Clinical Study

Myocardial Expression of PPARγ and Exercise Capacity in Patients after Coronary Artery Bypass Surgery

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Activation of PPARs may be involved in the development of heart failure (HF). We evaluated the relationship between expression of PPARγ in the myocardium during coronary artery bypass grafting (CABG) and exercise tolerance initially and during follow-up. 6-minute walking test was performed before CABG, after 1, 12, 24 months. Patients were divided into two groups (HF and non-HF) based on left ventricular ejection fraction and plasma proBNP level. After CABG, 67% of patients developed HF. The mean distance 1 month after CABG in HF was 397±85 m versus 420±93 m in non-HF. PPARγ mRNA expression was similar in both HF and non-HF groups. 6MWT distance 1 month after CABG was inversely correlated with PPARγ level only in HF group. Higher PPARγ expression was related to smaller LVEF change between 1 month and 1 year (R = 0.18, p < 0.05), especially in patients with HF. Higher initial levels of IL-6 in HF patients were correlated with longer distance in 6MWT one month after surgery and lower PPARγ expression. PPARγ expression is not related to LVEF before CABG and higher PPARγ expression in the myocardium of patients who are developing HF following CABG may have some protecting effect.

1. Introduction

Heart failure (HF) is characterized by reduced reserve of the cardiac output. Impaired functional capacity in patients with heart failure is common and results from inability to achieve sufficient oxygen and nutrients delivery and altered washout of metabolites from working muscles.

Decreased stroke volume, altered chronotropic reaction, insufficient increase of myocardial contractility, and altered left ventricular-aortic coupling constitute the major central cardiovascular abnormalities, while decreased capillary density, endothelial dysfunction, and lowered oxygen extraction create the peripheral basic pathomechanisms leading to insufficient oxygenation of skeletal muscles [1, 2]. In addition, chronic HF is related to dysfunctional metabolism of the skeletal muscles, changes in fibre composition, and progressive muscular atrophy [3, 4]. Overproduction of proinflammatory cytokines like TNF-α or IL-6, which is characteristic for chronic HF, may be responsible for altered muscular structure and function [5, 6]. These cytokines have an influence on normal physiology of the skeletal muscle cells but also affect proper function of endothelium by promoting generation of free oxygen radicals and decreasing availability of nitric oxide that altogether are responsible for insufficient vasorelaxatory function of the blood vessels [7]. The unfavorable effects of chronic inflammation in heart failure may be opposed by different endogenous mechanisms. One of these mechanisms may be related to...
the function of peroxisome proliferator-activated receptor gamma (PPARγ), which gained attention in recent decade mainly because of its metabolism-improving activities. Thiazolidinediones, the pharmacological activators of PPARγ, are used in patients with diabetes to decrease insulin resistance and improve glycemic control. Some positive effects of this therapy were also noted in relation to the cardiovascular system. Thiazolidinediones improved function of the endothelium, enhanced fatty acid oxidation in the cardiac muscle, decreased myocardial fibrosis, and diminished the risk of myocardial infarction and stroke [8]. Numerous experimental studies using rodents showed protective activity of PPAR agonists on cardiac function [9–12]. However the significance of PPAR activation is not univocal, as pioglitazone failed to provide any protective effect on the myocardium after ischemia-reperfusion in pigs [13] and rosiglitazone failed to prevent cardiac remodeling and caused increased mortality after acute infarction in rats [14]. Moreover, a meta-analysis of clinical studies showed increased risk for developing heart failure in patients with diabetes treated with PPARγ agonist pioglitazone [15]. The mechanisms leading to development of heart failure in patients treated with pioglitazone remain vague. These data were further blurred by improvement of aerobic capacity and skeletal muscle energy metabolism in patients with metabolic syndrome treated with pioglitazone [16].

In the present study we aimed to evaluate the relationship between expression of PPARγ in the myocardium, plasma levels of IL-6, and exercise tolerance in patients with ischemic heart disease undergoing coronary artery bypass grafting (CABG) before the operation and during the follow-up.

2. Methods

Patients with angiographically confirmed multivessel coronary artery disease, qualified to the CABG, were recruited to the study. Only subjects with normal blood levels of NT-proBNP and preserved cardiac function in resting echocardiographic examination and without clinical manifestations of heart failure or diabetes mellitus were included.

The clinical examination, biochemical tests, resting echocardiography, and six-minute walk test (6MWT) were performed before CABG and at 3-time points during the follow-up: one, twelve, and twenty-four months after the operation.

6MWT is a submaximal exercise test for evaluation of physical functional capacity measured in walked distance. The methodology of the examination was in agreement with the published guidelines [17]. Briefly, before the test patients were informed about the procedure and were allowed to rest in a sitting position for 10 minutes. Then they were asked to walk as fast and long as possible on a 50-meter walkway. Patients were allowed to stop and rest or reduce their walking speed if they felt fatigue. The dyspnea was estimated using the Borg scale. Samples of the left ventricular myocardium were harvested during the CABG procedure, and tissue fragments were placed in the “RNA later” solution (Qiagen) immediately after surgery and stored until RNA isolation. Expression of PPARγ mRNA was determined in these samples by means of quantitative real-time PCR using Taq-Man probes as previously described [18].

Blood samples were drawn initially and during each follow-up step. Serum concentrations of IL6 were measured using solid phase sandwich enzyme-linked immunosorbent assay kits (HS600B, R&D Systems) according to the manufacturer’s guidelines.

Patients were examined during the follow-up and the measures of heart failure development were sought. During follow-up all patients were divided into two groups: with heart failure (HF) and without heart failure (non-HF). The criteria for the diagnosis of heart failure were left ventricular ejection fraction (LVEF) < 40% or NT-proBNP > 400 pg/mL. Presence of any of the abovementioned values during the follow-up was considered a marker of heart failure.

2.1. Ethics Statement. The procedures followed in the study were conducted ethically according to the principles of the World Medical Association Declaration of Helsinki and ethical standards in sport and exercise science research. All procedures were approved by the Ethics Committee of the Regional Medical Chamber in Warsaw [IK NP-0021/13/998/2007]. Informed consent was obtained from all participants.

2.2. Statistics. Data are presented as mean ± SD for quantitative variables or percent of study group for qualitative variables. Specific parameters of both groups (group with and without heart failure at baseline) and change in parameter values during follow-up were compared using chi square test and ANOVA with post hoc analysis. Correlations between variables were tested using Pearson’s method. A value of \( p < 0.05 \) was considered statistically significant. Analysis was performed using Statistica 9.0PL.

3. Results

157 patients were qualified to the study. All patients did not have heart failure before CABG. After 1 month of CABG, 67% of patients developed heart failure. During 2-year follow-up the number of patients has been reduced in 1 year to 124 and in 2 years to 86 because of the loss of connection. In the HF group one patient died because of myocardial infarction in 1 year after CABG. The baseline characteristics of the study group were presented in the previous paper [18]. One month after CABG 106 patients (67%) were diagnosed with heart failure based on NT-proBNP exceeding 400 pg/mL or LVEF < 40%. Mean NT-proBNP concentration in this group was 675.2 ± 134.7 pg/mL and increased NT-proBNP was the most frequent indicator of HF. Only 13 subjects out of 106 had LVEF < 40%. The initial distance in 6MWT was 439 ± 73 m (408 ± 61 m in HF group and 458 ± 59 m in non-HF group). Patients developing HF during the follow-up had insignificantly lower exercise capacity 1 month after CABG than patients without HF. The mean 6MWT distance in HF group was 397 ± 85 m versus 420 ± 93 m in patients without HF. The distance improved significantly during the follow-up only in patients without HF (\( p = 0.002 \)) and 24 months after CABG it was significantly longer than in HF group (410 ± 134 m in HF group versus 522 ± 82 m in non-HF...
Figure 1: Changes of distance in 6-minute waking test in patients with (HF) and without heart failure (non-HF) during the follow-up. The improvement of the distance 24 months after CABG was observed only in non-HF group, and the distance was significantly longer as compared to the HF patients.

Figure 2: 6MWT distance 1 month after CABG was negatively correlated with PPARγ only in patients with HF during follow-up ($R = -0.24; p < 0.05$).

Figure 3: There were no significant correlations between PPARγ and either left ventricular end-diastolic dimension ($R = -0.11, p = \text{NS}$, the main graph) or left ventricular ejection fraction (LVEF; $R = 0.05, p = \text{NS}$, small graph) after CABG.

4. Discussion

The six-minute walk test (6MWT) is a submaximal exercise test for evaluating physical functional capacity. Fiorina et al. suggest that 6MWT is feasible and well tolerated in adult and older patients shortly after uncomplicated cardiac surgery and provides reference values for distance walked after cardiac surgery [19]. In our observations patients diagnosed with heart failure after CABG had a shorter distance in 6MWT, than patients without heart failure. In the HF group there was no significant improvement of the distance in 6MWT, while in patients without HF the distance increased significantly. Differences in exercise capacity can be attributed to the altered cardiac function; however there were no correlations between distance in 6MWT and LVEF or LV diastolic dimension. It should be emphasized that the abovementioned two parameters are related to the systolic function of the heart and poorly related to its diastolic performance.

Literature describing potential links between myocardial PPARγ expression and cardiac function or exercise capacity after CABG is very scant. In addition existing experimental
Table 1: Temporal changes of parameters related to six-minute walk test (6MWT) in patients with heart failure (HF) and without heart failure (non-HF).

|                          | Before CABG n = 157 | Before CABG n = 106 | HF 1 month n = 106 | HF 1 year n = 91 | HF 2 years n = 54 | P  | Non-HF 1 month n = 33 | Non-HF 1 year n = 33 | Non-HF 2 years n = 32 | P   |
|--------------------------|---------------------|---------------------|--------------------|-----------------|-----------------|----|----------------------|-----------------------|-----------------------|-----|
| **6MWT distance (±SD) [m]** | 439 (±73)           | 408 (±61)           | 397 (±85)          | 456 (±110)      | 410 (±134)      | NS | 458 (±59)           | 420 (±93)             | 499 (±87)             | 522 (±82) | 0.002 |
| **Rate of perceived exertion scale (Borg)** |                     |                     |                    |                 |                 |    |                      |                       |                       |       |
| 6–12                     | 90%                 | 75%                 | 80%                | 75%             | NS              | 80%| 85%                 | 90%                  | NS                    |     |
| 12–16                    | 10%                 | 20%                 | 15%                | 20%             | NS              | 20%| 15%                 | 10%                  | NS                    |     |
| 17–20                    | 0%                  | 5%                  | 5%                 | 5%              | NS              | 0% | 0%                  | 0%                    | 0%                    |     |
| **Respiratory rate/min.** |                     |                     |                    |                 |                 |    |                      |                       |                       |       |
| <14                      | 90%                 | 75%                 | 80%                | 75%             | NS              | 80%| 85%                 | 90%                  | NS                    |     |
| <20                      | 10%                 | 20%                 | 15%                | 20%             | NS              | 20%| 15%                 | 10%                  | NS                    |     |
| <25                      | 0%                  | 5%                  | 5%                 | 5%              | NS              | 0% | 0%                  | 0%                    | 0%                    |     |
| **HR/min.**              |                     |                     |                    |                 |                 |    |                      |                       |                       |       |
| <100                     | 90%                 | 75%                 | 80%                | 75%             | NS              | 80%| 85%                 | 90%                  | NS                    |     |
| <120                     | 10%                 | 20%                 | 15%                | 20%             | NS              | 20%| 15%                 | 10%                  | NS                    |     |
| <160                     | 0%                  | 5%                  | 5%                 | 5%              | NS              | 0% | 0%                  | 0%                    | 0%                    |     |
Figure 4: LVEF change between 1- and 12-month follow-up was significantly correlated with myocardial PPARγ in patients, in whom heart failure was diagnosed ($R = 0.25$, $p < 0.05$).

Figure 5: Significant negative correlation between serum IL-6 level before CABG and expression of PPARγ ($R = -0.31$, $p < 0.05$) was observed only in patients in whom later in the follow-up heart failure was diagnosed.

Higher levels of PPARγ in myocardium of patients who developed HF after CABG were correlated with smaller attenuation of LVEF, reduced plasma level of IL-6, and worsening of exercise tolerance. These results indicate that PPARγ expression in the myocardium was not related to left ventricular systolic function before CABG. However higher levels of PPARγ gene transcript in the myocardium of patients who develop heart failure following CABG may have some protecting effect on cardiac contractility, which seem not to be directly related to exercise capacity.

Additional Points

Study Limitations. Significant number of patients was lost during follow-up: 12% 1 month after CABG, 18% after one year, and 36% after two years. The significance of the PPARγ expression may not be directly translated into its activity and biological role. The correlations presented in the work do not

5. Conclusions

Higher levels of PPARγ in myocardium of patients who developed HF after CABG were correlated with smaller attenuation of LVEF, reduced plasma level of IL-6, and worsening of exercise tolerance. These results indicate that PPARγ expression in the myocardium was not related to left ventricular systolic function before CABG. However higher levels of PPARγ gene transcript in the myocardium of patients who develop heart failure following CABG may have some protecting effect on cardiac contractility, which seem not to be directly related to exercise capacity.

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imply the causality and may be accidental; however it is not possible to evaluate them in the clinical observational study.

Conflicts of Interest
The authors declare that they have no conflicts of interest.

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References
[1] B. P. Dhakal, R. Malhotra, R. M. Murphy et al., “Mechanisms of exercise intolerance in heart failure with preserved ejection fraction: the role of abnormal peripheral oxygen extraction,” Circulation: Heart Failure, vol. 8, no. 2, pp. 286–294, 2015.
[2] R. Arena, J. Myers, and M. Guazzi, “The clinical importance of cardiopulmonary exercise testing and aerobic training in patients with heart failure,” Revista Brasileira de Fisioterapia, vol. 12, no. 2, pp. 75–87, 2008.
[3] D. Harrington, S. D. Anker, T. P. Chua et al., “Skeletal muscle function and its relation to exercise tolerance in chronic heart failure,” Journal of the American College of Cardiology, vol. 30, no. 7, pp. 1758–1764, 1997.
[4] H. Drexler, U. Riede, T. Münnel, H. König, E. Funke, and H. Just, “Alterations of skeletal muscle in chronic heart failure,” Circulation, vol. 85, no. 5, pp. 1751–1759, 1992.
[5] S. D. Anker, P. P. Ponikowski, A. L. Clark et al., “Cytokines and neurohormones relating to body composition alterations in the wasting syndrome of chronic heart failure,” European Heart Journal, vol. 20, no. 9, pp. 683–693, 1999.
[6] T. Tsujiyaku, C. Ebisu, J. Fujita et al., “Muscle undergoes atrophy in association with increase of lysosomal cathepsin activity in interleukin-6 transgenic mouse,” Biochemical and Biophysical Research Communications, vol. 207, no. 1, pp. 168–174, 1995.
[7] V. M. Conraads, E. M. Van Craenenbroeck, C. De Maeyer, A. M. Van Berendonck, P. J. Beckers, and C. J. Vrints, “Unraveling new mechanisms of exercise intolerance in chronic heart failure. Role of exercise training,” Heart Failure Reviews, vol. 18, no. 1, pp. 65–77, 2013.
[8] W. S. Lee and J. Kim, “Peroxisome proliferator-activated receptors and the heart: lessons from the past and future directions,” PPAR Research, vol. 2015, Article ID 271983, 18 pages, 2015.
[9] D. Kamimura, K. Uchino, T. Ishigami, M. E. Hall, and S. Umemura, “Activation of Peroxisome Proliferator-activated Receptor γ Prevents Development of Heart Failure With Preserved Ejection Fraction; Inhibition of Wnt-β-catenin Signaling as a Possible Mechanism,” Journal of Cardiovascular Pharmacology, vol. 68, no. 2, pp. 155–161, 2016.
[10] T. Shiomi, H. Tsutsui, S. Hayashidani et al., “Pioglitazone, a peroxisome proliferator-activated receptor-γ agonist, attenuates left ventricular remodeling and failure after experimental myocardial infarction,” Circulation, vol. 106, no. 24, pp. 3126–3132, 2002.
[11] H. Ito, A. Nakano, M. Kinoshita, and A. Matsumori, “Pioglitazone, a peroxisome proliferator-activated receptor-γ agonist, attenuates myocardial ischemia/reperfusion injury in a rat model,” Laboratory Investigation, vol. 83, no. 12, pp. 1715–1721, 2003.
[12] T. Honda, K. Kaikita, K. Tsujita et al., “Pioglitazone, a peroxisome proliferator-activated receptor-gamma agonist, attenuates myocardial ischemia-reperfusion injury in mice with metabolic disorders,” Journal of Molecular and Cellular Cardiology, vol. 44, no. 5, pp. 915–926, 2008.
[13] Y. Xu, M. Gen, L. Lu et al., “PPAR-gamma activation fails to provide myocardial protection in ischemia and reperfusion in pigs,” American Journal of Physiology. Heart and Circulatory Physiology, vol. 288, no. 3, pp. H1314–H1323, 2005.
[14] C. A. Lygate, K. Hulbert, M. Monfared, M. A. Cole, K. Clarke, and S. Neubauer, “The PPARγ-activator rosiglitazone does not alter remodeling but increases mortality in rats post-myocardial infarction,” Cardiovascular Research, vol. 58, no. 3, pp. 632–637, 2003.
[15] H.-W. Liao, J. L. Saver, Y.-L. Wu, T.-H. Chen, M. Lee, and B. Ovbiagele, “Pioglitazone and cardiovascular outcomes in patients with insulin resistance, pre-diabetes and type 2 diabetes: A systematic review and meta-analysis,” BMJ Open, vol. 7, no. 1, Article ID e013927, 2017.
[16] T. Yokota, S. Kinugawa, K. Hirabayashi et al., “Pioglitazone improves aerobic capacity and skeletal muscle energy metabolism in patients with metabolic syndrome,” Journal of Cardiac Failure, vol. 22, no. 9, p. S205, 2016.
[17] ATS Committee on Proficiency Standards for Clinical Pulmonary Function Laboratories, “ATS statement: guidelines for the six-minute walk test,” American Journal of Respiratory and Critical Care Medicine, vol. 166, no. 1, pp. 111–117, 2002.
[18] I. Wojtkowska, A. Tysarowski, K. Seliga et al., “PPAR gamma expression levels during development of heart failure in patients with coronary artery disease after coronary artery bypass-grafting,” PPAR Research, Article ID 242790, 242790 pages, 2014.
[19] C. Fiorina, E. Vizzardi, R. Lorusso et al., “The 6-min walking test early after cardiac surgery. Reference values and the effects of rehabilitation programme,” European Journal of Cardiothoracic Surgery, vol. 32, no. 5, pp. 724–729, 2007.
[20] N. H. Son, T. S. Park, H. Yamashita et al., “Cardiomyocyte expression of PPARgamma leads to cardiac dysfunction in mice,” Journal of Clinical Investigation, vol. 11, no. 10, pp. 2791–2801, 2007.
[21] R. K. Vikramadithyan, K. Hirata, H. Yagyu et al., “Peroxisome proliferator-activated receptor agonists modulate heart function in transgenic mice with lipotoxic cardiomyopathy,” Journal of Pharmacology and Experimental Therapeutics, vol. 313, no. 2, pp. 586–593, 2005.
[22] M. A. Burke, S. Chang, H. Wakimoto et al., “Molecular profiling of dilated cardiomyopathy that progresses to heart failure,” JCI Insight, vol. 1, no. 6, 2016.
[23] H. Cernecke, G. Doka, J. Srankova et al., “Ramipril restores PPARβ/δ and PPARγ expressions and reduces cardiac NADPH oxidase but fails to restore cardiac function and accompanied myosin heavy chain ratio shift in severe anthracycline-induced cardiomyopathy in rat,” European Journal of Pharmacology, vol. 791, pp. 244–253, 2016.
[24] J. Grewal, R. B. McCully, G. Kane, C. Lam, and P. A. Pellikka, “Left ventricular function and exercise capacity,” JAMA, vol. 301, no. 3, pp. 286–94, 2009.
[25] Y. Ye, Z. Hu, Y. Lin, C. Zhang, and J. R. Perez-Polo, "Downregulation of microRNA-29 by antisense inhibitors and a PPAR-γ agonist protects against myocardial ischaemia–reperfusion injury," *Cardiovascular Research*, vol. 87, no. 3, pp. 535–544, 2010.

[26] A. Cabrero, J. C. Laguna, and M. Vázquez, "Peroxisome proliferator-activated receptors and the control of inflammation," *Curr Drug Targets Inflamm Allergy*, vol. 1, no. 3, pp. 243–248, 2002.

[27] L. Gullestad, T. Ueland, L. E. Vinge, A. Finsen, A. Yndestad, and P. Aukrust, "Inflammatory cytokines in heart failure: mediators and markers," *Cardiology*, vol. 122, no. 1, pp. 23–35, 2012.

[28] A. Deswal, N. J. Petersen, A. M. Feldman, J. B. Young, B. G. White, and D. L. Mann, "Cytokines and cytokine receptors in advanced heart failure: an analysis of the cytokine database from the Vesnarinone Trial (VEST)," *Circulation*, vol. 103, no. 16, pp. 2055–2059, 2001.

[29] J. Thierer, A. Acosta, N. Vainstein et al., "Relation of left ventricular ejection fraction and functional capacity with metabolism and inflammation in chronic heart failure with reduced ejection fraction (from the MIMICA Study)," *American Journal of Cardiology*, vol. 105, no. 7, pp. 977–983, 2010.

[30] M. M. Fernandes-Silva, G. V. Guimarães, V. O. Rigaud et al., "Inflammatory biomarkers and effect of exercise on functional capacity in patients with heart failure: insights from a randomized clinical trial," *European Journal of Preventive Cardiology*, vol. 24, no. 8, pp. 808–817, 2017.

[31] P. Muñoz-Cánoves, C. Scheele, B. K. Pedersen, and A. L. Serrano, "Interleukin-6 myokine signaling in skeletal muscle: a double-edged sword?" *The FEBS Journal*, vol. 280, no. 17, pp. 4131–4148, 2013.