European Review for Medical and Pharmacological Sciences 2023; 27: 4619-4625

Relationship of serum inducible and endothelial nitric oxide synthase with exercise in healthy adult males and patients with type 2 diabetes mellitus

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Abstract. – OBJECTIVE: The importance of physical activities in the control of diabetes mellitus disorder is well known but its correlation with endothelial nitric oxide synthase (eNOS) and inducible nitric oxide synthase (iNOS) in diabetic patients needs to be assessed. The aim of the study is to examine inducible and endothelial nitric oxide synthase in healthy adult male patients with type 2 diabetes mellitus (T2DM) in relation to exercise.

PATIENTS AND METHODS: This is a cross-sectional study that has been performed in the Department of Physiology, King Saud University, Riyadh, Saudi Arabia. Subjects were divided into Group 1: control (n=79), and Group 2: T2DM (n=85). Each group was subdivided into three subcategories: sedentary, moderate activity, and active subjects. Serum nitric oxide (NO), iNOS, eNOS, high sensitivity C-reactive protein (hs-CRP), nitrates, and nitrites, were compared between different groups.

RESULTS: We observed significant differences in iNOS in diabetic patients compared to the control [29.1 (2.8) *vs.* 22.4 (1.4), *p*=0.050] with a significant decrease in eNOS when compared to the control group [79.5 (4.8) *vs.* 101.8 (5.7), *p*=0.003]. In the control group, eNOS showed significant differences (increase) between subgroups; sedentary, moderate, and active control subjects [77.4 (9.2), 114.7 (9.3), and 105.6 (9.5), *p*=0.026]. It also showed insignificant differences between subgroups in iNOS [23.4 (2.5), 24.2 (2.2), and 20.4 (2.4), *p*=0.520]. In addition, hsCRP showed a decrease with exercise but with no statistically significant difference between the groups [4.0 (0.5), 3.3 (0.3), and 2.9 (0.4), *p*=0.271]. In the diabetic patients' group, a significant difference (decrease) between subgroups in iNOS [43.5 (4.8), 20.8 (3.9), and 19.8 (4.6), *p*<0.001] and hsCRP [5.7 (0.5), 3.8 (0.4), and 3.6 (0.5), *p*=0.006] was detected. In addition, eNOS showed a decrease with exercise but with no statistically significant difference between groups [82.9 (7.8), 74.6 (7.9), and 81.7 (10.2), *p*=0.741].

CONCLUSIONS: Patients with T2DM have shown significantly low eNOS and high iNOS levels. Furthermore, regular exercise can significantly decrease iNOS in patients with T2DM. Therefore, the importance of exercise for diabetic patients has to be emphasized to prevent long-term complications related to diabetes mellitus.

Key Words:
Type 2 diabetes mellitus, Exercise, Nitric oxide, iNOS eNOS.

Introduction

Metabolic disorders, especially diabetes mellitus, can affect multiple body systems, particularly the cardiovascular systems, through endothelial dysfunction[1,2]. Additionally, endothelial dysfunction might lead to metabolic disorders through endothelial nitric oxide synthase (eNOS) deficiency[3] which could lead to decreased glucose and insulin levels in the tissues and subsequently result in hyperglycemia and glucotoxicity[4].

Endothelial dysfunction can be caused by the reduction of eNOS through either nitric oxide (NO) reduction or by an elevated level of superoxide anion and subsequently reduction of endothelial NO[5,6]. Also, endothelial dysfunction leads to vascular inflammation and eventually increases the production of inducible nitric oxide synthase (iNOS)[7] which might lead to cardiovascular abnormalities in diabetic patients[8]. Controlling the level of iNOS might be used as a treatment key for cardiovascular dysfunction in diabetes mellitus[9,10].

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Several studies[11,12] suggested that NO can be stimulated by physical as well as chemical triggers that intensifies the activity of nitric oxide synthases (NOS). For example, exercise can increase the level of NO in the endothelial cells by physical modulation which triggers chemical mechanisms[12]. In addition, exercise improves the performance of blood vessels as well as myocardial perfusion in healthy and cardiovascular patients too[13,14].

The importance of physical activities in the control of diabetes mellitus disorder is well known but its correlation with eNOS and iNOS in diabetic patients needs to be assessed. Whether physical activity plays a role in eNOS and iNOS pathways in diabetes mellitus is still questionable. In this study, we examined serum inducible and endothelial nitric oxide synthase activities in healthy adult males and in patients with type 2 diabetes mellitus (T2DM) in relation to exercise.

Patients and Methods

The study has been performed in the Department of Physiology, King Saud University, Riyadh, Saudi Arabia from December 2020 to December 2021. The total of subjects was 164; 79 were healthy and 85 were diabetic. We grouped subjects into 1): control (n=79) and 2) T2DM (n=85). All subjects filled out a predesigned proforma regarding physical activities per week containing details of the physical activity in the last 6 months, duration, type, and intensity based on The Physical Activity Guidelines for Americans[15]. The subjects were divided into three subgroups: Group (1): sedentary group with no physical activity or less than moderate group (2): moderate group: 75 minutes of vigorous exercise or 150 minutes of moderate-intensity exercise (25 minutes of vigorous exercises such as jogging, swimming, rope jumping; 3 days per week or 150 minutes of moderate-intensity exercise such as 30 minutes of walking 5 days per week), and (3): active group: 150 minutes of vigorous exercise/300 minutes of moderate-intensity exercises (30 minutes of jogging, swimming, rope jumping 5 days per week or 60 minutes of walking 5 days per week)[15,16]. Serum iNOS, eNOS, high sensitivity C-reactive protein (hs-CRP), NO, nitrates, and nitrites were compared between different groups. Patients' data were collected from the diabetic clinic of the outpatient clinic of the King Khalid University Hospital. American Diabetes Association (ADA) criteria was used to recruit all the subjects. Serum nitrate (NO3-) anions, nitrite (NO2-) anions, and nitric oxide concentrations were assessed by the nitric oxide fluorometric Assay Kit of BioVision (Waltham, MA, USA). Subsequently, the total production of NO was determined after. Endothelial nitric oxide synthase (eNOS) kits (Catalog Number: E0868h) and inducible nitric oxide synthase (iNOS) kits (Catalog Number: E0837h) were obtained from USCN Life China (Wuhan, Hubei, China).

Statistical Analysis

SPSS Version 19 (SPSS Inc., Chicago, IL, USA) was utilized for data analysis. Mean ± Standard Error (SE) was calculated for descriptive variables. *t*-test was used to compare between control and diabetic groups. One-way ANOVA was used to compare subgroups. A p-value <0.05 was considered significant.

Results

Table I shows a significant increase in iNOS in diabetic patients when compared to the control [29.1 (2.8) *vs.* 22.4 (1.4) with p=0.050] with a significant decrease in eNOS when compared to the control group [79.5 (4.8) *vs.* 101.8 (5.7) with p=0.003].

Table II shows a comparison of demographic and biochemical profiles in all subjects between sedentary, moderate activity, and active groups (N=164). It showed significant differences in BMI between subgroups; sedentary, moderate, and active of all subjects [30.6 (0.7), 28.0 (0.6), and 27.6 (0.6) respectively with p=0.008]. It also showed significant differences (decrease) between subgroups in iNOS [35.5 (3.3), 22.5 (2.2), and 20.1 (2.5) with p<.001] and hsCRP [5.1(0.4), 3.6 (0.3), and 3.2 (0.3) with p<0.001]. In addition, eNOS showed an increase with exercises but with no statistically significant difference between groups [80.7 (5.9), 94.9 (6.6), and 94.4 (7.1) with p=0.230].

Table III shows a comparison of demographic and biochemical profiles in control subjects between sedentary, moderate activity, and active groups (n=79). eNOS shows significant differences (increase) between subgroups; sedentary, moderate, and active of control subjects [77.4 (9.2), 114.7 (9.3), and 105.6 (9.5) with p=0.026]. It also

Table I. Comparison of demographic and biochemical profile between Control and T2DM groups n = 164.

Variables	Control, n = 79	DM, n = 85	*p*-value
Age (years)	43.8 (1.2)	52.1 (1.0)	< .001*
BMI (Kg/m^2)	27.7 (0.6)	29.7 (0.5)	.018*
HbA1c (%)	5.1 (0.0)	7.7 (0.1)	< .001*
NO (µM)	56.8 (4.1)	52.2 (2.9)	.358
Nitrate (µM)	22.6 (2.0)	16.6 (0.8)	.006*
Nitrite (µM)	34.2 (2.8)	36.2 (2.3)	.590
iNOS (U/ml)	22.4 (1.4)	29.1 (2.8)	.050*
eNOS (U/ml)	101.8 (5.7)	79.5 (4.8)	.003*
hs-CRP (mg/L)	3.4 (0.2)	4.5 (0.3)	.007*

Data are represented as mean and standard error. Differences were assessed by *t*-test. *Is significant. nitric oxide (NO), endothelial nitric oxide synthase (eNOS), inducible nitric oxide synthase (iNOS), high sensitivity C-reactive protein (hs-CRP).

Table II. Comparison of demographic and biochemical profile in all subjects between sedentary, moderate activity and active groups n = 164.

Variables	Sedentary n = 53	Moderate n = 65	Active n = 46	*p*-value
Age (years)	45.8 (1.5)	49.7 (1.3)	48.5 (1.7)	.170
BMI(Kg/m^2)	30.6 (0.7)	28.0 (0.6)	27.6 (0.6)	.008*
HbA1c(%)	6.7 (0.2)	6.4 (0.2)	6.1 (0.2)	.280
NO (µM)	59.6 (5.0)	49.3 (3.2)	55.7 (4,9)	.212
Nitrate (µM)	21.1 (2.1)	17.8 (1.4)	20.0 (2.1)	.436
Nitrite (µM)	38.4 (3.7)	32.2 (2.3)	35.7 (3.4)	.340
iNOS (U/ml)	35.5 (3.3)	22.5 (2.2)	20.1 (2.5)	< .001*
eNOS (U/ml)	80.7 (5.9)	94.9 (6.6)	94.4 (7.1)	.230
hs-CRP (mg/L)	5.1 (0.4)	3.6 (0.3)	3.2 (0.3)	.001*
Physical activity (minute/week)				
Moderate activity	27.5	154	307.5	< .001*
Vigorous activity	12.5	72	140.5	< .001*

Data are represented as mean and standard error. Differences were assessed by ANOVA. *Is significant. nitric oxide (NO), endothelial nitric oxide synthase (eNOS), inducible nitric oxide synthase (iNOS), high sensitivity C-reactive protein (hs-CRP).

Table III. Comparison of demographic and biochemical profile in control subjects between sedentary, moderate activity and active groups n = 79.

Variables	Sedentary n = 21	Moderate n = 33	Active n = 25	*p*-value
Age (years)	43.0 (2.5)	44.7 (1.8)	43.3 (2.4)	.847
BMI (Kg/m^2)	28.6 (1.3)	27.8 (0.9)	26.8 (0.8)	.549
HbA1c(%)	4.9 (0.1)	5.1 (0.0)	5.1 (0.4)	.424
NO (µM)	60.7 (7.8)	53.1 (5.6)	58.6 (8.7)	.734
Nitrate (µM)	25.7 (4.6)	21.0 (2.5)	22.0 (3.8)	.639
Nitrite (µM)	34.9 (4.8)	32.1 (4.0)	36.5 (5.9)	.792
iNOS (U/ml)	23.4 (2.5)	24.2 (2.2)	20.4 (2.4)	.520
eNOS (U/ml)	77.4 (9.2)	114.7 (9.3)	105.6 (9.5)	.026*
hs-CRP (mg/L)	4.0 (0.5)	3.3 (0.3)	2.9 (0.4)	.271
Physical activity (minute/week)				
Moderate activity	25	165	312	< .001*
Vigorous activity	15	82	147	< .001*

Data are represented as mean and standard error. Differences were assessed by ANOVA. *Is significant. nitric oxide (NO), endothelial nitric oxide synthase (eNOS), and inducible nitric oxide synthase (iNOS), high sensitivity C-reactive protein (hs-CRP).

Table IV. Comparison of demographic and biochemical profile in patients with T2DM between sedentary, moderate activity and active groups n = 85.

Variables	Sedentary n = 32	Moderate n = 32	Active n = 21	*p*-value
Age (years)	47.6 (1.9)	54.8 (1.5)	54.8 (1.4)	.003*
BMI (Kg/m^2)	32.0 (0.8)	28.2 (0.9)	28.5 (0.9)	.006*
HbA1c (%)	7.8 (0.19)	7.7 (0.3)	7.4 (0.26)	.494
NO (µM)	58.8 (6.5)	45.4 (3.1)	52.5 (3.9)	.142
Nitrate (µM)	18.0 (1.7)	14.5 (0.7)	17.6 (1.3)	.123
Nitrite (µM)	40.7 (5.3)	32.4 (2.1)	34.8 (3.1)	.282
iNOS (U/ml)	43.5 (4.8)	20.8 (3.9)	19.8 (4.6)	< .001*
eNOS (U/ml)	82.9 (7.8)	74.6 (7.9)	81.7 (10.2)	.741
hs-CRP (mg/L)	5.7 (0.5)	3.8 (0.4)	3.6 (0.5)	.006*
Physical activity; minute/week				
Moderate activity	30	143	303	< .001*
Vigorous activity	10	62	134	< .001*

Data are represented as mean and standard error. Differences were assessed by ANOVA. *Is significant. nitric oxide (NO), endothelial nitric oxide synthase (eNOS), inducible nitric oxide synthase (iNOS), high sensitivity C-reactive protein (hs-CRP).

showed insignificant differences between subgroups in iNOS [23.4 (2.5), 24.2 (2.2), and 20.4 (2.4) with p=0.520]. In addition, hsCRP showed a decrease with exercises but with no statistically significant difference between groups [4.0 (0.5), 3.3 (0.3), and 2.9 (0.4) with p=0.271].

Table IV shows a comparison of demographic and biochemical profiles in patients with T2DM between sedentary, moderate activity, and active groups (n=85). It showed significant differences in BMI between subgroups; sedentary, moderate, and active of diabetic patients [32.0 (0.8), 28.2 (0.9), and 28.5 (0.9) with p=0.006]. It also showed significant differences (decrease) between subgroups in iNOS [43.5 (4.8), 20.8 (3.9), and 19.8 (4.6) with p<0.001] and hsCRP [5.7 (0.5), 3.8 (0.4), and 3.6 (0.5) with p=0.006]. In addition, eNOS showed a decrease with exercises but with no statistically significant difference between groups [82.9 (7.8), 74.6 (7.9), and 81.7 (10.2) with p=0.741].

Figure 1 shows a comparison of eNOS between subgroups in all, control, and DM subjects (sedentary, moderate, and active) while Figure 2 shows a comparison of iNOS between subgroups.

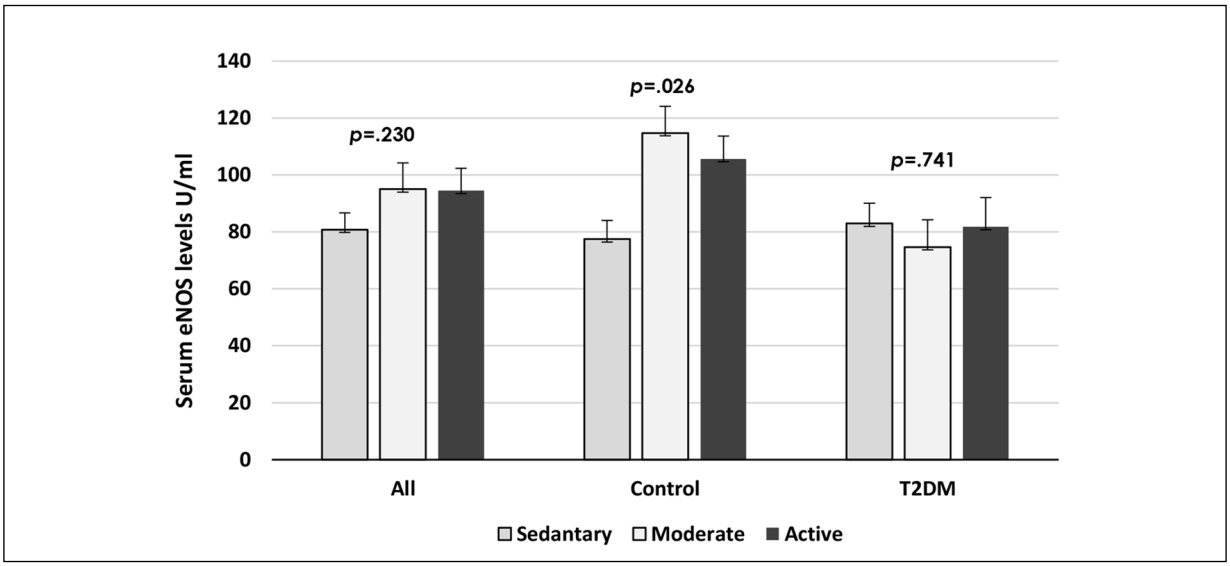

Figure 1. Serum eNOS comparison between all subjects, control, and DM subjects in relation to the exercise groups.

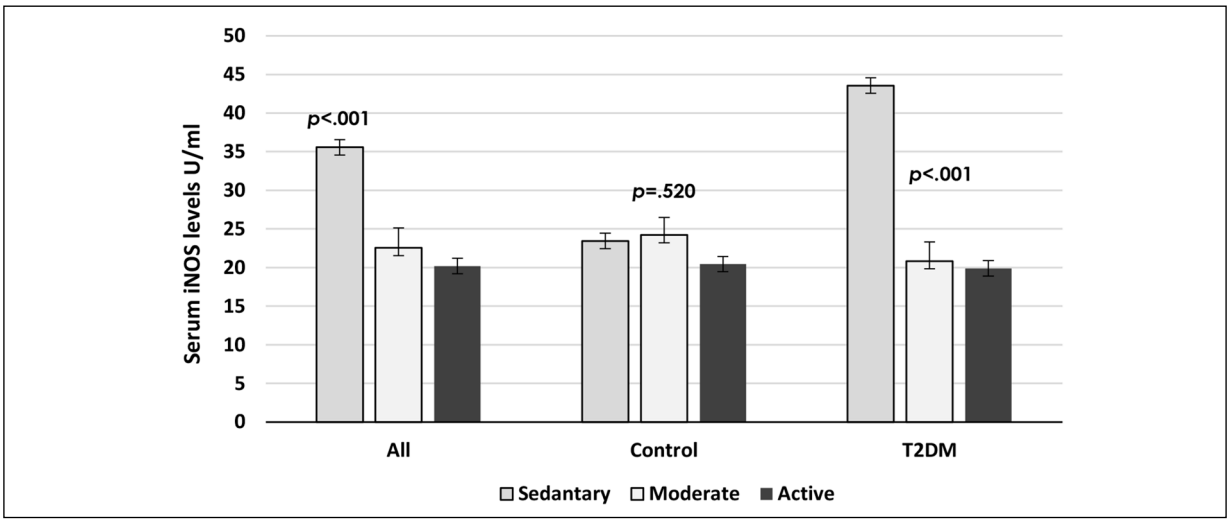

Figure 2. Serum iNOS comparison between all subjects, control, and DM subjects in relation to the exercise groups.

Discussion

Regardless of the tremendous healthcare organizations' efforts toward diabetes mellitus, it is still considered one of the most devastating health issues in the current century[17]. Approximately, 537 million were diagnosed with diabetes in 2021 amongst which 6.7 million died of diabetes. This number is expected to increase to 643 million by 2030[17,18].

It has been stated[19-23] in the literature that endothelial cell injury, oxidative stress, and macrophage dysfunction could lead to insulin resistance and DM. Therefore, nitric oxide synthase might be involved in the pathophysiology of diabetes mellitus[24].

Our study highlighted the importance of exercise in the pathway of nitric oxide synthase in diabetes mellitus. When diabetic patients were compared to the control, they showed higher levels of iNOS and lower levels of eNOS. However, our results showed a drop in iNOS as well as hsCRP in the exercise group of diabetes mellitus patients when they were compared to the sedentary group. This could lead to a decrease in the inflammatory process in diabetic patients. Elevation of iNOS and hsCRP in diabetic patients were consistent with the findings of our previous study[10] detecting the correlation of iNOS and hsCRP in diabetes mellitus. In addition, our results came with an agreement of the findings of Silva et al[25] who found that physical activity reduced iNOS. Our result also showed a reduction of NO production in exercise groups when compared to sedentary group diabetic patients (even though it was not statistically significant). This could be due to the decrease of iNOS expression in the exercise group of diabetes mellitus patients as mentioned earlier. These findings can be used as treatment procedures to abate the inflammatory process in diabetes mellitus. Also, it emphasized the importance of exercise as part of the control of this disorder. eNOS was elevated in the exercise group of the control group when compared to the sedentary group but was not elevated in diabetes mellitus group which needs further investigations.

Strengths and Limitations

It is the first study to assess the correlation of iNOS and eNOS to exercise in diabetic patients. In addition, we chose male subjects in order to have a lower confounding factor with the plan to have female subjects in the future for comparison. Also, we have collected decent sample sizes. The commitment of subjects to exercises and consistency was very difficult to control, which might be considered as a weakness. In addition, it is a cross sectional study.

Conclusions

Patients with T2DM have significantly low eNOS and high iNOS levels. However, regular exercise can significantly decrease iNOS in Patients with T2DM. Therefore, the importance of exercise with diabetic patients has to be emphasized to prevent long-term complications related to diabetes mellitus.

Conflict of Interest
The Authors declare that they have no conflict of interests.

Informed Consent
Signature of written consent forms was obtained from subjects.

Authors' Contribution
TK, SSH: designed the study, supervised, wrote, and edited the manuscript. HK, AS, AM, MH, JO, SMH: collected the data and analyzed the results.

Ethics Approval
The study was approved by the Institutional Review Board of King Saud University Medical City (KSUMC) with IRB No.: 10/2664.

Funding
The authors extend their appreciation to the Deputyship for Research & innovation, Ministry of Education in Saudi Arabia for funding this research work through the project (No. IFKSURG-83).

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