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Research Article

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The Effectof Exercise to Glomerular Filtration Barrier System in Diabetic Rat

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Introduction

People with diabetes mellitus are spread all over the world. Currently, it is estimated that nearly 425 million (8.8% of the world population), 20 - 79 years old, most (79%) are in low and middle income countries. The estimated number of people with diabetes will increase in 2045 to 625 million [1]. Along with these results the number of macro and microvascular complications is also expected to increase. About 40% of diabetics experience proteinuria in the form of microalbuminuria which is a sign of the progression of chronic kidney disease. Without special intervention 20-40% of patients with microalbuminuria will develop overtnepropathy and around 20% after 20 years theonsetof overt nepropathy will become CKD (Chronic kidney disease) [2].

Albuminuria in hyperglycemia or diabetes occurs from leakage of glomerular filtration barrier systems through the mechanism of activation of pathway polyol and hexosamine, increased advance glycation end-product (AGE), activation of protein kinase C (PKC) which will cause chronic low-grade inflammation and increased extracellular matrix by cell mesangial glomerulus and podocytes injury, resulting in glomerular hyper filtration, and increased vascular permeability [3]. Other circumstances causing albuminuria are insulin resistance, obese, hypertension, and dyslipidemia, alone or together and synergistically can cause kidney glomerular damage [4, 5].

Exercise is one of the cornerstones of controlling blood glucose in diabetes but can encourage proteinuria [6, 7]. Mild and moderate intensity exercise in people with diabetes mellitus will reduce albuminuria levels, but the mechanism is still unclear. This improvement is likely because mild to moderateintensity exercise in people with diabetes mellitus inhibits the progression of diabetic nephrophaty without affecting renal blood flow such as the absence of the ischemic process. These improvements are characterized by decreased albumin excretion that is parallel with the control of metabolic parameters, decreased flammation and oxidative stress, and improved renal glomerular morphological structure [8]. Allegedly, exercise in diabetes affects changes in renal hemodynamics and changes in negative pressure on glomerular capillary walls, and impacts maintenance of theglomerular filtration barriersystem [9, 10].

Albuminuria occurs due to a disruption of the glomerular filtration barrier system which has three components in the form of the glomerular endothelial cells (GEC) with fenestra, glomerular basement membrane (GBM), and podocytes, which form a selective filter. Podocytes injury as part of the glomerular filtration barrier plays an important role in the pathogenesis of albuminuria [11]. Normal individuals usually only release a small amount of protein in urine, an increase in albumin excretion (> 30 mg) a day is considered a marker of kidney damage [12]. Broadly speaking, the mechanism of albuminuria is due to glomerular disruption, where changes in glomerular permeability occur along with increased filtration of normal plasma proteins and impaired reabsorption of proximal tubular epithelial cells [13].

Persistent albuminuria is one of the high risk factors for cardiovascular disease in diabetes mellitus [14, 15]. Albuminuria can be controlled and inhibited by pharmacological therapy angiotensin-converting enzyme inhibitors (ACEI) or angiotensin II receptor blockers (ARBs) [13].

Currently, examining the ratio of urine creatinine albumin levels is recommended as a marker of damage to theglomerular filtration barrier system [12, 16]. Indeed, before the occurrence of albuminuria is preceded by podosituria as result of damage to glomerular podocytes which are one component of the glomerular filtration barrier [17]. Proteins which create podocyte such as nephrine, Synaptopodine, podocalyxin (PCX) and podocin in urine can be used as a marker for damage or podocytes injury. PCX as one of the proteins that create podocyte can be used as an initial marker of podocyte damage detected in the urine [11, 18]. There is no research that measures urine PCX levels as a marker of damage to podocytes as early detection before albuminuria after exercise in diabetic rats, For this reason, we conducted this study with the aim of looking at the impact of exercise on the damage to the glomerular filtration barrier system by measuring albumin and PCX levels in urine.

Material and Methods

24 male Wistar rats, 6-8 weeks and 150-300 gram weight, adaptation for 7 days by standard diet and placed one rat in a cage. Room temperature 22-25 0C, and in light for 12 hours and 12 hours in dark. Rats were divided into four groups: normal sedentary, normal exercise, diabetes sedentary, and diabetes exercise. Diabetes in rats was induced by giving a high-fat diet for two weeks, then injection of streptozotosine (STZ) 30 mg / kg (0.1 M citrate buffer, pH 4.5) intraperitoneal (IP) every week, for 2 weeks [19]. Blood glucose

levels were checked fourweeks after the last STZ injection and stated as diabetes if the fasting blood glucose >140 mg/dl or random blood glucose > 200 mg/dl [20].

Sedentary treatment, rats remain left in the cage whileexercise treatment using a special tool in the form of a treadmill rat (rodent-treadmill) produced by IDEAS Industry of Electronic and Software Bandung Indonesia, consisting of a transparent acrylic 2 line display chamber, drive motor, manual tilt angle regulator 0 - 450, speed controller 0 - 50 meters / minute, time processing 0-999 minutes and electric shock grid area to provide electric shock therefore the rats were not still. Six days a week for 10 weeks (chronic) and moderate intensity, beginning with a speed of 10 meters per minute for 10 minutes per day. The speed and duration of exercise were increased gradually every 2 weeks until it reached the length of exercise 1 hour per day with a speed of 27 meters per minute [21].

Using metabolic cage, urine was collected 24 hours before (pre) and after (post) sedentary and exercise treatment. Levels of albumin and podocalyxin (PCX) in 24-hour urine were measured by ELISA (Enzyme Linked Immunosorbent Assay) method (ELISA kit QAYEE-BIOF or Life Science). HOMA-IR was determined by formula, fasting serum insulin (mU/L) x fasting blood glucose (mmol/L) / 22.5 [22]. Level of blood glucose was measured by fluorometric method, (Glucose Assay Kit, Colorimetric/Fluorometric, ab65333), and level insulin was measured by ELISA method (ELISA kit QAYEE-BIO For Life Science).Hypothesis testing used paired t test, the data showed mean ± SD with p = <0.05 significant.

Result

Out of 24 rats during treatment and diabetes induction, three died and one was removed from the research because they did not want to eat. Thus, the number of rats included in the research and treated as many as 20 rats consisted of 5 rats in each group. Data analysis of the average value of 24-hour urine (albumin and PCX levels) and HOMA IR values of normal sedentary and exercise group are shown in table 1.

Tabel 1: Data Analysisof normal sedentary and exercise group

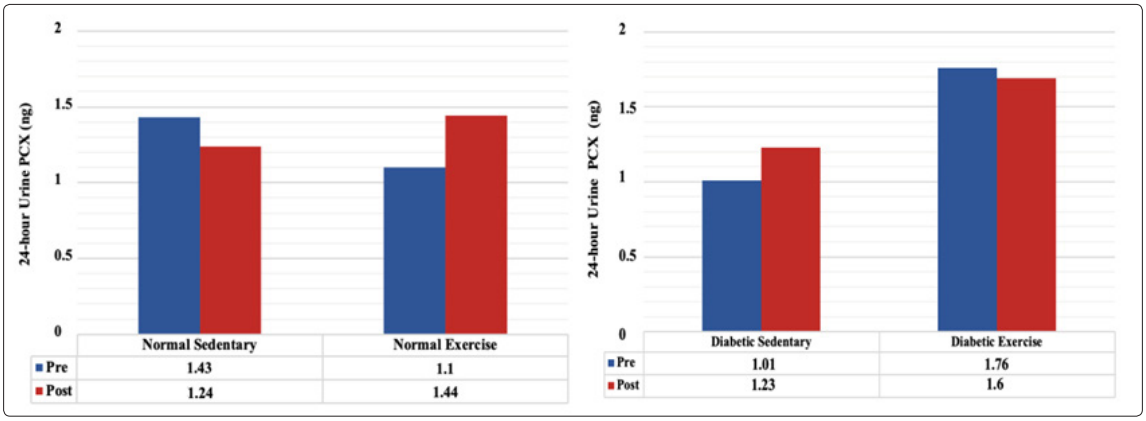
	Normal Sedentary			Normal Exercise		
	Pre (Mean ± SD)	Post (Mean ± SD)	p	Pre (Mean ± SD)	Post (Mean ± SD)	p
- 24-hour urine PCX (ng)	1.43 ± 0.03	1.24 ± 0.37	0.42	1.10 ± 0.14	1.44 ± 0.81	0.02
24-hour urine albumin (mg)	0.82 ± 0.12	1.00 ± 0.12	0.49	0.59 ± 0.09	0.89 ± 0.08	0.04
- HOMA-IR	0.56 ± 0.02	0.38 ± 0.07	0.04	0.70 ± 0.14	0.61 ± 0.06	0.59

Data analysis of the average value of 24-hour urine (albumin and PCX levels) and HOMA-IR values of diabetic sedentary and exercise group are shown in table 2.

Table 2: Data Analysis of diabetic sedentary and exercise group

	Normal Sedentary			Normal Exercise		
	Pre (Mean ± SD)	Post (Mean ± SD)	p	Pre (Mean ± SD)	Post (Mean ± SD)	p
- 24-hour urine PCX (ng)	1.01 ± 0.13	1.23 ± 0.52	0.47	1.31 ± 0.40	1.06 ± 0.39	0.32
24-hour urine albumin (mg)	0.69 ± 0.10	0.88 ± 0.29	0.27	1.76 ± 0.52	1..69 ± 0.51	0.71
- HOMA-IR	0.86 ± 0.40	1.03 ± 0.31	0.45	1.04 ± 0.34	0.98 ± 0.43	0.84

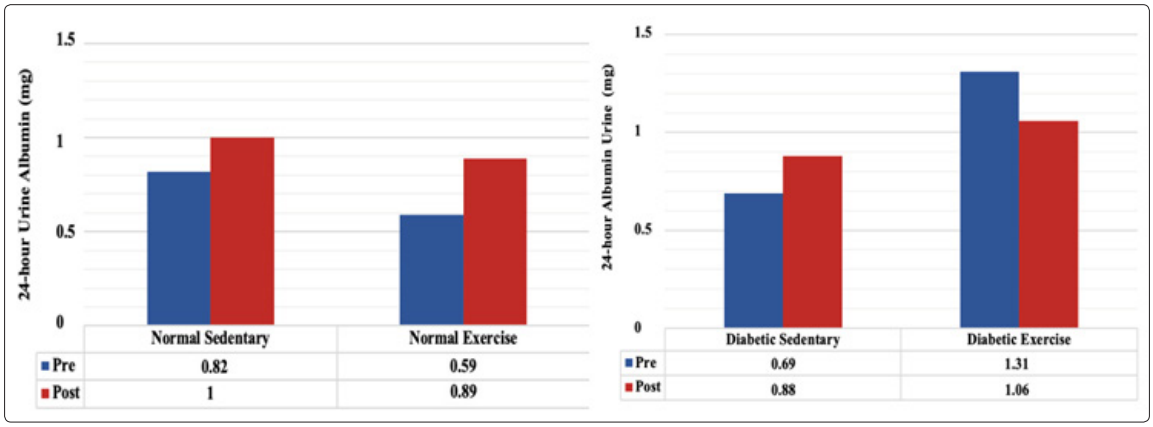
PCX levels (ng) before (pre) and after (post) treatment in normal sedentary group decreased 1.43 ± 0.03 vs 1.26 ± 0.37 (p = 0.42), while in normal exercise group increased 1.10 ± 0.14 vs 1.44 ± 0.81 (p = 0.20), in diabetic sedentary group increased 1.01 ± 0.13 vs 1.23 ± 0.52 (p = 0.47), while in diabetic exercise group decreased 1.76 ± 0.52 vs 1.69 ± 0.51 (p = 0.71). (Graph 1 and 2)



Graph 1: Comparison of normal rats24-hour urine PCX levels

Graph 2: Comparison of diabetic rats 24-hour urine PCX levels

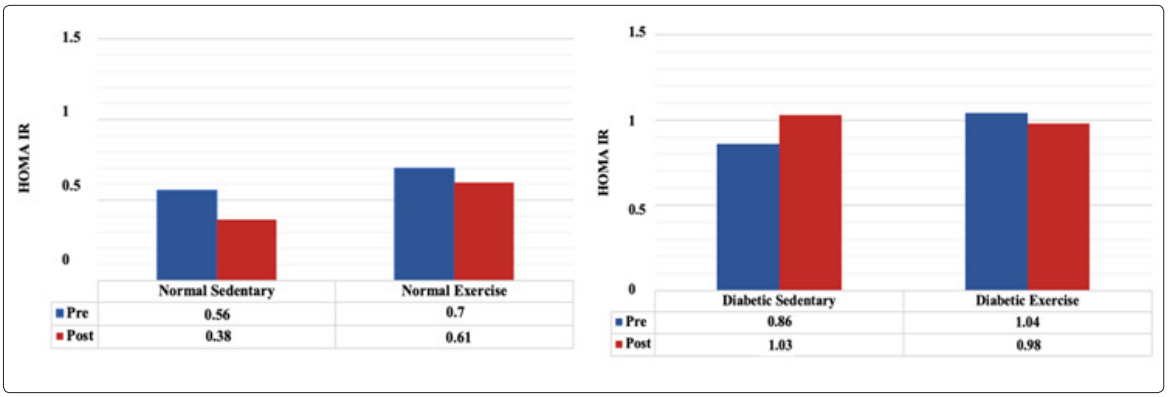
Albumin levels (mg) before (pre) and after (post) treatment in normal sedentary group increased 0.82 ± 0.12 vs 1.00 ± 0.12 (p = 0.49), while in normal exercise group increased 0.59 ± 0.09 vs 0.89 ± 0.08 (p = 0.04), in diabetic sedentary group increased 0.69 ± 0.10 vs 0.88 ± 0.29 (p = 0.27), while in diabetic exercise group decreased 1.31 ± 0.40 vs 1.06 ± 0.39 (p = 0.32). (Graph 3 and 4)



Graph 3: Comparison of normal rat's 24-hour urine albumin levels

Graph 4: Comparison of diabetic rat's 24-houruri ne albumin levels

HOMA-IR calculation before and after treatment in normal sedentary decreased0.56 ± 0.02 vs 0.38 ± 0.07 (p = 0.04) and while in normal exercise group decreased 0.70 ± 0.14 vs 0.61 ± 0.06 (p = 0.59), in diabetic sedentary group increased 0.86 ± 0.40 vs 1.03 ± 0.31 (p = 0.45), while in diabetic exercise group decreased 1.04 ± 0.34 vs 0.98 ± 0.43 (p = 0.84). (Graph 5 and 6)



Graph 5: Comparison of normal rats HOMA-IR.

Graph 6: Comparison of diabetic rats HOMA-IR.

Discussion

The albumin level in the diabetic sedentary rats group had a tendency to increase after sedentary treatment, as well as the urine PCX level with a tendency to increase in the group of diabetic sedentary rats after sedentary treatment equivalent to the tendency of increasing HOMA-IR. While the albumin level in the diabetic exercise group occurred a tendency to decrease after the exercise treatment, as well as the urine PCX level there was a decrease in the group of diabetic exercise rats after the exercise treatment, it was equivalent to the decrease tendency of HOMA-IR.

From all available data, it can be concluded that exercise appears more influential in the group of diabetic rats compared to normal rats, characterized by improvements in theglomerular filtrationbarrier system of diabetic rats after treatment which was measured in the presence of PCX and albumin in urine tend to decrease together with the improvement of metabolic parameters measured by HOMA-IR values. Decreasing blood glucose levels and blood insulin, which are components of the HOMA-IR value, will improve the level of inflammation and oxidative stress, followed by the improvement of the renal glomerular morphological structure, resulting in changes in renal hemodynamic and negative pressure changes in the glomerular capillary wall [8].

In contrast to the normal exercise group, proteinuria occurs due to leakage of the glomerular filtration barrier system due to the impact of theoretical exercise causing decreased blood flow to the kidney followed by constriction of afferent arteiol and glomerular efferent due to sympathetic nerve activity, increased levels of adrenaline / non adrenaline and auto regulation of kidney blood flow disruption which increases glomerular permeability and affects the occurrence of proteinuria. Proteinuria that occurs is temporary and usually returns to normal for 24 - 48 hours after exercise [3].

Thus, it can be suggested that chronic exercise with moderate intensity can reduce the progressive damage to the glomerular filtration barrier system in diabetics. Selecting the intensity and duration of selective exercise is needed to avoid damage to the glomerular filtration

system barrier in both normal individuals and individuals who have disease with a tendency to kidney complications such as diabetes.

As a result of the correlation between PCX and albumin in the urine, the presence of PCX in urine can also be used as an early detection of the occurrence of diabetic nepropathy before albuminuria in diabetes. Although podosituria can be used as an early marker of damage to the glomerular filtration barrier, urine albumin examination is still a recommended examination [23, 24]. Especially, examination of urine albumin creatinine ratio, besides affordable and easy in collecting random temporary urine as a sample also illustrates the extent to which albumin is excreted in the urine, compared to urine PCX examination that requires a 24-hour urine collection.

Conclusion

Moderat intensity exercise in diabetic rats has more influence on improving the value of urine albumin, urine PCX and HOMA-IR than normal rats.Moderat intensityexercise tends to decrease levels of albuminuria and PCX, together with the tendency to improve insulin resistance and other metabolic parameters in diabetic rats.

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