Marko Novaković

Vpliv različnih vrst telesne vadbe na izbrane kazalnike srčno-žilnega zdravja pri specifičnih skupinah srčno-žilnih bolnikov

DOKTORSKA DISERTACIJA

Influence of different types of exercise training on selected cardiovascular parameters in specific groups of cardiovascular patients

DOCTORAL DISSERTATION

Ljubljana, 2017
Marko Novakovič

Vpliv različnih vrst telesne vadbe na izbrane kazalnike srčno-žilnega zdravja pri specifičnih skupinah srčno-žilnih bolnikov

Influence of different types of exercise training on selected cardiovascular parameters in specific groups of cardiovascular patients

Imenovanje mentorja na seji senata dne: 15. september 2015
Komisija za oceno imenovana na seji senata dne: 10. april 2017
Komisija za zagovor imenovana na seji senata dne: 2. oktober 2017
Datum zagovora: 17. oktober 2017

Predsednik komisije: Prof. dr. Aleš Blinc, dr. med.
Članica: Prof. dr. Irena Keber, dr. med.
Član: Prof. dr. Andrew Nicolaides, dr. med.
There was never a night or a problem that could defeat sunrise or hope.

Sir Bernard Williams
Table of Contents

Abstract ........................................................................................................................................... 8
Povzetek ........................................................................................................................................... 11
1. Introduction ................................................................................................................................... 15
  1.1 Exercise in cardiovascular disease ................................................................................................. 15
     1.1.1 Definition ................................................................................................................................. 15
     1.1.2 Cardiovascular physiology and clinical impacts of exercise ....................................................... 15
  1.2 Tetralogy of Fallot .......................................................................................................................... 18
     1.2.1 Epidemiology, natural history, surgical management ................................................................. 18
     1.2.2 Adults with repaired tetralogy of Fallot .................................................................................... 20
        1.2.2.1 Right ventricular dysfunction .............................................................................................. 20
           1.2.2.1.1 Exercise performance ................................................................................................... 20
           1.2.2.1.2 Cardiac autonomic function .......................................................................................... 21
           1.2.2.1.3 Natriuretic peptides ..................................................................................................... 22
           1.2.2.1.4 Functional health status and quality of life ...................................................................... 22
        1.2.2.2 Ventricular dysrhythmias .................................................................................................... 23
        1.2.2.3 Vascular function ................................................................................................................. 23
        1.2.2.4 Exercise training in patients with tetralogy of Fallot ............................................................ 24
  1.3 Peripheral arterial disease ........................................................................................................... 25
     1.3.1 Definition and classification ...................................................................................................... 25
     1.3.2 Atherosclerosis and endothelial dysfunction ............................................................................ 26
     1.3.3 Exercise vs. other treatment modalities .................................................................................... 28
     1.3.1 Exercise training modalities ...................................................................................................... 32
  1.4 Evidence gaps .............................................................................................................................. 32
  1.5 Aims ................................................................................................................................................ 34
  1.6 Hypotheses ..................................................................................................................................... 35
     1.6.1 Primary hypotheses ................................................................................................................... 35
     1.6.2 Secondary hypotheses ............................................................................................................. 35
2. Methods .......................................................................................................................................... 37
  2.1 Study populations ......................................................................................................................... 37
     2.1.1 Patients with repaired tetralogy of Fallot .................................................................................. 37
     2.1.2 Patients with peripheral arterial disease ................................................................................... 38
  2.2 Study design ................................................................................................................................... 38
2.2.1 General considerations ........................................................................................................38
2.2.2 Patients with repaired tetralogy of Fallot ..........................................................................39
2.2.3 Patients with peripheral arterial disease ............................................................................40

2.3 Intervention ..............................................................................................................................42
2.3.1 Repaired tetralogy of Fallot ...............................................................................................42
2.3.2 Peripheral arterial disease .................................................................................................45

2.4 Observed variables ..................................................................................................................47
2.4.1 Clinical data ........................................................................................................................48
2.4.2 Exercise capacity ..................................................................................................................49
2.4.2.1 Cardiopulmonary exercise testing .................................................................................49
2.4.2.2 Six-minute walk test ......................................................................................................50
2.4.2.3 Walking testing ...............................................................................................................50
2.4.2.4 Ankle-brachial index .....................................................................................................50
2.4.3 Vascular function ................................................................................................................51
2.4.3.1 Flow-mediated dilation ...............................................................................................51
2.4.3.2 Carotid artery stiffness .................................................................................................51
2.4.4 Cardiac autonomic function ...............................................................................................52
2.4.4.1 Heart rate variability ....................................................................................................52
2.4.4.2 Heart rate recovery .......................................................................................................53
2.4.5 Biomarkers ........................................................................................................................53
2.4.6 Quality of life ....................................................................................................................54
2.4.6.1 Health related quality of life .........................................................................................54
2.4.6.2 Self-assessed physical activity level ..............................................................................55

2.5 Statistical methods ..................................................................................................................55
2.5.1 Sample size calculation .......................................................................................................55
2.5.2 Statistical analysis ..............................................................................................................56

3. Results ...........................................................................................................................................57
3.1 Repaired tetralogy of Fallot ......................................................................................................57
3.1.1 Exercise capacity ................................................................................................................59
3.1.2 Vascular function ...............................................................................................................62
3.1.3 Cardiac autonomic function ..............................................................................................64
3.1.3.1 Heart rate variability .....................................................................................................64
3.1.3.2 Heart rate recovery .......................................................................................................66
3.1.4 Biomarkers ........................................................................................................... 68
  3.1.4.1 Complete blood count ..................................................................................... 68
  3.1.4.2 Lipid profile .................................................................................................... 68
  3.1.4.3 Markers of inflammation, haemostasis and heart failure severity ................. 69
3.1.5 Quality of life ....................................................................................................... 70
3.2 Peripheral arterial disease ...................................................................................... 78
  3.2.1 Walking capacity ............................................................................................... 80
  3.2.2 Vascular function ............................................................................................... 82
  3.2.3 Cardiac autonomic function ............................................................................. 84
    3.2.3.1 Heart rate variability ................................................................................... 84
  3.2.4 Biomarkers ......................................................................................................... 84
    3.2.4.1 Markers of inflammation, hemostasis and heart failure severity ............... 85
  3.2.5 Quality of life ..................................................................................................... 86
4. Discussion .................................................................................................................. 90
  4.1 Safety and adherence ............................................................................................ 90
  4.2 Exercise/walking capacity ..................................................................................... 92
    4.2.1 Repaired tetralogy of Fallot ............................................................................. 92
    4.2.2 Peripheral arterial disease ............................................................................. 93
  4.3 Vascular function .................................................................................................. 95
    4.3.1 Repaired tetralogy of Fallot ............................................................................ 95
    4.3.2 Peripheral arterial disease ............................................................................. 96
  4.4 Cardiac autonomic function ................................................................................ 98
    4.4.1 Patients with repaired tetralogy of Fallot ..................................................... 98
    4.4.2 Peripheral arterial disease ............................................................................. 100
  4.5 Biomarkers .......................................................................................................... 101
    4.5.1 Repaired tetralogy of Fallot ............................................................................ 101
    4.5.2 Peripheral arterial disease ............................................................................. 103
  4.6 Quality of life ....................................................................................................... 103
    4.6.1 Repaired tetralogy of Fallot ............................................................................ 103
    4.6.2 Peripheral arterial disease ............................................................................. 104
5. Limitations ................................................................................................................. 105
6. Conclusions ............................................................................................................. 107
References .................................................................................................................. 108
Appendix A: Cardiovascular autonomic dysfunction and carotid stiffness in adults with repaired tetralogy of Fallot .................................................................................................................. 129
Appendix B: Exercise capacity, cardiac and vascular function in adults with repaired tetralogy of Fallot ............................................. 146
Appendix C: Clinical impact of exercise in patients with peripheral arterial disease .......................................................... 162
Appendix D: SF-36 questionnaire .................................................................................................................................................. 186
Appendix E: IPAQ questionnaire .................................................................................................................................................. 191
Abbreviations .............................................................................................................................................................................. 193
Acknowledgements ................................................................................................................................................................... 194
List of Figures

Fig 1. Anatomy of a normal heart and a heart of tetralogy of Fallot .................................18
Fig 2. Shunt surgery possibilities in infants with tetralogy of Fallot .................................19
Fig 3. Study design in patients with repaired tetralogy of Fallot according to CONSORT guidelines ........40
Fig 4. Study design in patients with peripheral arterial disease according to CONSORT guidelines ........41
Fig 5. Increase in intensity of training sessions for interval and continuous training protocols ..........44
Fig 6. Comparison of profiles of interval and continuous training protocols ...........................45
Fig 7. Exercise training (walking) protocols in patients with peripheral arterial disease ...............47
Fig 8. Enrolment and allocation of patients in a study of patients with repaired tetralogy of Fallot ........57
Fig 9. Collected data and count of patients for study groups in patients with repaired tetralogy of Fallot ...............................................................................................................58
Fig 10. Comparison of changes in workload in patients with repaired tetralogy of Fallot .............59
Fig 11. Comparison of changes in peak oxygen consumption for each group in the study of patients with repaired tetralogy of Fallot .............................................................................60
Fig 12. Comparison of changes in oxygen consumption at ventilatory threshold in patients with repaired tetralogy of Fallot ..................................................................................................................61
Fig 13. Comparison of changes in the 6-min walk test in patients with repaired tetralogy of Fallot .........62
Fig 14. Comparison of changes in flow-mediated vasodilation in patients with repaired tetralogy of Fallot ..............................................................................................................................63
Fig 15. Comparison of changes in pulse wave velocity in patients with repaired tetralogy of Fallot ..........64
Fig 16. Comparison of changes in rMSSD in patients with repaired tetralogy of Fallot .....................65
Fig 17. Comparison of changes in LFnu in patients with repaired tetralogy of Fallot .......................66
Fig 18. Comparison of changes in heart rate recovery after 2 minutes in patients with repaired tetralogy of Fallot ..........................................................................................................................67
Fig 19. Comparison of changes in high-density lipoprotein cholesterol in patients with repaired tetralogy of Fallot ..............................................................................................................................68
Fig 20. Comparison of changes in NT-proBNP levels in patients with repaired tetralogy of Fallot ........69
Fig 21. Comparison of changes in fibrinogen levels in patients with repaired tetralogy of Fallot ..........70
Fig 22. Comparison of changes in physical and mental component summary in patients with repaired tetralogy of Fallot ............................................................................................................................71
Fig 23. Enrolment and allocation of patients in a study of patients with peripheral arterial disease ........ 78

Fig 24. Collected data and count of patients for study groups in patients with peripheral arterial disease ................................................................................................................................. 79

Fig 25. Comparison of changes in claudication onset distance and maximal walking distance in patients with peripheral arterial disease .............................................................................................................. 81

Fig 26. Comparison of changes in ankle-brachial index in patients with peripheral arterial disease ........ 82

Fig 27. Comparison of mean changes in flow-mediated dilation in patients with peripheral arterial disease ........................................................................................................................................ 83

Fig 28. Comparison of changes in pulse wave velocity in patients with peripheral arterial disease ........ 84

Fig 29. Comparison of changes in fibrinogen levels in patients with peripheral arterial disease .............. 85

Fig 30. Comparison of changes in physical and mental component summary in patients with peripheral arterial disease .................................................................................................................................................. 86
List of Tables

Table 1. Fontaine and Rutherford classifications of peripheral arterial disease........................................... 26
Table 2. Literature review of exercise training modalities..................................................................................... 31
Table 3. Methods performed on patients in each of the two studies ................................................................. 48
Table 4. Baseline clinical data of patient with repaired tetralogy of Fallot who completed the study ........... 58
Table 5. Exercise training measurements for three studied groups before and after trainings, and changes in patients with repaired tetralogy of Fallot.................................................................................... 72
Table 6. Absolute changes and comparison among groups in patients with repaired tetralogy of Fallot .... 76
Table 7. Baseline clinical data of patients with peripheral arterial disease who completed the study......... 80
Table 8. Exercise training measurements for three studied groups before and after trainings, and changes in patients with peripheral arterial disease.................................................................................. 87
Table 9. Absolute changes and comparison among groups in patients with peripheral arterial disease.... 89
ABSTRACT

Introduction

Exercise training improves exercise capacity and reduces morbidity and mortality in patients with coronary heart disease and heart failure. However, recent research has started focusing on other cardiovascular conditions, such as grown-ups with congenital heart diseases and patients with peripheral artery disease (PAD). The former represent an ever growing population due to improved management of congenital diseases in childhood, and are characterised by low exercise capacity, which may have been exacerbated by discouraging them from exercising due to the perceived risk of exercise. The latter represent a substantial proportion of patients with atherosclerotic vascular disease, with exercise in form of walking providing both a measure for secondary prevention and symptom improvement. Nonetheless, both groups of patients remain underrepresented in studies of exercise training.

Therefore, we aimed to assess whether i) supervised moderate/lenient or ii) intensive/strenuous exercise training as compared to iii) usual care (no supervised exercise training) in I) adults with repaired tetralogy of Fallot (ToF) and II) in patients with PAD differentially affect a) exercise capacity, b) cardiac autonomic function, c) vascular function, d) disease-specific biomarkers and e) health-related quality of life.

Methods

Two interventional sub-studies were carried out, as follows: with adults with repaired ToF and with patients with PAD. Both groups of patients were randomised into 3 groups. Patients with repaired ToF were randomised to either high- and low-intensity interval or continuous moderate intensity training group (36 training sessions, 2-3 times per week of aerobic training with higher and lower intensities, or continuous intensity), or to a control group (advised to do normal everyday activities). Patients with PAD were randomised to either moderate-pain or pain-free training (36 training sessions, 2-3 times per week of walking on treadmill up to moderate-pain or up to two-thirds of claudication distance, with no inclination
and treadmill speed based on 70% of maximal predicted heart rate), or to a control group (advised to continue with previous activities). The following outcomes were determined at baseline and after the intervention period: exercise capacity (cardiopulmonary exercise testing) in patients with repaired ToF only; walking capacity (treadmill walk test) in patients with PAD only; vascular function (ultrasonographically assessed brachial artery flow-mediated dilation (FMD) and carotid pulse wave velocity), cardiac autonomic function (heart rate variability (HRV) and heart rate recovery (HRR)), biomarkers (lipid profile, D-dimer, fibrinogen, NT-proBNP) and health-related quality of life (SF-36 questionnaire).

Results

Twenty-seven patients with repaired ToF (mean age 39 (± 9) years, 37% of males) and 29 patients with PAD (mean age 64 (± 9) years, 72% of males) completed the study. All training modalities were safe; no adverse events were recorded.

In patients with repaired ToF, both interval and continuous training were associated with a significant improvement of work rate (from median 123 to 132 W, p=0.002 and from median 160 to 175 W, p=0.003, respectively). However, exercise capacity (determined with peak oxygen uptake, VO2peak) significantly improved after interval – but not after continuous – exercise training (from median 21.2 to 22.9 ml/kg/min, p=0.004 and from median 21.8 to 23.6 ml/kg/min, p=0.190, respectively). Interval training was associated with a significant increase in FMD (from 8.4 to 12.9%, p=0.019) and a decrease in pulse wave velocity (from 5.4 to 4.8 m/s, p=0.028), and a significant decrease in NT-proBNP (from a median 202 to 190 ng/L, p=0.032) and fibrinogen levels (from 2.67 to 2.46 g/L, p=0.018), as well as increase of HDL-cholesterol (from 1.26 to 1.33, p=0.030). Continuous training was not associated with any significant change in vascular function or biomarker concentrations. Conversely, continuous – but not interval – exercise training was associated with a significant improvement in cardiac autonomic function parameters, namely increase of HRR after 2 minutes (from 40 to 47, p=0.023) and reduction of low-frequency domain HRV (from 0.32 to 0.22, p=0.039), together with borderline significant improvement of a time-domain parameter of HRV, rMSSD (from 17.0 to
Moreover, continuous training was associated with significant improvements in mental-domains of quality of life (from 87 to 96, p=0.028).

In patients with PAD, walking capacity parameters, namely pain-free and maximal walking distance, were significantly increased with either moderate-pain (from 63 to 121 m, p<0.001 and from 109 to 199 m, p<0.001, respectively) and pain-free (from 43 to 116 m, p<0.001 and from 87 to 163 m, p<0.001) training. Moderate-pain – but not pain-free – training was associated with a significant increase in FMD (from 4.4 to 8.0 %, p=0.002), a significant decrease in pulse wave velocity (from 6.6 to 6.1 m/s, p=0.013), and a borderline significant decrease in fibrinogen levels (from 4.2 to 3.6 g/L, p=0.085). Neither training programme was associated with changes in cardiac autonomic function. Conversely, pain-free – but not moderate-pain – training was associated with improvements in several mental and physical domains of health-related quality of life.

**Conclusion**

Both lenient and strenuous exercise training modalities in adults with repaired ToF and in patients with PAD are safe and substantially improve exercise/walking capacity. Furthermore, our findings suggest that strenuous training modalities (i.e. interval exercise and moderate-pain walking, respectively) are more efficacious in improving vascular function and disease-specific biomarkers (NT-proBNP and fibrinogen levels, respectively), whereas lenient training modalities (i.e. continuous exercise and pain-free walking, respectively) are more efficacious in improving health-related quality of life. Additionally, interval training in patients with repaired ToF improves cardiac autonomic function. Overall, irrespective of differential physiologic effects, both strenuous and lenient training modalities safely and effectively improve exercise capacity and several indicators of cardiovascular health as compared to no exercise at all.
POVZETEK

Uvod

Telesna vadba izboljša telesno zmogljivost ter zmanjša zbolevnost in umrljivost bolnikov s koronarno srčno boleznijo in srčnim popuščanjem. Novejše raziskave pa vse več pozornosti usmerjajo v druge skupine posameznikov s srčno-žilnimi boleznimi, kot so odrasli s prirojenimi srčnimi napakami in bolniki s periferno arterijsko boleznijo (PAD). Prvi predstavljajo naraščajočo skupino bolnikov zaradi izboljšanega zdravljenja prirojenih napak v otroštvu, hkrati pa jih zaznamuje zmanjšana telesna zmogljivost, ki je delno povezana z odsvetovanjem telesne vadbe v preteklosti zaradi bojani pred možnimi posledicami telesnega napora. Drugi predstavljajo znaten delež bolnikov z aterosklerotično žilno boleznijo, za katere je telesna vadba eden izmed ključnih sekundarnopreventivnih ukrepov in hkrati ukrepov za lajšanje simptomov intermitentne klaudikacije.

Študije na omenjenih skupinah bolnikov so redke, še posebej glede ocenjevanja učinkovitosti in primerjave različnih vrst telesne vadbe, ne le za izboljšanje telesne dejavnosti oz. prehojenje razdalje, temveč tudi za izboljšanje žilne funkcije, avtonomne srčne funkcije, krvnih označevalcev in z zdravjem povezane kakovosti življenja.

Kljub temu so raziskave pri omenjenih skupinah bolnikov omejene. Namen doktorskega dela je bil zato preučiti vpliv nadzorovane i) zmerne in ii) intenzivne telesne vadbe v primerjavi z iii) običajno oskrbo brez vadbe I) pri odraslih po operaciji Fallotove tetraloglje (ToF) in II) pri bolnikih s PAD, in sicer na a) telesno zmogljivost, b) srčno avtonomno funkcijo, c) žilno funkcijo, č) za bolezen značilne biološke označevalce in d) z zdravjem povezano kakovost življenja.

Metode

Izvedli smo dve randomizirani kontrolirani raziskavi, in sicer pri odraslih po operaciji ToF in pri bolnikih s PAD. V vsaki skupini smo bolnike naključno razporedili v dve intervencijski in eno kontrolno skupino.
Pri bolnikih s ToF sta intervencijski skupini izvajali intervalno oziroma kontinuirano vadbo. Intervalna vadba se je izvajala v intervalih visoke in nizke intenzivnosti, medtem ko je bila intenzivnost pri kontinuirani vadbi ves čas treninga enaka in zmerna. Pri bolnikih s PAD sta intervencijski skupini izvajali hojo do zmerne klavdikacijske bolečine oziroma hojo do dveh tretjin klavdikacijske razdalje (hojo brez bolečine). Obe vrsti vadbe sta se izvajali na tekalni stezi brez naklona, s hitrostjo prilagojeno 70 % predvidene najvišje srčne frekvence. Vse štirje intervencijske skupine so opravile 36 treningov, in sicer od dva- do trikrat tedensko. Bolnikom v kontrolni skupini smo svetovali, da v času trajanja raziskave svoje vsakodnevne aktivnosti izvajajo kot običajno.

Vsem bolnikom smo izhodiščno in po intervencijskem obdobju določili: telesno zmogljivost s pomočjo spiroergometričnega obremenitvenega testiranja (samo pri bolnikih s ToF), obremenitveni test hoje na tekalni stezi z naklonom 12,5 % in s hitrostjo 3,2 km/h (samo pri bolnikih s PAD), žilno funkcijo (ultrazvočno ocenjeno od pretoka odvisno vazodilatacijo (FMD) brahialne arterije ter hitrost pulznega vala), avtonomno srčno funkcijo (variabilnost srčne frekvence (HRV) in upadanje srčne frekvence po najvišji obremenitvi (HRR)), za bolezen značilne označevalce (raven krvnih maščob, D-dimera, fibrinogena, NT-proBNP) ter z zdravjem povezano kakovost življenja s pomočjo vprašalnika SF-36.

Rezultati

Raziskavi je zaključilo 27 bolnikov s ToF (povprečna starost 39 (±9) let, 37 % moških) oziroma 29 bolnikov s PAD (povprečna starost 64 (±9) let, 72 % moških). Vse vadbe so bile varne, brez zapletov v intervencijskih skupinah.

Pri bolnikih s ToF sta obe vrsti vadbe statistično značilno izboljšali obremenitveno zmogljivost: intervalna s 123 na 132 W (p=0,002), kontinuirana s 160 W na 175 W (p=0,003). Največja poraba kisika (VO2peak) se je statistično značilno izboljšala po intervalni telesni vadbi (z 21,2 na 22,9 ml/kg/min, p=0,004), ne pa po kontinuirani vadbi (21,8 na 23,6 ml/kg/min, p=0,190). Intervalna (ne pa tudi kontinuirana) vadba je bila povezana tudi z značilnim izboljšanjem žilne funkcije (FMD je narasel z 8,4 na 12,9 %, p=0,019; hitrost
pulznega vala je upadla s 5,4 na 4,8 m/s, p=0,028) ter z upadom ravni NT-proBNP (z mediane 202 na 190 ng/L, p= 0,032) in fibrinogena (z 2,67 na 2,46 g/L, p=0,018) oziroma s porastom ravni holesterola HDL (z 1,26 na 1,33 mmol/L, p= 0,030). Nasprotno je bila kontinuirana (ne pa tudi intervalna) vadba povezana z izboljšanjem kazalnikov avtonomne srčne funkcije, in sicer z okrevanjem srčne frekvence dve minuti po koncu obremenitve (porast s 40 na 47 utripov/min, p=0,023), nizkofrekvenčnim označevalcem HRV (upad z 0,32 na 0,22, p=0,039) in kazalnikom časovne domene HRV rMSSD (statistično mejni porast mediane s 17,0 na 26,3, p= 0,087) ter z izboljšanjem mentalne domene z zdravjem povezane kakovosti življenja (z mediane 87 na 96, p=0,028).

Pri bolnikih s PAD sta tako vadba s hojo do zmerne bolečine kot vadba s hojo brez bolečine statistično značilno podaljšali prehoveno razdaljo: prva je podaljšala hojo brez bolečine s 63 na 121 m (p< 0,001) ter najdaljšo prehoveno razdaljo s 109 na 199 m (p<0,001); druga je podaljšala hojo brez bolečine s 43 na 116 m (p<0,001) ter najdaljšo prehoveno razdaljo s 87 na 163 m (p<0,001). Hoja do zmerne bolečine (ne pa tudi hoja brez bolečine) je značilno izboljšala FMD (s 4,4 na 8,0 %, p=0,002) in hitrost pulznega vala (s 6,6 na 6,1 m/s, p=0,013) ter mejno značilno znižala raven fibrinogena v krvi (s 4,2 na 3,6 g/L, p=0,085). Nobena vrsta vadbe ni značilno vplivala na kazalnike avtonomne srčne funkcije. Nasprotno je hoja brez bolečine (ne pa tudi hoja do bolečine) značilno izboljšala več kategorij duševne in telesne domene z zdravjem povezane kakovosti življenja.

Zaključki

Tako zmerna kot intenzivna telesna vadba sta pri odraslih po operaciji ToF in pri bolnikih s PAD varno izboljšali obremenitveno zmogljivost oziroma prehoveno razdaljo. Naši izsledki nakazujejo, da zahtevnejše vrste vadbe (tj. intervalna vadba oziroma hoja do zmerne bolečine) učinkoviteje izboljšajo žilno funkcijo in za bolezen značilne biološke označevalce (NT-proBNP oziroma fibrinogen), medtem ko manj zahtevne vrste vadbe (tj. kontinuirana vadba oziroma hoja brez bolečine) učinkoviteje izboljšajo z zdravjem povezano kakovost življenja. Poleg tega intervalna vadba pri odraslih po operaciji ToF izboljša srčno avtonomno
funkcijo. Kljub različnim fiziološkim učinkom tako zahtevnejše kot manj zahtevne vrste vadbe varno in učinkovito izboljšajo telesno zmogljivost in številne kazalnike srčno-žilnega zdravja v primerjavi s telesno nedeljavnostjo.
1. INTRODUCTION

1.1 EXERCISE IN CARDIOVASCULAR DISEASE

1.1.1 DEFINITION

Physical activity is broadly defined as any bodily movement involving skeletal muscles and consuming energy [1]. Conversely, exercise is defined as planned, structured, repetitive and purposeful physical activity aiming to improve fitness [2]. Sometimes, a loose distinction between exercise and training may also be employed, with training pertaining to a long-term goal, such as improved exercise capacity or health, rather than the process of exercising itself.

1.1.2 CARDIOVASCULAR PHYSIOLOGY AND CLINICAL IMPACTS OF EXERCISE

Exercise can be categorised based on mechanics or metabolism. Mechanics pertains to muscle contraction, which can be either isotonic (dynamic exercise) or isometric (static exercise) [3–5]. Metabolism pertains to main source of energy, which can derive either from oxidative processes (aerobic exercise) or glycolytic processes (anaerobic exercise) [6]. Although most exercise types are mixed in nature, a predominant combination can usually be identified for categorisation purposes (e.g. dynamic-aerobic).

Dynamic-aerobic exercise is characterised by a synchronised response of the cardiovascular, muscular and respiratory systems and is therefore advocated as safe, effective and preferential in terms of cardiovascular fitness and health [6,7].

In dynamic-aerobic exercise, increased skeletal muscle activity yields a coordinated response of the cardiovascular and respiratory systems. Demand-induced vasodilation in skeletal muscles activates the sympathetic system through the baroreceptor reflex and results in increased heart rate (HR) and cardiac contractility (cardiac output), and blood redistribution through selective vasodilation and vasoconstriction in different organs [7]. As opposed to static-anaerobic type of exercises, this response is coordinated and therefore favourable in terms of cardiovascular physiology [6].
During exercise, other changes also take place, namely oxidative stress [8], inflammatory response, hormonal rearrangement [7] and haemostatic changes [9]. These changes may be regarded as pathophysiologic in the acute setting, and have indeed been associated with an increase in the relative risk of cardiovascular events immediately after exercise [10]. However, long-term training with planned, structured and repetitive bouts of exercise yields longstanding favourable adaptations and improved health [11,12]. These include improved exercise capacity, glucose tolerance [13], lipid metabolism and blood pressure control, and decreased markers of inflammation [14].

Also, benefits of exercise training reflect in behavioural changes resulting in lower body weight, increased smoking cessation in smokers, lower levels of anxiety and lower prevalence of depression [15]. Overall, clinical benefits of long-term exercise translate in decreased morbidity, and cardiovascular and total mortality.

The seemingly discordant notion that exercise increases the risk of immediate cardiovascular events, but decreases long-term morbidity and mortality has been dubbed “the sports paradox” [16–18]. While maintaining that exercise provides health benefits, the sports paradox also acknowledges its potential immediate risks, especially in individuals with predisposing pre-existing conditions, such as cardiovascular disease [10]. In fact, cardiac rehabilitation programmes are based on the assumption that patients with cardiovascular disease on the one hand need medical supervision during exercise in order to prevent complications, while on the other hand they need a structured and planned programme to take advantage of evidence-based benefits of exercise in cardiovascular disease [19].

Cardiac rehabilitation has been shown to decrease cardiovascular and total mortality in patients with coronary artery disease and to decrease mortality and hospitalisation rates in patients with heart failure.

While patients with coronary artery disease and heart failure represent large patient population and have therefore historically been included in most trials, recent studies have started focusing on other cardiovascular patient populations. Studies have shown that exercise training improved exercise capacity
and physiologic parameters in patients with arterial hypertension, pulmonary hypertension, heart transplantation, after heart valve replacement, in grown-ups with congenital heart disease and peripheral arterial disease (PAD) [20–25].

Two groups of patients in particular merit attention. The first group are grown-ups with congenital heart diseases. On the one hand they represent an ever growing population due to improved management of congenital diseases in childhood, on the other hand they are characterised by residual high risk for exercise training and also by low exercise capacity, which may have been exacerbated by discouraging them from exercising due to the perceived risk of exercise. The second group are patients with peripheral artery disease. On the one hand, they represent a substantial proportion of patients with atherosclerotic vascular disease and exercise training provides a pivotal secondary preventive intervention in this setting. On the other hand, the main symptom of PAD (intermittent claudication) is both a limiting factor for, and the main target amendable by, regular exercise. Nonetheless, studies in these two groups of patient populations are limited, especially in terms of providing answers to what type of exercise should be preferable.
1.2 TETRALOGY OF FALLOT

1.2.1 EPIDEMIOLOGY, NATURAL HISTORY, SURGICAL MANAGEMENT

Tetralogy of Fallot (ToF), named after French physician Etienne-Louis Arthur Fallot (1850-1911), is a congenital heart disease comprising (i) ventricular septal defect, (ii) overriding aorta, (iii) obstruction of the right ventricle outflow and (iv) right ventricular hypertrophy (Fig 1) [26,27].

![Diagram of a normal heart and a heart of tetralogy of Fallot](image)

Fig 1. Anatomy of a normal heart (a) and a heart of tetralogy of Fallot (b)

ToF is the most prevalent among cyanotic congenital heart diseases, accounting for 7 to 10% of all congenital cardiac malformations [28]. Birth prevalence is estimated at 0.34 per 1000 live births in the world [29]. More males are born with ToF, with a male-to-female ratio of 1.44 [30]. Although the exact aetiology is heterogeneous, around 7% of ToF is associated with chromosomal malformations [31,32] and up to 27% of ToF is associated with genetic syndromes [32,33].

If untreated, ToF results in extremely high mortality in the first years of life, with very few affected individuals reaching adulthood [26,34]. Conversely, surgical management has dramatically reshaped the
natural course of the disease. The estimated 40-year survival rate of infants with ToF operated in 1980s and 1990s is currently 90% [35].

Surgical management includes palliative-then-corrective (staged) or straight-on corrective surgery [26]. While the first (originally introduced by Blalock-Taussig; Fig 2) provides immediate relief of cyanotic patophysiology with lower risk of neurologic complications (the child undergoes correction at a later stage in life) [36,37], the latter provides a comprehensive resolution of right ventricular overload [26]. The preference for each approach is individual, although recent trends are more prone to advocate straight-on corrective surgery in neonatal period [36].

![Fig 2. Shunt surgery possibilities in infants with tetralogy of Fallot](image)

(1) classic Blalock-Taussig shunt, (2) modified Blalock-Taussig shunt, (3) stenting of ductus arteriosus, (4) Potts-Smith-Gibbon's shunt and (5) Waterston-Cooley's shunt.
1.2.2 ADULTS WITH REPAIRED TETRALOGY OF FALLOT

With decreasing pre- and immediate post-operative morbidity and mortality rates, major challenges in repaired ToF occur decades after corrective surgery. Course of disease in first 10-20 postoperative years is relatively benign. Difficulties start with entering to the adulthood [38].

Long-term sequelae in adults after ToF repair mostly pertain to right ventricular (RV) dysfunction and ventricular dysrhythmias. Both are associated with pulmonary valve regurgitation and, to a lesser extent, postoperative tissue changes and may ultimately reflect in detrimental impairment not only of echocardiographic (and magnetic resonance) parameters, but also of exercise performance, vascular function, cardiac autonomic function, natriuretic peptides and quality of life [26,36].

1.2.2.1 RIGHT VENTRICULAR DYSFUNCTION

In patients with repaired ToF, both systolic and diastolic RV function are usually impaired [39]. Progression of RV dilatation due to pulmonary incompetence occurs even in adolescents, only around 10 years post-surgery [40], but can be tolerated for decades before the first symptoms [41–43].

1.2.2.1.1 EXERCISE PERFORMANCE

Exercise capacity is reduced in patients with repaired ToF. Two major contributors have been identified. One is RV dysfunction and its pathophysiologic ramifications [28], as progression of RV volume overload and pulmonary vascular resistance may worsen exercise capacity [44]. The other is attitude towards physical activity. Parental and environmental protection [45], together with cautious recommendations by healthcare professionals in the past [46] likely lead to sedentary lifestyle of grown-ups with congenital heart disease. In fact, observational studies indeed show this population to be less physically active and less engaged to sports, as compared to healthy peers [47–49].

Exercise capacity impairment in adults with ToF is estimated in the range of two-thirds of expected performance compared to a healthy age- and matched adult [50,51]. Exercise capacity in adults with
repaired ToF is associated with parameters of RV function, such as ejection function [52,53], stroke volume [52], peak systolic velocity [54] and RV mass [52], as well as with parameters of left ventricular function, such as stroke volume [52] and diastolic impairment [55].

1.2.2.1.2 CARDIAC AUTONOMIC FUNCTION

Cardiac autonomic function represents an interplay between sympathetic and parasympathetic activity. As such, it plays an important role in regulating the electrical stability in the heart. Studies have shown that cardiac autonomic function is impaired in patients with repaired ToF [56–59]. This impairment is associated with RV dysfunction [59] through mechanisms, such as denervation due to cardiac surgery [58], haemodynamic changes and consequent widening of the QRS complex [57], or as an after-effect of impaired vascular properties [60].

Cardiac autonomic function can be appraised with various methods, most often with heart rate variability (HRV) and heart rate recovery (HRR). HRV is the beat-to-beat variation in time of consecutive heartbeats. It is a result of the balance between the influences of sympathetic and parasympathetic (vagal) activity on the sinoatrial node's intrinsic rhythm [61]. Although induction of the HR is a relatively stable system, changes in the environment require dynamic response and adaptation. In that sense, increased HRV is an indicator of a healthy heart [61]. HRV has been shown to predict mortality and morbidity in healthy individuals [62] and patients with various cardiovascular diseases [61,63,64]. Increased sympathetic activity and reduced vagal activity, which are reflected in impaired HRV, cause the reduction of the threshold for ventricular tachycardia [57]. Therefore decreased HRV might be associated with higher risk of ventricular tachycardia and sudden cardiac death in patients with repaired ToF [65].

Post-exercise HRR is another parameter of cardiac autonomic function. While HRV is a marker of parasympathetic modulation, HRR reflects parasympathetic tone [66]. HRR is also a predictor of mortality in healthy individuals and patients with various cardiovascular diseases [67,68] including grown-ups with congenital heart disease [69].
1.2.2.1.3 NATRIURETIC PEPTIDES

Natriuretic peptides are biomarkers of cardiac function. Their plasma levels represent a direct response to cardiac pressure, volume expansion and increase in wall stress [70]. As a result, a synthesis cascade starts and include pre-pro brain natriuretic peptide (BNP) and finally biologically active BNP, together with biologically inactive amino-terminal prohormone BNP (NT-proBNP) [71]. Both are extensively used as diagnostic markers in heart failure [72] and other cardiovascular conditions [73–75] including grown-ups with congenital heart disease [70]. As diagnostic markers, values of BNP and NT-proBNP are mostly analogue, but are not interchangeable. NT-proBNP is shown to be a superior predictor of mortality and morbidity in patients with heart failure [76].

Natriuretic peptide levels are increased in patients with repaired ToF mainly because of RV dysfunction [70]. Even in asymptomatic individuals, levels of natriuretic peptides are increased and correlate well with their functional class. They are also shown to be predictors of mortality [77].

1.2.2.1.4 FUNCTIONAL HEALTH STATUS AND QUALITY OF LIFE

Adults with repaired ToF nowadays live relatively normal life with a satisfactory health status. Studies have shown that their social functioning is preserved, they mostly avoid harmful behaviour habits, such as cigarette smoking, alcohol and illicit drugs [78]. Emotional status of adults with repaired ToF is comparable to that of healthy siblings [78] and healthy peers [79,80]. Conversely, physical domains of quality of life seem to be decreased in this population [81], suggesting that there are certain aspects in which adults with repaired ToF face difficulties [78]. Impairment of physical domains of quality of life have been associated with RV dysfunction [82].

As patients with repaired ToF – as well as grown-ups with congenital heart disease in general – have reduced physical domains of quality of life, an exercise training programme would be expected to improve health-related quality of life. While some studies have indeed confirmed this assumption [83], others have not [23,84].
1.2.2.2 VENTRICULAR DYSRHYTHMIAS

Ventricular dysrhythmias are highly prevalent in adults long after ToF repair. Possible mechanisms include pulmonary regurgitation-related haemodynamic alterations [85] and reentry physiology associated with post-ventriculotomy scarring, ventricular septal defect and outflow patch [86].

Also, they are believed to have a direct causative effect on sudden cardiac death (SCD) in patients with repaired ToF [87]. In this population, SCD remains a major cause of mortality with an estimated yearly risk of 0.2 % [88]. However, risk is not evenly distributed among age groups, but increases over time [87]. Factors associated with SCD are numerous and are classified as surgical, clinical, electrocardiographic and morphofunctional [65].

QRS duration is proved to be associated with cardiomegaly [85]. Increasing RV dilatation causes slower conduction and increased risk for ventricular arrhythmias. Significant increase of QRS over time is a predictor of ventricular tachycardia and QRS [85]. When resting duration of QRS reaches 180 ms, patients are at high risk of ventricular tachycardia and SCD [86].

1.2.2.3 VASCULAR FUNCTION

A limited body of evidence suggests that children and adults with repaired ToF have impaired vascular function. Structural abnormalities in the vasculature (i.e. increased elastic fibre fragmentation and increase in collagen amount [89]) and complex cardiothoracic surgical procedures are main determinants of impaired vascular properties in patients with repaired ToF [90]. Also, autonomic cardiovascular dysfunction [91], cyanosis [26] and sedentary lifestyle [49] have been identified as possible contributors.

Flow-mediated dilation (FMD) has emerged as an indicator of endothelial function, an early marker of cardiovascular disease and a predictor of cardiovascular prognosis [92,93]. Endothelial function was shown to be impaired in children early after ToF repair [94], but was not confirmed in adults with repaired ToF.
Arterial stiffness, as another indicator of vascular health, is also increased in patients with repaired ToF [90].

1.2.2.4 EXERCISE TRAINING IN PATIENTS WITH TETRALOGY OF FALLOT

The latest European Society of Cardiology guidelines recommend exercise for grown-ups with congenital heart disease [96]. Recommendations stem from acknowledging prevalent exercise intolerance in grown-ups with congenital heart disease on the one hand, and from acknowledging potential benefits of exercise drawn from other diseases from the other. However, guidelines provide rather general recommendations. Approach to exercise prescription should be individualised based on considerate appraisal, which includes history and physical examination, echocardiographic assessment and exercise testing [96].

Thus, exercise intensity should ideally be individually recommended based on initial evaluation. As for the frequency and duration, a minimum of 3-4.5 hours of physical activities per week is recommended. Ideally, a session should last at least 30 min and should be performed daily or almost daily. The recommendation is based on guidelines for recommended physical activity level in the general population and in other groups of cardiovascular patients [19].

Literature reports on exercise training in grown-ups with congenital heart disease are characterised by heterogeneous population samples and interventions. Duppen and colleagues systematically collected data on effects of published articles regarding exercise training in patients with grown-ups with congenital heart disease in 621 subjects from 31 studies. Programmes were very heterogeneous in terms of patient groups, age, programme and session duration, supervision and intensity. According to their systematic review, duration of exercise training programmes lasted from 6 to 52 weeks (mostly 12 weeks) with an average number of 3 training sessions per week. Continuous aerobic training (with or without resistance training) was mostly used, with various intensities up to 85 % of peak oxygen uptake (VO2 peak) [23]. No sudden cardiac deaths were reported; one patient died, but his death was not related to the exercise itself.
Therefore, in general, exercise training programmes were safe. Furthermore, exercise training programmes were in most instances efficacious in improving fitness levels [23]. Effects of exercise training programmes on exercise performance in patients with ToF were only examined in few studies [97–99], while none performed a comparative assessment of various exercise types.

1.3 PERIPHERAL ARTERIAL DISEASE

1.3.1 DEFINITION AND CLASSIFICATION

Peripheral arterial disease (PAD) is broadly defined as a narrowing of the body arteries, excluding intracranial and coronary arteries [100]. Over the last decades, however, the definition has been narrowed to encompass impaired perfusion of the limbs due to atherosclerosis, and mostly refers to lower limb atherosclerotic vascular disease [101]. As such, it is primarily defined by an ankle-brachial index (ABI) below 0.9 [102].

PAD can be categorised clinically or anatomically. Clinical classifications, such as Fontaine and Rutherford, are based on symptoms and signs of ischemia (Table 1) [103]. Clinical classifications are employed to determine an optimal management strategy, and discern stable PAD presenting with oxygen depletion to limb muscles during effort (intermittent claudication), which can be improved by walking (provided walking distance is long enough for exercise to be implementable and efficacious) [103], from severe forms yielding to ischemic tissue loss (chronic critical limb ischemia), which warrants intervention [104].
Table 1. Fontaine and Rutherford classifications of peripheral arterial disease [105]

<table>
<thead>
<tr>
<th>Fontaine classification</th>
<th>Rutherford classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage</td>
<td>Symptoms</td>
</tr>
<tr>
<td>I</td>
<td>Asymptomatic</td>
</tr>
<tr>
<td>IIa</td>
<td>Mild claudication</td>
</tr>
<tr>
<td>IIb</td>
<td>Moderate to severe claudication</td>
</tr>
<tr>
<td>III</td>
<td>Ischaemic rest pain</td>
</tr>
<tr>
<td>IV</td>
<td>Ulceration or gangrene</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Conversely, segmental classification is based on the anatomic location of the occlusion: aortoiliac, femoropopliteal or infrapopliteal, and is usually employed when considering endovascular or surgical treatment [105,106].

1.3.2 Atherosclerosis and Endothelial Dysfunction

PAD is a manifestation of atherosclerotic vascular disease. As such, i) its pathophysiology needs to be addressed in the context of atherosclerotic disease processes, ii) its prevalence in industrialised countries is high and rising, iii) it reflects a systemic disease with unfavourable prognosis, and iv) it is strongly associated with traditional and non-traditional cardiovascular risk factors [107–111].

Firstly, in terms of atherosclerotic pathophysiology, PAD is associated with derangements of vascular function and morphology. While the latter represent definite pathologic changes, which can be employed in diagnostic appreciation (e.g. ultrasonographic detection of plaques [112]), the former represent early and/or subtle changes, which can be appreciated in early stages of atherosclerotic disease and may provide delectable short-term responses to interventions (e.g. various methods for detection of endothelial dysfunction and arterial stiffness). Endothelial dysfunction is believed to be an early manifestation of atherosclerotic disease. Endothelial dysfunction derives from disturbances in endothelial shear stress yielding reduced nitric oxide (NO) bioavailability, increased vascular cell-adhesion molecules expression, and impaired endothelial cell repair, inducing endothelial cell apoptosis [113–115]. Further pathogenetic
steps include subendothelial accumulation of low-density lipoprotein cholesterol (LDL-c), increase in reactive oxygen species and eventually proliferation of the smooth muscle cells [113,116]. Disturbances of endothelial shear stress also result in expression of chemoattractant and pro-inflammatory cytokines, promoting foam cell infiltration of the arterial wall, thus continuing the progression of atherosclerosis, further aggravated by other risk factors, such as cigarette smoking, hyperlipidemia, hypertension and diabetes mellitus [113,114].

Besides macrocirculation, microcirculation is also impaired in PAD [117–119]. Microcirculation comprises the capillaries, arterioles, venules, small lymphatic vessels and (in a wider sense) the endothelium that covers these structures and the circulating cells. Within this system, components interact with each other and together contribute to maintaining circulatory, metabolic and coagulative homeostasis [120]. Endothelial dysfunction affects microcirculation as well due to impaired endothelial vasodilators synthesis, such as NO [121], and impaired vasodilatation of microcirculatory structures, which have been determined as key elements in the pathogenesis of coronary artery disease and PAD [122].

Secondly, in terms of high and rising prevalence, literature reports determine prevalence of PAD to be around 1.3 % [109]. However, estimations of asymptomatic PAD may be much higher, between 3 and 10 % of the population, with up to 20 % in individuals older than 70 years [108]. In Slovenia, the estimated prevalence of PAD in 50- to 70-year-old individuals is 18.7 % (103). Gender distribution depends on age, with male preponderance in younger age and virtually equalized gender distribution in older populations (102).

Thirdly, in terms of systemic atherosclerotic vascular disease, strong overlapping of PAD, coronary and cerebrovascular disease has been consistently demonstrated [110,123]. Hence, unfavourable prognostic ramifications of other atherosclerotic vascular diseases also apply to PAD, with mortality rates 2 to 3 times higher as compared to healthy controls [124–126].
Fourthly, in terms of risk factors, atherosclerosis is strongly associated with a set of modifiable risk factors, such as hypertension (present in up to 92% of PAD patients [127]), dyslipidemia (in 77% of PAD patients [128]) and diabetes (in at least 20% of PAD patients [129]). More importantly, lifestyle-related risk factors – smoking (present in 50% of PAD patients [130–133]), obesity-related metabolic derangements and sedentary lifestyle [134] – represent potent, albeit changeable drivers of atherosclerosis disease progression [19].

Lastly, and most importantly, therapeutic approaches to PAD are similar to those for other atherosclerotic vascular disease. Therapeutic approaches consist of secondary prevention and interventions aimed at improving limb perfusion. Secondary prevention includes evidence-based interventions with proven efficacy in terms of morbidity and mortality reduction, namely pharmacologic therapy (antiplatelets, statins and renin-angiotensin-aldeosterone system inhibitors), risk factor control and lifestyle interventions (a Mediterranean-type diet, smoking cessation in smokers and exercise) [19]. Interventions improving limb perfusion and providing ischemic symptom relief include exercise (walking), and endovascular or surgical revascularisation procedures, depending on symptomatic and anatomic extent of PAD [102]. Of note, exercise falls into both secondary prevention and limb perfusion (symptom relief) improvement domains; as such it represents a pivotal therapeutic approach to PAD and deserves special attention [19,107,111].

1.3.3 EXERCISE VS. OTHER TREATMENT MODALITIES

Walking emerged as the most effective exercise for improving PAD symptoms. It is recommended by all relevant guidelines as the preferred form of exercise in rehabilitation of patients with PAD [135–137]. Supervised exercise training recommendations are backed with strong evidence (level of recommendation IA), while non-supervised exercise provides slightly lower effectiveness and is therefore recommended with a IB level [136]. Exercise (walking) – as opposed to percutaneous or surgical revascularisation – does not improve perfusion only directly (on a macrovascular level), but also tends to improve microvascular perfusion and collateral circulation (angiogenesis), and also promotes more efficient energy utilisation in
skeletal muscles (metabolic changes in muscle, which include improving oxidative phosphorylation at the molecular level, multiplication of mitochondria at the cellular level and changes in muscle fibres at the tissue level) [138].

Animal models have demonstrated direct effects of exercise on atherosclerotic disease [139]. Exercise training has been shown to improve atherosclerotic plaque stability [140,141], inflammatory [142] and antioxidative status in apolipoprotein E knockout mice [143]. As in humans, potential mechanism for these effects is explained with regulation of nitric oxide synthesis [143,144].

While walking is the preferred exercise modality for PAD patients, the body of evidence pertaining to frequency, intensity, type and duration of exercise programmes is heterogeneous. Exercise training modalities from the literature reports are summarised in Table 2.

Meta-analyses have shown that supervised programmes are more effective in improving walking distance compared to non-supervised walking [145]. However, Gardner and colleagues have shown that a well structured exercise at home, supported by electronic devices, may provide similar effectiveness as a supervised training in a hospital setting [146]. Similarly, McDermott and colleagues demonstrated that carefully structured exercise in a home environment can be adequately effective [147,148].

Also, some patients are unable to walk because of conditions, such as severe claudication pain, diabetic foot complications, non-vascular conditions (joint inflammations, degenerative diseases of the nervous system) and non-peripheral vascular diseases (stroke sequelae and congestive heart failure), or low pain threshold [149]. Guidelines do not address rehabilitation in terms of improving PAD symptoms in these patients. However, two meta-analyses (of a relatively small number of studies) suggest that PAD symptoms may be improved with exercise modalities other than walking, such as cycling, arm-cranking exercise, and progressive resistance training. Nonetheless, both meta-analyses concluded that further randomized trials are needed to better define appropriate frequency, intensity, type and duration of exercise in patients with PAD [149,150].
Invasive treatment modalities for treatment of PAD include endovascular and surgical procedures. Literature reports provide data about their efficacy, with or without comparison to exercise training. One study compared exercise training, peripheral artery revascularization with stenting and optimal medical care, and suggested that exercise training provides superior short-term and similar long-term effects as compared to stenting [151,152]. While meta-analyses failed to identify the best management options, they do suggest that exercise training programmes should be favoured and applied whenever possible [153].

PAD management consists of secondary prevention and interventions aimed at improving limb perfusion. Exercise training addresses both aspects. While revascularisation procedures may become increasingly important in advanced PAD disease (symptoms at rest or risk of critical limb ischemia), exercise plays a pivotal role in patients with intermittent claudication. Also, cost effectiveness analysis favours exercise as a cost-effective treatment modality [154,155], suggesting that exercise (walking) is even more cost-effective when combined with percutaneous revascularisation than revascularisation alone [155].

Therefore, current guidelines recommend exercise training as the first step for the management of most PAD patients (especially in earlier symptomatic stages). The exact modality of exercise, however, remains elusive.
Table 2. Literature review of exercise training modalities

<table>
<thead>
<tr>
<th>Type</th>
<th>Intensity</th>
<th>Rest</th>
<th>Time and progression</th>
<th>Frequency</th>
<th>Duration</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gardner <em>et al.</em></td>
<td>treadmill with speed 3.2 km/h at a slope</td>
<td>Slope adjusted at 50-80 % of the maximum load at exercise. Patients walked until the onset of pain (3-4 on a 5-point scale).</td>
<td>Until the pain was completely gone</td>
<td>3 times a week</td>
<td>6 months</td>
<td>[133,156–158]</td>
</tr>
<tr>
<td>Polish group</td>
<td>treadmill with speed 3.2 km/h at a slope adjusted to the claudication onset time</td>
<td>Slope adjusted to reach pain after 3-5 minutes</td>
<td>35-minute sessions prolonged for 5 minutes every 2 weeks</td>
<td>3 times a week</td>
<td>3 months</td>
<td>[159,160]</td>
</tr>
<tr>
<td>Australian group</td>
<td>treadmill with speed 3.2 km/h</td>
<td>Walking until pain 3-4 on a 5-point scale</td>
<td>No data</td>
<td>25-minute sessions with individually adjusted intensity (protocol not disclosed)</td>
<td>3 times a week;</td>
<td>6 and 12 months</td>
</tr>
<tr>
<td>Austrian group</td>
<td>treadmill speed adjusted to elicit claudication within 3-5 minutes; no data on slope</td>
<td>Walking until pain 3-4 on a 5-point scale</td>
<td>Brief resting period to resolve symptoms</td>
<td>35 minutes with gradual increase up to 50 minutes</td>
<td>2 times a week</td>
<td>6 months</td>
</tr>
<tr>
<td>Hodges <em>et al.</em></td>
<td>treadmill with speed 3.2 km/h at a slope</td>
<td>75 % of the maximum slope at exercise testing; stopped at severe pain</td>
<td>Until the pain was completely gone</td>
<td>30 minutes; no report on progression</td>
<td>2 times a week</td>
<td>3 months</td>
</tr>
<tr>
<td>Seatre <em>et al.</em></td>
<td>ordinary walking, no data on walking speed and slope</td>
<td>Walking until approximate maximum pain</td>
<td>Until the pain was completely gone</td>
<td>30-minute sessions prolonged for 5 minutes every week</td>
<td>2 times a week</td>
<td>2 months</td>
</tr>
<tr>
<td>Cucato <em>et al.</em></td>
<td>treadmill with speed 3.2 km/h at a slope based on claudication onset heart rate</td>
<td>Slope adjusted to reach heart rate, which was attained at claudication during exercise testing</td>
<td>2 minutes</td>
<td>15 cycles consisting of 2-minute walking</td>
<td>2 times a week</td>
<td>3 months</td>
</tr>
<tr>
<td>CLEVER study</td>
<td>treadmill speed and slope adjustment based on claudication onset time</td>
<td>Walking until pain; 3-4 on a 5-point scale</td>
<td>Until the pain was completely gone</td>
<td>50-minute sessions; progression based on claudication onset time</td>
<td>3 times a week</td>
<td>6 months</td>
</tr>
</tbody>
</table>
1.3.1 EXERCISE TRAINING MODALITIES

The American Heart Association/American College of Cardiology Foundation guidelines recommend supervised exercise to be carried out for 12 weeks or more, at least three times a week. Individual training should last 30 to 45 minutes [136]. Similarly, European guidelines recommend at least 12-week programme with a frequency of three times a week and each training lasting from 30 to 60 minutes. However, they emphasize the importance of continual and gradual increases in the intensity of training sessions throughout the programme [137].

Most studies of exercise programmes in PAD lasted from 1 to 12 months, most commonly 3 or 6 [156,159,162,163,170]. Trainings took place three times a week in most studies, in some only twice [133,161,166,171]. Studies in which the training intensity was increased during the programme, determined the progression by either a predefined protocol, i.e. on the number of training sessions, or the progress was assessed individually for each patient. In some studies, the intensity of training did not change during the duration of the programme [166].

1.4 EVIDENCE GAPS

Despite numerous studies supporting the efficacy of exercise training in terms of improved cardiovascular health, the body of evidence is still limited, especially when compared to research of pharmacotherapy or interventions. Historically, most studies have addressed the effects of exercise in patient populations with high levels of professional attention, such as coronary artery disease or heart failure patients [15,172]. Several patient populations were therefore excluded, but have recently gained deserved interest because of incurring epidemiological trends. Moreover, most studies of exercise training have either focused on a single exercise modality (compared to no exercise at all) and have thus failed to address possible specific merits and drawbacks of different exercise in terms of frequency, intensity, type and duration.
Our interests have specifically focused on two such patient populations – grown-ups with congenital heart disease and patients with PAD. While the former represent an ever growing population (due to effective management in childhood), the latter represent a substantial portion of patients with atherosclerotic disease, which is usually underrepresented in studies of atherosclerosis management. Also, these populations represent models of "cardiac" and "vascular" disease population, respectively; exercise provides distinctive cardiac and vascular effects and appraising differential cardiac and vascular effects of exercise in specific "cardiac" and "vascular" disease populations may provide a more comprehensive appreciation of exercise effects in the human body.

Furthermore, comparison of different exercise modalities may improve our current knowledge of exercise physiology and provide directions both in terms of patient management options and future research. In terms of intensity, exercise modalities can be broadly categorised as moderate or intensive. The first provides a moderate activation of the cardiovascular system (e.g. moderate rise in HR, moderate ischemic limb pain); as such, it is more comfortable and possibly safer for the patient, but its efficiency has been recently questioned. The second requires a near-maximal (patho)physiologic response (e.g. near-maximum rise in HR, maximal tolerable ischemic pain); as such it may provide superior training effects, but its safety remains a concern and its long-term effects yet to be proven.

Aerobic-dynamic exercise has been shown to be safest and most efficacious in cardiovascular patients; furthermore, different modalities of aerobic-dynamic exercise have also been studied. The most common distinction is between high-intensity interval and continuous exercise. High-intensity interval training pertains to alternating bouts of intense exercise with less intensive recovery periods. Continuous exercise training pertains to uninterrupted bouts of low- to moderate exercise without rest periods. Historically, continuous exercise training was the modality of choice for cardiovascular patients because of its postulated safety and demonstrated efficiency. However, recent studies suggest that high-intensity interval training is safe and may provide better results in terms of improve exercise capacity in patients with coronary artery disease and heart failure. As this training modality has not been carried out so far in
patients with ToF (and grown-ups with congenital heart disease in general), we decided to compare its efficacy with continuous training modality in terms of various “cardiac”, “vascular” and parameters of quality of life in patients with repaired ToF.

In patients with PAD, various exercise training (walking) protocols have already been assessed, with very few reports comparing two (or more) different training modalities. Unlike most studies, in which exercise intensity was mainly defined by the leg pain (claudication onset or moderate-to-maximum pain), we wanted to define exercise intensity with a HR range, as already performed in numerous exercise training reports on various cardiovascular conditions.

Guidelines suggest “walking to moderate-to-maximum claudication, alternating with periods of rest” (168). On the contrary, claudication onset could be regarded an unwanted ischemic event, suggesting that pain-free training protocols may be preferable. Comparison of these two training protocols is available (162), but without defining intensity by HR range that might induce changes in different cardiac and vascular parameters beyond walking capacity. Therefore, we decided to compare efficacy of moderate-pain and pain-free exercise training adjusted for intensity, which is defined by an exercise HR range defined as moderate (70 % of maximal predicted HR).

1.5 AIMS

The doctoral thesis explores the impact of different types of exercise training on selected parameters (exercise capacity, cardiac autonomic function, vascular function, disease-specific biomarkers and health-related quality of life) in different patient populations – adults with repaired ToF and patients with PAD. In terms of exercise training types, protocols have been selected to address the question whether moderate or intensive activation of the cardiovascular system brought about by exercise training provides specific effects. In terms of patient populations, adults with repaired ToF and patients with PAD have been selected because of their relative underrepresentation in previous studies of exercise training and because they
represent relative "cardiac" and "vascular" extremes of the cardiovascular disease spectrum. In terms of observable parameters, besides exercise capacity, disease-specific biomarkers and health-related quality of life, cardiac autonomic function and vascular function were selected because they may provide a differential appreciation of the cardiac and vascular effects of exercise in "cardiac" and "vascular" patients, respectively.

Thus, the aim of the doctoral thesis was to assess whether supervised moderate or intensive exercise training as compared to usual care (no supervised exercise training) in adults with repaired ToF and in patients with PAD differentially affect exercise capacity, cardiac autonomic function, vascular function, disease-specific biomarkers and health-related quality of life.

1.6 HYPOTHESES

1.6.1 PRIMARY HYPOTHESES

- Hypothesis 1a: Interval exercise training is associated with increased maximal oxygen uptake (magnitude of difference >1.5 ml/kg/min) as compared to continuous exercise training in patients with repaired ToF.
- Hypothesis 1b: Moderate-pain exercise training is associated with increased pain-free walking distance (magnitude of difference >100 m) as compared to pain-free exercise training in patients with PAD.

1.6.2 SECONDARY HYPOTHESES

- Hypothesis 2a: Interval exercise training is associated with a significant improvement of vascular function (increase in flow mediated dilation of brachial artery and decrease in pulse wave velocity) as compared to continuous exercise training in patients with repaired ToF.
- Hypothesis 2b: Moderate-pain exercise training is associated with a significant improvement of vascular function (increase in flow mediated dilation of brachial artery and decrease in pulse wave velocity) as compared to pain-free exercise training in patients with PAD.
- Hypothesis 3a: Interval exercise training is associated with a significant improvement of cardiac autonomic function (increase in heart rate variability and heart rate recovery) as compared with continuous exercise training in patients with repaired ToF.

- Hypothesis 3b: Moderate-pain exercise training is associated with a significant improvement of cardiac autonomic function (increase in heart rate variability) as compared to pain-free exercise training in patients with PAD.

- Hypothesis 4a: Interval exercise training is associated with a significant decrease in concentrations of NT-proBNP, D-dimer, fibrinogen as compared to continuous exercise training in patients with repaired ToF.

- Hypothesis 4b: Moderate-pain exercise training is associated with a significant decrease in concentrations of NT-proBNP, D-dimer, fibrinogen as compared to pain-free exercise training in patients with PAD.

- Hypothesis 5a: Interval exercise training is associated with a significant improvement of health-related quality of life (increased score on the SF-36 questionnaire) as compared to continuous exercise training in patients with repaired ToF.

- Hypothesis 5b: Moderate-pain exercise training is associated with a significant improvement of health related quality of life (increased score on the SF-36 questionnaire) as compared to pain-free exercise training in patients with PAD.
2. METHODS

2.1 STUDY POPULATIONS

2.1.1 PATIENTS WITH REPAIRED TETRALOGY OF FALLOT

Patients with repaired ToF were recruited from the Outpatient clinic for adults with congenital heart diseases at the Department of Cardiology, Division of Internal Medicine, University Medical Centre Ljubljana, Slovenia.

Inclusion criteria were:
- at least 18 years of age;
- complete correction of the defect in childhood.

Exclusion criteria were:
- known or symptomatic atherosclerotic disease;
- unstable cardiovascular disease or recent (<3 months prior to inclusion) cardiovascular events;
- acute illness or recent (<3 months prior to inclusion) non-cardiovascular diseases requiring hospital;
- emergency or unplanned specialist management;
- unstable or poorly controlled dysrhythmias;
- permanent atrial fibrillation;
- pregnancy;
- intellectual development disorder.
2.1.2 PATIENTS WITH PERIPHERAL ARTERIAL DISEASE

Patients with PAD were recruited from the Outpatient vascular disease clinic at the Department of Vascular Diseases, Division of Internal Medicine, University Medical Centre Ljubljana, Slovenia.

Inclusion criteria:
- at least 18 years of age;
- diagnosed peripheral arterial disease, classified as Fontaine stage II.

Exclusion criteria:
- unstable cardiovascular disease or recent (<3 months prior to inclusion) cardiovascular events;
- acute illness or recent (<3 months prior to inclusion) non-cardiovascular diseases requiring hospital;
- emergency or unplanned specialist management;
- unstable or poorly controlled dysrhythmias;
- permanent atrial fibrillation;
- contraindications for physical activity;
- pregnancy;
- intellectual development disorder.

2.2 STUDY DESIGN

2.2.1 GENERAL CONSIDERATIONS

Both studies were carried out at the Department of Vascular Diseases, Division of Internal Medicine, University Medical Centre Ljubljana, Slovenia between June 2015 and March 2017. Both cohorts, namely patients with repaired ToF and patients with PAD, were randomised into 3 groups (2 interventional and 1 control group) with a 1:1:1 ratio. Each cohort comprised 1 strenuous training-arm (interval and moderate-
pain in studies in patients with repaired ToF and PAD, respectively) and 1 lenient training-arm (continuous and pain-free in studies in patients with repaired ToF and PAD, respectively).

2.2.2 PATIENTS WITH REPAIRED TETRALOGY OF FALLOT

The study was designed according to the Consolidated Standards of Reporting Trials (CONSORT) guidelines [173], adapted for three parallel groups (Fig 3).

This open-label study was approved by the National Medical Ethics Committee. It has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. Written consent was obtained from all participants prior to their inclusion in the study. Interventional part of the study was registered at the clinicaltrials.gov with ID NCT02643810.
Fig 3. Study design of influence of exercise training in patients with repaired tetralogy of Fallot according to CONSORT guidelines

2.2.3 PATIENTS WITH PERIPHERAL ARTERIAL DISEASE

The study was designed according to the Consolidated Standards of Reporting Trials (CONSORT) guidelines [173], adapted for three parallel groups (Fig 4).
Fig 4. Study design of influence of exercise training in patients with peripheral arterial disease according to CONSORT guidelines.
This open-label study was approved by the National Medical Ethics Committee. It has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. Written consent was obtained from all participants prior to their inclusion in the study.

Interventional part of the study was registered at the clinicaltrials.gov with ID NCT02642276.

2.3 INTERVENTION

2.3.1 REPAIRED TETRALOGY OF FALLOT

Patients were randomised to either of the two intervention groups (interval training group and continuous training group) or control group.

Interventions consisted of 36 training sessions, 2-3 times per week. Patients were cycling and/or speed walking, according to their preferences. Intensity parameter was a percentage of HR peak reached on the exercise testing at the inclusion measurements. Control group was advised to continue with regular physical activities.

Protocol for the interval training group consisted of 5-min warm-up and 5-min cool-down periods at 50 % HR peak intensity. Main part had 8 cycles. Each cycle consisted of 1 minute exercise at the intensity of 80 % HR peak followed by 3 minute exercise at the intensity of 60 % HR peak. The whole training lasted 42 minutes. Intensity of the exercise during the main part was increased after 12th and 24th training session, each part of the interval was increased for 5 % points of HR peak.

We planned to use the same interval scheme as in the study of Rognmo and colleagues [174], but as patients were living mostly sedentary life and were physically inactive, we decided to modify the scheme of the intervals. Instead of 4 cycles consisting of 4 minutes at higher intensity followed by 3 minutes at lower intensity, we used 8 cycles consisting of 1 minute at higher intensity followed by 3 minutes at lower intensity.
Protocol for the continuous training group consisted of 8-minute warm-up and 7-minute cool-down periods at 50 % HR peak intensity. Main part was calculated in order to maintain the same total training load (total energy expenditure) as in the interval training group, as we considered that relationship between HR peak and peak workload (expressed as VO2 peak) is linear. This resulted in the main part of the training lasting 26 minutes. Similar to interval training protocol, intensity in the main part was increased for 5 % points of HR peak after 12th and 24th training session.

Both protocols were progressive in terms of intensity with time. Fig 5 depicts the increase in intensity after 12th and 24th training sessions for both interval and continuous training protocols. For the purpose of illustration, a short segment of 7 minutes for each of the two protocols is presented.
Fig 5. Increase in intensity of training sessions for interval (a) and continuous (b) training protocols.
Profiles of the two training protocols are visually compared on Fig 6. Overlapping of both protocols shows differences in intensity and time to scale, required for maintenance of the same total energy expenditure in both training groups.

![Fig 6. Comparison of profiles of interval and continuous training protocols](image)

### 2.3.2 PERIPHERAL ARTERIAL DISEASE

Patients were randomised to either of the two intervention groups (moderate-pain training group and pain-free training group) or control group.

Interventions consisted of 36 training sessions, 2-3 times per week. A single training session lasted around 60 minutes. Main part of the training session was walking on a treadmill, followed by active recovery by cycling on an exercise bike with no resistance. Intensity parameter was set on around 70% (±5%) of predicted maximal HR (HRmax), which was calculated with the formula: HRmax = 220 - age. In patients taking beta blockers, an effort was made to determine resting HR prior to beta blocker therapy (e.g. from available documentation/patients' history). If reliable information could be captured, the difference between beta-blocking therapy-free resting HR and actual resting HR was calculated and expressed as percentage. Predicted HRmax (calculated with the above mentioned formula) was then decreased for the
before mentioned percentage. If reliable information could not be captured, their predicted HRmax was calculated with the following formula: HRmax = 164 − 0.7 x age [175].

Patients randomised to the moderate-pain training group were walking on treadmill until feeling moderate pain (3 to 4 out of 5 on pain scale) in legs. Walking on a treadmill was followed by cycling on an exercise bike for at least 5 minutes or until the leg pain was gone. Setting the treadmill speed was based on intensity of 70% of predicted HRmax, without inclination (Fig 7). The same speed was used in following walking sessions until a patient improved. Improvement was defined if his/her HR during walking was less than 65% of predicted HRmax. In such case, treadmill speed was increased for 0.3 km/h.

Patients randomised to the pain-free training group were walking on a treadmill up to two-thirds of the claudication onset distance (Fig 7). Claudication onset distance was measured in 3 situations, as follows: (a) every 6th training session; (b) every time a treadmill speed was changed and (c) if a claudication pain showed up unexpectedly during walking on the treadmill. Also, walking on a treadmill was followed by cycling on an exercise bike for at least 5 minutes. The treadmill speed was based on 70% of predicted HRmax of a patient. Improvement was defined similarly as in a moderate-pain training protocol.

Control group was advised to continue with usual activities in the period of approximately three months.
2.4 OBSERVED VARIABLES

In order to analyse the effects of different types of exercise training, observed variables were assessed twice – before and after the intervention period. Selected set of variables was used to assess exercise capacity, vascular function, cardiac autonomic function, disease-related biomarkers and health-related quality of life. Table 3 shows which tests were performed in each study, namely patients with repaired ToF and patients with PAD.
Table 3. Methods performed on patients in each of the two studies

<table>
<thead>
<tr>
<th>Exercise capacity</th>
<th>Patients with repaired tetralogy of Fallot</th>
<th>Patients with peripheral arterial disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiopulmonary exercise testing</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>6 minute walk test</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Treadmill walking test</td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>Ankle-brachial index</td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>Vascular function</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flow-mediated dilation</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Carotid artery stiffness</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Cardiac autonomic function</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate variability</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Heart rate recovery</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Biomarkers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complete blood count</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Lipid profile</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Markers of inflammation, haemostasis and heart failure severity</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Quality of life</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SF-36 questionnaire</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>IPAQ questionnaire</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>

2.4.1 CLINICAL DATA

Patients underwent thorough clinical appraisal prior to inclusion, and inclusion and exclusion criteria were considered in details. Weight and height were measured and body mass index (BMI) was calculated as the ratio between weight (in kilograms) and square height (in meters). Blood pressure was assessed with sphygmomanometry and resting HR was assessed with electrocardiography. Medical history, current medication, and cardiovascular signs and symptoms were systematically recorded. Conditions, which may affect observable parameters, were specifically addressed, namely coronary artery disease (defined as history of myocardial infarction, evidence of myocardial ischemia on functional tests, evidence of coronary obstruction on angiographic examination), cerebrovascular disease (defined as history of stroke, transient ischemic attacks, amaurosis fugax, audible carotid bruits or ultrasonographic evidence of carotid plaques), chronic kidney disease (defined as estimated glomerular filtration rate <60 ml/min), diabetes mellitus (fasting blood glucose >7 mmol/L or postprandial blood glucose levels >11.1 mmol/L or management with antidiabetic medication), dyslipidemia (blood lipid levels above target levels based on current guidelines or
management with lipid-lowering medication), hypertension (blood pressure >140/90 mmHg or management with antihypertensive medication).

Patients with repaired ToF were additionally appraised for detailed history of cardiac surgeries, including age at and type of specific surgery.

Patients with PAD were additionally appraised for detailed history of intermittent claudication.

2.4.2 EXERCISE CAPACITY

2.4.2.1 CARDIOPULMONARY EXERCISE TESTING

Maximal cardiopulmonary exercise testing was carried out in a laboratory using cycle ergometer Schiller CS-200. Standardised exercise testing protocol consisted of 3 minute without workload and then gradual increase in workload by one tenth of maximal estimated workload per minute, which was calculated on the basis of age, gender and height.

All measurements were calibrated for environmental conditions, such as temperature, pressure and relative humidity. As part of calibration, spirometry was performed. Participants were wearing a mouthpiece, which was connected with the device, thus permanently measuring oxygen (O2) and carbon dioxide (CO2) flow during exercise (VO2 and VCO2, respectively). Anaerobic threshold (AT) was defined when ratio between VO2 and VCO2 was 1.0. Participants were ECG and HR monitored throughout exercise testing and during the cool-down period. Blood pressure was manually measured every 2 minutes. Patients gave their maximal effort before they stopped with cycling.

Following data were obtained from the exercise testing:

- peak workload (work rate), expressed in Watts;
- percentage of expected peak work rate, based on age, gender and BMI;
- calculated exercise capacity, estimated from the work rate and expressed in metabolic equivalents (METs);
- VO2peak – a measure of peak aerobic capacity during exercise testing. Officially, it is expressed in ml/min, but often normalised by body weight and expressed in ml/kg/min;
- percentage of expected VO2peak, based on age, gender and BMI;
- VO2 at AT, expressed in ml/kg/min;
- HR (resting, during peak exercise, and during recovery);
- systolic and diastolic blood pressure (resting, during peak exercise and during recovery);
- peak double product (DPpeak) calculated as the product of peak systolic blood pressure and peak HR during peak exercise (DPpeak = SBPpeak x HRpeak);

Chronotropic competence was calculated with the formula: HRpeak/(220 - age).

2.4.2.2 SIX-MINUTE WALK TEST

Six-minute walk test (6MWT) is a surrogate exercise test that has been validated in several cardiovascular observational studies and trials [23,176]; it is the distance that a patient is able to walk within 6 minutes. Testing was performed in a 35-m corridor. Turnaround points were visibly marked. During the test, patients were encouraged according to the guidelines [177].

2.4.2.3 WALKING TESTING

Walking capacity was assessed on a treadmill in morning hours with a standardized speed of 3.2 km/h and an inclination of 12.5 %. During the treadmill test, patients were asked to let us know on the onset of the claudication pain and walk further until the maximal pain (level 5 out of 5 on a pain scale). The distance they walked until the onset of the claudication pain and maximal walking distance were recorded and expressed in meters.

2.4.2.4 ANKLE-BRACHIAL INDEX

Ankle-brachial index (ABI) is a diagnostic parameter of PAD. It is evaluated using Doppler measurements of perfusion pressures on the right brachial artery and both dorsalis pedis and tibialis posterior arteries. ABI
of a leg is calculated by dividing higher of the pressures from the same leg by the systolic brachial pressure. ABI measurements were performed before and continuously after treadmill walking test, until pressures reached resting values.

2.4.3 VASCULAR FUNCTION

Aloka Prosound α7 ultrasound machine was used for assessment of vascular function. Selected parameters of the vascular function were measured: flow-mediated dilation and carotid artery stiffness.

2.4.3.1 FLOW-MEDIATED DILATATION

Flow-mediated dilation was measured on the right brachial artery. Participants were asked to lay still throughout the procedure. The first step was to visualize the artery approximately 5 cm above the antecubital fossa. After having the artery visualized in the horizontal position on the screen, 3 measurements of the arterial diameter were obtained \(d_1\). Next step was inflating the cuff just below the antecubital fossa with the pressure which is 50 mmHg above the systolic blood pressure. Ischemia was maintained for 4.5 minutes. Sixty seconds after deflation of the cuff, 3 measurements of the arterial diameter were obtained \(d_2\). FMD was calculated with the following formula:

\[
FMD = \left( \frac{d_2 - \overline{d_1}}{\overline{d_1}} \right) \cdot 100\%
\]

where \(\overline{d_1}\) and \(\overline{d_2}\) represent mean arterial diameters before and after cuff was inflated, respectively.

2.4.3.2 CAROTID ARTERY STIFFNESS

Carotid artery stiffness parameters, namely \(\beta\) stiffness coefficient and pulse wave velocity (PWV), were measured with dedicated echo-tracking, which allows us to determine carotid stiffness parameters through the analysis of the pulse waves. Measurements were performed on the right common carotid artery (RCCA). Patients had their head elevated of around 45° and 30° tilted to the left. After visualization of RCCA of around 2 cm before the bifurcation, the cursor pair was positioned on the anterior and posterior walls of
the artery. The software, which is an integral part of the device and is calibrated on the basis of systolic and
diastolic blood pressure values, analyzed RCCA’s diameter change waveforms and automatically calculated
β stiffness coefficient and PWV [178]. Calibration of the blood pressure was performed twice, during which
6 beats were made/taken. Therefore, β coefficient and PWV were calculated as means of 12 beats.

2.4.4 CARDIAC AUTONOMIC FUNCTION

2.4.4.1 HEART RATE VARIABILITY

HRV recordings were performed between 8 am and 10 am. Patients were lying in the supine position for at
least 10 minutes before measurements. Recordings were performed in a quiet room, with all electronic
devices and lights being turned off. Resting 5 minute high resolution ECG recordings were acquired using a
dedicated recording device (Cardiax®, IMED, Budapest, Hungary). ECG data were analysed using a
dedicated software package (Kubios v. 2.2, University of Eastern Finland, Kuopio, Finland). After correction
of premature beats and artefacts, HRV time and frequency domain parameters were calculated. Frequency
domain parameters were obtained using autoregressive model order 16, without factorisation.

Selected time domain parameters were used [61]:
- SDNN - standard deviation (SD) of all normal RR intervals (NN);
- rMSSD – square root of the mean of the sum of squares of successive NN interval difference and
  reflects vagal modulation;
- pNN50% - percentage of successive NN intervals differing by more than 50 ms reflecting vagal
  function.

Selected frequency domain parameters were used [61,179]:
- HFnu – normalised power in the high frequency range (0.15 Hz to 0.4 Hz), obtained with
  autoregressive method using the following formula: Hfnu = total power – low frequency power –
  very low frequency power;
- LFnu – normalised power in the low frequency range (0.04 Hz to 0.15 Hz), obtained with autoregressive method using the following formula: LFnu = total power – high frequency power – very low frequency power;

- LF:HF – ratio of normalised low- and high-frequency powers, which may reflect sympathovagal balance.

2.4.4.2 HEART RATE RECOVERY

HRR parameters were obtained during and immediately after cardiopulmonary exercise testing. After a patient reached his/her peak work rate, he/she remained seated on the cycle ergometer and HR was continuously measured in the following 4 minutes. HRR parameters were calculated using the following formula:

\[ \text{HRR}_X = \text{HR}_{\text{peak}} - \text{HR}_{\text{in the Xth minute after termination of the exercise testing}} \]

in which X takes values from 1 to 4 (HRR\(_1\), HRR\(_2\), HRR\(_3\) and HRR\(_4\)).

2.4.5 BIOMARKERS

All patients had venous blood samples taken after 30 minutes of rest in supine position from the cubital vein. For biochemical blood testing, 5 ml of blood was taken to the collection tube containing no anticoagulant. For complete blood count test, 3 ml of blood was taken to the collection tube containing ethylenediaminetetraacetic acid (EDTA). For haemostasis blood testing, 15 ml of blood was taken to the Beckton Dickinson Vacutainer\(^\circledR\) Citrate Tubes.

Complete blood count was obtained from the whole blood. Red blood cells, haemoglobin, haemotacrit, white blood cells with differential count and platelet counts were determined with haematologic analyser Comas Minos Stex\(^\circledR\), Roche; Basel, Switzerland.

Serum samples were obtained with centrifuging the whole blood at 3000 rpm for 10 min. Total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c) and triglycerides were colourimetrically determined on
the day of sampling (Ektachem 250 Analyzer®, Estman Kodak Company; Rochester, Minnesota, USA). LDL-c was determined with Friedewald equation [180]. Remaining serum for NT-proBNP analysis was stored at -70 °C. NT-proBNP levels were determined using sandwich chemiluminescent immunoassay with two antibodies (Roche diagnostics) and on an Elecsys 2010 analyser.

Plasma samples were prepared with centrifuging citrated blood at 3000 rpm for 30 min at 0 °C. Samples were then stored at -70 °C and later used for determination of D-dimer and fibrinogen. Fibrinogen (Multifibren U) and D-dimer (TriniLIA Auto-Dimer, Trinity Biotech, Bray, Ireland) were determined on an automated coagulation analyser (Behring Coagulation Timer, Dade Behring, Marburg, Germany).

2.4.6 QUALITY OF LIFE

2.4.6.1 HEALTH RELATED QUALITY OF LIFE

For quality of life assessment, the 36 item Short Form Survey (SF-36) was used. It is a public domain questionnaire, developed at the RAND Corporation as a part of the Medical Outcomes Study. SF-36 is widely used in various health conditions, including grown-ups with congenital heart disease [78,80,181] and exercise training interventional studies [84,182]. It is translated and validated in Slovenian language [183].

The questionnaire consists of two components, mental and physical. Mental domain is divided into the following four categories: social functioning, role emotional, vitality and mental health. Physical domain includes four categories: physical functioning, role physical, bodily pain and general health.

Social functioning assesses if a subject performs normal social activities and if mental and physical domains interfere with these activities. Role emotional refers to problems with work or other daily activities, which result from emotional problems. Vitality examines how energetic or tired a subject is. Mental health refers to the extent of nervousness and depression on one side, and happiness and calmness on another.

Physical functioning assesses if a subject performs all types of physical activities, or if they are limited and to what extent. Role physical examines if a subject is having problems with work or other daily activities as
a result of physical health. Bodily pain refers to the potential limiting pain. General health considers subject's perception of his/her personal health [184].

For calculation of categories and domain scores, German normatives for SF-36 questionnaire were used [185].

2.4.6.2 SELF-ASSESSED PHYSICAL ACTIVITY LEVEL

For determination of the physical activity level, we used short form of the International Physical Activity Questionnaire (IPAQ). It has been validated as a reliable measurement tool for assessing physical activity level. It is an open access questionnaire and can be obtained from the official homepage www.ipaq.ki.se.

The IPAQ consists of questions regarding vigorous, moderate and walking activities and volumes of each category. Resulting physical activity level is calculated with the following formula, suggested by its developers [186]:

\[
\text{Total MET-minutes/week} = \Sigma \text{walk} + \Sigma \text{moderate} + \Sigma \text{vigorou}\text{s}
\]

in which

\[
\Sigma \text{walk} = \text{days per week} \times \text{minutes per day} \times 3.3 \text{ METs};
\]

\[
\Sigma \text{moderate} = \text{days per week} \times \text{minutes per day} \times 4.0 \text{ METs};
\]

\[
\Sigma \text{vigorou}\text{s} = \text{days per week} \times \text{minutes per day} \times 8.0 \text{ METs}.
\]

2.5 STATISTICAL METHODS

2.5.1 SAMPLE SIZE CALCULATION

Sample size calculation suggested that 27 patients with repaired ToF (9 patients per group) should be included in order to detect a difference of at least 1.5 ml/kg/min (primary endpoint in the study with
repaired ToF) with a power of 0.8, at a level of statistical significance of less than 0.05 (two sided). We decided to include 30 patients, 10 to each group.

Sample size calculation in the study with patients with PAD suggested that 45 patients (15 patients per group) should be included in order to detect a difference of at least 100 m (primary endpoint in the study with PAD) with a power of 0.8, at a level of statistical significance of less than 0.05 (two sided). We decided to include 45 patients, 15 to each group.

2.5.2  STATISTICAL ANALYSIS

Normal distribution of variables were qualitatively determined and graphically described for each variable and confirmed with the Shapiro-Wilk test. Normally distributed continuous variables were described with mean values and standard deviations. Asymmetrically distributed continuous variables were described with medians and interquartile ranges. Categorical variables were described as numbers and/or percentages. If appropriate, asymmetrically distributed variables were log-transformed in order to obtain more symmetrical distributions throughout the statistical calculations.

Differences between groups were determined with the Student’s t-test for normally distributed variables and with the Mann-Whitney U test for asymmetrically distributed variables. Differences between groups of categorical variables (percentages) were assessed with chi-square test.

Effects of intervention were determined with the paired samples t-test for normally distributed variables and with the Wilcoxon U paired test for asymmetrically distributed variables.

The one-way analysis of variance (ANOVA) was used for comparison of variables among more than two groups with Bonferroni adjustment for multiple comparisons.

All data were analysed using IBM SPSS Statistics v. 20 software package. A p value less than 0.05 was considered statistically significant.
3. RESULTS

3.1 REPAIRED TETRALOGY OF FALLOT

A total of 62 patients were screened for eligibility, among which 30 were chosen to start with the intervention (Fig 8). Three patients did not complete the study, 1 from each group. A total of 27 patients have completed the study, 9 from each group (Fig 3).

Fig 8. Enrolment and allocation of patients in a study of patients with repaired tetralogy of Fallot

Some data for specific patients and measurements are not available due to (Fig 9):

- supraventricular tachycardia (SVT) - HRR measurements were not assessed (1 patient),
- permanent pacemaker – HRV measurements were not collected (1 patient).
Baseline data are summarised in Table 4. Average age of patients who completed the study was 38.5 (± 8.7) years, male:female ratio was 10:17. There were no significant differences among three groups in terms of age (p=0.557), gender (p=0.530) and BMI (p=0.676).

**Table 4. Baseline clinical data of patients with repaired tetralogy of Fallot who completed the study**

<table>
<thead>
<tr>
<th>Patients who completed the study (n=27)</th>
<th>Interval training group (n=9)</th>
<th>Continuous training group (n=9)</th>
<th>Control group (n=9)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>General data</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, mean (SD), years</td>
<td>38.5 (8.7)</td>
<td>36.2 (6.8)</td>
<td>40.1 (10.4)</td>
<td>38.4 (8.9)</td>
</tr>
<tr>
<td>Male:female</td>
<td>10:17</td>
<td>2:7</td>
<td>4:5</td>
<td>4:5</td>
</tr>
<tr>
<td>BMI, mean (SD), kg/m²</td>
<td>25.1 (5.8)</td>
<td>24.5 (6.2)</td>
<td>26.3 (6.0)</td>
<td>24.4 (5.6)</td>
</tr>
<tr>
<td><strong>Repair</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at repair, median (Q1-Q3), months</td>
<td>30 (24-58)</td>
<td>30 (17-57)</td>
<td>36 (24-72)</td>
<td>25 (24-50)</td>
</tr>
<tr>
<td>Time after repair, mean (SD), years</td>
<td>34.4 (7.2)</td>
<td>31.9 (6.0)</td>
<td>36.3 (8.2)</td>
<td>34.7 (7.4)</td>
</tr>
<tr>
<td>Total correction only, n (%)</td>
<td>12 (44.4)</td>
<td>5 (55.5)</td>
<td>4 (44.4)</td>
<td>3 (33.3)</td>
</tr>
<tr>
<td><strong>Clinical data</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>QRS, median (Q1-Q3), ms</td>
<td>150 (158-170)</td>
<td>160 (152-178)</td>
<td>158 (150-166)</td>
<td>158 (151-169)</td>
</tr>
<tr>
<td>Number of patients with NYHA class I/II/III</td>
<td>27/0/0</td>
<td>9/0/0</td>
<td>9/0/0</td>
<td>9/0/0</td>
</tr>
<tr>
<td>Number of patients with self-assessed physical activity level</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intensive/Moderate/Low</td>
<td>10/15/2</td>
<td>4/5/0</td>
<td>4/5/0</td>
<td>2/5/2</td>
</tr>
<tr>
<td>Patients with permanent pacemaker, n (%)</td>
<td>1 (3.7)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>1 (11.1)</td>
</tr>
<tr>
<td>Beta blockers, n (%)</td>
<td>6 (22.2)</td>
<td>1 (11.1)</td>
<td>3 (33.3)</td>
<td>2 (22.2)</td>
</tr>
</tbody>
</table>
Mean adherence to exercise training was 91.1% or 32.8 trainings on average out of 36. Adherence to interval training was 92.9%, (33.4 trainings on average), while adherence to continuous training was 89.2% (32.1 trainings on average). The difference in adherence between the two training groups was not statistically significant (p=0.448).

Both exercise training programmes were safe. There were no deaths or adverse events. Side effects of exercise, such as dyspnea, dizziness, chest pain or palpitations, were not reported. One patient from the interval training group had a hypothyroidism episode, which was resolved with increased dose of L-thyroxine.

3.1.1 EXERCISE CAPACITY

Data on exercise capacity improvement are summarised in Table 5a. Patients from both intervention groups have improved their exercise workload compared to baseline (from 123 W to 132 W on average in interval group, p=0.002, and from 160 W to 175 W on average in continuous group, p=0.003) (Fig 10). The difference in changes between two intervention group was not significant (p=0.483) (Table 6a).

Fig 10. Comparison of mean relative (%) changes in workload for each group in the study of patients with repaired tetralogy of Fallot. Values below groups/bars denote paired samples t-test significance.
Similarly, percentage of predicted exercise workload was also improved in both intervention groups (from 93 to 107% on average for interval training group (p=0.004), and from 93 to 101% on average in continuous training group). No significant difference was assessed between changes of both groups (p=0.382). Similar results were obtained in terms of METs (Table 5a and Table 6a).

Also, interval training led to improvement in VO2peak, from 21.2 to 22.9 ml/kg/min (p=0.004), while improvement in continuous training group was not significant (from 21.8 to 23.6 ml/kg/min, p=0.190 (Table 5a and Fig 11).

Fig 11. Comparison of mean relative (%) changes in peak oxygen consumption for each group in the study of patients with repaired tetralogy of Fallot. Values below groups/bars denote paired samples t-test significance.

Furthermore, variables of exercise capacity associated with VO2peak improvements, such as percentage of predicted VO2 peak, VO2 at AT (Fig 12), oxygen pulse and peak ventilation (VE), showed similar trends after training programmes (Table 5a and Table 6a).
There was a significant improvement in terms of 6MWT in both intervention groups: in interval training group the distance improved from 446 m to 464 m on average, \( p=0.001 \), while in continuous training group walking distance increased from 508 m to 529 m on average, \( p=0.038 \). However, the difference between changes was not significant (Table 5a, Table 6a and Fig 13).

Fig 12. Comparison of mean relative (%) changes in oxygen consumption at ventilatory threshold for each group in the study of patients with repaired tetralogy of Fallot. Values below groups/bars denote paired samples t-test significance.
3.1.2 VASCULAR FUNCTION

Both types of exercise training led to improvement in terms of endothelial function measured with FMD. In patients undergoing interval training, FMD increased from 8.4 to 12.9 %, p=0.019. In patients undergoing continuous training, FMD increased from 7.2 to 8.8 %, p=0.061 (borderline significant) (Table 5b). There was no difference between these two groups in terms of improvement of FMD. However, compared to controls, improvement of patients in interval training group was significant (p=0.015) (Table 6a and Fig 14).
Fig 14. Comparison of mean relative (%) changes in **flow-mediated vasodilation** for each group in the study of patients with repaired tetralogy of Fallot. Values below groups/bars denote paired samples t-test significance.

Interval exercise training lead to significant decrease of a stiffness parameter, namely PWV (from 5.36 to 4.71 m/s, p=0.028). In continuous training group, improvement of PWV was not significant (from 5.61 to 5.55 m/s, p=0.167) (Table 5b). The difference between groups in terms of improvement of PWV was not shown (Table 6a). However, there was a significant difference in improvements between interval training group and controls in terms of PWV (p=0.036) (Fig 15).
Fig 15. Comparison of mean relative (%) changes in pulse wave velocity for each group in the study of patients with repaired tetralogy of Fallot. Values below groups/bars denote paired samples t-test significance.

3.1.3 CARDIAC AUTONOMIC FUNCTION

3.1.3.1 HEART RATE VARIABILITY

As shown in Table 5b, interval training was not effective in improving HRV in adults with repaired ToF. Levels of time-domain parameters (SDNN, rMSSD and pNN50%) were even slightly lower compared to baseline (Table 5b). On the contrary, effects of interval training on frequency-domain parameters were beneficial – increased HFnu and decreased LFnu. All these changes were not statistically significant though (Table 5b).

On the contrary, continuous training showed better results in improving HRV. Time-domain parameters had higher values compared to baseline – SDNN has improved from median 26.71 to 39.84 ms (p=0.144), rMSSD from median 17.04 to 26.34 (p=0.087) and pNN50% from median 0.64 to 6.19 % (p=0.123). However, these improvements were not significant (improvement of rMSSD was borderline significant,
p=0.087 (Table 5b and Fig 16). Regarding frequency-domain parameters, effects were even more beneficial in a continuous training group – LFnu levels were significantly decreased from 0.32 to 0.22 (p=0.039) (Fig 17), HFnu was insignificantly increased from 0.14 to 0.17 (p=0.582) and LF:HF was decreased from median 3.35 to 1.36 (p=0.111). Improvements of HRV parameters after both training programmes were further compared (Table 6a). Time-domain parameters improvement after moderate training programme was significantly more beneficial compared to improvement after interval training programme: borderline significant difference in improvements of SDNN (p=0.072) and significant difference for pNN50% (p=0.043). Similarly, improvements after moderate training were borderline significant compared to controls for rMSSD (p=0.067). Differences between improvements of time-domain parameters between interval training group and controls were not significant. Also, there were no significant differences in improvements of frequency-domain among these groups.

Fig 16. Comparison of mean relative (%) changes in rMSSD for each group in the study of patients with repaired tetralogy of Fallot. Values below groups/bars denote paired samples t-test significance.
Fig 17. Comparison of mean relative (%) changes in LFnu for each group in the study of patients with repaired tetralogy of Fallot. Values below groups/bars denote paired samples t-test significance.

3.1.3.2 HEART RATE RECOVERY

Another cardiac autonomic function parameters were also examined before and after intervention programmes, and in controls. Data were obtained from 26 subjects instead of 27, as one subject from the interval training group had an episode of SVT just after the maximum workload during post-exercise test. As the HR in next 6 minutes was between 130 and 160/min, HRR parameters were not calculated.

Data are shown in Table 5b. Interval training programme did not lead to improvement of HRR parameters, levels of HRR1, HRR2 and HRR3 were even decreased on average, as compared to baseline levels. However, these differences were not significant (Table 5b). On the contrary, continuous programme led to increase of HRR parameters (Table 5b). Among them, only improvement of HRR2 was significant (from mean 40.2 to 46.9 beats, p=0.023) (Fig 18).
When improvements between two intervention groups are compared, results are as follows: improvement of HRR2 after continuous training programme is larger than improvement after interval training programme (p=0.057) (Table 6a).

![Comparison of mean relative (%) changes in heart rate recovery after 2 minutes for each group in the study of patients with repaired tetralogy of Fallot. Values below groups/bars denote paired samples t-test significance.](image)

Improvements in terms of other HRR variables were not significant when two intervention groups were compared. Also, comparison between improvements of interval training group and controls, and continuous training group and controls, showed no significant differences in terms of HRR variables.
3.1.4 BIOMARKERS

3.1.4.1 COMPLETE BLOOD COUNT

Table 5c depicts changes of complete blood count. No significant change was observed in any of the 3 groups. White blood cells count and neutrophil count were higher after moderate training programme (from 5.9 to 6.4, p=0.041 and from 3.0 to 3.4, p=0.059, respectively) (Table 5c).

3.1.4.2 LIPID PROFILE

Data on improvements of lipid status parameters are shown in Table 5c. As it can be seen, interval exercise training programme had a beneficial effect on lipid status – as levels of total cholesterol and triglycerides stayed almost unchanged, levels of LDL-c were decreased. HDL-c levels were increased from mean 1.26 to 1.33 mmol/L, p=0.030) and this change was statistically significant (Table 5c and Fig 19).

![Graph showing mean relative (%) changes in high-density lipoprotein cholesterol for each group in the study of patients with repaired tetralogy of Fallot. Values below groups/bars denote paired samples t-test significance.](image-url)
Continuous training programme also caused increase of levels of HDL-c, but it was not statistically significant (from mean 1.42 to 1.47 mmol/L, p=0.386) (Fig 19). Levels of LDL-c were slightly decreased, without statistical significance. On the contrary, levels of total cholesterol and triglycerides were slightly higher as compared to baseline, but without statistical significance (Table 5c). No significant differences in changes among groups were observed (Table 6b).

3.1.4.3 MARKERS OF INFLAMMATION, HAEMOSTASIS AND HEART FAILURE SEVERITY

Patients from interval training group had significantly lower post-exercise values of NT-proBNP compared to baseline values (from median 202.1 to 189.9 ng/L, p=0.032) (Table 5c). After continuous training, patients had slightly higher values of NT-proBNP compared to pre-exercise, but this increase was not statistically significant (from median 82.8 to 93.9 ng/L, p=0.720) (Fig 20). Also, the differences after training programmes were not significantly different between interval and continuous training groups (Table 6b).

Fig 20. Comparison of mean relative (%) changes in NT-proBNP levels for each group in the study of patients with repaired tetralogy of Fallot. Values below groups/bars denote paired samples t-test significance.
Interval training caused significant decrease of levels of fibrinogen (from 2.67 to 2.46 g/L, p=0.018), while the similar effect was not shown in patients who belonged to continuous training group (from 2.66 to 2.71 g/L, p=0.567) (Table 5c, Fig 21). The improvement rates were significantly different between two intervention groups (p=0.038).

Levels of D-dimer were not significantly changed after exercise training programmes in neither interval (from median 270 to 240 µg/L, p=0.846) nor continuous group (from median 190 to 150 µg/L, p=0.684) (Table 5c).

![Figure 21](image)

**Fig 21.** Comparison of mean relative (%) changes in fibrinogen levels for each group in the study of patients with repaired tetralogy of Fallot. Values below groups/bars denote paired samples t-test significance.

### 3.1.5 QUALITY OF LIFE

Interval training did not lead to improvement of either physical nor mental domain in SF-36 questionnaire (Table 5d and Fig 22). On the contrary, mental component was significantly improved after continuous
training programme (from 87 to 95 %, p=0.028) (Table 5d and Fig 22). The difference between mental domain score improvement was not significant between two intervention groups (p=0.895) (Table 6b).

Fig 22. Comparison of mean relative (%) changes in (a) physical component summary and (b) mental component summary for each group in the study of patients with repaired tetralogy of Fallot.

Values below groups/bars denote paired samples t-test significance.

Changes of all SF-36 categories are shown in Table 5d. As it can be seen, improvement of mental health category as a result of continuous training group was borderline significant (p=0.066) (Table 5d). There were no significant differences in change of mental health between continuous training group and controls (Table 6b).
Table 5a. Exercise training measurements for three studied groups before and after trainings, and changes in patients with repaired tetralogy of Fallot

<table>
<thead>
<tr>
<th>Exercise testing</th>
<th>Interval training group</th>
<th>Continuous training group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>p</td>
</tr>
<tr>
<td>Workload, W</td>
<td>123 (103-137)</td>
<td>132 (123-166)</td>
<td>0.002</td>
</tr>
<tr>
<td>Percentage of predicted workload, %</td>
<td>93.0 (25.4)</td>
<td>106.8 (28.8)</td>
<td>0.004</td>
</tr>
<tr>
<td>METs</td>
<td>8.27 (2.35)</td>
<td>9.29 (2.68)</td>
<td>0.005</td>
</tr>
<tr>
<td>VO2 at AT, ml/kg/min</td>
<td>19.9 (5.6)</td>
<td>22.6 (5.4)</td>
<td>0.012</td>
</tr>
<tr>
<td>VO2 peak, ml/kg/min</td>
<td>21.2 (18.4-31.3)</td>
<td>22.9 (21.1-35.4)</td>
<td>0.004</td>
</tr>
<tr>
<td>Percentage of predicted VO2peak, %</td>
<td>81.2 (21.2)</td>
<td>91.7 (24.2)</td>
<td>0.011</td>
</tr>
<tr>
<td>O2 pulse, ml/beat</td>
<td>10.4 (3.1)</td>
<td>11.9 (3.3)</td>
<td>0.048</td>
</tr>
<tr>
<td>VE peak, l/min</td>
<td>45.7 (32.7-67.4)</td>
<td>53.0 (33.9-66.1)</td>
<td>0.127</td>
</tr>
<tr>
<td>VE/VCO2</td>
<td>26.3 (3.5)</td>
<td>23.9 (3.7)</td>
<td>0.018</td>
</tr>
<tr>
<td>HR rest, beats/min</td>
<td>79.0 (10.5)</td>
<td>80.6 (9.3)</td>
<td>0.577</td>
</tr>
<tr>
<td>HR peak, beats/min</td>
<td>159.6 (20.6)</td>
<td>161.9 (22.3)</td>
<td>0.622</td>
</tr>
<tr>
<td>SBP rest, mmHg</td>
<td>105.8 (8.4)</td>
<td>107.6 (9.8)</td>
<td>0.476</td>
</tr>
<tr>
<td>DBP rest, mmHg</td>
<td>69.1 (9.0)</td>
<td>70.2 (6.9)</td>
<td>0.747</td>
</tr>
<tr>
<td>SBP max, mmHg</td>
<td>167.2 (22.8)</td>
<td>161.7 (33.0)</td>
<td>0.641</td>
</tr>
<tr>
<td>DBP max, mmHg</td>
<td>79.4 (4.3)</td>
<td>79.0 (4.4)</td>
<td>0.791</td>
</tr>
<tr>
<td>DP peak, mmHg/min</td>
<td>27031 (7289)</td>
<td>26571 (8250)</td>
<td>0.866</td>
</tr>
<tr>
<td>CHR competence</td>
<td>0.87 (0.10)</td>
<td>0.88 (0.12)</td>
<td>0.630</td>
</tr>
<tr>
<td>6MWT, m</td>
<td>446 (34.4)</td>
<td>464 (33.9)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Data are described as mean [standard deviation] or median (Q1-Q3). Columns p denote paired samples t-test significance.

MET – metabolic equivalent of task; VO2 – oxygen uptake; AT – anaerobic threshold; O2 – oxygen; VE – ventilation; VE/VCO2 – minute ventilation/carbon dioxide production slope; HR – heart rate; SBP – systolic blood pressure; DBP – diastolic blood pressure; DP peak – double product (HR peak*SBPmax); CHR – chronotropic; 6MWT – 6-minute walking test
Table 5b. Exercise training measurements for three studied groups before and after trainings, and changes in patients with repaired tetralogy of Fallot

<table>
<thead>
<tr>
<th>Vascular function</th>
<th>Interval training group</th>
<th>Continuous training group</th>
<th>Control group</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>p</td>
<td>Before</td>
</tr>
<tr>
<td>β</td>
<td>5.79 (5.16-6.29)</td>
<td>5.17 (3.94-6.73)</td>
<td>0.184</td>
<td>7.01 (5.77-8.20)</td>
</tr>
<tr>
<td>PWV, m/s</td>
<td>5.36 (5.02-5.65)</td>
<td>4.71 (4.09-5.72)</td>
<td>0.028</td>
<td>5.61 (5.32-6.78)</td>
</tr>
<tr>
<td>FMD, %</td>
<td>8.40 (3.55)</td>
<td>12.91 (4.31)</td>
<td>0.019</td>
<td>7.18 (3.20)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HRR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HRR1</td>
<td>33.56 (8.99)</td>
<td>30.00 (8.18)</td>
<td>0.256</td>
<td>27.22 (3.74)</td>
</tr>
<tr>
<td>HRR2</td>
<td>51.56 (12.55)</td>
<td>48.63 (7.51)</td>
<td>0.383</td>
<td>40.22 (8.51)</td>
</tr>
<tr>
<td>HRR3</td>
<td>60.67 (13.82)</td>
<td>56.00 (12.53)</td>
<td>0.312</td>
<td>53.22 (9.45)</td>
</tr>
<tr>
<td>HRR4</td>
<td>60.33 (13.56)</td>
<td>64.88 (12.79)</td>
<td>0.122</td>
<td>56.33 (11.02)</td>
</tr>
<tr>
<td>HRV</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SDNN</td>
<td>39.15 (23.39-59.05)</td>
<td>29.85 (25.42-38.92)</td>
<td>0.151</td>
<td>26.71 (24.47-39.97)</td>
</tr>
<tr>
<td>rMSSD</td>
<td>27.53 (14.76-52.86)</td>
<td>20.16 (17.56-36.35)</td>
<td>0.336</td>
<td>17.04 (12.57-25.16)</td>
</tr>
<tr>
<td>PNN50%</td>
<td>7.16 (0.86-31.01)</td>
<td>2.77 (0.70-16.01)</td>
<td>0.069</td>
<td>0.64 (0.27-7.71)</td>
</tr>
<tr>
<td>LFnu</td>
<td>0.32 (0.16)</td>
<td>0.29 (0.13)</td>
<td>0.578</td>
<td>0.32 (0.12)</td>
</tr>
<tr>
<td>HFnu</td>
<td>0.18 (0.11)</td>
<td>0.24 (0.17)</td>
<td>0.332</td>
<td>0.14 (0.12)</td>
</tr>
<tr>
<td>LF:HF</td>
<td>1.69 (0.88-4.25)</td>
<td>1.83 (0.56-2.82)</td>
<td>0.214</td>
<td>3.35 (1.36-7.49)</td>
</tr>
</tbody>
</table>

Data are described as mean (standard deviation) or median (Q1-Q3). Columns p denote paired samples t-test significance except for parameter PNN50% where Wilcoxon paired samples test.

β – beta stiffness coefficient; PWV – pulse wave velocity; FMD – flow-mediated dilation; HRR – heart rate recovery 1, 2, 3 or 4 minutes after exercise; HRV – heart rate variability; SDNN – standard deviation of all normal R-R (NN) intervals; rMSSD – root mean square of successive NN interval differences; PNN50% – percentage of successive NN intervals differing by >50ms; LFnu – power in the low-frequency range, normalized; HFnu – power in the high-frequency range, normalized; LF:HF – ratio of normalized low- and high-frequency power.
Table 5c. Exercise training measurements for three studied groups before and after trainings, and changes in patients with repaired tetralogy of Fallot

<table>
<thead>
<tr>
<th>Laboratory tests</th>
<th>Interval training group</th>
<th>Continuous training group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
</tr>
<tr>
<td>Glucose</td>
<td>4.70 (4.45-5.80)</td>
<td>4.80 (4.55-5.60)</td>
<td>0.253</td>
</tr>
<tr>
<td>TC</td>
<td>4.43 (0.77)</td>
<td>4.41 (0.64)</td>
<td>0.854</td>
</tr>
<tr>
<td>HDL-c</td>
<td>1.26 (0.19)</td>
<td>1.33 (0.23)</td>
<td>0.030</td>
</tr>
<tr>
<td>LDL-c</td>
<td>2.66 (0.69)</td>
<td>2.55 (0.56)</td>
<td>0.460</td>
</tr>
<tr>
<td>Trig.</td>
<td>1.14 (0.21)</td>
<td>1.16 (0.44)</td>
<td>0.874</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>2.67 (0.40)</td>
<td>2.46 (0.46)</td>
<td>0.018</td>
</tr>
<tr>
<td>D-dimer</td>
<td>270 (200-370)</td>
<td>240 (195-320)</td>
<td>0.846</td>
</tr>
<tr>
<td>NT-proBNP</td>
<td>202.1 (151.7-382.9)</td>
<td>189.9 (94.7-392.9)</td>
<td>0.032</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Haemogram</th>
<th>Before</th>
<th>After</th>
<th>Before</th>
<th>After</th>
<th>Before</th>
<th>After</th>
<th>p</th>
<th>p</th>
<th>p</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC</td>
<td>6.50 (1.31)</td>
<td>6.33 (1.48)</td>
<td>0.749</td>
<td>5.90 (1.43)</td>
<td>6.44 (1.47)</td>
<td>0.041</td>
<td>6.88 (1.54)</td>
<td>6.49 (1.78)</td>
<td>0.305</td>
<td></td>
</tr>
<tr>
<td>Lymph</td>
<td>1.90 (1.60-2.20)</td>
<td>1.80 (1.50-2.30)</td>
<td>0.297</td>
<td>1.70 (1.70-2.00)</td>
<td>1.90 (1.65-2.00)</td>
<td>0.641</td>
<td>2.10 (1.60-2.20)</td>
<td>1.80 (1.45-2.20)</td>
<td>0.378</td>
<td></td>
</tr>
<tr>
<td>Mono</td>
<td>0.60 (0.40-0.80)</td>
<td>0.50 (0.45-0.85)</td>
<td>0.393</td>
<td>0.50 (0.45-0.65)</td>
<td>0.50 (0.45-0.70)</td>
<td>0.450</td>
<td>0.60 (0.55-0.80)</td>
<td>0.60 (0.45-0.75)</td>
<td>0.464</td>
<td></td>
</tr>
<tr>
<td>Neu</td>
<td>3.90 (3.05-4.25)</td>
<td>3.50 (3.05-4.70)</td>
<td>0.772</td>
<td>3.00 (2.80-4.20)</td>
<td>3.40 (2.95-5.25)</td>
<td>0.059</td>
<td>3.80 (3.30-4.70)</td>
<td>3.60 (2.85-5.05)</td>
<td>0.114</td>
<td></td>
</tr>
<tr>
<td>RBC</td>
<td>4.86 (0.58)</td>
<td>4.77 (0.53)</td>
<td>0.261</td>
<td>4.87 (0.39)</td>
<td>4.93 (0.45)</td>
<td>0.373</td>
<td>4.90 (0.43)</td>
<td>4.82 (0.34)</td>
<td>0.385</td>
<td></td>
</tr>
<tr>
<td>HGB</td>
<td>142.6 (14.1)</td>
<td>140.0 (12.9)</td>
<td>0.228</td>
<td>136.9 (11.5)</td>
<td>138.6 (12.4)</td>
<td>0.349</td>
<td>139.9 (19.4)</td>
<td>136.1 (16.9)</td>
<td>0.131</td>
<td></td>
</tr>
<tr>
<td>Hct</td>
<td>0.42 (0.04)</td>
<td>0.41 (0.03)</td>
<td>0.169</td>
<td>0.42 (0.03)</td>
<td>0.43 (0.04)</td>
<td>0.154</td>
<td>0.42 (0.05)</td>
<td>0.41 (0.04)</td>
<td>0.548</td>
<td></td>
</tr>
<tr>
<td>PLT</td>
<td>203.9 (32.0)</td>
<td>198.6 (39.7)</td>
<td>0.281</td>
<td>230.2 (55.2)</td>
<td>228.7 (51.6)</td>
<td>0.669</td>
<td>237.9 (59.0)</td>
<td>241.6 (57.0)</td>
<td>0.710</td>
<td></td>
</tr>
</tbody>
</table>

Data are described as mean (standard deviation) or median (Q1-Q3). Columns p denote paired samples t-test significance.

TC – total cholesterol; HDL-c – high-density lipoprotein cholesterol; LDL-c – low-density lipoprotein cholesterol; Trig. – triglycerides; NT-proBNP – amino-terminal prohormone of brain natriuretic peptide;
WBC – white blood cell count; Lymph – absolute lymphocyte count; Mono – absolute monocyte count; Neu – absolute neutrophil count; RBC – red blood cell count; HGB – hemoglobin; Hct – hematocrit; PLT – platelet count
Table 5d. Exercise training measurements for three studied groups before and after trainings, and changes in patients with repaired tetralogy of Fallot

<table>
<thead>
<tr>
<th>SF-36</th>
<th>Interval training group</th>
<th>Continuous training group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>p</td>
</tr>
<tr>
<td>PCS</td>
<td>99 (62-108)</td>
<td>84 (65-111)</td>
<td>0.767</td>
</tr>
<tr>
<td>MCS</td>
<td>76 (71-87)</td>
<td>80 (68-92)</td>
<td>0.515</td>
</tr>
<tr>
<td>PF</td>
<td>90 (78-95)</td>
<td>85 (68-98)</td>
<td>0.667</td>
</tr>
<tr>
<td>RP</td>
<td>100 (34-100)</td>
<td>100 (50-100)</td>
<td>0.655</td>
</tr>
<tr>
<td>RE</td>
<td>67 (67-100)</td>
<td>100 (67-100)</td>
<td>0.666</td>
</tr>
<tr>
<td>VT</td>
<td>65 (60-70)</td>
<td>70 (48-78)</td>
<td>0.552</td>
</tr>
<tr>
<td>MH</td>
<td>76 (68-90)</td>
<td>84 (72-84)</td>
<td>0.725</td>
</tr>
<tr>
<td>SF</td>
<td>88 (69-100)</td>
<td>88 (69-100)</td>
<td>1.000</td>
</tr>
<tr>
<td>BP</td>
<td>80 (67-100)</td>
<td>100 (65-100)</td>
<td>0.733</td>
</tr>
<tr>
<td>GH</td>
<td>70 (58-75)</td>
<td>65 (55-80)</td>
<td>0.495</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Physical activity</th>
<th>IPAQ score</th>
<th>Before</th>
<th>After</th>
<th>p</th>
<th>Before</th>
<th>After</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2892 (2179-5374)</td>
<td>3492 (2050-5356)</td>
<td>0.924</td>
<td>2226 (1233-5724)</td>
<td>2430 (1786-2839)</td>
<td>0.497</td>
</tr>
</tbody>
</table>

Data are described as mean (standard deviation) or median (Q1-Q3). Columns p denote paired samples t-test significance.

SF-36 – short form health survey questionnaire; PCS – physical component summary; MCS – mental component summary; PF – physical functioning; RP – role functioning/physical; RE – role functioning/emotional; VT – vitality; MH – mental health; SF – social functioning; BP – bodily pain; GH – general health; IPAQ – international physical activity questionnaire
Table 6a. Absolute changes and comparison among groups in patients with repaired tetralogy of Fallot

<table>
<thead>
<tr>
<th>Exercise testing</th>
<th>Changes</th>
<th>Improvement comparisons (p values) *</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Interval training group</td>
<td>Continuous training group</td>
</tr>
<tr>
<td>Workload</td>
<td>19.7 (12.7)</td>
<td>11.3 (10.4)</td>
</tr>
<tr>
<td>Percentage or predicted workload</td>
<td>13.8 (10.2)</td>
<td>7.2 (5.9)</td>
</tr>
<tr>
<td>METs</td>
<td>1.02 (0.81)</td>
<td>0.50 (0.37)</td>
</tr>
<tr>
<td>VO2 at AT</td>
<td>2.7 (2.5)</td>
<td>1.2 (3.0)</td>
</tr>
<tr>
<td>VO2 peak</td>
<td>3.2 (2.9)</td>
<td>0.7 (1.6)</td>
</tr>
<tr>
<td>Percentage of predicted VO2 peak</td>
<td>10.4 (9.5)</td>
<td>2.7 (5.9)</td>
</tr>
<tr>
<td>O2 pulse</td>
<td>1.5 (2.0)</td>
<td>0.4 (1.1)</td>
</tr>
<tr>
<td>VE peak</td>
<td>-0.3 (18.1)</td>
<td>4.0 (4.0)</td>
</tr>
<tr>
<td>VE/VO2</td>
<td>-2.4 (2.4)</td>
<td>0.7 (2.0)</td>
</tr>
<tr>
<td>HR rest</td>
<td>1.6 (8.0)</td>
<td>0.3 (8.1)</td>
</tr>
<tr>
<td>HR peak</td>
<td>2.3 (13.6)</td>
<td>-0.1 (10.8)</td>
</tr>
<tr>
<td>SBP rest</td>
<td>1.8 (7.1)</td>
<td>7.2 (8.7)</td>
</tr>
<tr>
<td>DBP rest</td>
<td>1.1 (10.0)</td>
<td>2.8 (8.7)</td>
</tr>
<tr>
<td>SBP max</td>
<td>-5.6 (34.4)</td>
<td>0.0 (21.1)</td>
</tr>
<tr>
<td>DBP max</td>
<td>-0.4 (4.9)</td>
<td>5.6 (12.1)</td>
</tr>
<tr>
<td>DP peak</td>
<td>-460 (7921)</td>
<td>-9.4 (3318)</td>
</tr>
<tr>
<td>CHR competence</td>
<td>0.01 (0.08)</td>
<td>0.00 (0.06)</td>
</tr>
<tr>
<td>6MWT</td>
<td>18.9 (11.1)</td>
<td>21.1 (25.5)</td>
</tr>
<tr>
<td>Vascular function</td>
<td></td>
<td></td>
</tr>
<tr>
<td>β</td>
<td>-0.45 (1.27)</td>
<td>-0.36 (0.81)</td>
</tr>
<tr>
<td>PWV</td>
<td>-0.46 (0.51)</td>
<td>-0.26 (0.48)</td>
</tr>
<tr>
<td>FMD</td>
<td>4.50 (4.61)</td>
<td>1.59 (2.20)</td>
</tr>
<tr>
<td>HRR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HRR1</td>
<td>-3.25 (7.44)</td>
<td>1.56 (4.77)</td>
</tr>
<tr>
<td>HRR2</td>
<td>-3.25 (9.88)</td>
<td>6.67 (7.14)</td>
</tr>
<tr>
<td>HRR3</td>
<td>-4.75 (12.34)</td>
<td>1.22 (8.18)</td>
</tr>
<tr>
<td>HRR4</td>
<td>5.25 (8.45)</td>
<td>4.89 (8.58)</td>
</tr>
<tr>
<td>HRV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SDNN</td>
<td>-10.03 (17.07)</td>
<td>11.19 (16.03)</td>
</tr>
<tr>
<td>rMSSD</td>
<td>-8.09 (17.35)</td>
<td>10.02 (13.26)</td>
</tr>
<tr>
<td>PNN50%</td>
<td>-6.46 (14.06)</td>
<td>8.87 (13.84)</td>
</tr>
<tr>
<td>LFnu</td>
<td>-0.03 (1.42)</td>
<td>-0.10 (0.11)</td>
</tr>
<tr>
<td>HFnu</td>
<td>0.05 (0.15)</td>
<td>0.03 (0.16)</td>
</tr>
<tr>
<td>LF:HF</td>
<td>-1.14 (2.49)</td>
<td>-2.19 (3.55)</td>
</tr>
</tbody>
</table>

Changes are described as mean (standard deviation).

MET – metabolic equivalent of task; VO2 – oxygen uptake; AT – anaerobic threshold; O2 – oxygen; VE – ventilation; VE/VO2 – minute ventilation/carbon dioxide production slope; HR – heart rate; SBP – systolic blood pressure; DBP – diastolic blood pressure; DP peak – double product (HR peak*SBPmax); CHR – chronotropic; 6MWT – 6-minute walking test; β – beta stiffness coefficient; PWV – pulse wave velocity; FMD – flow-mediated dilation; HRR – heart rate recovery 1, 2, 3 or 4 minutes after exercise; HRV – heart rate variability; SDNN – standard deviation of all normal R-R (NN) intervals; rMSSD – root mean square of successive NN interval differences; PNN50% – percentage of successive NN intervals differing by >50ms; LFnu – power in the low-frequency range, normalized; HFnu – power in the high-frequency range, normalized; LF:HF – ratio of normalized low- and high-frequency power

* One-way ANOVA with Bonferroni correction
### Table 6b. Absolute changes and comparison among groups in patients with repaired tetralogy of Fallot

<table>
<thead>
<tr>
<th></th>
<th>Changes</th>
<th>Improvement comparisons (p values)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Interval training group</td>
<td>Continuous training group</td>
</tr>
<tr>
<td>Laboratory tests</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>0.23 (0.65)</td>
<td>0.14 (0.34)</td>
</tr>
<tr>
<td>TC</td>
<td>-0.03 (0.46)</td>
<td>0.06 (0.60)</td>
</tr>
<tr>
<td>HDL-c</td>
<td>0.08 (0.09)</td>
<td>0.06 (0.19)</td>
</tr>
<tr>
<td>LDL-c</td>
<td>-0.11 (0.44)</td>
<td>-0.06 (0.48)</td>
</tr>
<tr>
<td>Trig</td>
<td>0.02 (0.37)</td>
<td>0.14 (0.31)</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>-0.21 (0.21)</td>
<td>0.06 (0.28)</td>
</tr>
<tr>
<td>D-dimer</td>
<td>66.7 (365.4)</td>
<td>-2.2 (102.8)</td>
</tr>
<tr>
<td>NT-proBNP</td>
<td>-28.2 (34.8)</td>
<td>-1.0 (59.4)</td>
</tr>
<tr>
<td>Complete blood count</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WBC</td>
<td>-0.17 (1.51)</td>
<td>0.54 (0.67)</td>
</tr>
<tr>
<td>Lymph</td>
<td>-0.12 (0.35)</td>
<td>0.02 (0.17)</td>
</tr>
<tr>
<td>Mono</td>
<td>0.07 (0.24)</td>
<td>0.03 (0.15)</td>
</tr>
<tr>
<td>Neu</td>
<td>-0.11 (1.19)</td>
<td>0.49 (0.60)</td>
</tr>
<tr>
<td>RBC</td>
<td>-0.09 (0.22)</td>
<td>0.07 (0.21)</td>
</tr>
<tr>
<td>HGB</td>
<td>-2.56 (5.88)</td>
<td>1.7 (5.0)</td>
</tr>
<tr>
<td>Hct</td>
<td>-0.01 (0.02)</td>
<td>0.01 (0.02)</td>
</tr>
<tr>
<td>PLT</td>
<td>-5.3 (13.9)</td>
<td>-1.6 (10.5)</td>
</tr>
<tr>
<td>SF-36</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCS</td>
<td>-1.7 (12.3)</td>
<td>9.1 (18.8)</td>
</tr>
<tr>
<td>MCS</td>
<td>1.1 (20.4)</td>
<td>12.1 (19.7)</td>
</tr>
<tr>
<td>PF</td>
<td>-1.6 (9.2)</td>
<td>3.9 (10.2)</td>
</tr>
<tr>
<td>RP</td>
<td>-2.8 (19.5)</td>
<td>19.4 (39.1)</td>
</tr>
<tr>
<td>RE</td>
<td>3.7 (35.1)</td>
<td>18.5 (37.7)</td>
</tr>
<tr>
<td>VT</td>
<td>-2.2 (13.0)</td>
<td>6.7 (13.2)</td>
</tr>
<tr>
<td>MH</td>
<td>0.9 (14.1)</td>
<td>7.1 (10.9)</td>
</tr>
<tr>
<td>SF</td>
<td>0.0 (10.8)</td>
<td>9.7 (16.3)</td>
</tr>
<tr>
<td>BP</td>
<td>4.2 (25.2)</td>
<td>9.7 (18.6)</td>
</tr>
<tr>
<td>GH</td>
<td>-3.3 (12.7)</td>
<td>5.6 (13.8)</td>
</tr>
</tbody>
</table>

Changes are described as mean (standard deviation).

TC – total cholesterol; HDL-c – high-density lipoprotein cholesterol; LDL-c – low-density lipoprotein cholesterol; Trig. – triglycerides; NT-proBNP – amino-terminal prohormone of brain natriuretic peptide; WBC – white blood cell count; Lymph – absolute lymphocyte count; Mono – absolute monocyte count; Neu – absolute neutrophil count; RBC – red blood cell count; HGB – hemoglobin; Hct – hematocrit; PLT – platelet count; SF-36 – short form health survey questionnaire; PCS – physical component summary; MCS – mental component summary; PF – physical functioning; RP – role functioning/physical; RE – role functioning/emotional; VT – vitality; MH – mental health; SF – social functioning; BP – bodily pain; GH – general health; IPAQ – international physical activity questionnaire

* One-way ANOVA with Bonferroni correction
3.2 PERIPHERAL ARTERIAL DISEASE

As it can be seen from the Fig 23, a total of 55 patients were screened for eligibility, among which 36 were chosen to start with the intervention. Seven patients did not complete the study. A total of 29 patients have completed the study (Fig 4).

Fig 23. Enrolment and allocation of patients in a study of patients with peripheral arterial disease

As mentioned, some data for specific patients and measurements are not available due to various reasons (Fig 24).
Baseline data are summarised in Table 7. Average age of patients who completed the study was 64.4 (±9) years, male:female ratio was 21:8. There were no significant differences among three groups in terms of age (p=0.684), gender (p=0.526), BMI (p=0.287) and other clinical data (Table 7).

Mean adherence to exercise was 93.8 % or 33.8 trainings on average out of 36. Adherence to moderate-pain training was 92.5 % (33.3 trainings on average), while adherence to pain-free training was 95.0 % (34.2 trainings on average). The difference in adherence between the two training groups was not statistically significant (p=0.645).

Both exercise training programmes were safe. There were no deaths or adverse events. Side effects of exercise, such as dyspnea, dizziness, chest pain or palpitations, were not reported.
Table 7. Baseline clinical data of patients with peripheral arterial disease who completed the study

<table>
<thead>
<tr>
<th></th>
<th>Patients who completed the study (n=29)</th>
<th>Moderate-pain training group (n=10)</th>
<th>Pain-free training group (n=11)</th>
<th>Control group (n=8)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>General data</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, mean (SD), years</td>
<td>64.4 (9.0)</td>
<td>65.1 (7.6)</td>
<td>65.6 (11.0)</td>
<td>62.0 (8.3)</td>
<td>0.684</td>
</tr>
<tr>
<td>Male:female</td>
<td>21:8</td>
<td>6:4</td>
<td>9:2</td>
<td>6:2</td>
<td>0.526</td>
</tr>
<tr>
<td>BMI, mean (SD), kg/m²</td>
<td>28.7 (4.4)</td>
<td>30.4 (6.2)</td>
<td>27.3 (3.3)</td>
<td>28.5 (2.3)</td>
<td>0.287</td>
</tr>
<tr>
<td><strong>Smoking</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never, n (%)</td>
<td>5 (17.2)</td>
<td>2 (20.0)</td>
<td>3 (27.3)</td>
<td>0 (0)</td>
<td>0.139</td>
</tr>
<tr>
<td>Former, n (%)</td>
<td>16 (55.2)</td>
<td>5 (50.0)</td>
<td>7 (63.6)</td>
<td>4 (50.0)</td>
<td></td>
</tr>
<tr>
<td>Yes, n (%)</td>
<td>8 (27.6)</td>
<td>3 (30.0)</td>
<td>1 (9.1)</td>
<td>4 (50.0)</td>
<td></td>
</tr>
<tr>
<td><strong>Diabetes mellitus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No, n (%)</td>
<td>18 (62.1)</td>
<td>5 (50.0)</td>
<td>7 (63.6)</td>
<td>6 (75.0)</td>
<td>0.549</td>
</tr>
<tr>
<td>Yes, n (%)</td>
<td>11 (37.9)</td>
<td>5 (50.0)</td>
<td>4 (36.4)</td>
<td>2 (25.0)</td>
<td></td>
</tr>
<tr>
<td>Diet, n (%)</td>
<td>2 (6.9)</td>
<td>1 (10.0)</td>
<td>1 (9.1)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Peroral therapy, n (%)</td>
<td>6 (20.7)</td>
<td>3 (30.0)</td>
<td>1 (9.1)</td>
<td>2 (25.0)</td>
<td>0.509</td>
</tr>
<tr>
<td>Insulin, n (%)</td>
<td>3 (10.3)</td>
<td>1 (10.0)</td>
<td>2 (18.2)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td><strong>Drugs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspirin, n (%)</td>
<td>22 (75.8)</td>
<td>9 (90.0)</td>
<td>9 (81.8)</td>
<td>4 (50.0)</td>
<td>0.121</td>
</tr>
<tr>
<td>Beta blockers, n (%)</td>
<td>11 (37.9)</td>
<td>2 (20.0)</td>
<td>4 (36.4)</td>
<td>5 (62.5)</td>
<td>0.180</td>
</tr>
<tr>
<td>ACEI/ARB, n (%)</td>
<td>21 (72.4)</td>
<td>7 (70.0)</td>
<td>9 (81.8)</td>
<td>5 (62.5)</td>
<td>0.635</td>
</tr>
<tr>
<td>Ca blockers, n (%)</td>
<td>11 (37.9)</td>
<td>3 (30.0)</td>
<td>4 (36.4)</td>
<td>4 (50.0)</td>
<td>0.679</td>
</tr>
<tr>
<td>Diuretics, n (%)</td>
<td>14 (48.3)</td>
<td>6 (60.0)</td>
<td>5 (45.5)</td>
<td>3 (37.5)</td>
<td>0.620</td>
</tr>
<tr>
<td>Statins, n (%)</td>
<td>26 (89.7)</td>
<td>9 (90.0)</td>
<td>11 (100.0)</td>
<td>6 (75.0)</td>
<td>0.210</td>
</tr>
</tbody>
</table>

ACEI/ARB – angiotensin-converting enzyme inhibitors/angiotensin receptor blockers

3.2.1 WALKING CAPACITY

Both moderate-pain and pain-free training protocols lead to significant improvement of walking capacity parameters in patients with PAD.

In moderate-pain training group, claudication onset distance improved from median 63 m to 121 m (p<0.001). Maximal walking distance improved from median 109 m to 199 m (p<0.001) in the same group (Table 8a, Fig 25).

Pain-free training improved walking capacity: claudication onset distance improved from median 43 to 116 m (p<0.001), while maximal walking distance improved from median 87 to 163 m (p<0.001) (Table 8a, Fig 25).
Fig 25. Comparison of mean relative (%) changes in (a) claudication onset distance and (b) maximal walking distance for each group in the study of patients with peripheral arterial disease. Values below groups/bars denote paired samples t-test significance.

There were no significant differences between improvement rates, when two intervention protocols are compared (p=0.376 for claudication onset distance and p=1.000 for maximal walking distance). However, there was a significant difference in changes when moderate-pain training group and controls were compared in terms of claudication onset distance (p=0.007) and maximal walking distance (p=0.004). Differences in changes after pain-free training protocol and controls were also significant (p<0.001 for claudication onset distance and p=0.001 for maximal walking distance) (Table 9).

Resting ABI remained practically unchanged after moderate-pain training, while the pain-free training lead to a (borderline significant) increase in ABI (from mean 0.49 to 0.54, p=0.095) (Table 8a, Fig 26). The difference in changes after two training protocols was not significant though (p=0.936). Similarly, as compared to controls, changes were not significant either when moderate-pain training or pain-free training was compared to controls (Table 9). Continuous post-testing ABI measurement did not show any improvement in terms of time needed to normalise the pressure in legs – from mean 4.3 to 3.7 min,
p=0.423 for moderate-pain group, from mean 3.3 to 2.7 min, p=0.423 for pain-free group and from mean 4.0 to 3.5 min, p=0.500 for control group.

Fig 26. Comparison of mean relative (%) changes in ankle-brachial index for each group in the study of patients with peripheral arterial disease. Values below groups/bars denote paired samples t-test significance.

3.2.2 VASCULAR FUNCTION

Moderate-pain training protocol lead to improvement of FMD, a parameter of endothelial function, mean increase from 4.4 to 8.0 % (p=0.002). Improvement of FMD after pain-free training was borderline significant (from 4.6 to 6.9 %, p=0.066) (Table 8 and Fig 27). Differences in changes between training protocols were not significant (p=0.309). However, changes after both training modalities, compared to controls, were significant (p=0.004) for moderate-pain and borderline significant (p=0.077) for pain-free training) (Table 9).
Fig 27. Comparison of mean changes in flow-mediated dilation for each group in the study of patients with peripheral arterial disease. Values below groups/bars denote paired samples t-test significance.

Similarly, moderate-pain training significantly improved a parameter of arterial stiffness, PWV, which decreased from 6.6 to 6.1 m/s (p=0.013). Also, pain-free training also caused decrease in PWV (from 7.2 to 6.7 m/s), but this change was not statistically significant (p=0.127) (Table 8a and Fig 28). In addition, the difference in changes of PWV was not significant when two training groups were compared. Yet, improvements after both training groups were borderline significant as compared to control group (p=0.062 and p=0.054 for moderate-pain and pain-free training, respectively) (Table 9).
3.2.3 CARDIAC AUTONOMIC FUNCTION

3.2.3.1 HEART RATE VARIABILITY

Neither of the training groups led to significant improvements of HRV parameters. Both intervention groups led to increase of median values of rMSSD and pNN50%, but these changes were not significant (Table 8).

Comparison of the improvements between moderate-pain training group and controls in terms of pNN50% showed significant difference (p=0.042) (Table 9).

3.2.4 BIOMARKERS

None of the lipid profile parameters was significantly changed after exercise (walking) in patients with PAD (Table 8a).
3.2.4.1 MARKERS OF INFLAMMATION, HEMOSTASIS AND HEART FAILURE SEVERITY

As showed in Table 8a, there were no significant changes of NT-proBNP and D-dimer levels after either of the two training programmes.

Regarding fibrinogen, both training programmes led to improvement of fibrinogen levels. Improvement after moderate-pain training protocol was borderline significant (from average 4.2 to 3.6 g/L, p=0.085), while pain-free training protocol caused insignificant decrease in fibrinogen levels (from average 3.3 to 3.0 g/L, p=0.300) (Table 8a and Fig 29). However, this difference in improvements between training groups was not significant (p=0.501) (Table 9). Also, differences in changes after moderate-pain training and in controls, and after pain-free training and in controls were not significant (p=0.174 and p=0.367, respectively) (Table 9).

Fig 29. Comparison of mean relative (%) changes in **fibrinogen** levels for each group in the study of patients with peripheral arterial disease. Values below groups/bars denote paired samples t-test significance.
3.2.5 QUALITY OF LIFE

Moderate-pain training programme lead to improvement of physical domain in SF-36 questionnaire (from median 41.5 to 54.6 %, p=0.013) (Table 8b and Fig 30). On the contrary, mental component improvements were not statistically significant (from median 99.7 to 96.3 %, p=0.468) (Table 8b and Fig 30). Pain-free training programme led to borderline significant improvements in physical and mental domains (from median 58.2 to 67.1 %, p=0.067 and median 78.5 to 94.0 %, p=0.059, respectively).

Fig 30. Comparison of mean relative (%) changes in (a) physical component summary and (b) mental component summary for each group in the study of patients with peripheral arterial disease.

Values below groups/bars denote paired samples t-test significance.
<table>
<thead>
<tr>
<th>Vascular</th>
<th>Moderate-pain training group</th>
<th>Pain-free training group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
</tr>
<tr>
<td>β</td>
<td>8.8 (2.0)</td>
<td>7.6 (1.8)</td>
<td>10.1 (3.0)</td>
</tr>
<tr>
<td>PWV</td>
<td>6.6 (0.7)</td>
<td>6.1 (0.7)</td>
<td>7.2 (1.1)</td>
</tr>
<tr>
<td>FMD</td>
<td>4.4 (2.0)</td>
<td>8.0 (2.3)</td>
<td>4.6 (1.6)</td>
</tr>
<tr>
<td>ABI</td>
<td>0.51 (0.16)</td>
<td>0.51 (0.08)</td>
<td>0.49 (0.14)</td>
</tr>
<tr>
<td>Exercise testing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Claudication onset dist.</td>
<td>63 (30-84)</td>
<td>121 (78-203)</td>
<td>43 (35-58)</td>
</tr>
<tr>
<td>Maximal walking dist.</td>
<td>109 (74-160)</td>
<td>199 (124-482)</td>
<td>87 (66-100)</td>
</tr>
<tr>
<td>Laboratory tests</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>5.3 (5.1-11.7)</td>
<td>6.4 (5.1-11.0)</td>
<td>6.0 (5.2-8.9)</td>
</tr>
<tr>
<td>TC</td>
<td>3.9 (1.0)</td>
<td>4.2 (0.8)</td>
<td>4.3 (1.4)</td>
</tr>
<tr>
<td>HDL-c</td>
<td>1.2 (0.3)</td>
<td>1.3 (0.3)</td>
<td>1.3 (0.2)</td>
</tr>
<tr>
<td>LDL</td>
<td>2.0 (0.9)</td>
<td>2.1 (0.8)</td>
<td>2.3 (1.2)</td>
</tr>
<tr>
<td>Trig</td>
<td>1.7 (1.1)</td>
<td>1.5 (0.9)</td>
<td>1.7 (0.7)</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>4.2 (0.7)</td>
<td>3.6 (0.7)</td>
<td>3.3 (0.5)</td>
</tr>
<tr>
<td>D-dimer</td>
<td>340 (248-613)</td>
<td>370 (280-500)</td>
<td>450 (380-770)</td>
</tr>
<tr>
<td>NT-proBNP</td>
<td>215 (55-471)</td>
<td>196 (36-354)</td>
<td>196 (75-321)</td>
</tr>
</tbody>
</table>

Data are described as mean (standard deviation) or median (Q1-Q3). Columns p denote paired samples t-test significance.

β – beta stiffness coefficient; PWV – pulse wave velocity; FMD – flow-mediated dilation; ABI – ankle-brachial index; TC – total cholesterol; HDL-c – high-density lipoprotein cholesterol; LDL – low-density lipoprotein cholesterol; Trig. – triglycerides; NT-proBNP – amino-terminal prohormone of brain natriuretic peptide.
### 8b. Exercise training measurements for three studied groups before and after trainings, and changes in patients with peripheral arterial disease

<table>
<thead>
<tr>
<th></th>
<th>Moderate-pain training group</th>
<th>Pain-free training group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>p</td>
</tr>
<tr>
<td><strong>HRV</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SDNN</td>
<td>33.59 (23.33-43.33)</td>
<td>41.78 (24.69-56.14)</td>
<td>0.474</td>
</tr>
<tr>
<td>rMSSD</td>
<td>17.01 (13.36-30.78)</td>
<td>18.24 (12.40-37.42)</td>
<td>0.857</td>
</tr>
<tr>
<td>pNN50%</td>
<td>0.60 (0.34-9.62)</td>
<td>0.93 (0.20-16.92)</td>
<td>0.327</td>
</tr>
<tr>
<td>LFnu</td>
<td>0.27 (0.15)</td>
<td>0.25 (0.15)</td>
<td>0.707</td>
</tr>
<tr>
<td>HFnu</td>
<td>0.18 (0.16)</td>
<td>0.17 (0.19)</td>
<td>0.803</td>
</tr>
<tr>
<td>LFnu/HFnu</td>
<td>1.66 (0.57-6.05)</td>
<td>2.51 (0.59-4.55)</td>
<td>0.833</td>
</tr>
<tr>
<td><strong>SF-36</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCS</td>
<td>41.5 (22.4-74.8)</td>
<td>54.6 (48.6-77.1)</td>
<td>0.013</td>
</tr>
<tr>
<td>MCS</td>
<td>99.7 (70.6-103.8)</td>
<td>96.3 (73.4-98.9)</td>
<td>0.468</td>
</tr>
<tr>
<td>PF</td>
<td>43 (39-55)</td>
<td>58 (55-70)</td>
<td>0.007</td>
</tr>
<tr>
<td>RP</td>
<td>38 (0-100)</td>
<td>75 (25-100)</td>
<td>0.194</td>
</tr>
<tr>
<td>RE</td>
<td>100 (50-100)</td>
<td>100 (58-100)</td>
<td>0.157</td>
</tr>
<tr>
<td>VT</td>
<td>68 (50-80)</td>
<td>65 (55-80)</td>
<td>0.305</td>
</tr>
<tr>
<td>MH</td>
<td>86 (73-90)</td>
<td>86 (72-88)</td>
<td>0.752</td>
</tr>
<tr>
<td>SF</td>
<td>75 (75-100)</td>
<td>81 (59-100)</td>
<td>1.000</td>
</tr>
<tr>
<td>BP</td>
<td>45 (23-82)</td>
<td>66 (45-81)</td>
<td>0.028</td>
</tr>
<tr>
<td>GH</td>
<td>48 (35-63)</td>
<td>48 (34-69)</td>
<td>0.521</td>
</tr>
<tr>
<td><strong>Physical</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IPAQ score</td>
<td>5292 (1308-7279)</td>
<td>2826 (1772-6576)</td>
<td>0.950</td>
</tr>
</tbody>
</table>

Data are described as mean (standard deviation) or median (Q1-Q3). Columns p denote paired samples t-test significance except for parameter PNN50% where Wilcoxon paired samples test.

HRV = heart rate variability; SDNN = standard deviation of all normal R-R (NN) intervals; rMSSD = root mean square of successive NN interval differences; PNN50% = percentage of successive NN intervals differing by >50ms; LFnu = power in the low-frequency range, normalized; HFnu = power in the high-frequency range, normalized; LF:HF = ratio of normalized low- and high-frequency power; SF-36 = short form health survey questionnaire; PCS = physical component summary; MCS = mental component summary; PF = physical functioning; RP = role functioning/physical; RE = role functioning/emotional; VT = vitality; MH = mental health; SF = social functioning; BP = bodily pain; GH = general health; IPAQ = international physical activity questionnaire.
Table 9. Absolute changes and comparison among groups

in patients with peripheral arterial disease

<table>
<thead>
<tr>
<th>Changes</th>
<th>Improvement comparisons (p values) *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moderate pain training group</td>
<td>Moderate-pain vs. pain-free group</td>
</tr>
<tr>
<td>Vascular</td>
<td></td>
</tr>
<tr>
<td>β</td>
<td>-1.2 (1.2)</td>
</tr>
<tr>
<td>PWV</td>
<td>-0.5 (0.5)</td>
</tr>
<tr>
<td>FMD</td>
<td>3.7 (2.6)</td>
</tr>
<tr>
<td>ABI</td>
<td>0.001 (0.15)</td>
</tr>
<tr>
<td>Exercise testing</td>
<td></td>
</tr>
<tr>
<td>Claudication onset dist.</td>
<td>73.8 (49.7)</td>
</tr>
<tr>
<td>Maximal walking dist.</td>
<td>173.3 (191.1)</td>
</tr>
<tr>
<td>Laboratory tests</td>
<td></td>
</tr>
<tr>
<td>TC</td>
<td>0.1 (0.4)</td>
</tr>
<tr>
<td>HDL-c</td>
<td>0.1 (0.2)</td>
</tr>
<tr>
<td>LDL-c</td>
<td>0.1 (0.3)</td>
</tr>
<tr>
<td>Trig</td>
<td>-0.2 (0.6)</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>-0.4 (0.6)</td>
</tr>
<tr>
<td>D-dimer</td>
<td>13.3 (77.0)</td>
</tr>
<tr>
<td>NT-proBNP</td>
<td>89.2 (232.7)</td>
</tr>
<tr>
<td>HRV</td>
<td></td>
</tr>
<tr>
<td>SDNN</td>
<td>5.0 (16.6)</td>
</tr>
<tr>
<td>rMSSD</td>
<td>1.0 (5.1)</td>
</tr>
<tr>
<td>PNN50%</td>
<td>N/A</td>
</tr>
<tr>
<td>LFnu</td>
<td>-0.02 (0.15)</td>
</tr>
<tr>
<td>HFnu</td>
<td>-0.01 (1.09)</td>
</tr>
<tr>
<td>LF/HF</td>
<td>-0.4 (1.7)</td>
</tr>
<tr>
<td>SF-36</td>
<td></td>
</tr>
<tr>
<td>PCS</td>
<td>15.1 (12.2)</td>
</tr>
<tr>
<td>MCS</td>
<td>-0.2 (12.4)</td>
</tr>
<tr>
<td>PF</td>
<td>15.0 (10.3)</td>
</tr>
<tr>
<td>RP</td>
<td>15.0 (31.6)</td>
</tr>
<tr>
<td>RE</td>
<td>6.7 (14.0)</td>
</tr>
<tr>
<td>VT</td>
<td>3.5 (10.6)</td>
</tr>
<tr>
<td>MH</td>
<td>2.4 (12.2)</td>
</tr>
<tr>
<td>SF</td>
<td>0.0 (13.2)</td>
</tr>
<tr>
<td>BP</td>
<td>11.0 (12.6)</td>
</tr>
<tr>
<td>GH</td>
<td>2.5 (10.3)</td>
</tr>
<tr>
<td>Physical activity</td>
<td></td>
</tr>
<tr>
<td>IPAQ score</td>
<td>-723 (3408)</td>
</tr>
</tbody>
</table>

Changes are described as mean (standard deviation).

β – beta stiffness coefficient; PWV – pulse wave velocity; FMD – flow-mediated dilation; ABI – ankle-brachial index; TC – total cholesterol; HDL-c – high-density lipoprotein cholesterol; LDL-c – low-density lipoprotein cholesterol; Trig. – triglycerides; NT-proBNP – amino-terminal prohormone of brain natriuretic peptide; HRV – heart rate variability; SDNN – standard deviation of all normal R-R (NN) intervals; rMSSD – root mean square of successive NN interval differences; PNN50% – percentage of successive NN intervals differing by >50ms; LFnu – power in the low-frequency range, normalized; HFnu – power in the high-frequency range, normalized; LF/HF – ratio of normalized low- and high-frequency power; SF-36 – short form health survey questionnaire; PCS – physical component summary; MCS – mental component summary; PF – physical functioning; RP – role functioning/physical; RE – role functioning/ emotional; VT – vitality; MH – mental health; SF – social functioning; BP – bodily pain; GH – general health; IPAQ – international physical activity questionnaire

* One-way ANOVA with Bonferroni correction
4. DISCUSSION

4.1 SAFETY AND ADHERENCE

Exercise training is safe. In our study, both types of exercise – namely, strenuous (interval and moderate-pain, respectively) and lenient (continuous and pain-free, respectively) – resulted in no adverse events in patients with repaired ToF and PAD undergoing a supervised, planned, structured and repetitive programme of exercise training.

In the past, safety concerns may have precluded from more widespread utilisation of exercise in these patients. Although it was not the case in the past, systematic reviews and guidelines nowadays recommend exercise training as a part of congenital heart disease management [23,96]. However, exercise remains under-implemented. On the one hand, this is likely due to the reluctance of medical professionals to prescribe, and possibly lack of knowledge on positive effects of, exercise. On the other hand, under-implementation may likely derive from fear from adverse events and perceived risks of exercise in this population [23]. Our results have confirmed that exercise training is safe in adults with repaired ToF, which corroborate the results from the systematic review of Duppen and colleagues [23].

Of note, our study population of adults with repaired ToF consisted of stable and relatively asymptomatic patients. All included patients were classified as NYHA I; although higher NYHA class was not an exclusion criterion, only patients in NYHA I class responded and agreed to participate in our study. Conversely, higher NYHA classes are associated with higher risk of adverse cardiovascular events and safety issues must be differently addressed in such population of patients. Also, ventricular dysrhythmias represent a major cause of long-term mortality in adults with repaired ToF [65] and represented an exclusion criterion for participation in our study. Although none of the originally screened patients met this exclusion criterion, potential risks of exercise deriving from pre-existing ventricular dysrhythmias cannot be addressed. Therefore, our results on safety of exercise training programmes must be applied with due caution to high-risk adult populations with repaired ToF in terms of symptom severity and dysrhythmias.
Also in patients with PAD, no adverse events were recorded. However, safety issues regarding exercise training for patients with PAD are somehow different when compared to patients with repaired ToF. Patients with PAD are generally not at high risk for ventricular dysrhythmias and SCD. Also, exercise training protocols in patients with PAD in previous studies were focused on improvement of walking capacity [133,163,168,171], making it relatively less demanding and risky. However, these patients are on average older, often have numerous interplaying risk factors and likely have already developed generalised atherosclerosis, including coronary artery disease [110], which renders them at high risk for adverse cardiovascular events during exercise training. However, both training modalities for PAD employed in our study were safe, yielding no adverse events and thus confirming results reported in the literature [187].

In terms of adherence to participation, included participants in both subsets of patients and in all four exercise training programmes showed very high levels of adherence – in the range 90%. Previous studies have consistently shown that adherence to participation in exercise training programmes is one of the most important predictors of increased exercise capacity, improved physiologic parameters and reduced morbidity and mortality [172,188]. High adherence levels in our studies call for two issues to be addressed. Firstly, adherence to programme was feasible and attainable, and reflects a positive attitude towards the programme from a patient-centred perspective. Secondly, results in terms of efficiency of the exercise programme can only be inferred upon if such adherence levels are obtained. It should be noted, however, that this was a selected study population with a substantial number of potential enrollees declining study participation. Individuals who declined participation may have either done it due to low motivation (which would have likely resulted in failure to achieve improvements in observed parameters) or due to feasibility issues (such as transportation and timing), which represent a major barrier to adherence in exercise programmes [188]. Regardless of the reasons, high adherence levels should be regarded as a pivotal domain of our intervention and addressed when generalising our results.
4.2 EXERCISE/WALKING CAPACITY

4.2.1 REPAIRED TETRALOGY OF FALLOT

In patients with repaired ToF, any exercise training improves some domains of exercise capacity as compared to no exercise. However, in terms of maximal oxygen consumption (primary indicator of exercise capacity), interval training seems to improve VO2peak significantly (by an average magnitude of 1.7 ml/kg/min as compared to pre-training), while the improvement with continuous training failed to achieve statistical significance.

This is the first study in grown-ups with congenital heart disease, including those with repaired ToF, in which two types of exercise training were compared. Few previous studies only addressed safety [23] and efficacy of continuous training of different intensity; Avila [98] and Duppen [99,189] have shown that continuous exercise training increases peak workload, which is in line with our observation in the continuous training-arm in our study. However, while previous studies have also detected an improvement in VO2peak, our results only showed a tendency towards better oxygen consumption without significance (average increase 1.8 ml/kg/min, p=0.190). A smaller number of participants in our study may explain such discrepancy.

To the best of our knowledge, this is also the first study of interval training in grown-ups with congenital heart disease. As such, it provides novel insight in terms of training type for grown-ups with congenital heart disease. Interval training is safe and may be more efficacious in terms of exercise capacity gains (VO2peak), and as such may be recommended as a training modality in this population. However, the largest magnitude of improvement was detected in both exercise modalities as compared to no exercise (i.e. in terms of no statistically significant differences between post-exercise capacity in the interval and continuous group); in fact, our results suggest that there is no statistically significant difference in exercise capacity between interval and continuous training at the end of the intervention programme. Therefore,
either modality may be recommended for exercise capacity improvement depending on provider feasibility and patients’ preference.

Respective merits and drawbacks of interval and continuous training are a matter of controversy. Most previous studies on interval vs. continuous training were performed in patients with coronary artery disease or heart failure, and yielded conflicting results. Early smaller studies in coronary artery patients have shown that interval training may be more efficient in improving exercise capacity [190]; also, two meta-analyses [191,192] of 6 trials (229 patients) and 9 trials (206 patients), respectively, have suggested superiority of interval training compared to continuous in terms of exercise capacity. Conversely, the large randomised controlled SAINTEX-CAD trial in 200 coronary artery patients found comparable efficacy of interval and continuous training [193]. Moholdt and colleagues provided similar results [194]. Similarly, in patients with heart failure, interval training was suggested to be superior to continuous training in improving exercise capacity [195–197], but its long-term effects remain elusive.

Overall, interval training seems to provide more substantial improvements in exercise capacity, and this is in line with our results. However, long-term effects of interval training have not been determined yet, principally due to historic preference towards continuous training and only recent attention to the possibility that interval training may be as safe and more efficacious. Thus, our results fit in well with the existing evidence gap trying to address the merits and drawbacks of interval training in different cardiovascular conditions.

4.2.2 PERIPHERAL ARTERIAL DISEASE

Our study confirmed that exercise training (walking) improves walking performance in patients with PAD. However, previous data on different exercise modalities are scarce; thus, our results provide novel information, namely comparative efficacy of moderate-pain vs. pain-free exercise training. Our findings suggests that both exercise modalities comparably improve outcome measures – pain-free walking distance
and maximal walking distance – thus reinforcing the notion that most walking-based exercise programmes are efficient in improving walking capacity in patients with PAD [151,160,162,167,198–201].

Another specific feature of our study intervention is intensity being defined with percentage of maximum predicted HR. This, in turn, provides comparable cardiovascular intensity of both training modalities. Previous studies of exercise training in PAD patients have based exercise prescription on walking distance only, thus focusing on peripheral vascular symptoms (improved walking distance) rather than cardiovascular wellness. As the later is achieved through exercise prescription based on intensity derived from exercise capacity (VO2peak) or HRpeak, exercise training in PAD patients has been criticised for being “vascular” rather than “cardiovascular” in nature [202].

Our findings are consistent with previous research, which rather consistently demonstrated a favourable impacts on walking parameters in patients with PAD, irrespective of the outcome definition (pain-free walking distance, maximal walking distance, pain-free walking time and maximal walking time) [151,160,162,167,198–201]. These findings have also been upheld in systematic reviews and meta-analysis [203,204]. Improvements in walking performance have been shown to persist up to three years after exercise programme completion [205]. However, the effects of exercise training programmes persist only if the patient continues to exercise on his/her own for at least 60 minutes per week [206].

Walking parameters remain the most important outcome measure in studies on exercise training in patients with PAD. ABI was also a parameter widely used as a measure of disease severity and improvement. ABI is not only a diagnostic tool for diagnosis of asymptomatic PAD, but also a marker of diffuse atherosclerosis associated with coronary artery disease and cerebrovascular disease [110], and a strong predictor of cardiovascular mortality [126]. Of importance, in our study there was no improvement of ABI after either of the two exercise training programmes. Literature reports on this issue are conflicting, which is also reflected in a recent meta-analysis, suggesting that improvement of ABI after exercise training
in patients with PAD is borderline significant [24]. In some studies, improvement of ABI was associated with improvement of walking abilities [207], while in others no such association could be found [163].

4.3 VASCULAR FUNCTION

4.3.1 REPAIRED TETRALOGY OF FALLOT

Both exercise training modalities – interval and continuous – yield significant improvement of endothelial function, without significant between-modality differences.

This is the first study in patients with repaired with ToF, in which endothelial function is assessed as a dependent variable after an exercise training programme.

Patients with repaired ToF have impaired endothelial function, as shown in a study of de Groot and colleagues in paediatric population [94] and confirmed in our study examining adults with repaired ToF [Appendix B]. Potential mechanisms include lower physical activity in patients with repaired ToF [47,48], persistent haemodynamic abnormalities as a consequence of cardiac surgery in childhood, and oxidative stress [94].

Exercise is known to improve endothelial function not only in healthy individuals, but also various cardiovascular and metabolic patients [208,209]. Increased synthesis of NO as a result of repetitive shear stress, increased oxidative status, decreased production of pro-inflammatory molecules and promoted endothelial repair via endothelial progenitor cells mobilisation have all been suggested as possible mechanisms promoting improvement of endothelial function due to exercise training [208].

Brachial artery FMD, as a marker of endothelial dysfunction, is found to be a significant predictor of adverse cardiovascular events in apparently healthy subjects and individuals with cardiovascular diseases [210–213] and also a predictor of mortality [214]. Therefore, improving endothelial function is a potential mechanism how exercise training decreases adverse cardiovascular events in a population of adults with repaired ToF.
Arterial stiffness is another marker of vascular health. Results from studies of László and colleagues [90] suggest that arterial stiffness is impaired in patients with repaired ToF. Conversely, our results suggest that there are no differences between adults with repaired ToF and healthy controls in terms of arterial stiffness [56].

To date, effects of exercise training on arterial stiffness in adults with repaired ToF have not been studied. In our study, we have addressed possible changes in arterial stiffness with exercise and have demonstrated that interval training significantly decreases arterial stiffness, while improvement after continuous training only borderline significant. In a meta-analysis of 42 studies (1627 participants, either healthy individuals or patients with different conditions including cardiovascular disease), aerobic exercise training improved arterial stiffness [215], whereas other studies failed to detect significant improvements in prehypertensive and hypertensive patients, and patients after heart transplant with training [216,217]. Such discrepancies may be attributable to failure to discern different modalities of aerobic training, namely interval and continuous. In fact, interval – but not continuous – training has been associated with improvements in arterial stiffness in patients with cardiovascular and metabolic conditions [218].

Overall, in adults with repaired ToF, interval training seems to have a more profound impact on vascular function (as determined by FMD and arterial stiffness parameters) as compared to continuous training.

4.3.2 PERIPHERAL ARTERIAL DISEASE

Exercise training improves FMD in patients with PAD. However, while the improvement in moderate-pain training is substantial and significant, the improvement in pain-free training only showed a trend towards statistical significance.

Endothelial function, as determined by FMD [219], is impaired in patients with PAD and a marker of risk, as well as a predictor of symptom severity and impaired physical activity [220]. Improvement of FMD due to exercise training was shown in the majority of the studies [158,159,198], but not in all [221]. As suggested earlier, results on improved FMD might be explained by up-regulation of endothelial NO synthase and
consequently enhanced endothelial synthesis and release of NO, which is stimulated by an increase in blood flow due to exercise. In addition, improved blood perfusion can be at least partially explained by increased collateral flow, as has been suggested in studies that included coronary artery disease patients [222]. Also, exercise induces an increase in endothelial NO, which decreases the level of reactive oxygen species formation as well as inflammation [223,224].

In terms of effects of exercise on arterial stiffness in patients with PAD, ours is the first study to address this issue. Moreover, we studied the differential impact of two exercise modalities – pain-free and moderate-pain walking. Similar to endothelial function improvement (as determined by FMD), our results suggest that moderate-pain – but not pain-free – exercise improves arterial stiffness.

In patients with PAD, arterial stiffness is increased, and exercise training may be expected to decrease it, as shown in other populations, such as healthy individuals [225], patients with type 2 diabetes [226] and patients with heart failure [227]. This can be explained by several mechanisms, which may affect arterial stiffness and are, in turn, affected by exercise training – namely, a decrease in blood pressure, a decrease in inflammatory response and metabolic derangements and an increase in NO bioavailability. In our study, inflammation decrease (as suggested by lower post-exercise levels of fibrinogen) and increased NO bioavailability (as suggested by higher post-exercise FMD) may be regarded as potential mechanisms of an exercise-attributable decrease of arterial stiffness.

Of importance, several studies showed arterial stiffness to be increased in patients with PAD as compared to those without PAD [228,229]. Baseline arterial stiffness in our patients was rather high as compared to values reported in literature [178,230], and thus expectedly affected by an intervention. In fact, high arterial stiffness in PAD reflects systemic nature of arterial disease [228]. Potential mechanism for increased stiffness lays in “dual” origin of increased arterial stiffness: structural alterations and functional changes. PAD through arterial obstructions and decreased nitric oxide bioavailability affects both entities [231].
Overall, in patients with PAD, moderate-pain walking seems to have a more profound impact on vascular function (as determined by FMD and arterial stiffness parameters) as compared to pain-free walking. Drawing parallel features between our studies in PAD and adults after ToF repair, a strenuous vs. lenient exercise modality seems to have a larger impact on vascular function in two distinctive patient populations – those with predominantly “cardiac” derangements (ToF) and those with predominantly “vascular” derangements (PAD).

4.4 CARDIAC AUTONOMIC FUNCTION

4.4.1 PATIENTS WITH REPAIRED TETRALOGY OF FALLOT

Moderate continuous training significantly improved LFnu, a frequency-domain parameter, while improvement of time-domain parameters showed a trend towards significance. On the contrary, interval training caused no improvement of HRV. This benefit can be explained with increased parasympathetic and decreased sympathetic activity brought about by exercise training [61].

Although studies have shown that patients with repaired ToF have impaired HRV [57,58], which was confirmed in our study as well [56], this is the first study in this population to examine effects of exercise on HRV parameters.

Exercise has been suggested to improve HRV in various cardiovascular and cardiometabolic conditions, as summarised by a systematic review of Routledge and colleagues [61]. Moreover, in patients with heart failure, different types of exercise training (such as aerobic and resistance) have improved HRV. Similar findings are reported in patients after acute myocardial infarction [61].

Exercise affects HRV mainly through modulation of parasympathetic and sympathetic activities [61]. Buch and colleagues suggest that exercise training may increase vagal tone through angiotensin II and NO. On the one hand, exercise suppresses expression of angiotensin II, while angiotensin II, in turn, may inhibit
vagal activity. On the other hand, NO increases vagal tone either directly (nitric oxide synthase (NOS) isoforms are present in both central and peripheral vagal neurones) or indirectly (NOS isoforms are present in baroreflex receptors, and may modulate vagal responses) [232]. In dogs, infusion of a NOS inhibitor into the artery of the sinoatrial node resulted in increased HR and reduced HRV [233]. Therefore, exercise may increase NOS activation and improve NO bioavailability, which, in turn, may improve HRV. In our study, we have shown that exercise improved FMD, which reflects NO bioavailability and may therefore, at least in part, corroborate this hypothesis.

Another possible physiological pathway for beneficial effects of exercise to cardiac autonomic function is a decrease in circulating catecholamine levels. Rengo and colleagues have shown a significant decrease of the norepinephrine levels after exercise training programmes in patients with heart failure [234]. However, as our study did not measure circulating catecholamine levels, such mechanistic explanation remains speculative.

HRR is another parameter of autonomic cardiac function and a predictor of mortality in healthy individuals and different cardiovascular conditions, including adults with congenital heart disease [67–69]. We have shown that HRR is impaired in adults with repaired ToF [56]. Despite the fact that both HRV and HRR are markers of autonomic cardiac function, especially parasympathetic function, they represent different aspects of parasympathetic function – namely, HRR namely reflects parasympathetic tone, while HRV reflects parasympathetic modulation [66].

We have demonstrated that continuous – but not interval – exercise training improves HRR in adults with repaired ToF. This is a novel finding in adults with congenital heart disease. Reports from the literature in various cardiovascular diseases are conflicting. Some reports suggest that neither of the two modalities is successful in improving HRR [235], while others suggest that both may be efficient in improving abnormal HRR [236]. A study from Matsuo and colleagues found interval training to be more efficient in improving HRR [237], while in a study of Dimopoulos and colleagues continuous exercise training appeared to be
more efficient [238]. Results from the latter are in line with our results. Possible explanation for this result might be that exercise is being a stimulus for the changes in autonomic cardiac function only if it is vigorous enough and lasts long enough. Interval training comprises bouts of vigorous exercise, but they do not last long enough. On the other hand, continuous training might be less vigorous, but still above a hypothetical “threshold” intensity. Longer continuous duration of the vigorous enough training might be the reason why continuous training improved both HRV and HRR in our study. Insufficient involvement of the autonomic nervous system in terms of stimulus and time is a potential mechanism, suggested by Dimopoulos and colleagues [238].

Overall, in adults with repaired ToF, continuous training seems to have a more profound impact on cardiac autonomic function (as determined by HRV and HRR) as compared to interval training. This is, interestingly, in sharp contrast with interval training providing superior outcomes in terms of exercise capacity and vascular function in this group of patients.

4.4.2 PERIPHERAL ARTERIAL DISEASE

As opposed to adults with repaired ToF, neither exercise training (walking) modality in patients with PAD improved HRV parameters. This is in line with most literature reports [161,239] except one [240]. Potential explanation may lay in exercise intensity, which was not enough to cross a hypothetical “threshold” needed for activation of vagal modulation. Conversely, Chehuen and colleagues succeeded to show that walking might lead to improvement of HRV [240]. However, their patients did not take HR- and HRV-modulating medication, such as beta blockers, as this was an exclusion criterion of their study. Such an exclusion criterion questions generalisability of their results. As many as 40 % of our patients were taking beta blockers, mostly as secondary prevention or antianginal medication in the management of concomitant coronary artery disease, which is often present in patients with PAD [110].

Overall, in patients with PAD, neither moderate-pain nor pain-free walking seems to have a significant impact on cardiac autonomic function (as determined by HRV). It seems that despite adjusting for intensity,
duration (limited by claudication symptoms) may preclude PAD patients to achieve volumes of exercise, which would be necessary to induce changes in cardiac autonomic function. Of note, other exercise modalities not limited by claudication symptoms (e.g. cycling) may be warranted in PAD patients to provide “cardiac” exercise effects beyond “vascular” symptom improvements.

4.5 BIOMARKERS

4.5.1 REPAIRED TETRALOGY OF FALLOT

Our results show that interval, but not continuous training yields a decrease of the NT-proBNP levels in adults with repaired ToF. This is the first study in grown-ups with congenital heart disease patients showing that exercise may decrease NT-proBNP levels. In line with our results, previous studies with continuous training also showed no effect on NT-proBNP levels in ToF patients [98,189]. Although all our patients were in NYHA class I, values of natriuretic peptide levels were increased as compared to what would be expected in healthy individuals, and are in line with previous reports for asymptomatic patients with repaired ToF [77]. As natriuretic peptides predict mortality in adults with repaired ToF [77], a decrease in NT-proBNP levels after interval exercise training might also suggest a reduction in future risk of these patients. However, prospective studies are needed to confirm such considerations.

Previous studies [234,241] have shown that exercise training decreases NT-proBNP levels in patients with heart failure. Similarly to other acute effects of exercise, NT-proBNP levels also tend to rise during training [242,243], whilst chronic adaptations include long-term decreases in natriuretic peptide levels. While some authors have suggested structural cardiac improvements brought about by exercise may explain a fall in natriuretic peptides [234], most studies have failed to detect any changes in cardiac volumes or structure with exercise [99,189]. A change in cardiac function and/or haemodynamics is therefore a more likely explanation. In patients with heart failure before and after interval or continuous exercise training, no change in natriuretic peptide levels could be detected [244]. However, both baseline and post-exercise...
values were high and highly variable (as expected in patients with heart failure) and therefore small and subtle changes may have been impossible to capture.

Our results also show that interval, but not continuous training yields a decrease in fibrinogen level in patients with repaired ToF. This is the first study in grown-ups with congenital heart disease to assess influence of exercise on fibrinogen levels. Literature reports provide conflicting results in healthy individuals and patients with cardiovascular diseases: in a study with healthy individuals, continuous exercise did not lead to a decrease in fibrinogen levels [245]. However, Zanettini and colleagues demonstrated that continuous training caused significant reduction of fibrinogen levels in hypertensive patients [9]. Fibrinogen is an independent predictor of cardiovascular mortality in different populations [246–248]. Its (patho)physiological function is related to haemostasis, but may also be associated with inflammation and endothelial function [249]. In fact, our study confirmed both an improvement in endothelial function and a significant decrease in fibrinogen levels in patients undergoing interval training. Therefore, as suggested in a review of Kamath and Lip, exercise may exert its beneficial influence on cardiovascular events through a reduction of plasma fibrinogen levels [249]. These effects might be equivalent to a 15 % reduction in the risk of ischaemic heart disease [250].

We have demonstrated that interval – but not continuous – exercise training leads to a significant increase in HDL-c levels. This is the first study in grown-ups with congenital heart disease to demonstrate improvement of lipid profile after an exercise training programme. Previous studies have not provided consistent results in cardiovascular patients – some researchers suggested that both interval and continuous training modalities increase HDL-c levels [193], while in other found neither interval nor continuous training to improve HDL-c [194]. Possible explanation might lay in not reaching the necessary thresholds of exercise volume, duration and intensity, which induce changes in lipid profile, especially HDL-c [251,252].
4.5.2 PERIPHERAL ARTERIAL DISEASE

Moderate-pain training was associated with a trend towards significant reduction of fibrinogen levels. Literature reports provide conflicting results regarding influence of exercise on fibrinogen levels in PAD: some studies reported a decrease in fibrinogen levels [253], while others did not [165,254]. Fibrinogen is an important biomarker in PAD as it predicts future development of PAD, severity of PAD [255] and mortality in patients with PAD [256,257]. In contribution to the coagulation cascade [248], its role as an acute phase inflammation marker and its association with atherosclerotic plaque composition [249,255] may provide pathophysiologic explanations for such a role. Reducing its circulating levels might contribute not only to clinical improvement in patients with PAD, but also to reduction of cardiovascular morbidity and mortality in these patients.

Neither of the two training (walking) programmes led to significant improvement of lipid parameters. In this respect, also reports from the literature are inconsistent [159,254,258–260]. As suggested earlier, low volume and relatively short duration of intensive bouts of exercise might explain why neither training modality improved the lipid profile. Additionally, the vast majority (around 95 %) of patients were taking lipid-lowering medications; therefore any additional improvements of the lipid profile brought about by the training programme may have been too subtle to be detected in this setting [259]. Moreover, our patient population was by a large proportion of persisting smokers; as smoking is associated with decreased level of HDL-c, this may have blunt any effects of exercise on HDL-c [261,262].

4.6 QUALITY OF LIFE

4.6.1 REPAIRED TETRALOGY OF FALLOT

We have demonstrated that continuous – but not interval – exercise training improves health-related quality of life, predominantly its mental component in patients with repaired ToF. This result is mostly
opposite to other reports and meta-analyses, in which physical component improved more. Of importance, patients with repaired ToF are less physically active and have impaired exercise capacity, as compared to healthy peers [47,51]. It seems that continuous training, devoid of extreme (and unpleasant) high-interval bouts of exercise, led to improvement in exercise workload, but also had positive psychological effects. In that sense, results on improved HRV parameters and mental component of health-related quality of life suggest that cardiac autonomic function might also be partially a consequence of positive psychological effects [263].

4.6.2 PERIPHERAL ARTERIAL DISEASE

Both moderate-pain and pain-free exercise training improved quality of life as determined by the SF-36 questionnaire. Physical domains were affected the most and were predominantly driven by improvement of physical functioning and body pain in moderate-pain training, and by improvement of role functioning and body pain in pain-free training. In pain-free training, a trend towards significance in mental domain could also be appreciated, principally driven by mental health and social functioning, which both showed a tendency towards significance.

Intermittent claudication, as the most prominent symptomatic manifestation of PAD, is associated with limitations in everyday activities, such as walking. Therefore, improvements in pain-free walking distance were expected to translate in improved health-related quality of life measures. Most studies of exercise in patients with PAD used the SF-36 questionnaire, which has been shown to have good reliability and has been extensively validated; it is therefore the most widely used research tool for measurement of health-related quality of life in different diseases and conditions. Exercise training also leads to an improvement in the disease-specific quality of life as assessed by other questionnaires, such as walking impairment questionnaire (WIQ), The Peripheral Arterial Occlusive Disease 86 (PAVK-86), The Intermittent Claudications Questionnaire, VascuQol and others. A recent meta-analysis of 15 randomized trials (including data on 1257 participants) showed that along with increasing walking distance, the physical – but not mental – component of the SF-36 also increased significantly [264], which is partially in line with our results.
5. LIMITATIONS

We have identified some limitations in both of our sub-studies.

Firstly, a relatively small sample size. Sample size was primarily calculated to confirm or reject primary hypotheses. Therefore, it can be underpowered to detect differences in secondary observed variables and smaller differences in primary observed variables. Nonetheless, we did confirm an improvement in the primary observed variables (maximal oxygen uptake in patients with repaired ToF, and pain free walking distance in patients with PAD, respectively) with exercise; however, type of exercise did not affect improvements significantly, suggesting that exercise type either does not influence observed variables or the effect is so small in magnitude that our study did not detect it (being powered to detect a >1.5 ml/kg/min VO2peak difference and a >100 m difference, respectively) and hence may not be clinically relevant.

Secondly, we have identified only physiological outcomes as a consequence of intervention. Our study was underpowered to detect clinical endpoints, such as disease-related morbidity and mortality and a much larger sample size would be required to test such a hypothesis. Nonetheless, similar studies on exercise also face the problem of relatively small sample sizes and use physiological observed variables instead. It is noteworthy, however, that such physiological observed variables (such as exercise capacity and pain-free walking distance) represent strong predictors of morbidity and mortality and also, more importantly, patient-centred quality of life.

Thirdly, non-blind allocation of study participants cannot be adjusted for. As patients in all exercise and control groups were made aware of potentially favourable effects of exercise, an increase in home based activities in control group patients (“Howthorne effect”) cannot be ruled out. However, this remains an unavoidable issue in all studies on exercise training.

Fourthly, the study was a single-centre study. As the University Medical Centre Ljubljana is the national referral centre for both conditions, and the cardiovascular rehabilitation unit provides structural resources
for implementation of outpatient rehabilitation services, study population may be regarded as representative. However, our study may provide a starting point for larger multi-centre and multi-national studies aimed at confirming our study findings.

Fifthly, results from both sub-studies should remain limited to studied patients’ population. Hence, safety and efficacy of different types of exercise from our study can only be inferred upon patients with clinically stable NYHA I class patients after repaired ToF and clinically stable Fontaine IIa patients with PAD, respectively.

Overall, limitations of our study derive from a limited remit of our research questions, but also provide an opportunity for further research. Most of the limitations could be addressed by future larger studies providing larger patient populations, drawn from diverse clinical and demographic extraction, with interventions performed at different centres and thus allowing inference on health outcomes beyond exercise capacity and physiological endpoints.
6. CONCLUSIONS

Exercise training, be it strenuous (high-intensity interval/moderate-pain walking) or lenient (moderate-intensity continuous/pain-free walking) is safe in adults with repaired ToF and patients with PAD.

In adults with repaired ToF:

- High-intensity interval exercise training is more effective in improving exercise capacity, vascular function ("vascular benefit") and disease-specific biomarkers as compared to moderate-intensity continuous exercise training.
- Moderate-intensity continuous exercise training is effective in improving cardiac autonomic function ("cardiac benefit") and health-related quality of life as compared to high-intensity interval exercise training.

In patients with PAD:

- Both moderate-pain and pain-free exercise training (walking) are comparably effective in improving walking capacity.
- Moderate-pain exercise training is more effective in improving vascular function ("vascular benefit") and fibrinogen levels as compared to pain-free exercise training.
- Pain-free exercise training is more effective in improving quality of life as compared to moderate-pain exercise training.
- Neither exercise training modality improves cardiac autonomic function and NT-proBNP levels (no "cardiac benefit").
REFERENCES


113


[88] Khairy P, Van Hare GF, Balaji S, Berul CI, Cecchin F, Cohen MI, et al. PACES/HRS Expert Consensus Statement on the Recognition and Management of Arrhythmias in Adult Congenital Heart Disease: developed in partnership between the Pediatric and Congenital Electrophysiology Society (PACES) and the Heart Rhythm Society (HRS). Endorsed by the governing bodies of PACES, HRS, the American College of Cardiology (ACC), the American Heart Association (AHA), the European Heart Rhythm Association (EHRA), the Canadian Heart Rhythm Society (CHRS), and the International Society for Adult Congenital Heart Disease (ISACHD). Heart Rhythm 2014;11:e102–165. doi:10.1016/j.hrthm.2014.05.009.


Cardiovascular autonomic dysfunction and carotid stiffness in adults

with repaired tetralogy of Fallot

Marko Novaković 1,2, Katja Prokšelj 2,3, Vito Starc 4 and Borut Jug 1,2

1 Department of vascular diseases, University Medical Centre Ljubljana, Ljubljana, Slovenia
2 Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia
3 Department of cardiology, University Medical Centre Ljubljana, Ljubljana, Slovenia
4 Faculty of Medicine, Institute of Physiology, University of Ljubljana, Ljubljana, Slovenia

Published in Clinical Autonomic Research, March 8, 2017


doi: 10.1007/s10286-017-0411-0
Abstract

Purpose: Adults after surgical repair of tetralogy of Fallot (ToF) may have impaired vascular and cardiac autonomic function. Thus, we wanted to assess interrelations between heart rate variability (HRV) and heart rate recovery (HRR), as parameters of cardiac autonomic function, and arterial stiffness, as a parameter of vascular function, in adults with repaired ToF as compared to healthy controls.

Methods: In a case-control study of adults with repaired ToF and healthy age-matched controls we measured: 5-min HRV variability (with time and frequency domain data were collected), carotid artery stiffness (through pulse-wave analysis using echo-tracking ultrasound) and post-exercise HRR (cycle ergometer exercise testing).

Results: Twenty-five patients with repaired ToF (mean age 38±10 years) and ten healthy controls (mean age 39±8 years) were included. Selected HRR and HRV (time-domain) parameters, but not arterial stiffness were significantly reduced in adults after ToF repair. Moreover, a strong association between late/slow HRR (after 2, 3 and 4 minutes) and carotid artery stiffness was detected in ToF patients (r=-0.404, p=0.045; r=-0.545, p=0.005 and r=-0.545, p=0.005, respectively), with statistical significance retained even after adjusting for age, gender, resting heart rate and β-blockers use (r=-0.393, p=0.024 for HRR after 3 minutes).

Conclusion: Autonomic cardiac function is impaired in patients with repaired ToF, and independently associated with vascular function in adults after ToF repair, but not in age-matched healthy controls. These results might help in introducing new predictors of cardiovascular morbidity in a growing population of adults after surgical repair of ToF.

Keywords

Tetralogy of Fallot, autonomic nervous system, exercise test, heart rate, vascular stiffness
Introduction

Tetralogy of Fallot (ToF) is the most common cyanotic congenital heart disease, consisting of the following abnormalities: (1) ventricular septal defect, (2) overriding aorta, (3) obstruction of the right ventricle outflow, and (4) right ventricular hypertrophy. Surgical treatment in the childhood yields an increasing number of patients with ToF reaching adult age, with excellent long-term survival [1]. However, several long-term sequelae of ToF repair have been reported, including impaired cardiac autonomic [2, 3] and vascular function [4, 5]. Resting heart rate variability (HRV) and heart rate recovery (HRR) after exercise testing are two most used measures of cardiac autonomic function, and validated prognostic predictors of mortality in healthy individuals and several cardiovascular entities [6–8]. While in healthy subjects and patients with cardiovascular diseases HRV and HRR are interrelated [9–11], evidence on such association in adults after ToF repair is lacking. On the other hand, arterial stiffness has emerged as an important indicator of subclinical impairment of vascular function [12]. Stiffer carotid arteries might contribute to impaired activation of the baroreceptors situated around bifurcation of the common carotid artery and thus might lead to impaired cardiac autonomic control [13–15], thus providing a pathophysiologic rationale for cardiac autonomic and peripheral vascular function to be interrelated in adults after ToF repair.

In the present study, we sought to assess possible interrelationships between resting HRV, post-exercise HRR and carotid artery stiffness in patients with repaired ToF as compared to healthy adults. Thus, we tested the hypotheses that (1) carotid stiffness is associated with HRR and HRV parameters in patients with repaired ToF, and that (2) strength of correlation is different between adults after ToF repair and age-matched healthy controls (reflecting different pathophysiologic derangements).
Methods

Study design and subjects

This cross-sectional study was carried out at the Department of Vascular Diseases, Division of Internal Medicine, University Medical Centre Ljubljana, Slovenia. Twenty-five adults with repaired ToF were recruited from the Outpatient clinic for adults with congenital heart diseases at the Department of Cardiology, Division of Internal Medicine, University Medical Centre Ljubljana, Slovenia. Ten age-matched healthy controls were also included.

All patients with repaired ToF had a complete correction of the defect in the childhood and were at the time of data acquisition in the clinical class I according to New York Heart Association (NYHA) classification. Exclusion criteria included known or symptomatic atherosclerotic disease, unstable cardiovascular disease or recent (<3 months prior to inclusion) cardiovascular events, acute illness or recent (<3 months prior to inclusion) non-cardiovascular diseases requiring hospital, emergency or unplanned specialist management, pregnancy, unstable arrhythmias, chronic atrial fibrilation, permanent pacing and intellectual development disorder. This study was approved by the National Medical Ethics Committee. It has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. Written consent was obtained from all participants prior to their inclusion in the study.

Heart rate variability

HRV recordings were performed between 8 a.m. and 10 a.m. They were lying in the supine position for at least 10 min before measurements. Recordings were performed in a quiet room, with all electronic devices and lights turned off. Resting 5-minute-long high resolution ECG recordings were acquired using a 500 Hz dedicated recording device (Cardiax®, IMED, Budapest, Hungary) with no filter applied. ECG data were analyzed using a dedicated software package (Kubios v. 2.2, University of Eastern Finland, Kuopio, Finland). After correction of premature beats and artifacts, HRV time and frequency domain parameters were
calculated. Frequency domain parameters were obtained using autoregressive model order 16, without factorization.

Selected time domain parameters were used [16]: SDNN - standard deviation (SD) of all normal RR (NN) intervals; rMSSD – square root of the mean of the sum of squares of successive N-N interval difference, reflects vagal modulation; pNN50% - percentage of successive NN intervals differing by >50 ms, reflects vagal function.

Selected frequency domain parameters were used [16, 17]: HFnu – power in the high frequency range (0.15-0.4 Hz), obtained with autoregressive method using the following formula: HFnu = total power – low frequency power – very low frequency power; LFnu – power in the low frequency range (0.04-0.15 Hz), obtained with autoregressive method using the following formula: LFnu = total power – high frequency power – very low frequency power.

**Carotid artery stiffness**

Measurements of carotid artery stiffness parameters (β stiffness coefficient and pulse wave velocity (PWV)) were performed after heart rate variability measurement. Aloka Prosound α7 ultrasound machine was used, with dedicated echo-tracking used to determine carotid stiffness parameters through the analysis of the pulse waves. Measurements were performed on the right common carotid artery (RCCA). Patients had their head elevated of around 45° and 30° tilted to the left. After visualization of RCCA of around 2 cm before the bifurcation, the cursor pair was positioned on the anterior and posterior walls of the artery. The software, which is integral part of the device and calibrated on the basis of systolic and diastolic blood pressure values, analyzed RCCA’s diameter change waveforms and automatically calculated β stiffness coefficient and PWV [18]. Calibration of the blood pressure was performed twice, during which 6 beats were made/taken. Therefore, β coefficient and PWV were calculated as means of 12 beats.
Heart rate recovery

Maximal graded exercise testing followed previous two measurements. It was carried out in a laboratory room between 10 a.m. and 2 p.m. using cycle ergometer Schiller CS-200. Standardized exercise testing protocol consisted of gradual increase in workload by one tenth of maximal estimated workload per minute (on the basis of age and gender). Peak workload was then obtained, together with peak heart rate. After termination of the exercise testing, patients remained seated on the cycle ergometer and heart rate was continuously measured in the following 4 minutes. HRR parameters were calculated with the following formulas:

\[ \text{HRR}_X = \text{HR}_{\text{peak}} - \text{HR}_{\text{in the Xth minute after termination of the exercise testing}} \]

in which X had values from 1 to 4 (HRR\(_1\), HRR\(_2\), HRR\(_3\) and HRR\(_4\)).

Sample size calculation and statistical analysis

Sample size was assessed using standard deviation of the independent variable (β stiffness coefficient) as 2.2 [5] and of the dependent variable (HRR) as 10 beats [9]. As a ratio between these two values was around 4, minimal detectable difference (change in the dependent variable per unit change in the independent variable) was estimated to be 4 or less. We have performed calculations with minimal detectable difference as 3. Therefore, to detect a true difference of 3 beats in HRR for each 1 unit difference in β stiffness coefficient, sample size calculation suggested at least 21 patients should be included with a 0.05 two-sided significance level and a power of 0.80.

Data were analyzed using IBM SPSS version 20. To assess if the distribution of a variable is normal, eyeballing for normality was confirmed with the Shapiro-Wilk test. Data were presented as mean and SD for normally distributed variables, and median and interquartile range (Q1-Q3) for non-normally distributed variables. An independent samples T-test/Mann-Whitney U was used to determine differences of normally/non-normally distributed variables, respectively. Differences between categorical variables were
checked with chi-square test. Associations between two variables were assessed with Spearman’s correlation coefficient. Multivariate linear regression analysis was performed to determine parameters with strongest associations with HRR. A p value less than 0.05 was considered statistically significant.

Results

There were 25 patients with repaired ToF enrolled in our study, 12 of them were males. Mean age was 38 years. We have additionally included 10 healthy controls, 6 of them were males, mean age was 39 years. Other demographic and clinical characteristics of the participants, including HRR, HRV and carotid artery stiffness parameters of both groups are shown in the Table 1.

As shown in Table 1, there was a significant difference between groups in terms of SDNN, a parameter of HRV (32.8 vs. 46.8, p=0.012) and HRR2 (43.4 vs. 55.2, p=0.028). There was a trend towards significance in differences between groups in terms of HRR3 and HRR4 (52.8 vs. 63.4, p=0.081 and 57.6 vs. 69.0, p=0.076, respectively). There were no significant differences between groups in terms of parameters of carotid arterial stiffness, namely β stiffness coefficient and PWV (6.07 vs. 5.96, p=0.843 and 5.52 vs. 5.37, p=0.576, respectively).
Table 1. Demographic and clinical characteristics of the participants

<table>
<thead>
<tr>
<th></th>
<th>ToF (n = 25)</th>
<th>Healthy controls (n=10)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>General data</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, mean (SD), y</td>
<td>38.1 (9.8)</td>
<td>39.3 (8.2)</td>
<td>0.738</td>
</tr>
<tr>
<td>Males, n (%)</td>
<td>12 (48.0)</td>
<td>6 (60.0)</td>
<td>0.711</td>
</tr>
<tr>
<td>BMI, mean (SD), kg/m²</td>
<td>24.9 (5.4)</td>
<td>23.6 (2.5)</td>
<td>0.506</td>
</tr>
<tr>
<td><strong>Clinical data</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic pressure, mean (SD), mmHg</td>
<td>117.4 (15.8)</td>
<td>118.0 (10.3)</td>
<td>0.913</td>
</tr>
<tr>
<td>Diastolic pressure, mean (SD), mmHg</td>
<td>75.4 (9.6)</td>
<td>76.5 (7.5)</td>
<td>0.747</td>
</tr>
<tr>
<td>Time after repair, mean (SD), y</td>
<td>34.4 (8.5)</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>Age at repair, median (Q1-Q3), months</td>
<td>30 (19-58)</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>Single repair surgery, n (%)</td>
<td>10 (40.0)</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>HRR parameters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resting heart rate, mean (SD), bpm</td>
<td>73.6 (10.0)</td>
<td>75.1 (12.6)</td>
<td>0.706</td>
</tr>
<tr>
<td>Peak heart rate, median (Q1-Q3), bpm</td>
<td>160 (145-172)</td>
<td>167 (154-186)</td>
<td>0.201</td>
</tr>
<tr>
<td>Peak load, mean (SD), W</td>
<td>144.1 (67.3)</td>
<td>218.6 (48.8)</td>
<td>0.003</td>
</tr>
<tr>
<td>HRR1, mean (SD), bpm</td>
<td>29.8 (11.5)</td>
<td>31.0 (7.9)</td>
<td>0.758</td>
</tr>
<tr>
<td>HRR2, mean (SD), bpm</td>
<td>43.4 (13.2)</td>
<td>55.2 (15.2)</td>
<td>0.028</td>
</tr>
<tr>
<td>HRR3, mean (SD), bpm</td>
<td>52.8 (15.5)</td>
<td>63.4 (16.1)</td>
<td>0.081</td>
</tr>
<tr>
<td>HRR4, mean (SD), bpm</td>
<td>57.6 (16.6)</td>
<td>69.0 (16.6)</td>
<td>0.076</td>
</tr>
<tr>
<td>Carotid artery stiffness parameters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β coefficient, mean (SD)</td>
<td>6.1 (1.5)</td>
<td>6.0 (1.1)</td>
<td>0.843</td>
</tr>
<tr>
<td>PWV, mean (SD), m/s</td>
<td>5.5 (0.8)</td>
<td>5.4 (0.5)</td>
<td>0.576</td>
</tr>
</tbody>
</table>

HRR - heart rate recovery 1-4 minutes after exercise; HRV - heart rate variability; SDNN - standard deviation of all normal RR (NN) intervals; rMSSD - root of the mean of the sum of squares of successive N-N interval difference; pNN50% - percentage of successive NN intervals differing by >50 ms; HFnu - power in the high frequency range, normalized; LFnu - power in the low frequency range, normalized; bpm – beats per minute; PWV – pulse wave velocity; SD - standard deviation; Q1-Q3 - interquartile range

Association between HRR parameters and parameters of arterial stiffness increased from HRR1 to HRR4 (Fig. 1b). As shown on Fig. 1b, β stiffness coefficient was significantly associated with HRR2 (r=-0.404, p=0.045), HRR3 (r=-0.546, p=0.005) and HRR4 (r=-0.546, p=0.005) in patients with repaired ToF. Additionally, we have conducted a multivariate linear regression analyses with HRR2, HRR3 and HRR4 as dependent variables, and age, gender, resting heart rate, β-blockers use and β stiffness coefficient as independent variables (Table 2). According to our results, β stiffness coefficient was an independent predictor of HRR3, while regression power of β stiffness coefficient to predict HRR4 was borderline significant (p=0.05). Female gender was an independent predictor of HRR3, while resting heart rate, β-blocker use and age were not (Table 2).
Comparing two groups of patients with repaired ToF, those who had undergone single and those who had undergone multiple heart surgery procedures, there were no significant differences in terms of β stiffness coefficient (p=0.725) and PWV (p=0.375).

Fig 1. Association between heart rate recovery (HRR) 1, 2, 3 and 4 min after exercise with (a) time-domain parameters of heart rate variability (rMSSD, root of the mean of the sum of squares of successive N–N interval difference) and (b) carotid artery stiffness parameters.

* Difference between coefficients is in fourth decimal
Correlation between two entities of cardiac autonomic function, namely HRR and HRV was further assessed. HRR was not significantly correlated with any of the HRV parameters. Association between HRR parameters and time domain parameters of HRV increased from HRR to HRR, similarly as in previous paragraph, in patients with repaired ToF (Fig. 1a). HRR (Fig. 2a) and HRR were also associated with a frequency domain parameter of HRV, i.e. HFnu (r=0.513; p=0.009 and r=0.442, p=0.027, respectively). Associations between carotid artery stiffness and heart rate variability parameters were also determined. PWV, as a parameter of carotid artery stiffness, was significantly associated with SDNN (r=-0.417; p=0.038), rMSSD (r=-0.515; p=0.008), pNN50% (r=-0.465; p=0.019) and HFnu (r=-0.467; p=0.019) in patients with repaired ToF (Fig. 2b).

Fig 2. Association between heart rate variability parameters and (a) heart rate recovery 4 min after exercise testing (HRR); (b) carotid artery stiffness parameters (pulse wave velocity, PWV)
Table 2. Multiple linear regression analyses with heart rate recovery after 2, 3 and 4 minutes (HRR$_2$, HRR$_3$ and HRR$_4$) as dependant variables in patients with repaired ToF

<table>
<thead>
<tr>
<th>Model 1: Dependent variable HRR$_2$</th>
<th>B</th>
<th>Std. Error</th>
<th>Beta</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Constant)</td>
<td>96.249</td>
<td>23.756</td>
<td>-0.378</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age</td>
<td>-0.513</td>
<td>0.293</td>
<td>-0.378</td>
<td>0.096</td>
</tr>
<tr>
<td>Female gender</td>
<td>3.042</td>
<td>4.226</td>
<td>0.117</td>
<td>0.480</td>
</tr>
<tr>
<td>HR rest</td>
<td>-0.288</td>
<td>0.254</td>
<td>-0.219</td>
<td>0.271</td>
</tr>
<tr>
<td>β stiffness coef.</td>
<td>-2.507</td>
<td>1.710</td>
<td>-0.294</td>
<td>0.159</td>
</tr>
<tr>
<td>β blockers use</td>
<td>-7.841</td>
<td>6.664</td>
<td>-0.242</td>
<td>0.254</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Model 2: Dependent variable HRR$_3$</th>
<th>B</th>
<th>Std. Error</th>
<th>Beta</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Constant)</td>
<td>117.747</td>
<td>22.346</td>
<td>-0.302</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age</td>
<td>-0.480</td>
<td>0.275</td>
<td>-0.302</td>
<td>0.097</td>
</tr>
<tr>
<td>Female gender</td>
<td>9.089</td>
<td>3.975</td>
<td>0.299</td>
<td>0.034</td>
</tr>
<tr>
<td>HR rest</td>
<td>-0.468</td>
<td>0.239</td>
<td>-0.304</td>
<td>0.065</td>
</tr>
<tr>
<td>β stiffness coef.</td>
<td>-3.931</td>
<td>1.609</td>
<td>-0.393</td>
<td>0.024</td>
</tr>
<tr>
<td>β blockers use</td>
<td>-10.533</td>
<td>6.269</td>
<td>-0.277</td>
<td>0.109</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Model 3: Dependent variable HRR$_4$</th>
<th>B</th>
<th>Std. Error</th>
<th>Beta</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Constant)</td>
<td>102.304</td>
<td>29.181</td>
<td>-0.327</td>
<td>0.139</td>
</tr>
<tr>
<td>Age</td>
<td>-0.554</td>
<td>0.359</td>
<td>-0.327</td>
<td>0.139</td>
</tr>
<tr>
<td>Female gender</td>
<td>4.877</td>
<td>5.191</td>
<td>0.150</td>
<td>0.359</td>
</tr>
<tr>
<td>HR rest</td>
<td>-0.045</td>
<td>0.312</td>
<td>-0.027</td>
<td>0.887</td>
</tr>
<tr>
<td>β stiffness coef.</td>
<td>-4.407</td>
<td>2.101</td>
<td>-0.412</td>
<td>0.050</td>
</tr>
<tr>
<td>β blockers use</td>
<td>-4.446</td>
<td>8.186</td>
<td>-0.110</td>
<td>0.593</td>
</tr>
</tbody>
</table>

Discussion

In the present study, we confirmed an association between cardiac autonomic dysfunction and arterial stiffness in adults with repaired ToF. Although previous studies have reported interrelationships between HRV and artery stiffness [13], this is the first study to report on HRR in repaired ToF and its relationship with other cardiac autonomic function parameters as well as vascular function determined by carotid artery stiffness.

Our findings suggest that cardiac autonomic function (HRR and HRV), but not arterial stiffness is impaired in adults with ToF as compared to healthy controls. While reduced HRV in patients with repaired ToF has already been reported [2, 3], our results provide novel insight into a reduced HRR in patients with repaired ToF compared to healthy peers. As for vascular function, our finding of comparable carotid stiffness between adults after ToF repair and healthy controls may be explained by the older age of our study population (mean age 38 years), as most previous studies included younger patients (mean age 15–21
years) [5, 13, 19]. It is possible that, over time, increased arterial stiffness in patients after ToF repair equalizes to that of apparently healthy controls, as both ToF and healthy individuals are subject to vascular ageing and cardiovascular risk factors. Increased survival of this population only now reaching middle-age permits appreciation of such processes that may ultimately be unrelated to the congenital heart condition itself [1].

Despite comparable vascular function, adults after ToF repair — but not controls — had a significant association between HRR and arterial stiffness. Moreover, HRR was associated with arterial stiffness irrespective of age, gender, resting heart rate and β-blockers use. HRR is a marker of cardiovascular prognosis in both healthy individuals and patients with cardiovascular disease, and reflects autonomic nervous system conditioning [6, 20]. Also, HRR is affected by both, structural and functional vascular changes, explained in a study of Janic et al. [12]. On the one hand, structural interactions may arise from impaired HRR due to degenerative and atherosclerotic vascular disease [21]. On the other hand, correlation between HRR and endothelial dysfunction suggests a possible association between functional vascular component and HRR [22]. Decreased physical activity level, often seen in adults with congenital heart disease [23], may further unfavorably affect both HRR and arterial stiffness.

Of interest, only later phases of HRR (namely, 2 or more minutes post-exercise) — but not HRR₁ — were associated with carotid artery stiffness parameters in patients with repaired ToF. Although most studies on HRR focused on the recovery in the first minute after exercise, there are a few literature reports in which both early and late HRR have been examined [24, 25]. Additionally, different implications of exercise physiology can be appreciated with a comprehensive analysis of HRR beyond the first minute. After exercise, HRR in the first 30 seconds to 1 minute is predominantly driven by parasympathetic reactivation (early phase with rapid decline), whereas in the subsequent 2 or more minutes (late phase with slow decline) sympathetic withdrawal in addition to parasympathetic reactivation sets in as a pivotal determinant of heart rate decline [24]. Vascular function is determined by a complex interplay between both sympathetic and parasympathetic activity, and vascular dysfunction is associated with vegetative...
derangements such as sympathetic hyperactivity [26]; thus, HRR$_2$-HRR$_4$ (reflecting such vegetative derangements) may indeed be more strongly associated with vascular function than HRR$_1$, as suggested by our findings.

As expected, HRR was also associated with other parameters of cardiac autonomic function, i.e. HRV in patients with repaired ToF. To our knowledge, this is the first study in adults with congenital heart disease, in which association between these two parameters was assessed. In literature, associations between HRR and HRV remain controversial in cardiovascular patients [9–11]. Failure to detect an association between HRV and HRR in some of these studies may be explained by two differential aspects of cardiac parasympathetic function — namely, that HRR mainly reflects parasympathetic tone, while HRV is a marker of parasympathetic modulation [27]. As suggested in a study of Buchheit et al, these two aspects might depend on physical activity levels, but in a different way. Higher physical activity performance leads to reaching a plateau of HRV parameters ("saturation" effect), while HRR parameters are further increased [27]. This "saturation" effect of HRV parameters — but not of HRR parameters — may explain the lack of association between HRR and HRV in some studies. Adults with congenital heart disease tend to be less physically active [23] and less engaged to sports [28]; thus, "saturation" effect of HRV may not be reached and the association between HRV and HRR may therefore be retained, which is in line with our findings. On the contrary, Piotrowicz et al. [29] reported on improvements of HRV parameters — and not in HRR — in patients with chronic heart failure after physical training program, providing opposite results as in a study of Buchheit et al. [27]. These controversial results in different cardiovascular conditions ask for further prospective analyses on influence of exercise training on both aspects of cardiac parasympathetic function in a growing population of adults with repaired ToF.

As for the association between HRV and arterial stiffness in patients with repaired ToF, our findings corroborate previous observations, only association has been demonstrated for carotid artery distensibility, another parameter of carotid artery stiffness [13]. In our study, carotid artery stiffness parameters were inversely related with indicators of vagal modulation (rMSSD, pNN50 and HFnu) in patients with repaired
ToF. Possible explanations for this association include direct mechanistic sequelae of open-heart cardiac surgery [2, 3] as well as indirect non-cardiac issues associated with lifestyle of adults after ToF repair (e.g. lack of physical activity [23]). A causative association between arterial stiffness and HRV impairment pathogenesis should also be taken into account — stiffer carotid arteries affect afferent stimuli from the heart to the brain via carotid sinus baroreceptors [14], which could result in deranged autonomic modulation of the heart rate (represented by HRV).

Our study has a few limitations. Firstly, a relatively small sample size. As sample size was primarily calculated to detect a possible association between HRR and β stiffness coefficient, the study may have been underpowered to detect weaker associations and is therefore subject to limitations that are inherent to studies of patients with similarly rare conditions. Secondly, control group consisted of age- and gender-matched controls, but unadjusted for other differences that may have influenced our results. Thirdly, this was a case-control study and can only answer the question of association, not causality.

In conclusion, autonomic cardiac function is impaired in patients with repaired ToF, and independently associated with vascular function in adults after ToF repair, but not in age-matched healthy controls.

With results from the present study, we are able to better understand vascular and autonomic cardiac function in a novel population of patients with repaired ToF entering middle age. However, there are still challenges to better understand pathophysiological derangements between these two entities, to determine predictive values of arterial stiffness and autonomic cardiac function parameters in adults with congenital heart disease, and to introduce interventions in order to improve them, such as exercise training programs. Larger (multi-center) studies are needed to address these issues, while our findings nonetheless provide novel insight into the association between cardiac autonomic activity and vascular function in the growing population of patients with ToF surviving into adulthood.
Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

References


Exercise capacity, cardiac and vascular function in adults with repaired tetralogy of Fallot

Marko Novaković 1,2, Katja Prokšelj 2,3 and Borut Jug 1,2

1 Department of vascular diseases, University Medical Centre Ljubljana, Ljubljana, Slovenia
2 Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia
3 Department of cardiology, University Medical Centre Ljubljana, Ljubljana, Slovenia

Submitted for publication.
Abstract

Background: Exercise capacity and endothelial function are impaired in adults with repaired Tetralogy of Fallot (ToF). This may be related to pathophysiological determinants, such as cardiac and vascular impairment, or to a more sedentary lifestyle. Therefore, we sought to assess how cardiac and vascular function, and self-reported physical activity determine exercise capacity in adults with repaired ToF.

Methods: In a case-control study, we compared adults with repaired ToF and age- and gender-matched healthy controls in terms of exercise capacity (exercise workload in Watts and peak oxygen consumption - VO2 peak) and flow-mediated vasodilation (FMD) of the brachial artery. Additionally, we determined NT-proBNP levels, echocardiographic parameters of size, function and systolic pressure of the right ventricle (RV) and self-reported levels of physical activity (IPAQ questionnaire) as possible predictors of VO2peak in adults with repaired ToF.

Results: A total of 26 patients (mean age was 38±10 years, 46 % were males) and 10 controls were included. Patients with repaired ToF had reduced VO2peak (24.5 vs. 36.3 ml/kg/min, p<0.001) and FMD (7.6 vs. 10.9 %, p=0.010) compared to healthy controls. Exercise workload was significantly associated with FMD (r=0.428, p=0.029), RV systolic function (r=0.404, p=0.040) and RV systolic pressure (r=-0.438, p=0.028), while VO2 peak was associated with NT-proBNP (r=-0.523, p=0.006). In multivariate analysis, NT-proBNP (β=-0.505, p=0.007) and self-reported physical activity level (β=0.327, p=0.041), but not FMD emerged as independent predictors of VO2 peak.

Conclusions: NT-proBNP and self-reported physical activity level, but not FMD, are independent predictors of exercise performance in adults with repaired ToF. While the first indicates pathophysiological impairment, the latter underscores the importance of regular physical activity in adults with repaired ToF.

Keywords
Tetralogy of Fallot, exercise testing, echocardiography, endothelial function, natriuretic peptide
Introduction

Tetralogy of Fallot (ToF) is the most common cyanotic congenital heart defect, occurring in approximately 1 of 3500 births [1]. Despite improved survival to adult life, impaired cardiac and vascular function, persisting hemodynamic derangements, cardiac autonomic disturbances and sedentary lifestyle [2,3] may result in significant impairments of exercise capacity and quality of life [1]. Moreover, reduced exercise capacity, together with impaired cardiac and vascular function, has been associated with increased morbidity and mortality of patients after ToF repair [4–7].

Also, vascular structure and function is impaired in patients with repaired ToF. Congenital specifics of the arterial tree with impaired collagen/elastin ratio [8], autonomic cardiovascular dysfunction [3], cyanosis [9] and sedentary lifestyle [10] have been identified as possible contributors. Flow-mediated dilation (FMD) has emerged as an indicator of endothelial function, an early marker of cardiovascular disease and a predictor of cardiovascular prognosis [11,12]. Endothelial function impairment has been shown in children early after ToF repair [13], whereas its persistence in adults has not been confirmed [14].

Possible ramifications of cardiac and vascular impairments include exercise intolerance in adults with repaired ToF[15]. However, sedentary lifestyle may also be a contributing factor, as adults with congenital heart disease including ToF repair—also due to previously cautious recommendations by healthcare professionals—are less physically active and less engaged to sports compared to healthy peers [10,16,17].

Therefore, we sought to assess vascular function (FMD), cardiac function (echocardiographic and neurohormonal indices of RV function), self-reported physical activity and exercise performance, their intercorrelation, and their respective predictive impact on exercise capacity in adults with repaired ToF.
Methods

Participants

This was a case-control study carried out at the Department of the Vascular Diseases, Division of Internal Medicine, University Medical Centre Ljubljana, Slovenia. Consecutive patients with repaired ToF, referred for exercise testing were recruited from the national referral Outpatient clinic for adults with congenital heart diseases at the Department of Cardiology, Division of Internal Medicine, University Medical Centre Ljubljana, Slovenia.

Patients with repaired ToF had a complete surgical repair of the cardiac defect in the childhood. They were all in the clinical class I according to NYHA classification. Exclusion criteria included known or symptomatic atherosclerotic disease, unstable cardiovascular disease or recent (<3 months prior to inclusion) cardiovascular events, acute illness or recent (<3 months prior to inclusion) non-cardiovascular diseases requiring hospital, emergency or unplanned specialist management, pregnancy, unstable arrhythmias, chronic atrial fibrillation, permanent pacing and intellectual development disorder.

This study was approved by the National Medical Ethics Committee. Written consent was obtained from all participants prior to their inclusion in the study.

Vascular function

The examination was performed in the morning hours. Participants were lying in the supine position at least 10 minutes before measurements. Aloka Prosound α7 ultrasound machine was used for measuring FMD. Measurements were performed on the right brachial artery, participants were asked not to move at all. The first step was to visualize the artery approximately 5 cm above the antecubital fossa. After having the artery visualized in the horizontal position on the screen, 3 measurements of the arterial diameter were obtained (d1). Next step was inflating the cuff just below the antecubital fossa with the pressure which is 50 mmHg above the systolic blood pressure. Ischemia was maintained for 4.5 minutes. Sixty seconds after
deflation of the cuff, 3 measurements of the arterial diameter were obtained again (d₂). FMD was calculated with the following formula:

\[
FMD = \left(\frac{\text{mean } d₂ - \text{mean } d₁}{\text{mean } d₁}\right) \times 100 \%
\]

**Ergospirometry**

Maximal ergospirometry performance was carried out in a laboratory room between 10 a.m. and 2 p.m. using cycle ergometer Schiller CS-200. Standardized exercise testing protocol consisted of gradual increase in workload by one tenth of maximal estimated workload per minute (on the basis of age, gender and height). As a part of calibration, a spirometric measurement was performed. Participants were ECG monitored during whole ergospirometry testing and in the cool-down period. Oxygen and CO₂ flow were permanently measured during exercise. Anaerobic threshold was defined when ratio between O₂ and CO₂ flow was 1. Chronotropic competence was calculated with the formula: HR peak/(220 - age).

**Echocardiography**

Transthoracic echocardiography was performed using Phillips Epic 7c ultrasound system with 3D transducer (X5-1) at the Department of Cardiology, Division of Internal Medicine, University Medical Centre Ljubljana, Slovenia.

Measurements were performed according to current guidelines [18]. A RV diameter at the base, as a measure of RV size, was determined from the 4-chambers view, according to the report of the American Society of Echocardiography [18]. Velocity of the tricuspid annular systolic motion (RV S'), as an indicator of RV systolic function, was assessed with tissue Doppler using an apical 4-chamber window. RV systolic pressure (RVSP) was calculated from peak tricuspid regurgitation jet velocity, as described in details in the guidelines [18].
**Self-estimated physical activity level**

Self-estimated physical activity level was assessed using International Physical Activity Questionnaire (IPAQ), used in numerous studies on physical activity and exercise training. According to IPAQ guidelines, Metabolic equivalents of tasks (MET) were calculated, based on frequency and duration of the physical activity [19].

**Statistical analysis**

Data were analyzed using IBM SPSS version 20. Distribution of a variable was assessed with the Shapiro-Wilk test. Data were presented as mean and standard deviation (SD) for normally distributed variables, and median and interquartile range (Q1-Q3) for non-normally distributed variables. An independent samples t-test and Mann-Whitney U were used to determine differences of normally and non-normally distributed variables, respectively. Differences between categorical variables were checked with chi-square test. Multivariate linear regression analysis was performed to determine possible independent parameters of exercise performance. A p value less than 0.05 was considered statistically significant.

**Results**

A total of 26 patients with repaired ToF and 10 healthy age- and gender- matched controls were included (Table 1).

Patients with repaired ToF had significantly reduced exercise workload (146.5 vs. 249.7 W, p=0.010), lower percentage of the predicted workload (89.2 vs. 139.6 %, p=0.006), lower VO2peak level (25.0 vs. 36.3 ml/kg/min, p<0.001), VO2 at the anaerobic threshold (20.3 vs. 27.7 ml/kg/min, p=0.010) and VE/VCO2 slope (24.5 vs. 22.0, p=0.050) compared to healthy controls. Vascular function, determined with FMD, was also significantly decreased, as compared to healthy controls (7.6 vs. 10.8 %, p=0.009).
Table 1. Demographics and clinical data of the participants included in the study

<table>
<thead>
<tr>
<th>Demographics</th>
<th>ToF n=26</th>
<th>Healthy n=10</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, mean (SD), y</strong></td>
<td>37.7 (9.8)</td>
<td>39.3 (8.2)</td>
<td>0.649</td>
</tr>
<tr>
<td><strong>Male gender, n (%)</strong></td>
<td>12 (46.1)</td>
<td>6 (60.0)</td>
<td>0.710</td>
</tr>
<tr>
<td><strong>BMI, mean (SD), kg/m²</strong></td>
<td>24.9 (5.3)</td>
<td>23.7 (2.5)</td>
<td>0.480</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Clinical data</th>
<th><strong>Systolic pressure, median (Q1-Q3), mmHg</strong></th>
<th>115 (105-130)</th>
<th>120 (114-126)</th>
<th>0.720</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diastolic pressure, median (Q1-Q3), mmHg</strong></td>
<td>75 (70-80)</td>
<td>80 (70-80)</td>
<td>0.534</td>
<td></td>
</tr>
<tr>
<td><strong>Resting HR, mean (SD), min⁻¹</strong></td>
<td>72.9 (10.2)</td>
<td>75.1 (12.6)</td>
<td>0.628</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Drugs</th>
<th><strong>Beta blockers, n (%)</strong></th>
<th>6 (23.1)</th>
<th>2 (20.0)</th>
<th>1.000</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>ACE-i, n (%)</strong></td>
<td>4 (15.4)</td>
<td>0 (0.0)</td>
<td>0.559</td>
</tr>
<tr>
<td></td>
<td><strong>Statins, n (%)</strong></td>
<td>2 (7.7)</td>
<td>0 (0.0)</td>
<td>1.000</td>
</tr>
</tbody>
</table>

| Vascular function | **FMD, mean (SD), %** | 7.6 (3.3) | 10.8 (2.3) | 0.009 |

<table>
<thead>
<tr>
<th>Ergospirometry</th>
<th><strong>Maximal workload, mean (SD), W</strong></th>
<th>146.5 (60.8)</th>
<th>249.7 (99.7)</th>
<th>0.010</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Percentage of predicted workload, mean (SD), %</strong></td>
<td>89.2 (24.1)</td>
<td>139.6 (44.9)</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td><strong>VO₂ peak, mean (SD), ml/kg/min</strong></td>
<td>25.0 (6.6)</td>
<td>36.3 (6.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td><strong>Percentage of predicted VO₂ peak, mean (SD), %</strong></td>
<td>79.9 (18.7)</td>
<td>110.3 (26.0)</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td><strong>Anaerobic threshold, mean (SD), ml/kg/min</strong></td>
<td>20.3 (5.2)</td>
<td>27.7 (7.1)</td>
<td>0.010</td>
</tr>
<tr>
<td></td>
<td><strong>VE/VO₂ slope, mean (SD)</strong></td>
<td>24.5 (4.4)</td>
<td>22.0 (2.6)</td>
<td>0.050</td>
</tr>
</tbody>
</table>

BMI – body mass index; HR – heart rate; ACE-i – angiotensin converting enzyme inhibitors; FMD – flow-mediated dilation; VO₂ peak – peak oxygen uptake during exercise testing; VE/VO₂ – minute ventilation/carbon dioxide production slope

Patients with repaired ToF were divided into 2 subgroups according to exercise testing performance, as in previous reports [20, 21]. Subgroup 1 consisted of patients who reached 85 % or more of expected VO₂ peak during exercise testing. Subgroup 2 consisted of patients who did not reach 85 % of expected VO₂ peak. Table 2 shows differences between subgroups of patients with repaired ToF.

As it is shown in Table 2, patients with impaired exercise performance had expectedly lower values of exercise testing parameters, such as maximal workload (112.6 vs. 200.7 W, p=0.001), VO₂ peak (21.4 vs. 30.7 ml/kg/min, p<0.001), VO₂ at anaerobic threshold (17.3 vs. 25.0 ml/kg/min, p<0.001) and chronotropic competence level (81.1 vs. 92.3 %, p=0.014), as compared to those who had reached at least 85 % of expected exercise performance. The difference in terms of FMD was not significant, but showed a trend towards significance (9.0 vs. 6.7 %, p=0.081). Those with poorer exercise performance also had significantly higher NT-proBNP values (227.7 vs. 87.3 ng/L, p=0.007). Among examined echocardiographic parameters,
only RV S', as a parameter of RV systolic function, was significantly higher in patients with retained exercise capacity (10.5 vs. 12.3 mm, p=0.042). There were no differences between two subgroups in terms of self-estimated physical activity level (2930.5 vs. 1844.0 METs, p=0.170).

**Table 2. Comparison of the clinical data between two examined subgroups of patients with repaired ToF**

<table>
<thead>
<tr>
<th>Clinical data</th>
<th>All ToF patients (n=26)</th>
<th>Subgroup 1 (VO2 peak ≥ 85 % predicted) (n=10)</th>
<th>Subgroup 2 (VO2 peak &lt; 85 % predicted) (n=16)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD), y</td>
<td>37.7 (9.8)</td>
<td>41.6 (10.4)</td>
<td>35.3 (8.9)</td>
<td>0.129</td>
</tr>
<tr>
<td>Male gender, n (%)</td>
<td>12 (46.1)</td>
<td>7 (70.0)</td>
<td>5 (31.3)</td>
<td>0.105</td>
</tr>
<tr>
<td>BMI, mean (SD), kg/m²</td>
<td>24.9 (5.3)</td>
<td>27.0 (5.1)</td>
<td>23.6 (5.2)</td>
<td>0.123</td>
</tr>
<tr>
<td>Time after repair, mean (SD), y</td>
<td>34.4 (8.3)</td>
<td>37.7 (8.5)</td>
<td>32.3 (7.8)</td>
<td>0.122</td>
</tr>
<tr>
<td>Age at repair, median (Q1-Q3), months</td>
<td>30.0 (22.5-55.5)</td>
<td>36.0 (27.0-61.5)</td>
<td>24.0 (18.0-52.5)</td>
<td>0.244</td>
</tr>
<tr>
<td>QRS duration, median (Q1-Q3), ms</td>
<td>160 (152-171)</td>
<td>159 (156-163)</td>
<td>165 (150-176)</td>
<td>0.874</td>
</tr>
<tr>
<td>Cardiopulmonary exercise testing</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximal workload, mean (SD), W</td>
<td>146.5 (60.8)</td>
<td>200.7 (55.8)</td>
<td>112.6 (33.3)</td>
<td>0.001</td>
</tr>
<tr>
<td>VO2peak, mean (SD), ml/kg/min</td>
<td>25.0 (6.6)</td>
<td>30.7 (5.5)</td>
<td>21.4 (4.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Anaerobic threshold, mean (SD), ml/kg/min</td>
<td>20.3 (5.2)</td>
<td>25.0 (4.1)</td>
<td>17.3 (3.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VE/VO2 slope, mean (SD)</td>
<td>24.5 (4.4)</td>
<td>23.9 (4.4)</td>
<td>24.9 (4.5)</td>
<td>0.586</td>
</tr>
<tr>
<td>Resting HR, mean (SD), min⁻¹</td>
<td>72.9 (10.2)</td>
<td>71.7 (6.4)</td>
<td>73.6 (12.1)</td>
<td>0.604</td>
</tr>
<tr>
<td>Resting SBP, median (Q1-Q3), mmHg</td>
<td>115 (105-130)</td>
<td>125 (109-133)</td>
<td>110 (105-128)</td>
<td>0.141</td>
</tr>
<tr>
<td>Resting DBP, median (Q1-Q3), mmHg</td>
<td>75 (70-80)</td>
<td>80 (68-85)</td>
<td>70 (70-80)</td>
<td>0.202</td>
</tr>
<tr>
<td>HR max, median (Q1-Q3), min⁻¹</td>
<td>162 (146-174)</td>
<td>161 (153-179)</td>
<td>162 (143-170)</td>
<td>0.292</td>
</tr>
<tr>
<td>SBP max, median (Q1-Q3), mmHg</td>
<td>170 (158-200)</td>
<td>205 (189-220)</td>
<td>160 (145-170)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DBP max, median (Q1-Q3), mmHg</td>
<td>80 (79-85)</td>
<td>88 (80-100)</td>
<td>80 (71-80)</td>
<td>0.005</td>
</tr>
<tr>
<td>Max HR*Max SBP, mean (SD), mm Hg/min</td>
<td>27348 (6786)</td>
<td>33477 (4554)</td>
<td>23518 (4861)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Chronotropic competence, mean (SD), %</td>
<td>85.4 (13.1)</td>
<td>92.3 (7.0)</td>
<td>81.8 (14.4)</td>
<td>0.014</td>
</tr>
<tr>
<td>Vascular function</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FMD, mean (SD), %</td>
<td>7.6 (3.3)</td>
<td>9.0 (2.9)</td>
<td>6.7 (3.4)</td>
<td>0.081</td>
</tr>
<tr>
<td>Echocardiography</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RV base diameter, mean (SD), mm</td>
<td>4.7 (0.7)</td>
<td>4.4 (0.5)</td>
<td>4.8 (0.8)</td>
<td>0.198</td>
</tr>
<tr>
<td>RV S', mean (SD), mm</td>
<td>2.1 (0.4)</td>
<td>12.3 (2.0)</td>
<td>10.5 (2.2)</td>
<td>0.042</td>
</tr>
<tr>
<td>RV SP, mean (SD), mm Hg</td>
<td>36.0 (10.6)</td>
<td>32.3 (9.0)</td>
<td>38.3 (11.2)</td>
<td>0.149</td>
</tr>
<tr>
<td>Biomarkers</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NT-proBNP, median (Q1-Q3), ng/L</td>
<td>180.9 (88.9-337.9)</td>
<td>87.3 (35.9-171.5)</td>
<td>227.7 (118.3-459.3)</td>
<td>0.007</td>
</tr>
<tr>
<td>Physical activity level</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IPAQ score, median (Q1-Q3), METs</td>
<td>2263 (1370-4089)</td>
<td>2930 (1751-5547)</td>
<td>1844 (1350-3202)</td>
<td>0.170</td>
</tr>
</tbody>
</table>

BMI – body mass index; VO2 peak – peak oxygen uptake during exercise testing; HR – heart rate; SBP – systolic blood pressure; DBP – diastolic blood pressure; FMD – flow-mediated dilation; IPAQ – self-estimated physical activity score; RV – right ventricle; RV S’ - tissue Doppler-derived right ventricular systolic excursion velocity; RV SP – right ventricular systolic pressure

Associations between exercise capacity, endothelial function, NT-proBNP levels and echocardiographic parameters of RV in patients with repaired ToF are shown in Table 3. FMD, as a marker of endothelial dysfunction, was significantly correlated with exercise workload (p=0.428, p=0.029) (Fig 1).
Fig 1. Association between exercise workload and endothelial dysfunction (FMD)

Associations of NT-proBNP and both exercise workload and exercise performance were significant ($r=-0.557$, $p=0.003$ and $r=-0.523$, $p=0.006$, respectively) (Fig 2). RV $S'$, as a parameter of RV systolic function and RVSP were significantly associated with exercise workload ($0.404$, $p=0.040$ and $r=-0.438$, $p=0.028$, respectively) (Fig 3a). RV base diameter, as a parameter of RV size, was associated with neither exercise workload nor exercise performance (Table 3).

Fig 2. Association between natriuretic peptides (NT-proBNP) and exercise capacity (VO2 peak)
Table 3. Associations between exercise capacity, endothelial function and cardiac function in patients with repaired ToF

<table>
<thead>
<tr>
<th></th>
<th>Exercise workload (W)</th>
<th>Exercise performance (VO2 peak, ml/kg/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p</td>
</tr>
<tr>
<td>FMD (%)</td>
<td>0.428</td>
<td>0.029</td>
</tr>
<tr>
<td>Log NT-proBNP (ng/L)</td>
<td>-0.557</td>
<td>0.003</td>
</tr>
<tr>
<td>RV base diameter (mm)</td>
<td>-0.144</td>
<td>0.483</td>
</tr>
<tr>
<td>RV S’ (mm)</td>
<td>0.404</td>
<td>0.040</td>
</tr>
<tr>
<td>RV SP (mm Hg)</td>
<td>-0.411</td>
<td>0.037</td>
</tr>
</tbody>
</table>

r – Spearman’s Rho/coefficient; FMD – flow-mediated dilation; NT-proBNP – amino-terminal prohormone brain natriuretic peptide; RV – right ventricle; RV S’ – tissue Doppler-derived RV systolic excursion velocity; SP – systolic pressure

Fig 3. Associations of echocardiographic parameters with
(a) exercise capacity and (b) NT-proBNP levels
As expected, NT-proBNP levels were significantly correlated with echocardiographic parameters of RV size and systolic pressure ($r=0.623$, $p=0.001$ and $r=0.438$, $p=0.028$), while association with RV systolic function (RV S’) showed a trend towards statistical significance ($r=-0.377$, $p=0.058$) (Fig 3b).

We have further conducted a multivariate regression analysis with VO2 peak as a dependant variable in patients with repaired ToF (Table 4). NT-proBNP ($\beta=-0.505$, $p=0.007$) and physical activity level ($\beta=0.327$, $p=0.041$) are proven to be independent predictors of exercise performance (Table 4).

Table 4. Multivariable linear regression analysis with maximal oxygen uptake (VO2 peak) as a dependent variable in patients with repaired ToF

<table>
<thead>
<tr>
<th>Dependent variable: VO2 peak</th>
<th>B</th>
<th>Std. error</th>
<th>Beta</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model (Constant)</td>
<td>36.641</td>
<td>12.719</td>
<td>0.010</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>-0.015</td>
<td>0.102</td>
<td>-0.023</td>
<td>0.883</td>
</tr>
<tr>
<td>Male gender</td>
<td>-3.724</td>
<td>2.611</td>
<td>-0.287</td>
<td>0.170</td>
</tr>
<tr>
<td>BMI</td>
<td>-0.222</td>
<td>0.215</td>
<td>-0.178</td>
<td>0.313</td>
</tr>
<tr>
<td>Log NT-proBNP</td>
<td>-7.674</td>
<td>2.559</td>
<td>-0.505</td>
<td>0.007</td>
</tr>
<tr>
<td>Log IPAQ-score</td>
<td>4.854</td>
<td>2.218</td>
<td>0.327</td>
<td>0.041</td>
</tr>
<tr>
<td>FMD</td>
<td>0.140</td>
<td>0.302</td>
<td>0.071</td>
<td>0.647</td>
</tr>
</tbody>
</table>

BMI – body mass index; NT-proBNP – amino-terminal prohormone brain natriuretic peptide; IPAQ-score – self-estimated physical activity score obtained with IPAQ questionnaire; FMD – flow-mediated dilation

Discussion

Vascular function (FMD) and exercise capacity are impaired in adults after ToF repair as compared to healthy age- and gender-matched controls. Moreover, vascular function impairment and exercise capacity are strongly associated. However, when analyzing predictors of VO2peak, residual cardiac dysfunction (NT-proBNP levels) and self-reported levels of physical activity, but not vascular function emerged as independent predictors of exercise capacity in adults after ToF repair.
Previous studies have shown impaired exercise performance and suggested impaired vascular function in adults after ToF repair. Trojnar ska et al. reported exercise capacity reduction in the range of two-thirds of expected performance [22]. Similarly, our results have shown that exercise capacity is reduced to 80% of expected achievements, with low symptom burden (all our participants were in NYHA class I) and age (38 years) possibly explaining the difference in attained performance. In terms of impaired vascular function, available evidence is less consistent. Studies with cyanotic adults, including subsets of patients with repaired ToF [22,23] have shown impaired FMD. Also in children early after ToF repair, FMD is significantly impaired [13]. Conversely, studies in adults with cyanotic congenital heart disease [24] and repaired ToF only [14] have not demonstrated persistent peripheral vascular dysfunction, although a smaller number of participants may provide some explanation. In fact, pathophysiologic substrate in terms of disarranged vascular architecture with impaired collagen/elastin ratio, derangements in cardiac-vascular hemodynamic coupling and post-surgery sequelae may predispose adults after ToF repair to peripheral vascular impairments.

A further, novel finding is the association between FMD and exercise capacity. Although endothelial dysfunction has been associated with impaired exercise performance in patients with coronary artery disease [25] and heart failure [26], such association in adults with repaired ToF has not been reported to date. Adequate vasodilatation is a pivotal response to exercise; impaired vascular function may therefore likely thwart appropriate exercise performance. In addition to vascular function, markers of cardiac dysfunction — namely echocardiographic indices of systemic (right) ventricular performance and NT-proBNP levels — were also strongly associated with exercise capacity of our study participants. This is in line with previous reports on the association between exercise capacity and ventricular function in imaging [27,28] and neurohumoral studies [15,20,22].

An important question is whether exercise performance is hampered by pathophysiologic vascular and cardiac derangements, or is it rather a consequence of an interplaying confounder, affecting both vascular function and exercise capacity, such as sedentary lifestyle. In this respect, a possible merit of our study is
quantification of self-reported physical activity. Our results suggest that cardiac function (as determined by NT-proBNP levels) and self-reported physical activity, but not vascular impairments (as determined by FMD) predict exercise performance. Exercise training improves endothelial function [29]; therefore, decreased FMD in adults with repaired ToF likely reflects physical inactivity rather than causing it. This is a plausible finding and also an important suggestion of the importance of regular physical activity in patients after ToF repair.

Our study provides novel insight into the complex association between cardiac and vascular function, exercise performance and physical activity in adults after ToF repair. However, we identified some limitations. Firstly, a relatively small sample size and case-control design of the study requires caution, but reflects a relatively low prevalence of adults with repaired ToF. Secondly, healthy controls were matched for age and gender, but were not adjusted for other differences, which may have influenced our results. Thirdly, cardiac function was estimated echocardiographically, whereas advanced imaging methods (such as with magnetic resonance) may provide better appreciation of the right ventricular function and its association with exercise performance. Nonetheless, our findings improve understanding of complex pathophysiologic phenomena in a population of patients after ToF that has only recently started reaching middle age and require better appreciation of possible cardiovascular risks embedded in its specific disease trajectory.

In conclusion, exercise capacity and vascular function are impaired in adults after ToF repair. Both endothelial (as determined by FMD) and cardiac dysfunction (as determined by echocardiography and natriuretic peptide levels) are strongly associated with exercise performance. However, NT-proBNP levels and self-reported physical activity level, but not FMD, independently predict of exercise capacity in adults with repaired ToF. This implies that vascular dysfunction in patients with ToF may be a consequence rather than a cause of impaired exercise performance. Moreover, while association of natriuretic peptides with exercise capacity indicates pathophysiological causes of exercise intolerance, the association of self-
reported physical activity levels with exercise performance underscores the importance of regular physical activity in adults with repaired ToF.

References


[19] The IPAQ Group. Guidelines for Data Processing and Analysis of the International Physical Activity Questionnaire [Internet]. [cited 2017 Jun 1]. Available from: https://docs.google.com/viewer?a=v&pid=sites&srcid=ZGVmYXVsdGRvbWFpbnx0aGVpcGFxfGd4OjE0NDgxMDk3NDU1YWRlZTM


Clinical impact of exercise in patients with peripheral arterial disease

Marko Novaković 1,2, Borut Jug 1,2 and Helena Lenasi 3

1 Department of vascular diseases, University Medical Centre Ljubljana, Ljubljana, Slovenia
2 Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia
3 Faculty of Medicine, Institute of Physiology, University of Ljubljana, Ljubljana, Slovenia

Published in Vascular, November 9, 2016

(Vascular 2017; 25: 412-22)

doi: 10.1177/1708538116678752
**Abstract**

Increasing prevalence, high morbidity and mortality, and decreased health-related quality of life are hallmarks of peripheral arterial disease (PAD). About one third of PAD patients have intermittent claudication with deleterious effects on everyday activities, such as walking. Exercise training improves PAD symptoms and is recommended as first line therapy for PAD. This review examines the effects of exercise training beyond improvements in walking distance, namely on vascular function, parameters of inflammation, activated hemostasis and oxidative stress, and quality of life. Exercise training not only increases walking distance and physiologic parameters in patients with PAD, but also improves the cardiovascular risk profile by helping patients achieve better control of hypertension, hyperglycemia, obesity and dyslipidemia, thus further reducing cardiovascular risk and the prevalence of coexistent atherosclerotic diseases. American guidelines suggest supervised exercise training, performed for a minimum of 30-45 minutes, at least 3 times per week, for at least 12 weeks. Walking is the most studied exercise modality and its efficacy in improving cardiovascular parameters in patients with PAD has been extensively proven. As studies have shown that supervised exercise training improves walking performance, cardiovascular parameters and quality of life in patients with PAD, it should be encouraged and more often prescribed.

**Keywords**

Peripheral arterial disease, intermittent claudication, exercise, walking, rehabilitation, cardiovascular risk
Background

Ageing population and increasing prevalence of non-communicable chronic diseases represent a challenging socio-economic burden. Cardiovascular diseases and atherosclerotic vascular disease in particular, are becoming one of the most significant health problems in the elderly [1]. Peripheral arterial disease (PAD) is defined as atherosclerotic occlusion of the body arteries, excluding intracranial and coronary arteries [2]. Recent research has estimated that prevalence of PAD exceeds 200 million people in the world. PAD is generally perceived as a problem of older adults because the prevalence of PAD increases with age and peaks in the sixth and seventh decades of life [3,4]. In the Slovenian population aged between 50 and 70 years, prevalence of PAD was found to be 18.7 % [5]. In people older than 70 years, estimation ranges from 15 to 20 % of the world’s population [6]. Despite its high prevalence, PAD is still underdiagnosed and undertreated disease [7].

PAD is associated with significant morbidity and mortality. Mortality in patients with PAD is around two- to three-fold higher than in age-matched controls, with a five-year mortality of about 30 % [8]. In a large longitudinal study of 811 patients with PAD and 778 age- and gender-matched controls, patients with PAD retained a borderline increase in all-cause mortality (3.3 vs. 1.8 % at 2 year-follow up, p=0.059) and a significant increase in non-fatal cardiovascular event rate (20.1 vs. 3.6 %, p<0.001) despite optimal state-of-the-art preventive management [9].

Clinical manifestations of PAD range from asymptomatic atherosclerotic plaques to intermittent claudication, critical limb ischemia and limb loss. The most frequent symptom of PAD is intermittent claudication (i.e. exercise-dependent pain in the lower limb muscles that resolves with rest). Symptoms appear due to atherosclerotic plaques in lower limb arteries, causing reduced blood flow and impaired skeletal muscle perfusion resulting in ischemia-induced debilitating pain associated with walking [10]. It is estimated that intermittent claudication affects around one third of patients with PAD [11].
PAD is easily detected by estimating ankle-brachial index (ABI). It is calculated by dividing the systolic pressure on dorsalis pedis or posterior tibial artery (measured by Doppler ultrasound blood flow detector) with the systolic pressure on the brachial artery. An ABI below 0.9 represents a diagnostic criterion for PAD, and also a strong prognostic predictor of adverse cardiovascular events and mortality [12,13].

Exercise training improves cardiovascular performance in both, healthy individuals and in patients with different cardiovascular diseases. In the past, it was believed that the benefits of exercise were solely due to improving traditional cardiovascular risk factors (e.g. high blood pressure, increased plasma lipid levels, increased plasma glucose, and obesity) [14]. However, epidemiological studies reported that less than one half of the beneficial effects of exercise can be attributed to risk factors modification [15]. The other 50% benefits arise from inducing resistance against currently existing harmful levels of risk factors, as concluded in the study of Santos-Parker et al. [14].

Recent in vivo and in vitro studies emphasize the role of endothelial shear stress (ESS) as a key factor in the pathogenesis of atherosclerosis [16-18]. ESS is the tangential stress due to the friction of the flowing blood on the endothelial surface of the arterial wall [16]. In a healthy normal vessel, blood flow is laminar, making the ESS unidirectional. On certain regions in the bloodstream, such as curvatures, arterial branch ostia and bifurcations, blood flow becomes nonlaminar, resulting, among others in decreased ESS, which further results in reduced nitric oxide synthesis, increased vascular cell-adhesion molecules (VCAM-1) expression, impaired endothelial cell repair and induces endothelial cell apoptosis [16-18]. These entities are defined as endothelial dysfunction. Next pathogenetic steps include subendothelial accumulation of low-density lipoprotein cholesterol, increase in reactive oxygen species (ROS) and smooth muscle cell proliferation [16]. Low ESS is also associated with increased expression of several chemoattractant chemokines and pro-inflammatory cytokines, which induce transmigration of monocytes into the intima. After infiltrating beneath the endothelium, monocytes differentiate to macrophages and evolve to foam cells, which sustain the progression of atherosclerosis, further aggravated by other risk factors, i.e. hypertension, cigarette smoking, hyperlipidemia, hyperhomocysteinemia and diabetes mellitus [16,17].
Animal models on effects of exercise on atherosclerosis have been extensively studied. Apolipoprotein E knockout (apoEKO) mice were mostly examined, as they have been shown to have similar atherosclerosis pathogenesis and cardiovascular disease progression as in humans [19]. Exercise training has been shown to improve atherosclerotic plaque stability [20,21], atherosclerosis lesions area, inflammatory [22] and antioxidative status in apoEKO mice [23]. Similar to humans, potential mechanism for these effects lies in regulation of nitric oxide synthesis [23,24].

The review focuses on the effects of exercise training on vascular function, parameters of inflammation, walking performance and quality of life in patients with PAD. The effects of exercise on other cardiovascular risk factors and atherosclerotic diseases are further discussed.

**Influence of exercise on vascular function**

Endothelial dysfunction is the earliest event in the development of atherosclerosis [16-18,25]. Patients with PAD have impaired endothelial function in comparison with healthy peers [26] that correlates with their walking performance [27]. Flow-mediated dilation (FMD), as a widely used method for assessing endothelial function [28], was performed in studies that included patients with PAD and it was confirmed that FMD could serve as a risk marker for symptom severity and impaired physical activity in patients with PAD [27]. Improvement of FMD due to exercise training was shown in the majority of the studies [29-31], but not in all [32]. Results on improved FMD might be explained by upregulation of endothelial nitric oxide synthase and consequently enhanced endothelial synthesis and release of nitric oxide, which is stimulated by an increase in blood flow due to exercise. In addition, improved blood perfusion can be at least partially explained by increased collateral flow, as has been suggested in studies that included coronary artery disease (CAD) patients [33].

Besides macrocirculation, microcirculation is also affected in PAD [34-36]. Microcirculation comprises the arterioles, capillaries, venules, initial lymphatic vessels and, in a more wide sense, the endothelium that
covers these vascular structures and the circulating cells. It is a system in which components interact with each other and contribute to maintaining circulatory, coagulative and metabolic homeostasis [37]. Endothelial dysfunction occurs in microcirculation as well due to impaired synthesis of endothelial vasodilators, such as nitric oxide [38], and impaired microcirculatory vasodilatation, which have been singled out as key elements in the pathogenesis of PAD and CAD [39]. Exercise has a beneficial effect on microcirculation, which is confirmed not only in healthy population [40], but also in cardiovascular patients [25,41].

**Influence of exercise on parameters of inflammation**

PAD, as all other forms of atherosclerotic disease, is associated with low-grade inflammation. During repetitive bouts of peripheral ischemia, patients with PAD undergo episodes of low-grade inflammatory response reflected in increased values of plasma markers of inflammation [42,43]. A strong association between markers of inflammation with walking performance [44,45], as well as with calf musculature oxygen saturation has been reported [45]. Markers of activated coagulation and fibrinolysis (D-dimer and thrombin-antithrombin III complex), and endothelial stimulation (von Willebrand factor) are also increased in PAD due to endothelial damage and pathologic changes at the atherosclerotic sites [43]. As previously stated, exercise leads to reduced production of proinflammatory cytokines, such as interleukin (IL)-1, IL-6 [46] and adhesion molecules [47], but in the study of Nowak et al., some cytokines achieved surprisingly higher values after the exercise training program was finished [48] as compared to pre-exercise levels. Studies have mostly shown that exercise training induced a decrease in the C-reactive protein (CRP) or high sensitive CRP (hs-CRP) values in patients with PAD [29,49], but there are also studies in which the improvement was not reached [50]. Fibrinogen, as another marker of inflammation was also examined, but possible effects of exercise on the fibrinogen values are also controversial [49,50]. Apart from its effects on suppression of production of cytokines, exercise induces an increase in endothelial nitric oxide, which
decreases the level of ROS formation and inflammation [25, 51]. Besides possible methodological issues in detection and measurement of markers of inflammation, and especially markers of activated hemostasis or oxidative stress, basic principles of long-term adaptation to short-term exercise-associated physiologic derangements should be taken into account. For instance, exercise is associated with transient inflammation, spill-over of ROS and activated coagulation and fibrinolysis, while chronic adaptation is associated with a favorable decrease in these biomarkers; the timing of detection (i.e. preexercise, immediately post-exercise, after long-term exercise training or at rest) may therefore significantly influence blood levels of these markers [52].

**Influence of exercise on walking performance**

Numerous studies have shown beneficial effects of exercise training on walking performance in patients with PAD. Outcome measures used as ‘walking parameters’ were pain-free walking distance, maximal walking distance, pain-free walking time and maximal walking time [30,42,47,48, 53-56].

Although some studies failed to prove a favorable association between exercise training and walking parameters [57], various meta-analyses confirmed beneficial effects of exercise training [58,59]. Walking performance has been shown to improve not only during exercise training programs or shortly after them, but even three years after cessation of the exercise program [60]. However, the effects of exercise training programs persist only if the patient continues to exercise on his/her own for at least 60 minutes per week [61].

Walking parameters remain the most important outcome measure in studies on exercise programs in patients with PAD. ABI was also a parameter widely used as a measure of disease severity and improvement. ABI is not only a diagnostic tool for diagnosis of asymptomatic PAD, but also a marker of diffuse atherosclerosis associated with CAD and cerebrovascular disease (CVD) [62], and a strong predictor of cardiovascular mortality [8]. In a meta-analysis of 30 studies on exercise training in PAD, exercise was
associated with an 0.05 increase in ABI as compared to placebo/optimal medical care; the effect size was relatively small in magnitude, but highly significant due to a large number of patients (N=1822) [63]. In some studies, improvement in ABI was correlated with improvement in walking abilities [64], while in some the improvement in the walking performance was not followed by the improvement in ABI [65].

**Influence of exercise on quality of life**

Numerous authors have shown that, due to positive effects of exercise training programs on walking abilities and vascular function, quality of life of patients with PAD has also improved. Intermittent claudication as the most prominent symptomatic manifestation of PAD is associated with limitations in everyday activities, such as walking. Therefore, improvements in pain-free walking distance are expected to translate in improved health-related quality of life measures. Most studies of exercise in patients with PAD used the Short Form (36) Health Survey (SF-36), a 36-item general health-related quality of life questionnaire comprising physical and mental domains. SF-36 has been shown to have good reliability and has been extensively validated; it is therefore the most widely used research tool for measurement of health-related quality of life in different diseases and conditions. Exercise training also leads to an improvement in the disease-specific quality of life as assessed by other questionnaires, such as walking impairment questionnaire (WIQ), The Peripheral Arterial Occlusive Disease 86 (PAVK-86), The Intermittent Claudication Questionnaire, VascuQol and others. A recent meta-analysis of 15 randomized trials (including data on 1257 participants) showed that along with increasing walking distance, the physical – but not mental – component of the SF-36 also increased significantly [66].
Supervised vs. non-supervised exercise training programs

In terms of supervision, exercise programs are divided into two subgroups: supervised exercise programs and home-based programs, the latter with two entities where different approaches are examined: different structured home-based exercise programs and “go home and walk” policy.

Supervised exercise programs are organized in the hospitals and health-care clinics [67] and recommended by all relevant guidelines for the treatment of patients with PAD [68-70]. As professional support and supervision provide early recognition of possible complications, supervised exercise programs are generally safe [71]. Moreover, extremely low complication rates suggest that even cardiovascular screening is superfluous when the exercise program provides necessary supervision [71]. Nonetheless, the American Heart Association/American College of Cardiology (AHA/ACC) guidelines encourage electrocardiographic, heart rate, blood pressure, and blood glucose monitoring [70]. The most important exclusion criteria, which might be used as contraindications for exercise commencement, are summarized in the study of McDermott et al. and are the following: major amputation, critical limb ischemia, surgery or a myocardial infarction within the past three months, dementia, foot ulcers, inability to walk on a treadmill, and poorly controlled arterial hypertension [72].

Comparison between supervised and home-based training programs was performed in several studies and reviews [73-75]. It has been shown that supervised exercise is superior to both entities of home-based exercise (i.e. the “go home and walk” and the structured home-based exercise [73]) in terms of walking performance [73,74]. In two cost-effectiveness reports [76,77], supervision was marked as a value for money as compared to unsupervised. It has been shown to stimulate and increase daily physical activity when the supervision was finished [78]. However, in a study of Gardner et al. [79], accurately monitored home-based exercise program was shown to be equally efficacious in terms of walking performance compared to supervised exercise program. Unlike “go home and walk” policy, structured home-based
exercise programs seem to be an attractive alternative to supervised exercise programs and should be offered to patients when supervised exercise training is unavailable or impractical [67].

Low adherence to exercise was mostly emphasized as a main problem in home-based programs in comparison with supervised programs. Obviously, psychosocial aspects play an important role when comparing the two. Supervised programs are often organized for more patients. Thus, patients are given an opportunity to interact with others, which improves adherence to exercise, as reported in one meta-analysis [80]. Social aspect of the program and positive entertainment associated with it, together with the supportive role played by the supervision are probable answers why supervision programs were superior compared to home-based programs [81].

**Exercise modalities**

Walking was the most common exercise training modality, applied in thousands of patients participating in numerous studies and has been shown to be an effective treatment modality in patients with PAD. That is why walking is recommended by the AHA/ACC [70]. However, due to different conditions, such as severe claudication pain, diabetic foot complications, arthritis, osteoporotic vertebral fractures, stroke and severe cardiorespiratory or degenerative neurological diseases, some patients are unable (or unwilling) to take part in the walking programs. Alternative efficient exercise modalities for these patients might be varieties of Nordic walking, cycling, arm-cranking exercise, and progressive resistance training [82].

Most often, walking exercise programs in the available studies lasted for three to six months [29-31,49,83,84], although some programs lasted (and were supervised) for even 12 months [56,85]. Results from the study of Gardner et al. [86] suggest that the first two months in a longer exercise training program are crucial for improvement of walking performance. After the fourth month, improvement is also significant. On the last follow-up after the sixth months of exercise program, there was no significant improvement as compared to the follow-up after the fourth month [86]. Therefore, hospitals with limited
human and financial resources could still organize qualitative and efficient exercise training program of shorter duration.

As for the frequency, three sessions per week were mostly seen in the programs, although in some reports the frequency was two sessions per week [50,87]. Progressive exercise programs were performed in most of the studies [30,31,84].

Intensities of the training programs were mostly defined as percentage of the maximal workload and ranged from 40 to 80 % [31,83,86]. A less precise way of defining the intensity was the onset of claudication pain or its severity [29,30,50,85,88]. Results from the study of Gardner et al. [83] indicate that more intensive exercise is not associated with superior improvement compared to low intensity exercise in terms of walking performance, tissue oxygen uptake and quality of life.

An optimal treatment modality has not been identified yet. In AHA/ACC guidelines, recommended modality includes supervised exercise training, performed for a minimum of 30 to 45 minutes, in sessions performed at least 3 times per week for a minimum of 12 weeks (Table 1) [70].

| Table1. Exercise training modalities which improve walking performance in patients with peripheral arterial disease |
|---------------------------------------------------------------|---------------------------------------------------------------|
| **Exercise training modality**                               | **Preferred/recommended**                                      | **Alternative modalities**                                    |
| Walking [70]                                                 | Nordic walking, cycling, arm-cranking, progressive resistance training [82] |
| **Program duration**                                         | 12 weeks or more [70]                                         |
| **Frequency**                                                | 3 times per week or more [70]                                |
| **Duration of a session**                                    | At least 30 – 45 min [70]                                    |
| **Supervision**                                              | Supervised [73,74]                                           |
| **Definition of intensity**                                  | Max workload (40-80 %)                                        |
|                                                            | [31,83, 86]                                                  |
|                                                            | Claudication pain onset or pain intensity[29,30,50]           |
Besides from exercise training as an intervention, previous reports provide data about efficacy of other treatment modalities, with or without comparison to exercise training. In one study, exercise training, peripheral artery revascularization with stenting, and optimal medical care were compared. Exercise training mostly showed superior short-term effects as compared to stent vascularization intervention [55]. Long term benefits of exercise were equal to benefits of revascularization procedure [89]. In various meta-analyses on this issue authors also do not answer the question about superior intervention model, suggesting that exercise training programs should be favorized and applied whenever possible [90]. British guidelines explicitly give priority to exercise training in the treatment hierarchy [69].

Cost-effectiveness also plays an important role in deciding on appropriate therapy. Results from several cost-effectiveness studies give priority to exercise, as there were no significant differences in the effectiveness between endovascular revascularization compared to exercise, but the costs were significantly higher for revascularization procedures [91,92]. Results from cost-effectiveness studies emphasize exercise as the most cost-effective first line therapy [92,93], adding that exercise training is even more cost-effective when combined with endovascular revascularization than endovascular revascularization alone [93].

Influence of exercise training on cardiovascular risk factors, and coexistence with coronary artery disease, cerebrovascular disease and the metabolic syndrome

Due to their common etiopathogenesis in regard to atherosclerosis, the coexistence of PAD, CAD and CVD is not a surprise and has been proved in many studies. Prevalence of CAD in patients with PAD is estimated to range from 55% [94] to 62% [95]. Prevalence of CAD in patients with PAD is around 43% [94]. Association between complex PAD and complex CAD was shown in a study of Aykan et al. [96]. The coexistence is associated with more severe morbidity profiles - it has been estimated that patients with
PAD are three to six times more likely to experience myocardial infarction and stroke than patients without PAD [97].

Conversely, the prevalence of PAD is also high (36–42%) in patients with established CAD or CVD, and in individuals at high cardiovascular risk (i.e. with two or more risk factors). More importantly, half of the PAD is asymptomatic but nonetheless associated with an increased cardiovascular risk [62].

Approximately 50 to 92% of patients with PAD have arterial hypertension [98], 77% have hyperlipidemia [99], 20% of symptomatic patients with PAD have diabetes (which is probably underestimated, as there are more asymptomatic than symptomatic PAD patients) [100].

Numerous studies have demonstrated beneficial effects of exercise on CAD and CVD [101-103]. Reducing cardiovascular risk factors due to exercise is very well documented [104,105]. Therefore, we strongly believe that exercise training is not helpful only in PAD, but also in conditions associated with it. Exercise training should decrease the incidence and prevalence of other two coexisting atherosclerotic diseases (CAD and CVD) in patients with PAD, as well as other cardiovascular risk factors that worsen prognosis of PAD (hypertension, hyperlipidemia, diabetes) (Fig 1).

![Effects of exercise training on atherosclerotic diseases](image)

**Fig1. Effects of exercise training on atherosclerotic diseases**
Some of these hypotheses have also been confirmed in the literature. Effects on lipid profiles differed from study to study. In some, exercise had a lowering effect on values of total cholesterol [49, 88], LDL cholesterol [49, 88] and triglycerides [49], and an increasing effect on the values of HDL [49] in patients with PAD. On the other hand, these effects of exercise were not shown in the study of Januszek et al. [29]. The effects of exercise on significant systolic blood pressure reduction were documented in the study of Izquierdo-Porrera et al. [88].

Relationship between tobacco smoking and PAD has been described in numerous studies and meta-analyses [106,107]. It is indicated that the prevalence of patients with PAD, attributed to smoking, is around 50 % [108]. In a report of Gardner et al., patients with PAD who were smokers had significant impairments in terms of walking performance, calf muscles blood flow and quality of life, as compared to patients with PAD who were not cigarette smokers. However, exercise training was beneficial almost equally for both groups of patients. That is why smokers are indicated as prime candidates for exercise, due to their low baseline physical performance. It seems that their ability to regain impaired walking performance is not lost due to cigarette smoking and that exercise is an efficient way to regain it [84].

Usually, patients with PAD have poor nutrition in terms of quality, rich in saturated fats and sodium, and low in fibres, vitamin E and folate intake [54]. In the study of Leicht et al., supervised exercise training has been successful in improving walking performance in patients with PAD, but this improvement has not been followed with healthier eating habits. Therefore, future studies are needed to examine the impact of combining dietary interventions with supervised exercise in PAD patients [85].

Sedentary lifestyle is associated with increased risk of PAD [109]. In patients who already have PAD, sedentary lifestyle is more common than in healthy controls [110] and is associated with higher mortality rate [111]. Body mass index also increases the mortality risk in patients with PAD [111]. Similar result, namely that obesity is associated with cardiovascular events in patients with PAD was obtained in the study of Golledge et al. [112]. In their study, only waist circumference was used to define obesity. In the same
study, obesity was associated with more impaired walking performance. In several reports, it was indicated that exercise training leads to a decrease in body fat percentage [86] and body weight [49].

Metabolic syndrome (MetS) is an entity defined by the USA National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP) III as having at least three out of the five following components: abdominal obesity, elevated triglycerides, reduced high-density lipoprotein cholesterol, elevated blood pressure and elevated fasting glucose [113]. Results from several studies, in which patients exhibiting both PAD and MetS were included, report that prevalence of MetS in patients with PAD range from 52 % to 58 % [114,115]. Patients with PAD, who have MetS, have increased risk of cardiovascular events [112], limited quality of life [116] and walking performance [112, 116], compared to patients with PAD without MetS. Results from the study of Gardner et al. suggest that having more MetS components leads to worsening of walking performance, health-related quality of life and peripheral circulation in patients with PAD. Obesity and elevated fasting glucose are components with highest predictive value [117]. However, results from the study of Ambrosetti et al. suggest that MetS is not associated with poor response to exercise in patients with PAD [118]. Therefore, due to their increased morbidity from one side and good response to exercise from the other, patients with PAD and MetS should be a target population to commence an exercise training program.

Conclusion

PAD is becoming increasingly important in terms of public health. Firstly, increased morbidity and mortality, and severely impaired quality of life in patients with PAD call for a sustained improvement in the management of patients with PAD. Secondly, a widespread risk factors yielding to PAD represent a potentially modifiable, albeit complex etiopathologic interplay. Exercise training provides an effective management strategy for PAD. Several guidelines have pointed out the supervised exercise training as the first line therapy in patients with PAD. On the one hand, exercise training improves the most common
symptomatic manifestation of PAD – intermittent claudication. On the other hand, exercise training helps in the management of cardiovascular risk factors, such as diabetes, dyslipidemia and hypertension, which are important for other two atherosclerotic entities – CAD and CVD. Lastly, exercise training provides an effective vehicle for delivery of lifestyle intervention beyond exercise itself – including a healthy diet and smoking cessation.

**Funding**

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

**Conflict of interest**

The authors declare that there is no conflict of interest.

**References**


[107] Lu L, Mackay DF, Pell JP. Meta-analysis of the association between cigarette smoking and peripheral arterial disease. *Heart* 2014; 100: 414-423.


APPENDIX D: SF-36 QUESTIONNAIRE

SF-36 VPRAŠALNIK O ZDRAVJU

**NAVODILA:** Vprašalnik sprašuje o tem, kako gledate na svoje zdravje. Iz odgovorov bomo zvedeli, kako se počutite in kako dobro lahko opravljate svoja običajna opravila.

Odgovorite na vsa vprašanja tako, da izberete in označite odgovor. Če niste prepričani, kako odgovoriti na katero od vprašanj, izberite odgovor, ki je povašem mnenju najboljši.

1. Ali bi lahko rekli, da je vaše zdravje na splošno:  

   (obkrožite eno številko)
   - Odlično........................................................................................................1
   - Zelo dobro......................................................................................................2
   - Dobro...........................................................................................................3
   - Ne dobro, ne slabo.......................................................................................4
   - Slabo...........................................................................................................5

2. Kako bi sedaj na splošno ocenili svoje zdravje, če ga primerjate s svojim zdravjem pred enim letom?

   (obkrožite eno številko)
   - Veliko boljše kot pred enim letom...............................................................1
   - Nekoliko boljše kot pred enim letom..........................................................2
   - Približno enako kot pred enim letom...........................................................3
   - Nekoliko slabše kot pred enim letom...........................................................4
   - Veliko slabše kot pred enim letom...............................................................5
3. Naslednja vprašanja zadevajo stvari, ki jih najbrž počnete ob običajnih dnevih. Vas zdaj vaše zdravstveno stanje pri the dejavnostih ovira? Če vas ovira, ocenite v kakšni meri:

(v vsaki vrsti obkrožite eno število)

<table>
<thead>
<tr>
<th>DEJAVNOSTI</th>
<th>Da, zelo mo ovira</th>
<th>Da, malo mo ovira</th>
<th>Ne, sploh me ne ovira</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Težka opravila, recimo tek, vzdigovanje težkih predmetov, naporni športi</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>b. Zmerno težka opravila, recimo premikanje mize, porivanje sesalca po sobi, balinanje ali gobarjenje</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>c. Vzdigovanje ali prenašanje vrečk s hrano iz trgovine</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>d. Vzpenjanje peš po stopnicah nekaj nadstropij</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>e. Vzpenjanje peš po stopnicah eno nadstropje</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>f. Sklanjanje, poklekanje, počepanje</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>g. Hoja dije kot en kilometer</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>h. Hoja nekaj stometrov daleč</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>i. Hoja sto metroval daleč</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>j. Samostojno kopanje ali oblačenje</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

4. Ste imeli v preteklih 4 tednih zaradi telesnega zdravja pri delu ali pri drugih rednih dnevnih opravilih katerega od naštetih problemov?

(v vsaki vrsti obkrožite eno število)

<table>
<thead>
<tr>
<th>DA</th>
<th>NE</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Ste morali skrajšati čas namenjen delu ali drugim dejavnostim?</td>
<td>1</td>
</tr>
<tr>
<td>b. Ste postorili manj kot bi želeli?</td>
<td>1</td>
</tr>
<tr>
<td>c. Ste bili omejeni tako, da nistemogli opravljati določene vrste dela ali dejavnosti?</td>
<td>1</td>
</tr>
<tr>
<td>d. Ste s težavo opravljali delo ali druge dejavnosti (tako, da je bil na primer potreben dodaten napor)?</td>
<td>1</td>
</tr>
</tbody>
</table>
5. Ste imeli v preteklih 4 tednih zaradi čustvenih težav (recimo da ste bili potri ali zelo zaskrbljeni) pri svojem delu ali pri drugih rednih dnevnih opravilih katerega od naštetih problemov?

(v vsaki vrsti obkrožite eno številko)

<table>
<thead>
<tr>
<th></th>
<th>DA</th>
<th>NE</th>
</tr>
</thead>
<tbody>
<tr>
<td>a.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ste morali <strong>skrajšati čas</strong> namenjen delu ali drugim dejavnostim?</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>b.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ste <strong>postorili manj</strong> kot bi želeli?</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>c.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ste bili pri delu ali drugih opravilih <strong>manj skrbni</strong> kot sicer?</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

6. Koliko so v preteklih 4 tednih vaše telesno zdravstveno stanje ali čustvene težave ovirale vaše običajno družabno življenje v krogu družine, prijateljev, sosedov, ali drugih ljudi?

(obkrožite eno številko)

- Prav nič .............................................................................................................. 1
- Nekoliko ............................................................................................................ 2
- Zmerno ............................................................................................................... 3
- Precej ................................................................................................................... 4
- Skrajno ............................................................................................................... 5

7. Ste v preteklih 4 tednih v telesu čutili kakšne bolečine?

(obkrožite eno številko)

- Nobenih ............................................................................................................ 1
- Zelo blage .......................................................................................................... 2
- Blage .................................................................................................................. 3
- Zmerne .............................................................................................................. 4
- Hude ................................................................................................................... 5
- Zelo hude .......................................................................................................... 6
8. Koliko so vas v preteklih 4 tednih bolečine ovirale pri vašem običajnem delu (tako izven doma kot pri hišnih opravilih)?

<table>
<thead>
<tr>
<th></th>
<th>Vesčas</th>
<th>Večino časa</th>
<th>Precej časa</th>
<th>Nekaj časa</th>
<th>Malo časa</th>
<th>Nikoli</th>
</tr>
</thead>
</table>
a. Polni življenja     | 1      | 2           | 3           | 4          | 5         | 6      |
b. Zelo živčni         | 1      | 2           | 3           | 4          | 5         | 6      |
c. Tako na tleh, da vas nič ni moglo spraviti v dobro voljo | 1      | 2           | 3           | 4          | 5         | 6      |
d. Mirni in sproščeni | 1      | 2           | 3           | 4          | 5         | 6      |
e. Polni energije      | 1      | 2           | 3           | 4          | 5         | 6      |
f. Malodušni in pottri | 1      | 2           | 3           | 4          | 5         | 6      |
g. Izčrpani            | 1      | 2           | 3           | 4          | 5         | 6      |
h. Srečni              | 1      | 2           | 3           | 4          | 5         | 6      |
i. Utrujeni            | 1      | 2           | 3           | 4          | 5         | 6      |

(obkrožite eno številko)

9. Ta vprašanja sprašujejo o tem, kako ste se počutili in kako vam je šlo v preteklih 4 tednih. Za vsako vprašanje poiščite odgovor, ki najbolje opisuje, kako ste se počutili. Koliko časa v zadnjih 4 tednih ste bili: (v vsaki vrsti ob krožite eno številko)
10. Koliko časa so vas v preteklih 4 tednih vaše telesno zdravstveno stanje ali čustvene težave ovirale pri družabnem življenju (na primer pri srečanjih s prijatelji, sorodniki, itd.)?

(obkrožite eno številko)

<table>
<thead>
<tr>
<th>Vse časa</th>
<th>Večino časa</th>
<th>Nekaj časa</th>
<th>Malo časa</th>
<th>Nikoli</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

11. Koliko vsaka od naslednjih stvete za vas DRŽI ali NE DRŽI?

(v vsaki vrsti obkrožite eno številko)

<table>
<thead>
<tr>
<th></th>
<th>Polovina drži</th>
<th>V glavnem drži</th>
<th>Ne vem</th>
<th>V glavnem ne drži</th>
<th>Nikakor ne drži</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Zdi se mi, da malo hitreje obolevam kot drugi</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>b. Tako zdrav sem kot vsi drugi ljudje, ki jih poznam</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>c. Pričakujem, da se mi bo zdravje poslabšalo</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>d. Moje zdravje je odlično</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>
IPAQ – mednarodni vprašalnik o telesni aktivnosti

Zanima nas, na kakšen način in v kolikšni meri so posamezniki telesno aktivni v svojem vsakdanju. Naslednja vprašanja se nanašajo na pogostnost vaše telesne aktivnosti v zadnjih 7 dneh. Prosimo vas, da odgovorite na vsa vprašanja, čeprav zase menite, da niste telesno aktivni; razmislite o vseh oblikah telesne aktivnosti, ki jih počnete pri vašem delu, pri hišnih in/ali vrtnih opravilih, pri dnevnih opravkih (npr. hoja v trgovino) ter v svojem prostem času – rekreacijo, šport in telesno vadbo.

A. Naslednja vprašanja se nanašajo na zelo naporno telesno aktivnost v zadnjih 7 dneh. Zelo naporna telesna aktivnost pomeni aktivnost, ki zahteva veliko napora in zaradi katere se zadihate. Zanimajo nas zgolj tiste oblike aktivnosti, ki ste jim posvetili po vsaj 10 minut naenkrat.

1. Koliko krat ste v zadnjih 7 dneh opravljali zelo naporno telesno aktivnost, kot je npr. dvigovanje težjih bremen, prekopavanje, hiter tek ali kolesarjenje?

□ _________ dni na teden

☐ Če niste imeli nobenih hudih telesnih aktivnosti ➔ preskočite na vprašanje 3

2. Holiko časa običajno opravljate izjemno telesno aktivnost v teh dneh?

□ _________ ur na dan

□ _________ minut na dan

☐ Ne vem, nisem prepričan(a)

B. Vprašanja se nanašajo na zmerno telesno aktivnost v zadnjih 7 dneh. Zmerna telesna aktivnost pomeni aktivnost, ki zahteva zmeren napor in zaradi katere ste nekoliko bolj zadihani kot običajno. Zanimajo nas zgolj tiste oblike aktivnosti, ki ste jih počeli po vsaj 10 minut naenkrat.


□ _________ dni na teden

☐ Če niste imeli nobenih zmernih telesnih aktivnosti ➔ preskočite na vprašanje 5

4. Koliko časa dnevno ste bili zmerno telesno aktivni v teh dneh?

□ _________ ur na dan

□ _________ minut na dan

☐ Ne vem, nisem prepričan(a)
C. Naslednja vprašanja se nanašajo na hojo v zadnjih 7 dneh. To pomeni hojo doma, v službi, po opravkih (npr. v trgovino) in sprehanjanje kot oblika rekreacije, športa, telesne vadbe ali sproščanja.

5. Koliko dni v tednu ste hodili vsaj po 10 minut naenkrat?
   _________ dni na teden

6. Koliko časa dnevno hodite v teh dneh?
   _________ ur na dan
   _________ minut na dan
   □ Ne vem, nisem prepričan(a)

D. Naslednje vprašanje se nanaša na sedenje v zadnjih 7 dneh. To pomeni sedenje v službi, doma ali med prosočasnimi aktivnostmi (npr. branje, gledanje televizije, obiskovanje prijateljev ipd.)

7. Koliko časa dnevno presedite?
   _________ ur na dan
   _________ minut na dan
   □ Ne vem, nisem prepričan(a)

IME IN PRIIMEK ___________________________________________________________

Hvala!
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>6MWT</td>
<td>six-minute walk test</td>
</tr>
<tr>
<td>ABI</td>
<td>ankle-brachial index</td>
</tr>
<tr>
<td>ACEi/ARB</td>
<td>angiotensin-converting enzyme inhibitors/angiotensin receptor blockers</td>
</tr>
<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
</tr>
<tr>
<td>AT</td>
<td>anaerobic threshold</td>
</tr>
<tr>
<td>β</td>
<td>beta stiffness coefficient</td>
</tr>
<tr>
<td>BMI</td>
<td>body mass index</td>
</tr>
<tr>
<td>BNP</td>
<td>brain natriuretic peptide</td>
</tr>
<tr>
<td>BP</td>
<td>bodily pain</td>
</tr>
<tr>
<td>CHR</td>
<td>chronotropic</td>
</tr>
<tr>
<td>CO2</td>
<td>carbon dioxide</td>
</tr>
<tr>
<td>CONSORT</td>
<td>Consolidated Standards of Reporting Trials</td>
</tr>
<tr>
<td>DBP</td>
<td>diastolic blood pressure</td>
</tr>
<tr>
<td>DP peak</td>
<td>peak double product</td>
</tr>
<tr>
<td>EDTA</td>
<td>ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>FMD</td>
<td>flow-mediated dilation</td>
</tr>
<tr>
<td>GH</td>
<td>general health</td>
</tr>
<tr>
<td>Hct</td>
<td>haematocrit</td>
</tr>
<tr>
<td>HDL-c</td>
<td>high-density lipoprotein cholesterol</td>
</tr>
<tr>
<td>HFnu</td>
<td>power in the high-frequency range, normalized</td>
</tr>
<tr>
<td>HGB</td>
<td>haemoglobin</td>
</tr>
<tr>
<td>HR</td>
<td>heart rate</td>
</tr>
<tr>
<td>HRR</td>
<td>heart rate recovery</td>
</tr>
<tr>
<td>HRV</td>
<td>heart rate variability</td>
</tr>
<tr>
<td>IPAQ</td>
<td>International Physical Activity Questionnaire</td>
</tr>
<tr>
<td>LDL-c</td>
<td>low-density lipoprotein cholesterol</td>
</tr>
<tr>
<td>LF:HF</td>
<td>ratio of normalized low- and high-frequency powers</td>
</tr>
<tr>
<td>LFnu</td>
<td>power in the low-frequency range, normalized</td>
</tr>
<tr>
<td>Lymph</td>
<td>absolute lymphocyte count</td>
</tr>
<tr>
<td>MCS</td>
<td>mental component summary</td>
</tr>
<tr>
<td>MET</td>
<td>metabolic equivalent of task</td>
</tr>
<tr>
<td>MH</td>
<td>mental health</td>
</tr>
<tr>
<td>Mono</td>
<td>absolute monocyte count</td>
</tr>
<tr>
<td>Neu</td>
<td>absolute neutrophil count</td>
</tr>
<tr>
<td>NN</td>
<td>RR intervals of normal beats</td>
</tr>
<tr>
<td>NO</td>
<td>nitric oxide</td>
</tr>
<tr>
<td>NOS</td>
<td>nitric oxide synthase</td>
</tr>
<tr>
<td>NT-proBNP</td>
<td>amino-terminal prohormone of brain natriuretic peptide</td>
</tr>
<tr>
<td>O2</td>
<td>oxygen</td>
</tr>
<tr>
<td>PAD</td>
<td>peripheral arterial disease</td>
</tr>
<tr>
<td>PCS</td>
<td>physical component summary</td>
</tr>
<tr>
<td>PF</td>
<td>physical functioning</td>
</tr>
<tr>
<td>PLT</td>
<td>platelet count</td>
</tr>
<tr>
<td>pNN50%</td>
<td>percentage of successive NN intervals differing by more than 50 ms</td>
</tr>
<tr>
<td>PWV</td>
<td>pulse wave velocity</td>
</tr>
<tr>
<td>RBC</td>
<td>red blood cell count</td>
</tr>
<tr>
<td>RCCA</td>
<td>right common carotid artery</td>
</tr>
<tr>
<td>RE</td>
<td>role functioning/emotional</td>
</tr>
<tr>
<td>rMSSD</td>
<td>square root of the mean of the sum of squares of successive NN interval difference</td>
</tr>
<tr>
<td>RP</td>
<td>role functioning/physical</td>
</tr>
<tr>
<td>RV</td>
<td>right ventricular</td>
</tr>
<tr>
<td>SBP</td>
<td>systolic blood pressure</td>
</tr>
<tr>
<td>SCD</td>
<td>sudden cardiac death</td>
</tr>
<tr>
<td>SD</td>
<td>standard deviation</td>
</tr>
<tr>
<td>SDNN</td>
<td>standard deviation of all normal RR (NN) intervals</td>
</tr>
<tr>
<td>SF</td>
<td>social functioning</td>
</tr>
<tr>
<td>SF-36</td>
<td>36 item Short Form Survey</td>
</tr>
<tr>
<td>SVT</td>
<td>supraventricular tachycardia</td>
</tr>
<tr>
<td>TC</td>
<td>total cholesterol</td>
</tr>
<tr>
<td>ToF</td>
<td>Tetralogy of Fallot</td>
</tr>
<tr>
<td>VCO2</td>
<td>carbon dioxide flow during exercise</td>
</tr>
<tr>
<td>VE</td>
<td>ventilation</td>
</tr>
<tr>
<td>VE/VCO2</td>
<td>minute ventilation/carbon dioxide production slope</td>
</tr>
<tr>
<td>VO2</td>
<td>oxygen flow during exercise</td>
</tr>
<tr>
<td>VT</td>
<td>vitality</td>
</tr>
<tr>
<td>WBC</td>
<td>white blood cell count</td>
</tr>
</tbody>
</table>
ACKNOWLEDGEMENTS

A thesis such as this would not have been finished without the assistance of many people. I can only hope that they are aware how grateful I am for their assistance in preparing this PhD thesis.

First of them is Borut Jug1, my mentor and supervisor in so many senses. I thank him for believing in me, for a huge intellectual input and for always having a positive attitude for all my ideas. I am honoured for a chance to be a PhD student under his mentorship. His contribution towards this thesis is enormous. As there are so many research plans to be done, I look forward for mutual collaboration in future.

Katja Prokšelj2 is another important person for this PhD thesis, as she is the one who allocated the whole cohort of tetralogy of Fallot patients. Her unlimited positive energy and clinical knowledge is something I will always remember when thinking of my PhD thesis.

Uroš Rajkovič3 had an irreplaceable role in data analysis and visual data interpretation. His ideas of visualising obtained data were endless. Thank you!

No matter that we live in an individualised world, my deepest gratitude is reserved for the entire Centre for preventive cardiology - physicians, nurses, physiotherapists and administrators. Such a huge project is always associated with numerous technical, logistical and substantive issues, but thanks to perfect coordination abilities of Katja Janša Trontelj4, these issues did not affect my work.

Without contribution of the following persons, I would have difficulties with allocating patients for the study, data collection and/or measurement performance (listed in alphabetical order): Mojca Bervar, MD; Prof. Aleš Blinc, MD, PhD; Vinko Boc, MD; Mojca Božič Mijovski, PhD; Urška Bregar, MD, PhD; Marta Cvijič, MD; Prof. Zlatko Fras, MD, PhD; Miodrag Janič, MD, PhD; Majda Joras, MD, PhD; Amelia Kabaklič, MD, PhD; Prof. Matija Kozak, MD, PhD; Barbara Krevel, MD; Monika Kurtiš, RN; Urška Osredkar, RN; Nina Ostaševski Fernandez, MD; Rok Perme, MD; Prof. Vito Starc MD, PhD; Jerneja Tasič, MD, PhD; Gregor Tratar, MD, PhD; Martina Turk, MD; Prof. Dušan Štajer, MD, PhD and Tjaša Vižintin Cuderman, MD, PhD.

I am grateful to all the patients for their participation in the study. The primary aim of the PhD thesis was an improvement of their health and quality of life.

I also thank Ms. Doris Sattler and The Public Scholarship, Development, Disability and Maintenance Fund of the Republic of Slovenia for funding my PhD studies.

Last but most importantly, I thank my mother and sister for limitless support throughout all these years. Very last thoughts and words of the thesis are reserved for my father, who recently passed away.

Marko

---

1 Assistant Professor Borut Jug, MD, PhD
2 Assistant Professor Katja Prokšelj, MD, PhD
3 Associate Professor Uroš Rajkovič, PhD
4 Katja Janša Trontelj, RN