ORIGINAL ARTICLE

Effect of Morning and Afternoon Exercise on the Improvement of Endothelial Dysfunction in Type-2 Diabetes Mellitus Patients

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ABSTRACT

Introduction: Endothelial dysfunction, an early precursor to cardiovascular complications in Diabetes Mellitus (DM) patients, can be effectively managed through exercise, a non-pharmacological approach. However, it is significant to consider the timing of exercise as it can influence the benefits obtained in the management of vascular complications. This is because various systems in the body, including endothelial function, undergo fluctuations according to the circadian rhythm. This study aimed to explore the differences between morning and afternoon exercise in improving vascular endothelial dysfunction in the production of endothelial Nitric Oxide Synthase (eNOS) and Endothelin-1 (ET-1). **Methods:** Twenty two Type-2 Diabetes Mellitus (T2DM) patients were randomly divided into morning and afternoon exercise groups, and they were administered 10 weeks of Persadia diabetes gymnastics treatment. Before treatment, venous blood was taken as pre-exercise data, and after 10 weeks, it was obtained again for post-exercise examination. The examined data consisted of blood glucose, eNOS, and ET-1 levels. The data was processed statistically using the t-test. **Results:** eNOS level increased significantly in both samples at 6.66 ± 0.56 ng/ml and 5.46 ± 0.9 ng/ml ($p<0.001$). However, the increment was higher in the morning group. Whereas, the level of ET-1 decreased significantly in both samples at 58.08 ± 4.01 pg/ml vs. 34.84 ± 4.75 pg/ml ($p<0.001$), but the decrement was greater in the morning group. **Conclusion:** This study indicates that morning exercise is more influential in improving endothelial dysfunction by increasing the eNOS enzyme and significantly decreasing ET-1 in T2DM patients.
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Keywords: Exercise, Circadian Rhythm, Diabetes Mellitus, Endothelial Nitric Oxide Synthase, Endothelin-1

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INTRODUCTION

The increasing number of Diabetes Mellitus (DM) patients in the future is expected to cause a significant economic burden due to the profound disease impact on human resource quality, which decreased work productivity, and increased healthcare costs. According to International Diabetes Federation , global healthcare spending for diabetes patients was estimated at 966 billion USD in 2019. However, this figure is projected to increase to 1.03 and 1.05 trillion USD in 2030 and 2045, respectively (1-3).

DM is a multisystem disease that can lead to both micro and macrovascular complications (3,4). Furthermore, endothelial dysfunction plays a key part in initiating the inflammatory mechanism that underlies these complications. It has evolved as a characteristic and predictor of cardiovascular disease, highlighting the need for DM management to focus on improving endothelial function and preventing complications (5-7).

The pathophysiological mechanism of endothelial dysfunction is represented by a decrease in the production and bioavailability of Nitric Oxide (NO) as well as an increase in the synthesis and secretion of Endothelin-1 (ET-1) (8,9). Specifically, the reduction in the production and bioavailability of NO is attributed to a decrease in the expression and movement of the Endothelial NO Synthase (eNOS) enzyme and an increase in NO degradation due to its reaction with superoxide (10). Consequently, a decrease in eNOS level in the blood plasma is one important indicator of endothelial dysfunction (11).

Exercise is one of the pillars in the management of T2DM. T2DM is the most common type of diabetes and

is often associated with a sedentary lifestyle. Some of the beneficial effects of exercise on T2DM: (1) Increases insulin sensitivity. Poor insulin sensitivity is one of the characteristics of T2DM, where the body does not respond well to insulin. Regular exercise can increase the body's sensitivity to insulin; (2) Lose weight. Exercise helps burn calories and reduce weight. Healthy weight loss can help prevent or control DM; (3) Increases glucose metabolism. Exercise can increase the body's ability to regulate blood glucose better; (4) Reduces the risk of macro and microvascular complications (4,12,13).

Exercise is a lifestyle intervention that has been proven to improve endothelial dysfunction (6). This is because shear stress can increase constitutive eNOS, thereby increasing NO production (5,14,15). Therefore, engaging in aerobic exercise consistently yields beneficial outcomes for arterial endothelial function, primarily by augmenting NO circulation and diminishing ET-1 levels (16).

The circadian rhythm of the body fluctuates over 24 hours, which affects the functions of various systems, including endothelial function (17). Notably, ET-1 exhibits its highest and lowest levels at 08:00 a.m. and 04:00 p.m., respectively. In contrast, the most heightened and lowest levels of eNOS level were recorded at 08:00 p.m. and 08:00 a.m., respectively (18). Melatonin and body antioxidants also follow the circadian rhythm, with higher levels in the morning (19).

Several studies have attempted to elucidate the impact of morning and evening exercise on various physiological responses (20). However, the results regarding the influence of timing on exercise outcomes remained a subject of debate. There is no sufficient data to analyze and draw firm conclusions about CVD attacks which are higher in the morning. This lack of data raised unanswered questions, particularly concerning when individuals with CVD risk factors should engage in "recreational" sports, either at morning or evening (21,22). Therefore, aligning exercise time with the circadian rhythm of the body is expected to provide greater benefits, especially for T2DM patients who are already at risk of cardiovascular disease.

MATERIALS AND METHODS

The present study received ethical approval from the Health Research Ethics Committee of Dr. Moewardi Hospital in Surakarta, number 85/II/HREC/2021. To determine the effect of exercise timing on endothelial function in T2DM patients, a total of 22 female participants aged 55-65 years, with a BMI of 23-25 kg/m2, were selected. The female sex was chosen due to the high prevalence of cardiometabolic diseases including DM and cardiovascular complications, in the gender (55.8%) than in males (23). They were randomly assigned to either morning or afternoon exercise levels. Those who received insulin therapy and had severe complications were excluded from the study.

Persadia diabetes gymnastics was utilized as a form of exercise, which was specifically designed for DM patients. This was made by the Ministry of Health and was conducted under the supervision of a certified coach. The excercise was moderately intense and was performed at 64% -76% of the maximum HR. The frequency was 3 times a week, with each training session lasting for 50 minutes. This type of gymnastic movement employed combines aerobics with intermittent weight training. The entire gymnastics training program spanned a duration of 10 weeks. Morning and afternoon exercise was conducted from 07:00 to 08:00 a.m. and 04:00 to 05:00 p.m., respectively.

Participants were provided with a detailed explanation of the purpose and procedures of the study. These include information about the allowed and prohibited activities, food, and drink during treatment, in accordance with informed consent. Furthermore, blood samples were obtained from the median cubital vein before and behind the completion of the 10th week of exercise, following the established sampling protocol.

Biochemical Assessments

The biochemical assessment included the measurement of Random Plasma Glucose levels, measured in mg/dl using the hexokinase practice. Meanwhile, eNOS and ET-1 were analyzed using the Abbexa ELISA kit with standard 96-well plates. To minimize variability, duplicate samplings were processed on each plate, and the average of 2 aliquots was utilized as the cytokine concentration measurement.

Statistical Test

Data in this study were presented as mean ± SD, and normality and variance were analyzed using the test of Shapiro-Wilk and Lavene, respectively. For the morning group, a dependent t-test was employed to examine the difference between pre-and post-exercise eNOS data, which were normally distributed and homogeneous, while the Wilcoxon test was used for the afternoon eNOS data of the group, which were not normally distributed. The difference in eNOS levels between the morning and afternoon classes was tested using the Mann-Whitney U test. ET-1 data for both groups were normally distributed, and the different test between pre- and post-exercise was evaluated using paired t-test. The difference in ET-1 between the morning and afternoon groups, which were normally distributed and homogeneous, was analyzed using an unpaired t-test. The statistical analysis was performed using the SPSS program (version 25), and statistical significance was considered when $p<0.05$.

RESULTS

The study subjects were recruited from participants in the Persadia Palur diabetes gymnastics, located at Karanganyar, Central Java, Indonesia, and their characteristics included age, BMI, blood glucose level, normally distributed and homogeneous (p>0.05), as shown in Table I.

Table I: Morning and Afternoon Group Characteristics (mean ± SD)

Variable	Morning Group N = 11	Afternoon Group N =11	p
Age (years)	61.09 ± 3.59	60.64 ± 3.64	0.77*
BMI (kg/m²)	23.99 ± 0.58	24.35 ± 0.55	0.15*
Random blood glucose (mg/dl)	226.98 ± 40.15	230.98 ± 45.75	0.72 #
Weight (kg)	56.64 ± 1.29	55.36 ± 1.69	0.70 #
Height (cm)	153.55 ± 2.88	150.73 ± 3.20	0.98 #
Duration of Suffering from DM (years)	13.91 ± 3.02	14.92 ± 4.18	0.74 #

*Unpaired t-test, # Mann Whitney U test

eNOS is an enzyme in the endothelium that plays a crucial role in catalyzing L-Arginine into NO as a major vasodilator in the vasculature. The statistical test of pre- and post-exercise eNOS levels showed a significant increase in this endothelial enzyme, both in the morning and afternoon group, as presented in Figure 1. eNOS level in the morning and afternoon group increased from 14.22 ± 0.24 ng/mL to 20.87 ± 0.48 ng/mL (p<0.001) and 14.73 ± 0.31 ng/mL to 20.19 ± 0.83 ng/mL (p=0.003), respectively. The difference in this increase was significant (p=0.001), with a greater rise observed in the morning group.

ET-1 was a potent vasoconstrictor peptide produced by endothelial cells. Its levels at pre and post-exercise were observed using T-test, and the results showed a significant decrease in this vasoconstrictor substance from 109.59 ± 3.71 pg/ml to 51.51 ± 3.01 pg/ml and 110.75 ± 3.39 pg/ml to 75.92 ± 3.55 pg/ml in both morning and afternoon group, respectively. The difference in ET-1 decrease was significant (p<0.001) (Figure 2).

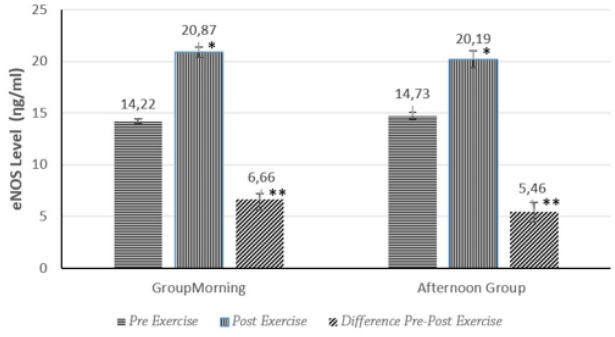

Figure 1: The impact of morning and afternoon exercise on eNOS Level. Data were presented in mean ± SD (n=11 people/group) and the measurement of eNOS level used the ELISA method. * p<0.05 compared to the pre-exercise group (morning group with a paired t-test, while afternoon group with Wilcoxon test). ** p<0.05 between morning and afternoon group (Mann-Whitney test)

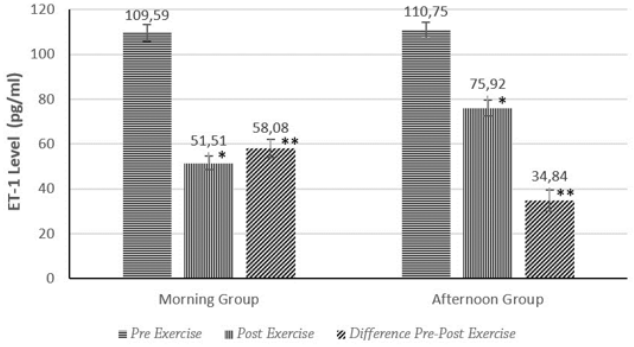

Figure 2: Effect of morning and afternoon exercise on ET-1 Level. Data were presented in mean ± SD (n=11 people/group) and the measurement of ET-1 level used the ELISA method. * p<0.05 compared to the pre-exercise group (paired t-test). ** p<0.05 between morning and afternoon group (unpaired t-test)

The morning pre-exercise glucose group had an initial average of 226.98 ± 40.18 mg/dl, which decreased significantly to 194.19 ± 25.76 mg/dl (p=0.003). Similarly, the afternoon pre-exercise glucose group started with an average of 230.98 ± 45.75 mg/dl, which decreased to 199.87 ± 29.23 mg/dl (p=0.003). This implied that exercise can reduce GDS levels in both morning and evening sports. The difference in blood sugar reduction in the morning group was greater than in the afternoon counterpart (32.79 ± 16.08 (mg/dl) VS 29.79 ± 16.98 (mg/dl), but not statistically significant (p=0.82).

DISCUSSION

Endothelial cells normally have antithrombotic, anti-inflammatory, and vasodilatory properties to maintain blood flow as well as prevent thrombosis and leukocyte diapedesis. However, they can lose their protective properties, leading to endothelial dysfunction (24). Vascular endothelial cells produce NO, which is a potent vasodilator and has anti-atherosclerotic properties. They also produce ET-1, which is a potent vasoconstrictor peptide and has proliferative activity on Vascular Smooth Muscle Cells (VSMC) (25). This molecule is an effective marker of endothelial dysfunction (16).

A previous study has proposed oxidative stress and inflammation as the majority of mechanisms underlying the pathogenesis of endothelial dysfunction. NADPH oxidase is a source of O2- that causes vascular oxidative stress (26). Furthermore, free radicals reduce eNOS stimulation in endothelial cells resulting in decreased NO production, impaired relaxation of smooth muscle cells, and predisposition to atheromatous plaque formation (14). Another mechanism of endothelial dysfunction is eNOS uncoupling, which increases O2- production. Superoxide can react with NO to form peroxynitrite (ONOO-) and nitrogen dioxide (NO2), contributing to

vascular inflammation, decreasing NO availability, and triggering thromboembolic processes (14).

eNOS is a member of the NOS family of 3 isoforms that catalyze the oxidation of the terminal guanidino group of L-arginine to produce NO and L-citrulline. It is activated by increasing laminar shear stress on endothelial cells during exercise, which will subsequently increase the production of NO (7). In addition to being a vasodilator, NO also has anti-atherogenic activity, including inhibiting the adhesion of monocytes, leukocytes, and platelets, antioxidants, and inhibiting VSMC proliferation (14).

The results showed a substantial increase in the plasma eNOS level after regular Persadia gymnastics for 10 weeks in two morning and afternoon groups. These support previous studies on both humans and test animals. For example, a study on pre-DM Wistar rats showed that regular exercise for 8 weeks increased eNOS activity and expression as well as eNOS protein in the aorta (27). Additionally, exercise for 8 weeks improved endothelial dysfunction by increasing eNOS levels in the hearts of db/db diabetic mice (28). Furthermore, a study on DM test animals showed that 8 weeks of exercise increased the expression of eNOS and nNOS proteins in the medulla and cortex of the kidneys (29). Finally, those using mice also showed that 3 weeks of exercise increased eNOS protein expression in the aorta (15).
After 4 weeks of exercise, T2DM patients exhibited an increase in eNOS mRNA expression, which was further enhanced after 6 months (30). Furthermore, 3 months of combined aerobic and resistance exercise led to a significant improvement in FMD (Flow Mediated Dilation) among T2DM patients (29,30). For CAD patients, engaging in regular physical activity for 4 weeks resulted in a 2-fold increase in eNOS protein expression (33).

Regular exercise has been shown to increase blood flow and shear stress, which in turn leads to improved production and bioavailability of NO. One of the mechanisms responsible for this improvement is the up-regulation of eNOS, both in messenger ribonucleic acid (mRNA) and protein expression levels, under laminar shear stress (5). This effect is related to the mechanotransduction of shear stress, which is responsible for converting mechanical forces into molecular changes and improving endothelial function (34,35).

Exercise can also enhance glucose uptake in muscle cells via the GLUT4 transporters, leading to increased insulin sensitivity (36). Insulin has a direct effect on endothelial cells and regulates vascular tone. Meanwhile, its impact on the vascular process is mediated by NO and ET-1.
Endothelial cells produce ET-1, which is a potent vasoconstrictor with inotropic and mitogenic effects. It affects water and salt homeostasis and stimulates the renin-angiotensin-aldosterone system as well as the sympathetic nervous technique. Studies have shown that ET-1 can activate several transduction signal pathways related to cellular hypertrophy, migration, growth, and proliferation of various cell types including cardiac and cardiomyocyte tissue, as well as renal mesangial cells (37).

A significant decrease in the ET-1 level was observed after 10 weeks of regular Persadia gymnastics in two morning and afternoon groups. This means that standard exercise can improve endothelial dysfunction by reducing the ET-1 level. These are consistent with previous studies that aerobic exercise 4 times/week for 12 weeks can significantly decrease the potent peptide in hypertensive patients (38). Additionally, Donghui (2019) showed an increase in the ratio of plasma NO/ET-1 levels in obese individuals after 6 weeks of exercise (16).

This study showed a greater increase in eNOS level and a more significant decrease in the ET-1 in the morning exercise group. This indicated a greater improvement in vascular endothelial dysfunction at morning exercise than afternoon. This may be attributed to higher levels of melatonin which is secreted in the morning and antioxidants. Previous studies have shown that morning exercise can elevate melatonin levels. Furthermore, its increase from baseline to post-exercise is greater in the morning (11.1 ± 8.7 pg/ml) than in the afternoon (5.1 ± 5.7 pg/ml) (39).

Melatonin plays a beneficial role in glucose metabolism. Its oral administration in rats can reverse insulin resistance and improve glucose metabolism (40). Furthermore, Mc Mullan et al. (2013) reported that a decrease in melatonin secretion is associated with an improved risk of T2DM (41). This is because the hormone increases insulin secretion in human pancreatic cell cultures (42), and has powerful antioxidant and anti-inflammatory abilities. Melatonin can scavenge two Reactive Nitrogen Species (RNS) and Reactive Oxygen Species (ROS), including peroxynitrite (ONOO-), and block transcription factors that produce pro-inflammatory cytokines (43). In the morning, antioxidant levels are higher, and this protects against the negative effect of free radicals on eNOS in endothelial cells, resulting in decreased NO production. A previous study reported that morning exercise can enhance oxidative stress conditions by improving the antioxidant enzyme glutathione peroxidase-1 (GPx-1) and decreasing malondialdehyde (MDA) (44).

Exercise in the morning can expose participants to UVB rays, which serve as the primary source of vitamin D3 for most of the population. It is worth noting that patients with T2DM tend to have lower levels of vitamin D3 than the control group (45). This vitamin can improve insulin resistance and the function of β-Langerhans cells (46). Furthermore, endurance exercise can stimulate the secretion of Vitamin D3 metabolites, which possess

antioxidant, and anti-inflammatory properties (47).

The regulations of this report are the small sampling size due to the Covid-19 pandemic and the food of participants' intake not being strictly controlled. However, the results support previous studies that exercise supplies good benefits in the form of the improvement in endothelial dysfunction which is greater in morning exercise.

CONCLUSION

Exercising in the morning and evening can improve endothelial dysfunction by increasing eNOS levels and decreasing ET-1 levels. For T2DM patients, the improvement was significantly greater with morning exercise.

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