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The effectiveness of yoga exercise toward blood pressure and endothelial-derived hyperpolarizing factor (EDHF) level in hypertensive diabetic population

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ABSTRACT

Introduction: Diabetes and its complications are the leading causes of premature death worldwide. Diabetes causes complications such as hypertension and an increased risk of cardiovascular diseases. Endothelial dysfunction is the primary pathophysiology that underlies complications of diabetes mellitus. EDHF (Endothelial-Derived Hyperpolarizing Factor) is a relaxing factor that targets current therapy in vascular dysfunction and insulin resistance in obesity and diabetes mellitus. Yoga training is a physical activity that has been shown to play a role in decrease blood pressure, but to our knowledge, there are no studies that prove the effectiveness of yoga in increasing EDHF levels. Therefore, this study aimed to determine the efficacy of yoga to decrease blood pressure and increase EDHF levels in type II diabetes mellitus

patients with hypertension.

Methods: This research was a randomized control trial that involved 39 hypertensive diabetic respondents and was conducted in Denpasar, Bali, Indonesia. Yoga exercise was carried out two times a week for eight weeks, with a duration of 60 minutes per session. EDHF levels were examined using the ELISA technique.

Results: There was a significant difference in systolic, diastolic and EDHF levels after the intervention between the yoga and control groups and large effect size.

Conclusion: In conclusion, yoga training effectively reduced blood pressure and prevented endothelial dysfunction by increasing the blood vessel relaxing factor, namely EDHF.

Keywords: postures, breathing control, relaxing factor, endothelial dysfunction, complication.

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INTRODUCTION

The incidence of diabetes mellitus is continuously increasing. The prevalence of diabetes mellitus has quadrupled in the last three decades. It is estimated that 1 in 11 (415 million) adult populations aged 20-79 years worldwide have diabetes, 90% of them are type 2 diabetes mellitus (T2DM) and estimated to increase to 642 million by 2040. Asia is a major area of the rapidly emerging global type 2 diabetes mellitus (T2DM) epidemic.¹ The mortality of cardiovascular disease in diabetic patients was found to be higher in men than in women. The relative risk of cardiovascular disease morbidity and mortality in diabetic patients ranges from 1-3 in women and 2-5 in men compared to the population without diabetes.²

Endothelial dysfunction underlies various

diabetic-related complications and hypertension is one of the most prevalent.³ Type 2 diabetes mellitus (TD2M) is associated with higher mortality and premature morbidity due to hypertension. A study shows that the prevalence of hypertension in patients with type 2 diabetes mellitus (T2DM) was relatively high, at 59.5%, and most had poor blood pressure control.⁴ When patients with type 2 diabetes experience hypertension, the risk for cardiovascular disease is even greater. Systolic blood pressure greater than 140 mmHg in patients with type 2 diabetes is related to an increased risk of coronary heart disease, stroke, and other mortality factors.⁵

EDHF is a therapeutic targeting that can prevent vascular dysfunction such as hypertension and coronary heart diseases, which are the leading causes of morbidity and mortality in patients with

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type 2 diabetes mellitus (T2DM).⁶ Understanding and overcoming endothelial dysfunction is a major focus in the prevention of vascular complications associated with diabetes. Targetting endothelial dysfunction in diabetes is the latest treatment strategy for preventing cardiovascular disease due to diabetes.³ The balance or homeostasis between endothelial relaxing and endothelial constricting factors is impaired in insulin resistance conditions, including obesity, type 2 diabetes mellitus (T2DM) and hypertension.⁷ One of the endothelial relaxing factors that play a role is the endothelium-derived hyperpolarizing factor (EDHF). Endothelium-derived hyperpolarizing factors (EDHF) are the potential therapeutic targets of vascular dysfunction in obesity and insulin resistance in diabetes.⁶

The first objective in any hypertensive diabetic should be to initiate lifestyle changes that improve hypertension and EDHF levels. The easiest and most feasible method is by doing exercise regularly. Evidence showed that regular exercise positively affects endothelial dysfunction through increased EDHF response.⁸ Various studies on the effects of yoga combined with breathing techniques and mental relaxation as a lifestyle modification therapy reported positive benefits of those regiments in lowering blood pressure in hypertensive patients,⁹ but no studies are examining the effectiveness of yoga in increasing levels of EDHF. Because EDHF is a potential therapeutic target for vascular dysfunction in insulin resistance, our study's objective was to determine yoga's effectiveness to improve hypertension and EDHF levels in hypertensive diabetic populations.

METHODS

Materials

The hypertension was diagnosed using The Eighth Joint National Committee (JNC 8) criteria, which use systolic and diastolic blood pressure. Those criteria include normal blood pressure, prehypertension, stage I hypertension and stage II hypertension. The following is a classification of hypertension based on JNC 8¹⁰ (Table 1).

Table 1. BMI criteria based on JNC8 classification¹⁰

Classification	Blood pressure (mmHg)
Normal	Systole BP < 120 mmHg AND Diastole BP < 80 mmHg
Prehypertension	Systole BP 120-139 mmHg OR Diastole BP 80-90 mmHg
Stage I HTN	Systole BP 140-159 mmHg OR Diastole BP 90-99 mmHg
Stage II HTN	Systole BP \geq 160 mmHg OR Diastole BP \geq 100 mmHg

Notes: BP, Blood Pressure; HTN, Hypertension

Blood pressure measurement was assessed using Onemed® sphygmomanometer. EDHF level examination was carried out before and after treatment (yoga exercise). We used human Endothelium-Derived Hyperpolarizing Factor (EDHF) ELISA Kit 96T catalog no EA0163Hu Merk BT LAB.

Subject recruitment

This research involved 39 respondents who grouped using a simple random sampling method with a random number table. From 42 eligible respondents, three were excluded because they refused to join and participate in the study. The study involved two arms (yoga group and control group). Respondents were divided into two groups randomly. Blocked randomization was used to group the respondents. The block size was not stated in the protocol, so the investigators were blinded. Twenty-one respondents were allocated to the yoga group and 18 were assigned to the control group. Some respondents discontinued the intervention because they did not attend yoga exercise more than three times ($n = 4$). Therefore, the number of respondent data analyzed in the yoga group was 17, while the control group was 18 respondents. Respondent's recruitment strategy is summarized using the CONSORT statement flow diagram presented in Figure 1.

Study design

This research was a randomized control trial in which respondents were hypertensive diabetic population and conducted at Denpasar, Bali, Indonesia. Ethical clearance for this study had been issued by the research ethics commission Medical Faculty of Udayana University/Sanglah Hospital Denpasar with ethical clearance number 1737/UN14.2.2.VII.14/LT/2020. An explanation of the study's procedures and benefits was conducted on all respondents before the study began. All of the subjects were asked to fill the informed consent form if they agree to participate. The yoga group was given yoga exercise for eight weeks, two times a week, and the total duration of yoga exercise of 60 minutes. The control group was a negative control that was not given any intervention.

Respondents in this study were diabetic hypertensive population in Denpasar city, Bali. The respondents were established according to eligibility criteria such as aged 50-70 years, has a history of controlled diabetes at least in the last six months, has hypertension history at least in the last six months, and can attend and perform the exercise accordingly. Respondents were excluded if they have the following events: has emergency hypertension

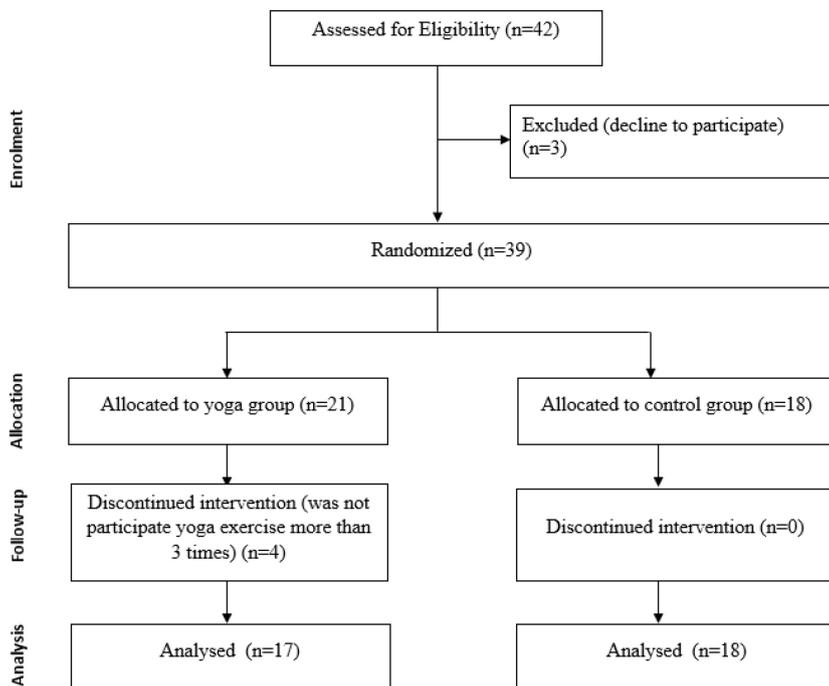


Figure 1. CONSORT statement diagram of respondent recruitment

Table 2. Details of yoga exercise intervention

Practices	Duration	Repetition	Total duration
Warming up	5 minutes	1	5 minutes
Tadasana	1 minutes	5	5 minutes
Virabhadrasana	1 minutes	5	5 minutes
Dandasana	1 minutes	5	5 minutes
Supta tadasana	1 minutes	5	5 minutes
Supta padangustasana	1 minutes	5	5 minutes
Urdva hastasana	1 minutes	5	5 minutes
Ardha uttanasana	1 minutes	5	5 minutes
Baddha konasana	1 minutes	5	5 minutes
Virasana	1 minutes	5	5 minutes
Swastikasana	1 minutes	5	5 minutes
Cooling down (Shavasana)	5 minutes	1	5 minutes

during the exercise, participate in a physical exercise program other than the intervention, have a history or are currently experiencing injuries, have physical disabilities, or have balance disorders. Respondents were dropped out when they were unable to complete the treatment given, suffered injuries during the study, or resigned by themselves.

The yoga group was given a yoga exercise, which was done twice a week for eight weeks. Yoga exercises were carried out for 60 minutes each session, twice a week for eight intervention weeks. Yoga exercise was under the supervision of a yoga therapist and medical team. Yoga exercise began with active stretching as a warming-up for 5 minutes and consisted of 10 postures which each

posture was done for 1 minute with five repetitions. Yoga postures, accompanied by breathing control (Table 2). At the end of the session, the respondents did the relaxation technique, namely, Shavasana posture, for 5 minutes.

Blood sampling was taken twice, the day before yoga exercise (pretest) and the day after it (posttest). Venous blood taken using a syringe was left standing for 1-2 hours, so serum/plasma and other blood components were separated. After 1-2 hours, a new 300 rpm centrifuge was carried out for 10 minutes. The supernatant was put in a 1.5 ml microcentrifuge tube and stored in the freezer -20/-80°C. EDHF measurement was conducted using the ELISA technique and Endothelium-Derived Hyperpolarizing Factor (EDHF) ELISA Kit 96T catalog no EA0163Hu Merk BT LAB was used for analysis. Preparation of reagents, samples, standard solutions and assay procedures had been carried out according to standard EDHF measurement procedures.

Statistical Analysis

All of the data were compiled into excel and spss format in preparation for analysis. The data analyzed descriptively to obtain wider picture and the distribution of each variable in both groups. Then, normality test (shapiro-wilk) was performed to determine which bivariate analysis should be used to compare the mean blood pressure and EDHF. Finally, bivariate analysis was conducted to compare mean systolic and diastolic blood pressure as well as mean EDHF level. The effect size was also calculated in both groups. P-value < 0.05 was considered significant.

RESULTS

The baseline characteristics of respondents based on age, systole blood pressure, diastole blood pressure, BMI, fasting blood glucose and fasting blood cholesterol are shown in table 3.

According to gender, a higher proportion of females were enrolled in this study compared to males. Additionally, the majority of the subjects had type II HTN with no prehypertension. Twenty-one subjects had obese, while the rest of the subjects had normal and overweight.

The number of respondents based on gender is that there are more female respondents than men. Total respondents based on the hypertension category were that most of the respondents in both groups were classified as grade 2 hypertension and obese BMI category (Figure 2).

Bivariate analysis was conducted to assess yoga exercise's effect on the subject's blood pressure and EDHF (Table 4). Subjects in the yoga group

Table 3. Characteristics of respondents by age, systole blood pressure, diastole blood pressure, BMI, fasting blood glucose and fasting blood cholesterol

VARIABLES	MEAN	MIN-MAX	SD	CI
AGE				
Yoga group	63.00	56-68	3.70	61.09-64.91
Control group	63.24	56-69	4.72	60.81-65.66
BMI				
Yoga group	25.18	19.60-33.60	3.81	23.22-27.14
Control group	26.53	21.30-31.60	2.95	25.01-28.05
FASTING BLOOD GLUCOSE				
Yoga group	201.82	90-469	124.76	137.68-265.97
Control group	137.18	80-339	61.47	105.57-168.78
BLOOD CHOLESTEROL				
Yoga group	216.12	134-298	49.394	190.72-241.51
Control group	189.24	152-232	26.70	175.50-202.97
SYSTOLE				
Yoga group	153.53	140-170	10.57	148.09-158.96
Control group	156.47	140-180	12.21	150.19-162.75
DIASTOLE				
Yoga group	92.94	90-100	4.697	90.53-95.36
Control group	92.94	90-100	4.697	90.53-95.36

Notes: min-max: Minimum-Maximum; SD: Standard Deviation; CI: Confidence Interval

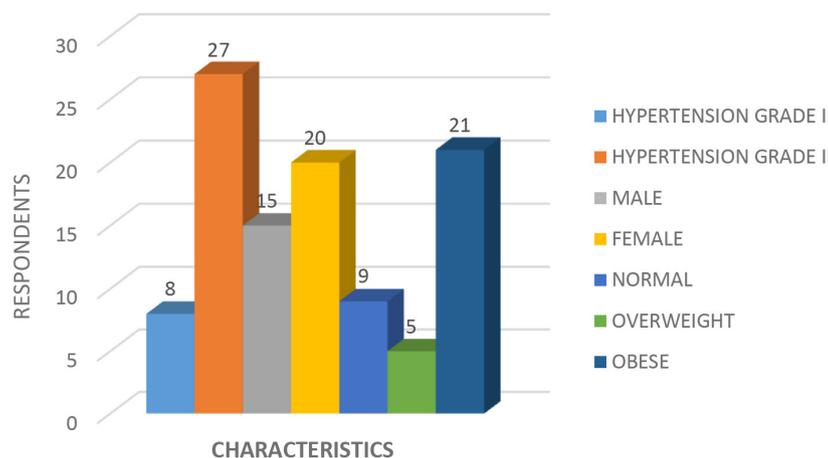


Figure 2. The number of the respondents based on gender, hypertension grade and BMI category

had a significantly lower mean of systolic blood pressure (yoga vs. control: 153.53 ± 12.18 mmHg vs. 156.47 ± 11.60 mmHg; $p=0.000$) compared to control at the end of the intervention. The mean diastolic blood pressure was also significantly lower in the yoga group than the control group (yoga vs. control: 81.76 ± 8.09 mmHg vs. 90.00 ± 6.12 mmHg; $p=0.002$). Finally, the mean EDHF level was also increased to almost twice compared to the control group (yoga vs. control: 517.84 ± 21.17 mmHg vs. 173.91 ± 4.91 mmHg; $p=0.000$).

To better understand the extent of blood pressure improvement, an intra-group analysis was conducted where the blood pressure and EDGF

level before and after yoga exercise were analyzed and compared. The result showed that both yoga and control groups had lower blood pressure after yoga, but a higher magnitude was observed in the yoga group in both systolic and diastolic blood pressure. The EDHF also increased dramatically in the yoga group with a mean difference at 342.72 ± 22.23 , while the control group experienced almost no change in EDHF level (Table 4).

A large effect size ($>0,8$) was observed in the mean of systole blood pressure, diastole blood pressure, and EDHF when comparing the yoga group and control group (Figure 3A). A similar finding was also observed in the pre-post analysis of those parameters (Figure 3B). These findings indicate that the differences observed in this study are important and likely to be beneficial for the targeted population.

DISCUSSION

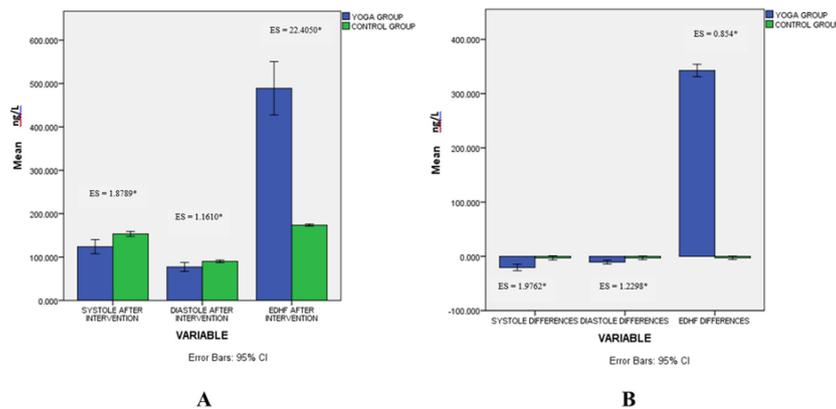
The incidence of type 2 diabetes mellitus increases with age due to natural physiological changes associated with the aging process. The aging process is associated with a reduced ability of the body to regenerate β cells of the pancreas.¹¹ Decreased pancreatic β cell replication is associated with reduced expression of cell transcription factors. Reduced replication of pancreatic β cells in the aging process is accompanied by an increase in the expression of cell cycle inhibiting factors, causing a decrease in the ability of pancreatic β cell regeneration.¹²

In our study, more female respondents than men

Table 4. Systolic blood pressure, diastolic blood pressure, EDHF levels and differences of all parameters in yoga and control group before and after the intervention

VARIABLES	MEAN	MIN-MAX	SD	p	CI
SYSTOLE					
Before intervention					
Yoga group	153.53	140-170	10.57	0.546	148.09-158.96
Control group	156.47	140-180	12.21		150.19-162.75
After intervention					
Yoga group	131.18	110-150	12.18	0.000*	124.91-137.44
Control group	152.94	130-170	11.60		146.98-158.91
DIASTOLE					
Before intervention					
Yoga group	92.94	90-100	4.69	0.918	90.53-95.36
Control group	92.94	90-100	4.69		90.53-95.36
After intervention					
Yoga group	81.76	70-90	8.09	0.002*	77.61-85.92
Control group	90.00	80-100	6.12		86.85-93.15
EDHF					
Before intervention					
Yoga group	175.12	168.13-179.78	3.46	0.369	173.33-176.90
Control group	176.54	167.91-185.27	5.99		173.46-179.62
After intervention					
Yoga group	517.84	498.54-561.71	21.17	0.000*	506.96-528.73
Control group	173.91	167.14-180.22	4.91		171.39-176.44
SYSTOLE DIFFERENCE					
Yoga group	-21.76	-40-0	11.31	0.000*	-27.58-(-15.95)
Control group	-3.53	-10-10	7.01		-7.14-0.08
DIASTOLE DIFFERENCE					
Yoga group	-11.18	-20-0	6.96	0.001*	-14.76-(-7.59)
Control group	-2.94	10-10	6.86		-6.47-0.59
EDHF DIFFERENCE					
Yoga group	342.72	319.42-387.21	22.23	0.000*	331.29-354.16
Control group	-2.63	-11.53-10.55	5.93		-5.68-0.419

Notes : min-max : Minimum-Maximum; SD, Standard Deviation; p, p-value; *, p < 0,05; CI, Confidence Interval



Notes: ES, Effect Size; *, ES>0,8

Figure 3. A. The mean and effect size of systole blood pressure, diastole blood pressure, EDHF levels after intervention between yoga and control group. B. Mean and effect size of the differences of systole blood pressure, diastole blood pressure, EDHF after intervention between yoga and control group

(Figure 2) were linear with another study that found that more women had type 2 diabetes mellitus than men. This phenomenon is caused by less muscle mass, which results in lower glucose uptake and higher estrogen and progesterone levels in women. High levels of estrogen and progesterone play a significant role in decreasing insulin sensitivity throughout the body.¹³ The respondents' BMI was mostly in the obese category (Figure 2), with a mean of 25.18 kg / m2 in the yoga group and 26.53 kg / m2 in the control group (table 3), which is also in line with the fact that obesity strongly linked to various biomarkers of cardiovascular disease and type 2 diabetes mellitus. Obese has also been linked to higher blood pressure systole, diastole, HbA1C, triglycerides, and LDL.¹⁴

We also check the respondents' fasting blood sugar, where monitoring blood glucose is one of the diabetes treatments to prevent complications due to diabetes. Most of our respondents' total cholesterol

levels were high. Blood cholesterol management can help manage risk factors for dyslipidemia and reduce the condition's adverse effects.¹⁵ This finding is in line with a study that found 30.7% of respondents with diabetes had total cholesterol levels above 200 mg/dL, and there was a significant correlation between cholesterol, triglycerides and low density lipoprotein (LDL) levels.¹⁶

Most of the respondents' blood pressure was categorized as Grade II hypertension (Figure 2). This is consistent with a study that found a higher prevalence of hypertension in the elderly population with diabetes. Hypertension is a common comorbidity in patients with diabetes and its prevalence increases with increasing age. In a population with type 2 diabetes mellitus, hypertension is a major risk factor for cardiovascular disease.¹⁷ Diabetes mellitus and hypertension are the main risk factors for cardiovascular disease, which can be modified with lifestyle changes, namely, regular physical activity. Therefore, in this study, we assessed yoga exercise's effectiveness in improving these main risk factors.

The effectiveness of yoga in reducing systolic and diastolic blood pressure has been proven through various studies. Yoga affects blood pressure by modulating the body's physiological system and stabilizing the autonomic function characterized by a significant reduction in systolic and diastolic blood pressure.¹⁸ Some reports also showed that yoga exercise consisting of various asana postures and breathing control techniques interacts with somato-neuro-endocrine mechanisms which affect autonomic function.¹⁹

In line with the previous study, our study also consisted of 10 asana postures and breathing control (pranayama) but with different exercise regiments that would provide the same physiological adaptations. We considered that the dose of yoga exercise that we implemented was sufficient to induce the body's adaptation process to reduce systolic and diastolic blood pressure in our respondents. Our finding is also linear. A study reported that yoga exercises consisting of multiple asana postures, pranayama, and meditation effectively reduced systolic and diastolic pressure in patients with type 2 diabetes mellitus with hypertension. This study explains that various asana postures in yoga can increase the sensitivity of baroreceptors in blood vessels, so that blood pressure control becomes effective. This study's meditation was believed to provide a relaxing effect that reduces psychological stress, where psychological stress is a risk factor for hypertension.²⁰

In our study, yoga exercise does not include meditation, but a relaxing effect can be obtained

through breathing exercise (pranayama). Respiratory exercise in our study was performed with slow, deep breathing, which has been shown to have a therapeutic effect on autonomic tone. Slow and deep breathing exercise can strengthen the cardio respiration system, increase parasympathetic activity, decrease blood pressure and increase baroreflex sensitivity.²¹

Our study also found significant differences in EDHF levels and differences in EDHF levels after the intervention in the yoga and control groups (table 4), with an effect size of more than 0.8. Based on previous research, resistance exercise (the combination of isotonic and isometric exercise) effectively modified endothelial function in a population with systemic hypertension. It was thought that resistance exercise improved endothelium-dependent vasodilation.²² Asanas in our study are similar to isotonic and isometric exercises at an early stage. However, asanas in yoga are not equivalent to isotonic and isometric exercises because asanas maintain posture, but keeping certain positions comfortably with the mind is focused and relaxed. When the asana is performed slowly, with steadiness and accompanied by regulating breathing, the asana is carried out into subconscious activities so that the physical effects of yoga are accompanied by mental modification control.²³

A previous study found that Bikram yoga performed in a high-temperature environment (40.5°C) was just as effective as Bikram yoga performed at normal temperature (23°C) in increasing endothelial-dependent vasodilation. Asana, accompanied by breathing control, is a factor underlying endothelial-dependent vasodilation changes. This discovery proved that yoga postures' effectiveness without heat conditions can improve blood vessel health.²⁴ Our study is also in line with the new findings presented in this study, wherein this study measured one of the factors that play a role in endothelial relaxing, namely EDHF. As far as we know, no studies have examined the effectiveness of yoga in increasing the relaxing endothelial factor in relation to the prevention of endothelial dysfunction that occurs due to complications of diabetes mellitus. We conclude that yoga exercise effectively reduces systolic and diastolic blood pressure in diabetes mellitus patients with hypertension.

CONCLUSIONS

In conclusion, yoga exercise effectively increased EDHF levels and reduced systolic and diastolic blood pressure in the diabetic population. These findings indicate that yoga may have a beneficial

effect in preventing type 2 diabetic-related complications. We recommend that future studies involve a larger number of respondents to be generalized to a broader population.

ETHICS APPROVAL

This study had been approved by Ethics Committee of Udayana University with Ethical Clearance Number 1737/UN14.2.2.VII.14/LT/2020.

AUTHOR CONTRIBUTION

All authors contributed equally in writing and revising this article.

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CONFLICT OF INTEREST

The author reports no conflicts of interest in this work.

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