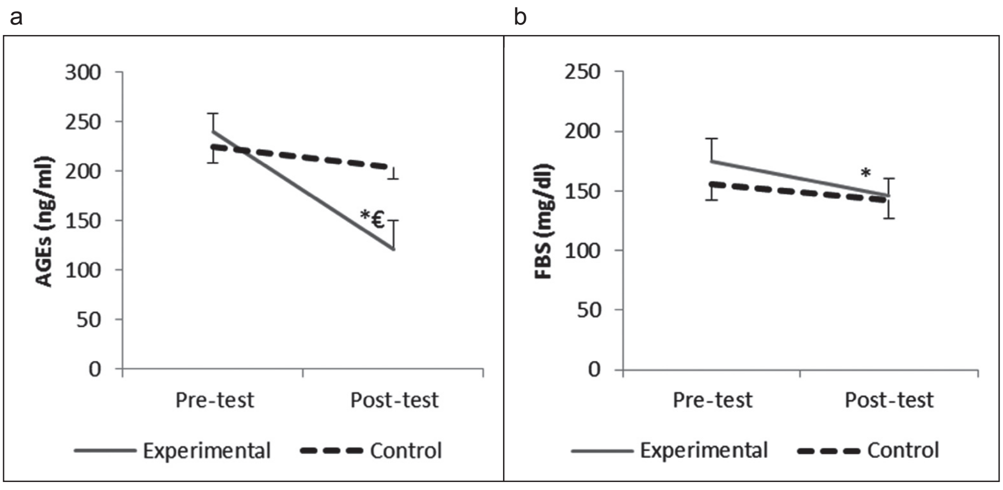
Table 1. General characteristics of the subjects (age, body mass, body height and BMI)

		Pre-test	Post-test
Age (years)	Experimental	53±5	
	Control	50.9±6	
Height (cm)	Experimental	153±4	
	Control	153.8±6	
Mass (kg)	Experimental	67±11	65.7±11
	Control	69.6±12	70±11
BMI (kg/m^2)	Experimental	29.4±4	29±4
	Control	29.3±3	29.8±3

Table 2. Changes of strength in the experimental and control groups

		Pre-test	Post-test
Chest press (kg)	Experimental	6.2±1	11±2 *€
	Control	6.5±1	6±1
Military dumbbell press (kg)	Experimental	9.9±3	12.8±2 *€
	Control	10.2±2	8.8±1
Biceps curls (kg)	Experimental	6.8±2	10.3±2 *€
	Control	6.9±2	6.1±2
Lat pull-down (kg)	Experimental	36.4±3	39.3±3
	Control	34.4±3	32±3
Leg extension (kg)	Experimental	7.4±3	12±2 *€
	Control	7.3±2	6.5±1.5
Hamstring curls (kg)	Experimental	7.6±2	12.3±3 *€
	Control	7.1±2	6.7±2

*: Significant changes compared to the pre-test, €: Represents the difference between the two groups at the post-test. The significance level was set at α<.05.



*: Significant changes compared to the pre-test, €: Represents the difference between the two groups at the post-test. The significance level was set at α<.05.

Figure 1. Changes in advanced glycation end product (AGEs) (a), and fasting blood sugar (FBS) (b) following eight weeks of resistance training in experimental group or non- exercise control group.

significant differences between the two groups in the post-test; hence, the experimental group scored higher on strength (p<.05). The experimental group also showed considerable within-group changes (p<.05), so that in the post-test compared to the pre--test there was a significant increase in bench press, leg extensions, hamstring curls, military dumbbell press and standing EZ-curls values.

Discussion and conclusions

The study results illustrated that eight weeks of RT had a favorable effect on AGEs serum concentrations and FBS in postmenopausal women with type II diabetes.

Eves and Plotnikoff (2006) state in their review study that exercise leads to lower blood sugar and glycosylated hemoglobin. In addition, it is pointed out that the glycation of hemoglobin persists (and the subsequent formation of glycation end products), except when the blood sugar levels drop. It has been claimed that exercise, by increasing the number of glucose transporters (GLUT4), promotes glucose metabolism and can reduce the amount of glycated hemoglobin (Frosig & Richter, 2009). The exercise protocol in the present study significantly increased improvement of 1RM in all resistance exercises. Subsequently, it could be stated that the present protocol has created the necessary neuromuscular adaptation, although the mechanisms described previously resulted in blood glucose control and a reduction in AGEs.

In a short-term study it was found that acute intensive running exercise has no effect on AGEs (Mydlík, et al., 2012). On the other hand, Maiorana and colleagues (2002) investigated the effect of two months of resistance exercise on glucose metabolism and glycosylated hemoglobin in males and females aged 60-80 years. They reported no change in this indicator, which is in conflict with the present study findings. It appears that age of participants and type of exercise (resistance, endurance or combined training) could be the reason for this difference. In the present study, we applied low-intensity resistance training. Moreover, a combination of aerobic and resistance training program was used.

Our findings are consistent with several previous studies (Choi, et al., 2009; Teixeira de Lemos, et al., 2009; Radak, et al., 2004; Panteleeva & Rogozkin., 2001). The results of these studies have shown a significant reduction in markers of glycation end products including transverse cross-links. Decrease of NF-kB was also observed, which in turn led to a reduction in oxidative stress and inflammatory factors such as IL-6 and TNF-α (Radak, et al., 2004). Eriksson et al. (1997) stated that there is a negative relationship between muscle mass and glycosylated hemoglobin. Glycosylated hemoglobin, as mentioned previously, is one of the precursors of AGEs. In the present study, maximal strength increased in the experimental group, but these changes might be related to neural adaptations, not muscular adaptations (hypertrophy). However, in line with previous findings, the results of our study showed that RT induced a decrease in AGEs, as well as in FBS levels. Furthermore, a review of the literature suggests that the duration of the training protocol should be at least eight weeks or longer to show a positive impact of RT on glycation products. On this subject, Ishii et al. (1998) announced that four to six weeks of moderate intensity resistance training (five times per week) will not cause a change in glycosylated hemoglobin (Ishii, et al. 1998). The Irvine and Taylor's (2009) study also confirmed this matter and added that although the effects of resistance exercise on skeletal muscle glucose transporter proteins plays an important role in glucose disposal, RT for short terms will not alter glycated hemoglobin and training duration should be longer than eight weeks. Their finding is that high-volume RT for 16-26 weeks leads to a decrease in glycated hemoglobin and eight weeks of RT is effective in improving glycemic control.

Overall, there may be differences in various studies due to the variety of RT programs (different intensities and volumes of resistance training), the use of multiple interventions (combination of exercise and diet), use of a combination of aerobic and resistance training, types of subjects (healthy, ill, or obese individuals), lack of a control group and sampling time after the last training session (Poehlman, Dvorak, Denino, Brochu, & Ades, 2000; Bastard, Jardel, Bruckert, Vidal, & Hainque, 2000).

In general, previous studies have examined the effect of regular physical activity or aerobic exercise on AGEs serum concentrations which is consistent with the present findings (Magalhães, et al., 2008; Yoshikawa, et al., 2009; Boor, et al., 2009; Goon, et al., 2009). Physical activity can improve blood sugar levels and reduce complications in patients with type II diabetes in a variety of ways. It plays an important role in improving insulin efficiency, and, due to increased glucose uptake by the exercising muscle, slows down blood sugar levels and improves blood flow, as well as muscle tonus (Ledbetter & Phipps, 2007). Yoshikawa et al. (2009) report that carboxy methyl lysyn (CML), which is known as one of the specific AGEs, correlates negatively with high-density lipoprotein (HDL-C). On the other hand, weight, sex, and duration of the intervention are important factors in the response of HDL to exercise (Fahlmen, Boardley, Lambert, & Flynn, 2002). Thus, regular physical activity in general, particularly RT, can have an impact on reducing AGEs by changing blood sugar and CML values, as well as by improving lipid profile.

It can be concluded, based on the present findings, that eight weeks of RT (with 70% 1RM, three times a week) significantly reduce AGEs levels in postmenopausal women with type II diabetes by reducing the blood sugar level. In addition, the findings of previous studies have pointed out that short-term RT programs (less than eight weeks) do not cause changes in glycosylated hemoglobin (Takahashi, et al, 2000). Thus, moderate intensity RT should be performed for at least eight weeks to be effective in reducing advanced glycation end products (AGEs). Nowadays, long-term RT is recommended as a treatment strategy for the control of blood sugar to improve insulin function, increase muscle mass (for better utilization of glucose) and prevent certain complications such as osteoporosis and diabetes. Our findings show, in line with the aforementioned, that there was a reduction in fasting blood sugar levels and glycated products following eight weeks of RT, which reaffirms the effectiveness of such an exercise training program in type II diabetic patients.

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