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Response of nitric oxide to resistive exercise for quadriceps muscle in hypertensive women

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ABSTRACT

Background: Shear stress on endothelial cells is a potent stimulus for Nitric oxide production, (NO) exerts important vasodilator. Exercising quadriceps musclewas associated with a significant increase in mean nitric oxide concentration. **Objective:** to evaluate the effect of resistance exercise forquadriceps muscle on Nitric oxide levels in hypertensive women. **Methods:**Twenty women were randomly assigned into two groups, study group consist of ten patients they participated in a supervised resistive exercises program performed 3 times per week for 8 weeks. Using moderate work load method (60 % of maximum voluntary contraction (MVC), control group included Ten patients received their medications only without participating in any exercise program. all patients to be assayed later for measurement of nitric oxide, before the initiation of the training program and after the completion of the study (after 8 weeks).**Results:** the control group showed minimal effects on NO concentration (-0.52 (2.11%) µmol/dl), while the exercising muscles the NO concentration, showed marked differences as compared to the control group, That is, quadriceps showed biggest effect in the form of increase in NO levels of 4.7 (19.18%) µmol/dl.

Key word:*Nitric oxide -Resistive exercise- mild hypertension-large muscles.*

INTRODUCTION

Nitric oxide, known as the 'endothelium-derived relaxing factor', or 'EDRF', is biosynthesized endogenously from L-arginine, oxygen, and NADPH by various nitric oxide synthase (NOS) enzymes. Reduction of inorganic nitrate may also serve to make nitric oxide. The endothelium (inner lining) of blood vessels uses nitric oxide to signal the surrounding smooth muscle to relax, thus resulting in vasodilation and increasing blood flow [1].

The endothelial NO synthase (eNOS) gene may play a part in the development of blood pressure and left ventricular mass. Endothelial cells play an important local regulatory role by secreting substances that control both vascular tone and structure, including NO, which is derived from the metabolism of l-arginine by NO synthase, a constitutive enzyme that is present in endothelial cells. NO is produced and released under the influence of endothelial agonists—including acetylcholine, bradykinin, and others—acting on specific receptors, and by mechanical forces, such as shear stress. Experimental evidence indicates that almost the totality of cardiovascular risk factors, such as aging and hypertension, are characterized by the presence of endothelial dysfunction, which is mainly induced by the production and release of oxygen-derived free radicals, which cause NO breakdown [2,3].

Nitric oxide (NO) contributes to vessel homeostasis by inhibiting vascular smooth muscle contraction and growth, platelet aggregation, and leukocyte adhesion to the endothelium. Humans with atherosclerosis, diabetes, or hypertension often show impaired NO pathways. A high salt intake was demonstrated to attenuate NO production in patients with essential hypertension, although bioavailability remains unregulated[4].

In patients with essential hypertension, one of the main mechanisms leading to impaired endothelium-dependent vasodilatation is the production of oxidative stress, which reduces NO availability. When oxidative stress is removed by a scavenger such as vitamin C, NO availability is restored [5].

In the presence of physical exercise, physical and chemical stimuli control NO production. In the endothelial cells, exercise stimulates NO synthesis through chemical mechanisms. Chemical mechanisms involve interaction of endogenous/exogenous agonists (acetylcholine, bradykinin, and ATP) with the specific receptors on the endothelial cells. Evidence suggests that physical exercise stimulates release of these molecules [6].

Emerging evidence suggests that resistive exercise improves endothelial function and reduces blood pressure in hypertensive patients through the release of endothelium-derived relaxing factors such as nitric oxide, which is stimulated mainly by the rise in shear stress occurring during exercise [7].

The aim of this study was to evaluate the effect of resistance exercisefor quadricepsmuscles on Nitric oxide levels in hypertensive women.

METHODS

Twenty sedentary postmenopausal hypertensive female was selected for this study ,with 4-5 years at least of menopause, Their ages ranged 50-60 years ,with mild hypertension from 139/89 to 159/99mmHg, their body mass index ranged from 25 to 29.9 (kg/ m2) none of them receiving hormonal therapy replacement. subjects were under medical control they received antihypertensive drugs in form of angiotensin Inhibitor (ACE). subjects are all house wife's. subject were selected with muscle power ranged from grade 4 to grade 5.subjects were divided into two group control group (ten patients) received medication only without exercise program ,study group(ten patients underwent resistive exercise program for quadriceps muscle for eight weeks, All patients underwent a physical examination and a review of their medical histories and all participants were given a written informed consent for all procedures.

A) Instrumentation for evaluation

Weight and height scale were used to measure body weight and height (to calculate body mass index), disposable plastic syringes were used to draw venous blood samples, disposable sterile syringes, Rap, Gloves, page of swabs, Ice box, Disposable plastic sterile dropper, Disposable plastic cons, Rack collection and Plaster all in the appendices.

Analyzing chemicals & commercial kits (R &D systems, total Nitric oxide kit) were used to measure the level of NO in the -blood Stat Fax (2100) Micro plate reader for blood sample analysis and CN 180 centrifuge.

B) Therapeutic materials

Weights were used for resistive training of quadriceps muscle; the weight is determined by an assessment session to test one maximum repetition (highest load that the individual could move during the entire movement).Sand bags weights ranged from half to two kilos (1/ 2, 1, 11/ 2, 2 kilos)

A)Procedure for evaluation

Measurement of body weight and height and body mass index:weight and height of each patient was measured using weight and height scale and then BMI for each patient was calculated using the following formula:

BMI= weight (kg) / [height (m)] 2 (WHO, 2004)

Muscle power test were done before the program to thecontrol group and studygroup (which exercised the quadriceps muscle) to determine the power of the muscle (which grade) to be sure that the subjects are able to do the exercise against gravity with moderate resistance, subject included in these study their muscle power ranged from grade 4 to grade 5.

A three milliliter-sample of venous blood will be drawn from the antecubital vein from all patients to be assayed later for measurement of nitric oxide before the initiation of the training program and after the completion of the study (after 8 weeks).

One examination session were done to the study group which exercised the quadriceps muscle to determine the greatest amount of tension the muscle can generate and hold then take 60 % of MVC maximum voluntary contraction

B) Treatment procedures

Control group (ten patients) received medication only without exercise program, while study group (tenpatients) participated in a supervised resistive exercises program exercised for specific part which is right side quadriceps muscle. Used moderate work load method (60 % of maximum voluntary contraction MVC).

The exercise program performed 3 times per week for 8 weeks. Medications are taken hours before the exercising at clockto avoids any interfere between the effect of medication and the effect of exercise. (patients exercise in the morning between 9 to 12 AM.

The program consistsof 4 sets each set consist of 60 repetitions. Duration: of the program about 30 min (five min warm up , and five min cooling down in form of light aerobic exercise), Intensity: moderate intensity (60%MVC), Frequency: 3days / wks. for 8 weeks and Mode: resistive exercise in form of weight training exercise.

Study Group (quadriceps exercise →knee extension): patient in sitting position knee flexed, weight are positioned above the ankle from anterior part, fixation is done above the knee to avoid hip raising from the plinth after warming up patient start first set, patients are asked to extend the knee to the end it takes about 1 second of contraction then asked to relax and rest for two seconds patient asked to do 15 repetition and rest for two minutes then do another 15 repetition. Each set consist of 30 repetitions.Then patient is asked to rest and relax for two minutes before starting the second set.

The second, third and fourth set is the same as first set. Each set takes aboutthree and half minutes.After finishing the four sets patient asked to do cooling down in form of light aerobic exercise.

After the completion of the study (after 8 weeks) a three milliliter-sample of venous blood will be drawn from the antecubital vein from all patients to be assayed later for measurement of nitric oxide.

RESULTS

Twenty cases were included in the study distributed among two groups of 10 cases each. These were the Controls, and the Quadriceps, Table (1 and 2) shows the mean and Standard error of some baseline measurements.

The total period of training program was eight weeks, three session /per week. All medical investigation was performed before training program; body mass index was calculated height weight and nitric oxide for the two groups.

Table 1:Pre-exercise means of baseline measurements between the two groups (age and BMI).

Group	Age (years) Mean±SD	BMI Mean±SD
Controls	54.3 ± 2.9	28.4 ± 1.1
Quadriceps training group	54.5 ± 3.4	28.2 ± 1.4

Paired t-test was used to test the null hypothesis that there are no differences in the means of the age, BMI,and nitric oxide concentration between the two groups.Indeed, results confirmed that there was not enough evidence to reject the null hypothesis as evidenced by the high p values displayed in Table 3. That is, the means of these variables across the two groups were not significantly different prior to exercising.

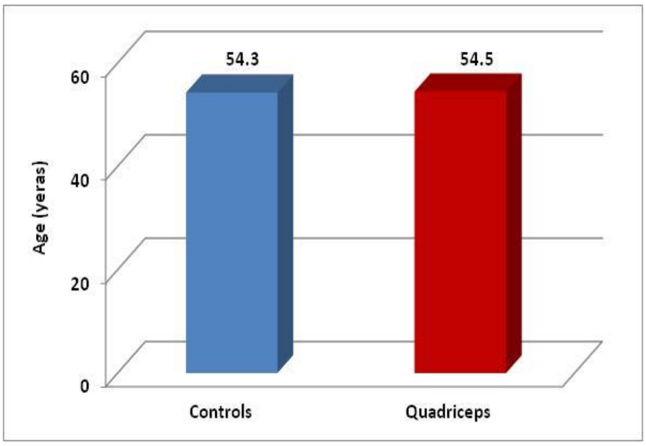


Fig. 1:age distribution within the two groups

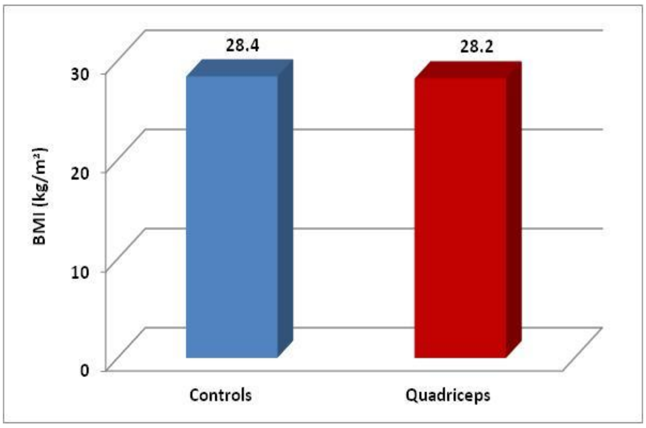


Fig. 2:BMI distribution within the two groups.

Table 2: Pre-exercise baseline measurements between the two groups (Baseline nitric oxide concentration, and Baseline muscle strength)

Group	Baseline nitric oxide concentration (µmol/dl) Mean±SD	Baseline muscle strength (grade) Median
Controls	24.7 ± 0.7	4
Quadriceps	24.5 ± 0.9	4

Kruskal–Wallis test were used to test the null hypothesis that there are no differences in the medians of the muscle strength between the two groups. Indeed, results confirmed that there was not enough evidence to reject the null hypothesis as evidenced by the high p values displayed in Table (4). That is, the medians of these variables across the two groups were not significantly different prior to exercising.

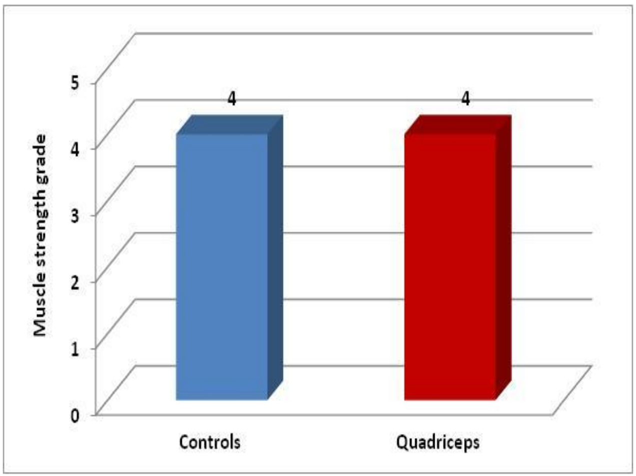
Table 3: Paired t-test was used to test the null hypothesis that there are no differences in the means of the age, BMI, ,and nitric oxide concentration between the two groups

Variable	T-value	P-value
Age	0.4	0.77
BMI	0.6	0.60
Baseline Nitric Oxide concentration	0.2	0.87

Table 4:Kruskal–Wallis results for comparison of baseline measurement of muscle strength between two groups.

Variable	χ2 statistic	p-value
Baseline muscle strength	0.42	0.93

Fig. 3: Median values of muscle strength within the two groups



Training Program results: Following exercise, new measurements were taken including the nitric oxide concentration Comparison between the baseline and post-exercise measurement are displayed in table (5) below together with results of the paired t test of significance for comparing the results before and after exercise in each group.

From the table above, we can see that the increase in NO concentration post-exercise is highly significant in the quadriceps groups of exercising patients compared to baseline measurements, while it is low significant in the control group compared to baseline measurements (p0.02).

In order to assess objectively the effect of the training program on the aforementioned variables, one new variable were computed to examine these effects taking into consideration the change in measurements per each case. These variables include the "Increase in NO concentration" variable in µmol/dl computed by subtracting the pre-exercise NO concentration from the post-exercise NO concentration. These variables aim to measure the effect difference in NO concentration levels achieved through exercising. Table (6) shows the mean difference and percent of change in blood pressure and NO concentration.

Fig. 4: increase in NO concentration in µmol/dl across the two groups.

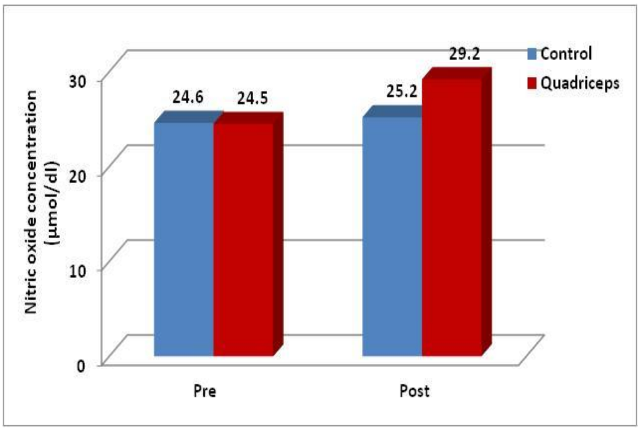


Table 5: Comparison of baseline and post-exercise NO concentration in each group.

Group	Baseline nitric oxide concentration (µmol/dl) Mean ± SD	Post-exercise nitric oxide concentration (µmol/dl) Mean ± SD	t - value	p – value
Controls	24.6 ± 0.7	25..2 ± 0.9	-2.8	0.02
Quadriceps	24.5± 3.8	29.2± 3.8	-25.8	<0.001

Fig. 5: Comparison of baseline and post-exercise NO concentration in each group.

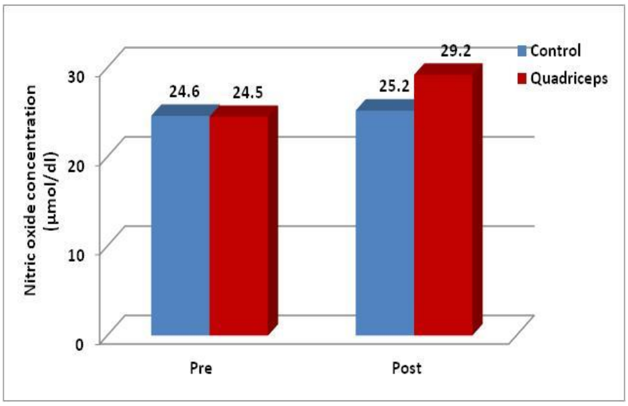
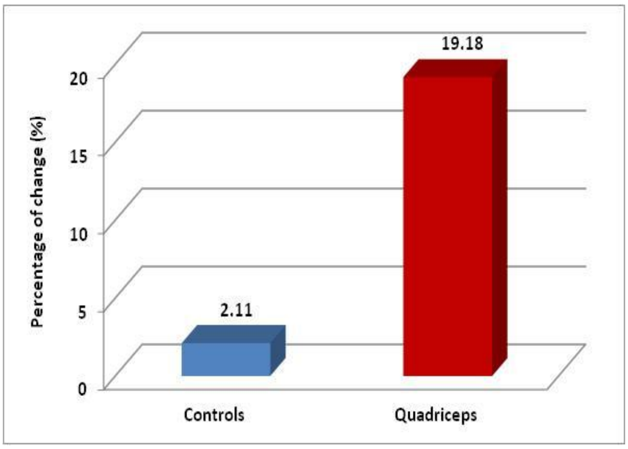


Table 6: Computed effects post-exercise for NO concentration between the two groups

Group	Increase in NO concentration (µmol/dl) Mean difference	% of increase
Controls	-0.52	2.11
Quadriceps	-4.7	19.18

Fig. 6: Percent of change in blood pressure and NO concentration across the two groups.



Looking at the above data, it’s clear that the control group showed minimal effects (low significant) on NO concentration (-0.52 (2.11%) µmol/dl).

The quadriceps showed biggest effect in the form of the increase in NO levels of 4.7 (19.18%) µmol/dl.

DISCUSSION

This study examined the effects of resistance exercise protocol for quadriceps muscle on nitric oxide in hypertensive women. the primary findings of this investigation was the increase of NO production in moderate resistance exercise group ($p < 0.05$). The response to resistance exercise protocol showed a high significant increase between the values obtained before and after the exercise program in quadriceps group ($p < 0.05$) in compare to the control group the response were low significant.

Endothelial function becomes progressively more impaired as blood pressure increases, and the degree of dysfunction is related to the severity of hypertension. Therefore, it is expected that endothelial dysfunction will be improved by lowering bloodpressure. Although clinically effective anti-hypertensive therapies, such as angiotensin converting enzyme inhibitors and aerobic exercise, have restored resistance artery endothelial function of forearm circulation in patients with essential hypertension [8].

Exercise has been implicated as an important factor in the up-regulation of both endothelialnitric oxide (NO) syntheses (eNOS) and neuronal NOS (nNOS) while lowering blood pressure. Physical exercise is a powerful stimulus to increase blood flow in vascular beds and consequently the beneficial effects of physical training on cardiovascular diseases are strongly associated with increase in NO production and/or its bioavailability in human [8].

Vascular formation of NOx is directly facilitated by increased shear stress.During a session of physical exercise, cardiac output increases and blood redistribute to the exercising muscles. The exercise-induced increase of blood flow elicits an increase in shear stress, thereby providing a possible coupling between exercise and endogenous NO formation. Although exercise training involving repetitive bouts of exercises over weeks or months up-regulates endothelial bioactivity [9].

Recent experimental studies have demonstrated that continued exercise augmented vasodilation evoked by the endothelium-dependent vasodilator acetylcholine (ACh) in dogs and rats . We found that physical training enhanced endotheliumdependentvasodilation in forearm circulation in hypertensivepatients as well as in healthy individuals. A 12-week moderate resistive exercise program improved endothelium-dependent vasodilation with Ach [8].

Nitric oxides were utilized in to assess the effect of nitric oxides release by moderate resistive exercises as a vasodilator on endothelium cell of blood vessels that increases blood flow while lowering blood pressure [11]. Firstly according to the obtained result and analytical statistics cleared that moderate exercise intensity succeeded to increase as pretreatment mean value for nitric oxides was 24.33±1.98 (µmo/L) and increased to31.46±2.5 (µmo/L) for study group. While the control group was23.53±1.35 (µmo/L) and increased to25.8±2.04 (µmo/L).One of the physiological mechanisms that could explain the influence of muscle mass on blood pressure after resistance exercise is the reduction in vascular resistance, caused by the liberation of vasodilating endothelial substances (e.g. nitric oxide and prostaglandins) [10,11].

Another study examined the effects of different resistance exercise protocols on markers of muscle damage, lipid peroxidation and nitric oxide response in the plasma of the sedentary men. One of the primary findings of this investigation was the increase of NO production in high resistance exercise (HR) group ($p < 0.05$) [12].

Short term resistance training also has been shown to increase nitric oxide production in healthy older men. Eight young males were randomized to participate in two studies, each consisting of two bouts of lower limb exercise, separated by a 30-min recovery. Results in a generalized increase in NO bioactivity through the tissue [13].

17 training patients (T) and 18 control patients (C),the aim of this study was to investigate the effects of exercise training on the endothelial function in relation to the expression of eNOS.Patients in T had a 2-fold higher eNOS protein expression. Exercise training in leads to an improved agonist-mediated endothelium-dependent vasodilatory capacity. The change in acetylcholine-induced vasodilatation was closely related to a shear stress-induced eNOS [14].

Moderate resistive exercises are known as stimulators of NO release; considering this fact, the resistive exercise can be considered as a protective factor against cardiovascular diseases [10].

CONCLUSION

In the present study, it was found that resistive exercise program with moderate intensity, 3 days per week, for 8 weeks for thirty minutes per session led to significant improvement in the study group. It's clear that the control group showed minimal effects on NO concentration (-0.52 (2.11%) µmol/dl). For the exercising muscles, the NO concentration, again it showed marked difference as compared to the control group. That is, quadricep showed biggest effect in the form of the increase in NO levels 4.7 (19.18%) µmol/dl.

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الملخص العربى

استجابة أكسيد النيتريك لتمرينات المقاومة في عضلة الفخذ لدى السيدات ذات الضغط المرتفع

هدف البحث: - تقييم تأثير تمارين المقاومة في عضلة الفخذ لمستويات أكسيد النيتريك للسيدات في مرحلة ما بعد انقطاع الطمث واللاتي يعانون من ارتفاع ضغط الدم. **تصميم البحث: -** برنامج علاجى باستخدام تمارين المقاومة فى عضلة الفخذ وتأثيره على نسبة أكسيد النتريك فى الدم. **مقاييس النتائج الرئيسية: -** قياس نسبة أكسيد النيتريك بالدم، الوزن والطول. **طريقة البحث: -** شارك في الدراسة 20 سيدة في مرحلة ما بعد انقطاع الطمث ب 3-5 سنوات على الأقل تراوحت أعمارهن بين 50-60سنةو كان المتوسط الحسابي± الانحراف المعياري(54.4± 2.9) جميعهن يعانون من ارتفاع ضغط الدم المتوسط ويعالجون بالأدوية ولا يوجد بينهم سيدات يتلقين العلاج الهرموني. أجريت لكل السيدات المشاركة قبل بدء البرنامج قياسات تشمل قياس نسبة أكسيد النيتريك بالدم، الوزن والطول. قسمت السيدات المشاركة عشوائياً إلى مجموعتين المجموعة الضابطة تشمل 10 سيدات تلقين العلاج الدوائي فقط ومجموعة الدراسة وتشمل 10. تقوم المجموعة بعمل برنامج تدريبي يشمل تمارين المقاومة متوسطة الشدة باستخدام رفع أوزان تمثل 60% من الوزن الأقصى لكل عضلة لمدة شهرين بواقع 3 جلسات أسبوعياً كل جلسة 30 دقيقة وتشمل مجموعة الدراسة عضلة الفخذ رباعية الرؤوستم عمل تمرينات المقاومة متوسطة وبعد انتهاء البرنامج أعيدت القياسات السابقة لكل السيدات. **النتائج: -** أظهرت النتائج زيادة في نسبة أكسيد النيتريك في الدراسة بالمقارنة للمجموعة الضابطة حيث كان المتوسط الحسابيالمعياري للزيادة في نسبة أكسيد النيتريك في مجموعة عضلة الفخذ 4.7 ± 0.6 و 2.3±0.5 ميكرومول/لتروذلك بالمقارنة بالمجموعة التي تلقت دواء علاجي فقط. **الخلاصة: -** بحسب نتائج هذه الدراسة تقوم تمرينات المقاومة متوسطة الشدة لعضلة الفخذ رباعية الرؤوس للسيدات في مرحلة ما بعد انقطاع الطمث واللاتي يعانون من ارتفاع ضغط الدم بزيادة نسبة أكسيد النيتريك فى الدم.

مفتاح كلمات البحث: - أكسيد نيتريك،تمرينات المقاومة، ارتفاع ضغط الدم.