

*Original article*

# Impact of Exercise Training on Dipeptidyl Peptidase 4 and Insulin-Like Growth Factor Binding Protein 1 in Patients with Coronary Artery Disease: Relationship to Nitric Oxide Response

Dejan Simonović<sup>1</sup>, Marina Deljanin-Ilić<sup>1,2</sup>, Gordana Kocić<sup>3</sup>, Gordana Lazarević<sup>4</sup>, Branka Đorđević<sup>3</sup>, Sandra Šarić<sup>1</sup>, Sanja Stojanović<sup>1</sup>, Stevan Ilić<sup>5</sup>

<sup>1</sup>*Institute of Cardiology „Niška Banja”, Niška Banja, Serbia*

<sup>2</sup>*University of Niš, Faculty of Medicine, Niš, Serbia*

<sup>3</sup>*University of Niš, Faculty of Medicine, Institute of Biochemistry, Niš, Serbia*

<sup>4</sup>*Clinic for Cardiovascular Diseases, Clinical Center Niš, Niš, Serbia*

<sup>5</sup>*Cardiointernal Specialist Ordination Niš, Niš, Serbia*

## SUMMARY

Dipeptidyl peptidase 4 (DPP4) is a proteolytic enzyme, involved in a wide range of different cellular functions and expressed on the surface of most cell types, including the endothelium. The circulating level of insulin-like growth factor (IGF) binding protein 1 (BP1) is associated with insulin resistance and diabetes, but it also affects the prognosis and mortality of CV diseases.

The aim of the study was to evaluate the effects of exercise training on DPP4 and circulating IGF BP1, and to assess their relationship to nitric oxide (NOx), a circulating marker of endothelial function, in patients with stable coronary artery disease (CAD).

An overall of 48 subjects was involved in the study, including 28 patients with stable CAD (CAD group, 59.2 ± 8.2 years) and 20 healthy controls (C group, 57.1 ± 8.2 years). At baseline, DPP4, IGF BP1 and NOx, as well as an exercise test, were performed in both groups. After the initial study, CAD group underwent a supervised three-week exercise training at a residential center, and after that period DPP4, IGF BP1, NOx and exercise tolerance were determined again.

At baseline, both DPP4 and IGF BP1 were significantly ( $p = 0.023$  and  $p = 0.021$ ) higher in CAD group, compared to the C group. Since both DPP4 and IGF BP1 significantly decreased in CAD group ( $p = 0.002$  and  $p = 0.026$ , respectively) after three weeks of exercise, there were no significant differences between CAD and C groups in DPP4 and IGF BP1 at the end of the study. NOx was significantly lower in CAD group at baseline compared to the C group ( $p = 0.032$ ). Since it significantly increased in CAD group ( $p = 0.028$ ), there were no significant differences between groups after three weeks of exercise. Exercise capacity was significantly lower

in CAD group at baseline compared to the C group ( $p < 0.001$ ). However, it significantly increased in CAD group during the study ( $p < 0.001$ ) and therefore, at the end of the study there were not registered any significant differences between CAD and C group. The DPP4 decrease positively correlated with IGF BP1 decrease ( $r = 0.920$ ,  $p < 0.001$ ), NOx increase ( $r = 0.965$ ,  $p < 0.001$ ) and exercise capacity increase ( $r = 0.818$ ,  $p < 0.001$ ); IGF BP1 decrease significantly correlated with NOx increase ( $r = 0.890$ ,  $p < 0.001$ ) and exercise capacity increase ( $r = 0.878$ ,  $p < 0.001$ ), and NOx increase significantly correlated with exercise capacity increase ( $r = 0.827$ ,  $p < 0.001$ ).

Regular exercise significantly improves exercise tolerance and endothelial function in stable CAD patients, since it significantly reduces DPP4 and IGF BP1 and increases NOx after three weeks of supervised exercise.

**Key words:** exercise, coronary artery disease, dipeptidyl peptidase 4, insulin-like growth factor binding protein 1, nitric oxide

Corresponding author:

**Dejan Simonović**

E-mail: drdejan76@gmail.com

## INTRODUCTION

Arterial blood pressure is regulated by an adequate endothelial function. In addition, endothelium is responsible for human homeostasis, distribution of nutrients and hormones, as well as providing a smooth surface that modulates coagulation, fibrinolysis and inflammation. Endothelial dysfunction is defined as a pathological state. Low level systemic inflammation of endothelium is responsible for the expression of pro-vasoconstrictive mediators but also for abnormal plasma clot formations. It was demonstrated to be a major underlying mechanism for coronary artery disease (CAD), and can be a consequence of various pathophysiological mechanisms (1). Endothelial vasomotor reactivity is altered and impaired in CAD patients, but it has been shown that exercise training can reverse impaired vasomotor responsiveness. Exercise training improves endothelial vasomotor function of coronary vasculature (2). Moreover, there is evidence that the reduction of physiological distress can improve long-term cardiac prognosis (3). Cardiac rehabilitation (CR) is very important in the treatment of CAD patients, as it improves depression, functional capacity, anxiety, quality of life, and long-term prognosis (4). The beneficial effects of exercise include an improvement of endothelial function through an increase in nitric oxide (NO) release and/or inhibition of NO degradation (5, 6). NO contributes to beneficial effects in the vasculature. It has been shown that vasodilatation and fibrinolysis are enhanced. Atherothrombosis can be suppressed by inhibiting platelet aggregation and leukocyte adhesion. Also, there is smooth muscle cell proliferation inhibition (7, 8).

Dipeptidyl peptidase 4 (DPP4) is a proteolytic enzyme. It is expressed on the surface of the most cell types. It is also associated with immune regulation affecting growth factors and chemokines. DPP4 affects neuropeptides and vasoactive peptides (9), cell signal transduction and apoptosis. Glucose metabolism depends on DPP4 activity through the degradation of incretins (glucagon-like-peptide 1, GLP-1), and it is shown that DPP4 activity is elevated in diabetes mellitus (10), so nowadays it is a target for antidiabetic therapy. DPP4 can be measured in plasma as it is released from the plasma membrane via proteolytic activity (11 - 15). Vascular endothelium expresses the highest amount of DPP4, and thus contributes to soluble pool of plasma DPP4. It can be also found in the intestinal endothelium. DPP4 activity is present in many cells, such as lymphocytes and monocytes, but in many organs as well

(kidney, liver, lung, spleen) (16). It is shown that there are other pathological states with increased DPP4 activity, such as nonalcoholic fatty liver and CV diseases. When cardiovascular diseases are concerned, the cardioprotective role of GLP-1 analogues and DPP4 inhibition is well-known in acute myocardial infarction (MI) as well. It is interesting what happens with DPP4 activity in myocardium during ischemia, since it is present in the endothelium of myocardial vasculature. During acute ischemia, DPP4 expression and activity decreases within the infarction area, but microvascular tissue factor expression is increased. After acute MI, there is a dysfunction of myocardial microvasculature and remodeling of left ventricle. No-reflow phenomenon and loss of DPP4 may contribute to this dysfunction. A larger infarct size, in general, correlates with poor clinical outcome.

GLP1 has multiple physiologic roles in the body. It stimulates pancreas to release insulin and maintain glucose hemostatic level without any hyperglycemia. In addition, GLP-1 is rapidly degraded by the enzyme DPP-4 and it is no longer available in active form (17). In DM patients, the GLP1 level is reduced and the DPP4 level is increased and this in turn will cause the glucose hemostatic imbalance. At present, inhibiting DPP4 and increasing GLP1 levels are among the most important methods of controlling and managing type 2DM (18). The results show that in type 2DM patients, frequent muscle contractions, in the absence of insulin, will ease the absorption and consumption of glucose into the muscle cells. According to researchers, exercise activities have the following three influences on the body: an increase in transporting of glucose to the muscles, an increase in insulin performance on the involved muscles and positive adjustment in message paths stimulated by the insulin. In addition, physical activity acts as a pseudo insulin activity by reducing the intercellular fat reservoir, increasing fat oxidation and protein expression, which will lead to an increase in muscle capacity and regulation of the amount of glucose in circulation (19).

Insulin-like growth factor binding protein 1 (IGF BP1) is a secretory protein and belongs to a family of proteins that bind IGFs. It is produced mainly in the liver and kidney; it is also present in other tissues, but expression is smaller (20) and has influence on metabolism through IGF dependent and independent mechanisms. IGF BP-1 correlates with insulin sensitivity in older subjects, with glucose intolerance and DM. It is known that IGF BP1 expression is very low in normal vessels, and circulating IGF BP1 is associated with insulin resistance and DM (21, 22); IGF BP-1 has been

further proposed as a marker of insulin resistance. Some studies have shown that IGF BP1 can affect prognosis and mortality from CV diseases, independently of diabetes (23 - 25), which can be related to direct regulation of vascular endothelium and smooth muscle cells. Since, IGF BP1 is increased in atherosclerotic plaques (26), it is assumed that it can contribute to the plaque stability through its local expression. Some studies (27 - 28) have also shown that increased IGF BP1 levels can protect against atherosclerosis, and on the other hand, low levels of IGF BP1 could be a marker of increased CAD risk. IGF BP-1 can be used for CV disease risk assessment, since it was shown that low IGF BP-1 concentrations are associated with increased prevalence of CV diseases and metabolic syndrome.

There is a connection between insulin-like growth factor I (IGF-I) and IGF BP-1. IGF BP-1 can inhibit anabolic effects of IGF-I, while catabolic state increases IGF BP-1. Low levels of IGF-I can contribute to development of CV diseases and congestive heart failure (CHF) through its effect on myocardial contractility, resistance to ischemia and myocyte aging. In some studies, IGF-I is increased in CHF patients; an elevated level of IGF-I is also linked to increased mortality after MI in elderly patients (29 - 33). Many chronic diseases are caused by physical inactivity, and it is assumed that physical activity is associated with IGF-1 and IGF BP1 levels. Among established CV risk factor, sedentary lifestyle is well positioned, so well designed exercise training program, in previously sedentary adults, has the potential to reduce the CV risk.

The aim of the present study was to evaluate the effects of structured exercise training on DPP4 and circulating IGF BP1, as well as to assess its relationship to NOx, a circulating marker of endothelial function, in patients with stable CAD.

## PATIENTS AND METHODS

### Patients

The present study was carried out in 48 subjects, including 28 patients with stable CAD, admitted for CR (CAD group,  $59.2 \pm 8.2$  years), and 20 healthy controls (C group,  $57.1 \pm 8.2$  years).

### Methods

#### Baseline assessments

A detailed medical evaluation was performed at baseline, recording the underlying risk factors, comorbi-

ditities and previous medical history. According to previously diagnosed comorbidities, concomitant treatment may have included regular use of cardioprotective (antiplatelets,  $\beta$ -blockers, angiotensin-converting enzyme inhibitors and/or angiotensin-receptors blockers), (oral hypoglycemic drugs) and lipid-lowering drugs (statins).

The examined biochemical markers, including DPP4, IGF BP-1, and NOx were determined at baseline, as well as exercise test in order to evaluate exercise capacity. After the initial study, CAD group underwent a supervised three-week aerobic exercise training at a residential center and after that period, DPP4, IGF BP-1, and NOx and exercise capacity were determined again. Venous blood samples for biochemical analyses were taken from the antecubital vein after an overnight fast. An exercise test was performed, using standardized protocol, on electronically braked bicycle (KETTLER ergocycle, Germany).

Dipeptidyl peptidase IV (DPP4) was detected by using the DPP4 Activity Assay Kit (Fluorometric) (ab204722) purchased from Abcam. The assay is based on the detection of released quenched fluorescent group AMC (7-Amino-4-Methyl Coumarin) from substrate via the action of DPP-4. It was detected at Ex/Em = 360/460 nm. The DPP4 detection minimum activity is 3  $\mu$ U per well.

The concentration of human insulin-like growth factor (IGF)-binding protein-1 was detected by using Human IGFBP-1 DuoSet ELISA (DY871) assay, purchased from R&D Systems (Minneapolis, USA). The range of detection via sandwich ELISA assay was from 31-2,000 g/mL.

NOx production was assessed through the changes of stable end-products of NOx and determined using the Saville-Griess method (34 - 36).

### Statistical analysis

Continuous variables are expressed as mean  $\pm$  standard deviation (SD); categorical variables are presented as counts and percentages. The distribution of variables was checked by Shapiro-Wilk and Kolmogorov-Smirnov tests. Between-group characteristics were compared by independent-sample t-test, Mann-Whitney rank sum test or Chi Square test, as appropriate; and for in-group repeated measures, paired-sample t-test and Wilcoxon signed-rank test were used. Pearson correlation was used to explore the strength of the relationship between two continuous variables. The correlation coefficient (Pearson  $r$ ) provided an indication of the linear relationship between variables. The strength was defined through Pearson's  $r$

coefficient ((.90 to 1.00 (-.90 to -1.00) - very high positive (negative) correlation; .70 to .90 (-.70 to -.90) - high (strong) positive (negative) correlation; .50 to .70 (-.50 to -.70) - moderate positive (negative) correlation; .30 to .50 (-.30 to -.50) - low positive (negative) correlation; .00 to .30 (.00 to -.30) and negligible correlation)). A P-value < 0.05 was considered statistically significant. Statistical analyses were performed using the SPSS package version 21.0 (Chicago, L, USA).

## RESULTS

The main characteristics of all enrolled participants are shown in Tables 1 - 2.

At baseline, both DPP4 and IGF BP1 were significantly ( $p = 0.023$  and  $p = 0.021$ ) higher in CAD group compared to the C group (Tables 3 - 4). Since both DPP4 and IGF BP1 significantly decreased in CAD group ( $p = 0.002$  and  $p = 0.026$  respectively) after three weeks of exercise, there were no significant differences between CAD and C groups in DPP4 and

IGF BP1 at the end of the study (Tables 3-4).

NOx was significantly lower in CAD group at baseline compared to the C group ( $p = 0.032$ ). Since it significantly increased in CAD group after three weeks of exercise ( $p = 0.028$ ), there were no significant differences between groups at the end of the study (Table 5).

Exercise capacity was significantly lower in CAD group at baseline, compared to the C group ( $p < 0.001$ ). However, it significantly increased in CAD group during the study, ( $p < 0.001$ ) and therefore, at the end of the study there were not registered any significant differences between CAD and C groups (Table 5).

The DPP4 decrease positively correlated with IGF BP1 decrease ( $r = 0.920$ ,  $p < 0.001$ ), NOx increase ( $r = 0.965$ ,  $p < 0.001$ ) and exercise capacity increase ( $r = 0.818$ ,  $p < 0.001$ ) (Graph 1). IGF BP1 decrease significantly correlated with NOx increase ( $r = 0.890$ ,  $p < 0.001$ ) and exercise capacity increase ( $r = 0.878$ ,  $p < 0.001$ ) (Graph 1). NOx increase significantly correlated with exercise capacity increase ( $r = 0.827$ ,  $p < 0.001$ ) (Graph 1).

**Table 1. Baseline characteristics in CAD and C group**

|                                             | CAD group(N = 28) | C group(N = 20)  | P value           |
|---------------------------------------------|-------------------|------------------|-------------------|
| <b>Demographics</b>                         |                   |                  |                   |
| Male gender, n (%)                          | 21 (75.0%)        | 12 (65.0%)       | 0.430             |
| Age (years), mean ( $\pm$ SD)               | 59.2 $\pm$ 8.2    | 57.1 $\pm$ 8.2   | 0.504             |
| BMI (Kg/m <sup>2</sup> ), mean ( $\pm$ SD)  | 26.4 $\pm$ 3.1    | 24.7 $\pm$ 2.7   | 0.058             |
| <b>Physical examination</b>                 |                   |                  |                   |
| Systolic BP (mmHg), mean ( $\pm$ SD)        | 116.8 $\pm$ 12.2  | 124.1 $\pm$ 11.7 | <b>0.045</b>      |
| Diastolic BP (mmHg), mean ( $\pm$ SD)       | 70.2 $\pm$ 7.4    | 78.2 $\pm$ 6.8   | <b>&lt; 0.001</b> |
| Heart rate (bpm), mean ( $\pm$ SD)          | 75.8 $\pm$ 15.1   | 77.1 $\pm$ 10.3  | 0.734             |
| <b>Laboratory</b>                           |                   |                  |                   |
| Haemoglobin (g/L), mean ( $\pm$ SD)         | 151.4 $\pm$ 8.4   | 147.8 $\pm$ 11.5 | 0.226             |
| Hematocrit (L/L), mean ( $\pm$ SD)          | 0.42 $\pm$ 0.02   | 0.42 $\pm$ 0.03  | 0.962             |
| Potassium (mEq/L), mean ( $\pm$ SD)         | 4.9 $\pm$ 0.4     | 4.5 $\pm$ 0.6    | <b>0.003</b>      |
| Creatinine ( $\mu$ mol/L), mean ( $\pm$ SD) | 98.5 $\pm$ 16.2   | 88.5 $\pm$ 15.9  | <b>0.039</b>      |
| Cholesterol (mmol/L), mean ( $\pm$ SD)      | 4.4 $\pm$ 0.9     | 5.4 $\pm$ 0.9    | <b>&lt; 0.001</b> |
| HDL cholesterol (mmol/L), mean ( $\pm$ SD)  | 1.1 $\pm$ 0.2     | 1.5 $\pm$ 0.3    | <b>&lt; 0.001</b> |
| LDL cholesterol (mmol/L), mean ( $\pm$ SD)  | 2.6 $\pm$ 0.7     | 3.9 $\pm$ 0.8    | <b>&lt; 0.001</b> |
| Triglycerides (mmol/L), mean ( $\pm$ SD)    | 1.7 $\pm$ 0.6     | 1.7 $\pm$ 1.1    | 0.988             |
| Glucose (mmol/L), mean ( $\pm$ SD)          | 6.4 $\pm$ 1.2     | 5.4 $\pm$ 0.5    | <b>&lt; 0.001</b> |

CAD group, coronary artery disease group; C group, healthy controls; BMI, body mass index; SD, standard deviation; BP, blood pressure; HDL cholesterol, high-density lipoprotein cholesterol; LDL cholesterol, low-density cholesterol.

Table 2. Baseline characteristics in CAD group

|                               | CAD group(N=28) |
|-------------------------------|-----------------|
| <b>Medical history, n (%)</b> |                 |
| Hypertension                  | 23 (82.1)       |
| Diabetes                      | 5 (17.8)        |
| Coronary artery disease       | 28 (100)        |
| Dyslipidemia                  | 26 (92.8)       |
| Smoking (current, previous)   | 20 (71.4)       |
| <b>Therapy, n (%)</b>         |                 |
| Beta-blocker                  | 27 (96.4)       |
| ACE-i or ARB                  | 28 (100)        |
| Ca blocker                    | 4 (14.2)        |
| Aspirin                       | 28 (100)        |
| Clopidogrel                   | 19 (67.8)       |
| Statin                        | 28 (100)        |
| Nitrates                      | 6 (21.4)        |
| Diuretics                     | 11 (39.2)       |
| Aldosterone antagonist        | 3 (10.7)        |

CAD group, coronary artery disease group; ACE-i, angiotensin converting enzyme inhibitor; ARB, angiotensin II receptor blocker; Ca blocker, calcium blocker

Table 3. DPP4 in CAD and C groups at baseline and at the end of the study

|                  | Before DPP4 (ng/mL) | After DPP4 (ng/mL) | P       |
|------------------|---------------------|--------------------|---------|
| <b>CAD group</b> | 751,42 ± 250,86     | 617,87 ± 216,14    | = 0,002 |
| <b>C group</b>   | 597,65 ± 155,57     | 597,65 ± 155,57    | /       |
| <b>P</b>         | = 0,023             | ns                 |         |

DPP4, Dipeptidyl peptidase 4; CAD group, Coronary artery disease group; C group, Control group

Table 4. IGF BP1 in CAD and C groups at baseline and at the end of the study

|                  | Before<br>IGF BP1 (pg/mL) | After<br>IGF BP1 (pg/mL) | P       |
|------------------|---------------------------|--------------------------|---------|
| <b>CAD group</b> | 908,93 ± 554,88           | 745,77 ± 567,95          | = 0,026 |
| <b>C group</b>   | 523,92 ± 234,93           | 523,92 ± 234,93          | /       |
| <b>P</b>         | = 0,021                   | ns                       |         |

IGF BP1, insulin like growth factor binding protein 1; CAD group, Coronary artery disease group; C group, Control group.

Table 5. NOx in CAD and C groups at baseline and at the end of the study

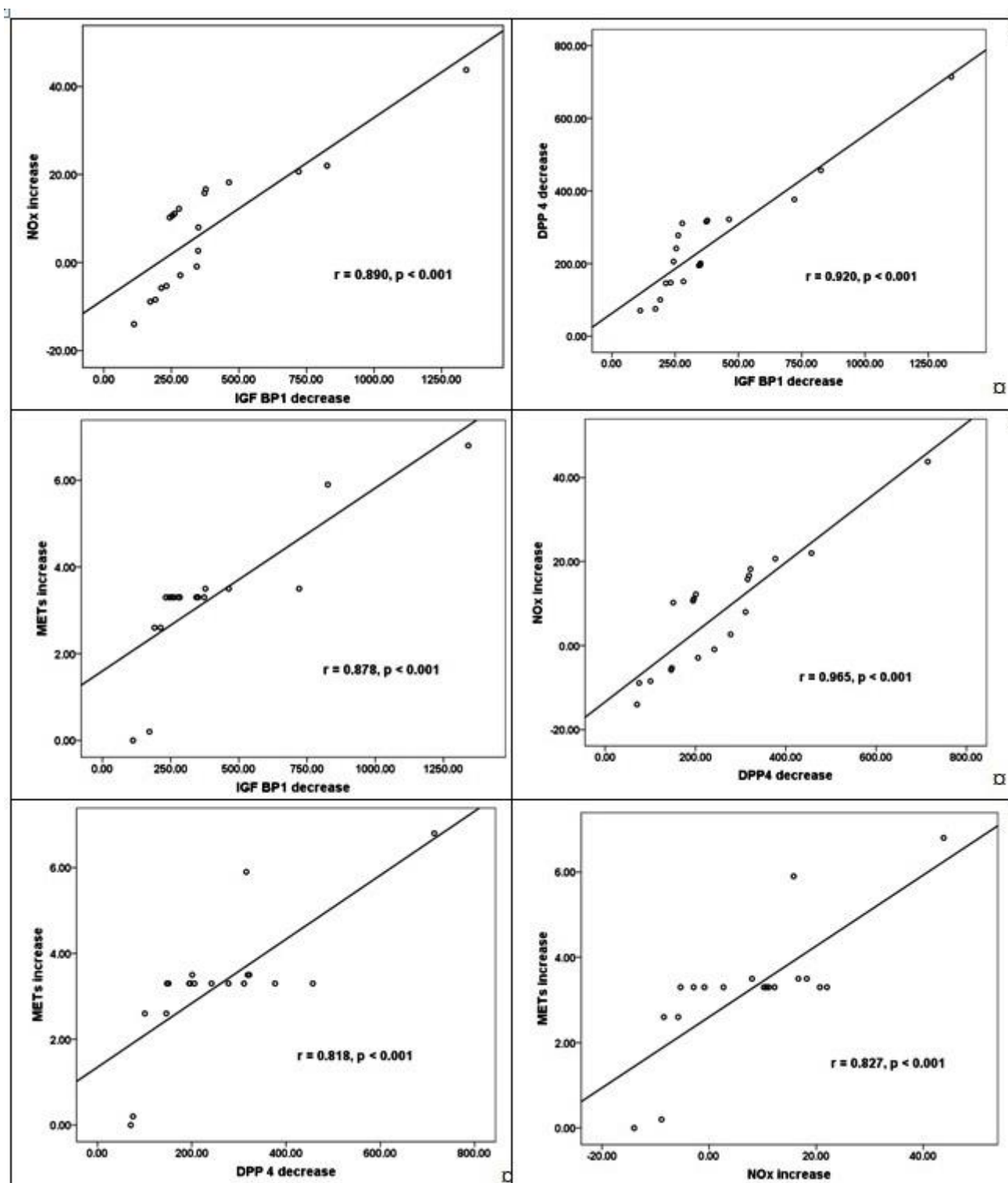
|                  | Before<br>NOx ( $\mu\text{mol/L}$ ) | After<br>NOx ( $\mu\text{mol/L}$ ) | P       |
|------------------|-------------------------------------|------------------------------------|---------|
| <b>CAD group</b> | 60,81 $\pm$ 10,93                   | 68,49 $\pm$ 8,11                   | = 0,028 |
| <b>C group</b>   | 77,28 $\pm$ 29,86                   | 77,28 $\pm$ 29,86                  | /       |
| <b>P</b>         | = 0,032                             | ns                                 |         |

NOx, nitric oxide; CAD group, Coronary artery disease group; C group, Control group.

Table 6. Exercise capacity in CAD and C groups at baseline and at the end of the study

|                  | Before<br>METs   | After<br>METs    | P       |
|------------------|------------------|------------------|---------|
| <b>CAD group</b> | 7,19 $\pm$ 2,33  | 8,92 $\pm$ 2,83  | < 0,001 |
| <b>C group</b>   | 11,08 $\pm$ 3,91 | 11,08 $\pm$ 3,91 | /       |
| <b>P</b>         | <0,001           | ns               |         |

METs, Metabolic equivalents of task; CAD group, Coronary artery disease group; C group, Control group.



Graph 1. Correlation between examined biochemical markers and exercise capacity (according to their change during rehabilitation period)

## DISCUSSION

It is shown that CR reduces CV mortality, the incidence of MI and other CV events. CR is a very complex process, and it must be multidisciplinary and well designed for each patient individually (37). The primary goal of any CV rehabilitation is to achieve optimal physical, psychological and social well-being. In addition, CR can stabilize atherosclerotic processes in all blood vessels, and after that, slow or even reverse atherosclerosis, which leads to the reduction of CV morbidity and mortality (38, 39). It is well known that primary and secondary CV prevention cannot be realized without regular physical activity, despite regular drug treatment and modification of other CV risk factors. In spite of this, regular physical activity is still below the required level. Everyday physical activity is very important because of cardiorespiratory fitness improvement and additional cardiac protection in many subjects. Sedentary behaviors must be reduced, since decreasing the total daily time of physical activity may be insufficient to prevent other CV events and decrease mortality. As noted in the existing recommended guidelines, moderate or intensive physical activities are associated with more pronounced health benefits, and also with mandatory reducing sedentary behaviors (40). Despite that, it is still unknown why regular physical activity in many CAD patients is underestimated, and CAD patients are still insufficiently engaged in physical activity. It is shown that one-third of patients with chronic CAD practice less physical activity than recommended in the current guidelines (41). In addition, it is determined that sedentary patients can be divided in two groups, those related to individual health and those related to race group, country and level of education (42). At the beginning of our study, exercise capacity was significantly lower in CAD group compared to the C group ( $p < 0.001$ ). However, it significantly increased in CAD group during the study ( $p < 0.001$ ), and therefore, there were not registered any significant differences between CAD and C groups after three weeks of exercise. Moreover, an exercise capacity increase positively correlated with DPP4 and IGF BP1 decrease ( $r = 0.818$ ,  $p < 0.001$  and  $r = 0.878$ ,  $p < 0.001$  respectively) as well as with NOx increase ( $r = 0.827$ ,  $p < 0.001$ ).

As previously demonstrated, exercise training can reduce plasma DPP-4 levels. Beneficial effects of physical training in obese subjects with metabolic syndrome might be in connection with better regulation of insulin sensitivity and fat oxidation (43). Therefore, a reduction of DPP-4, after physical training program,

might be essential in glucose and lipid metabolism regulation. Also, it is known that leptin levels decrease following exercise and weight loss. Knowing that leptin is associated with higher DPP-4 concentrations, it is reasonable to assume that DPP-4 decreases following exercise. It is already published that lifestyle modification, physical activity, weight loss, reduced dietary fat intake can reduce DPP-4 in overweight children, so the reduction of DPP-4 in our patients is related to the published results. (44). The authors, however, recognized that they could not causally determine if exercise alone reduced DPP-4, given that examined cohort lost approximately 8% body weight and suggested that the future work might be needed to test the independent effects of physical training on DPP-4 levels. As the results of our study demonstrated, DPP4 was significantly ( $p = 0.023$ ) higher in CAD group, compared to the C group at baseline. Since it significantly decreased in CAD group ( $p$ ) after three weeks of exercise, there were no significant differences between CAD and C groups in DPP4 at the end of the study. The DPP4 decrease positively correlated with IGF BP1 decrease ( $r = 0.920$ ,  $p < 0.001$ ), NOx increase ( $r = 0.965$ ,  $p < 0.001$ ) and exercise capacity increase ( $r = 0.818$ ,  $p < 0.001$ ). These results confirmed that exercise alone, without reduced dietary intake, was effective in lowering DPP4, and therefore might be proposed as one of the most important non-pharmacological tools for reducing DPP4, improving insulin sensitivity and reducing cardiometabolic risk. Since the decrease in DPP4 followed a significant increase in NOx, a marker of endothelial function, the decrease of DPP4 might be suggested as a potential mechanism for exercise in improving insulin sensitivity and cardiometabolic risk through its effects on NOx.

The circulating level of IGF BP1 is associated with insulin resistance, DM (21 - 22), adverse prognosis and CV mortality in patients without DM and glucose intolerance (23 - 25), and the low level of IGF BP1 can be considered a marker of cardiometabolic risk (27 - 28). Physical inactivity is associated with IGF-1 and IGF BP1 levels. Also, physical inactivity is one of the main causes of many chronic diseases, including CV diseases. As we know, sedentary lifestyle and physical inactivity are well known and established CV and metabolic risk factors, so physical training programs in previously sedentary adults has the potential to reduce this risk. Studies confirmed that total serum IGF-1 concentrations increase with endurance and strength exercise. In the case of a local increase (ex. muscle), IGF-1 constantly increases after exercise, but it depends on the length and intensity

of exercise. However, the published results are undetermined for peripheral IGF-1, because endurance or resistance training can increase or decrease it, or IGF-1 can be unchanged (45) and therefore the IGF BP 1 levels associated with exercise were different as well. Effects of IGF BP1 on IGF-1 bioactivity can be different, from inhibition to stimulation. But, in some cell types, IGF BP1 can affect cellular processes independently of IGFs (20). It is shown that IGF BP1 concentrations can correlate with insulin sensitivity (46 - 47); also, low levels of IGF BP1 concentrations can predict the development of glucose intolerance and DM (48 - 49). There is an inverse relationship between IGF BP1 concentrations and CV risk. In addition, carotid artery intima thickness and macrovascular diseases are in inverse relationship to IGF BP1. Some authors have published that expression of human IGF BP1 in mice was associated with increased vascular NO production and release, causing lower blood pressure (20). In the same study, on mouse models, elevation of IGF BP1 levels improved insulin sensitivity and glucose tolerance. Also, elevation of IGF BP1 can lower blood pressure through endothelial NO production, and it is published that atherosclerosis is less likely with low levels between IGF BP1 (27). However, the results are again unclear when it comes to relationship of IGF BP1 concentrations and CV risk. Higher levels of IGF BP1 can predict the development of heart failure after MI (50 - 51). Therefore, further studies

on IGF BP1 and its molecular effects are required. As for the results of the present study, at baseline, exercise capacity was significantly lower in CAD group compared to C group ( $p < 0.001$ ) and was followed with significantly higher IGF BP1 in CAD group compared to C group ( $p = 0.021$ ). After three weeks of structured, regular exercise training, a significant decrease in IGF BP1 in CAD group ( $p = 0.026$ ) was followed with significant increase in exercise capacity ( $p < 0.001$ ). A decrease in IGF BP1 positively correlated not only with exercise capacity increase ( $r = 0.878$ ,  $p < 0.001$ ), but also with DPP4 decrease ( $r = 0.920$ ,  $p < 0.001$ ) and NOx increase ( $r = 0.890$ ,  $p < 0.001$ ).

In conclusion, regular exercise significantly improves exercise tolerance and endothelial function in stable CAD patients, since it significantly reduced DPP4 and IGF BP1 and increased NOx after three weeks of supervised exercise.

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## Conflict of interests

Authors report no conflicts of interest.

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## Uticaj fizičkog treninga na dipeptidil peptidazu 4 i insulinu sličan faktor rasta vezujući protein 1 kod bolesnika sa koronarnom bolešću: povezanost sa odgovorom azot oksida

Dejan Simonović<sup>1</sup>, Marina Deljanin-Ilić<sup>1,2</sup>, Gordana Kocić<sup>3</sup>, Gordana Lazarević<sup>4</sup>,  
Branka Đorđević<sup>3</sup>, Sandra Šarić<sup>1</sup>, Sanja Stojanović<sup>1</sup>, Stevan Ilić<sup>5</sup>

<sup>1</sup>Institut za kardiologiju „Niška Banja“, Niška Banja, Srbija

<sup>2</sup>Univerzitet u Nišu, Medicinski fakultet, Niš, Srbija

<sup>3</sup>Univerzitet u Nišu, Medicinski fakultet, Institut za biohemiju, Niš, Srbija

<sup>4</sup>Klinika za kardiovaskularne bolesti, Klinički centar Niš, Niš, Srbija

<sup>5</sup>Cardiopoint internistička specijalistička ordinacija, Niš, Srbija

### SAŽETAK

Dipeptidil peptidaza 4 (DPP4) je proteolitički enzim koji je uključen u više različitih ćelijskih funkcija i eksprimovan na površini višecelijskih tipova, uključujući i endotel. Nivo cirkulišućeg insulinusličnog faktora rasta (IGF) vezujućeg proteina 1 (BP1) povezan je sa insulinskom rezistencijom i dijabetesom, ali takođe utiče i na prognozu i mortalitet od kardiovaskularnih bolesti.

Cilj ove studije je da evaluiira efekat fizičkog treninga na DPP4 i cirkulišući IGF-BP1 i proceni njihovu povezanost sa azot oksidom (NOx) kao cirkulišućim markerom endotelne funkcije, kod bolesnika sa stabilnom koronarnom bolešću (KB).

Ukupno 48 ispitanika bilo je uključeno u istraživanje, od čega 28 bolesnika sa stabilnom KB (CAD grupa, 59,2 godine  $\pm$  8,2 godine) i 20 zdravih ispitanika (C grupa, 57,1 godina  $\pm$  8,2 godine). Na početku ispitivanja određeni su, DPP4, IGF-BP1 i NOx; urađen je i test fizičkim opterećenjem u obe grupe. Nakon inicijalnog pregleda ispitanika, CAD grupa je uključena u program tronedelnog fizičkog treninga, pod nadzorom lekara u stacionarnim uslovima, a nakon tog perioda ponovo su određivani DPP4, IGF-BP1 i NOx, i ponovo je urađen test fizičkim opterećenjem.

Na početku ispitivanja, nivoi DPP4 i IGF-BP1 bili su značajno veći u CAD grupi u odnosu na C grupu ( $p = 0,023$  i  $p = 0,021$ ). Nakon 3 nedelje fizičkog treninga nivoi DPP4 i IGF-BP1 značajno su se snizili u CAD grupi ( $p = 0,002$  i  $p = 0,026$ ), ali nije bilo značajne razlike u odnosu na vrednosti u C grupi, na kraju ispitivanja. Nivo NOx bio je značajno niži u CAD grupi u odnosu na C grupu ( $p = 0,032$ ), na početku ispitivanja. Vrednosti NOx značajno su se snizile u CAD grupi ( $p = 0,028$ ), ali nije bilo značajne razlike između grupa na kraju ispitivanja. Kapacitet za fizički trening bio je značajno niži u CAD grupi na početku ispitivanja, u odnosu na C grupu ( $p < 0,001$ ). Ipak se kapacitet za fizički trening značajno povećao u CAD grupi tokom trajanja ispitivanja ( $p < 0,001$ ), te se na kraju ispitivanja nije značajno razlikovao u odnosu na C grupu. Sniženje DPP4 pozitivno korelira sa sniženjem IGF-BP1 ( $r = 0,920$ ,  $p < 0,001$ ), povećanjem NOx ( $r = 0,965$ ,  $p < 0,001$ ) i povećanjem kapaciteta za fizički trening ( $r = 0,818$ ,  $p < 0,001$ ). Sniženje IGF-BP1 značajno korelira sa povećanjem NOx ( $r = 0,890$ ,  $p < 0,001$ ) i povećanjem kapaciteta za

fiziki trening ( $r = 0,878$ ,  $p < 0,001$ ); povećanje NOx značajno korelira sa kapacitetom za fizički trening ( $r = 0,827$ ,  $p < 0,001$ ).

Redovan fizički trening značajno poboljšava kapacitet za fizički trening i endotelnu funkciju kod bolesnika sa stabilnom koronarnom bolešću, sobzirom da značajno redukuje DPP4 i IGF-BP1 i povećava NOx 3 nedelje nakon kontrolisanog fizičkog treninga.

*Ključne reči:* fizička aktivnost, koronarna bolest, dipeptidil peptidaza 4, faktor rasta sličan insulinu, vezujući protein 1, azot oksid