The acute effect of exercise on flow-mediated dilation in young people with cystic fibrosis

Dissertation

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Abstract

Introduction: Cystic fibrosis (CF) is a genetic disease which disrupts the function of an epithelial surface anion channel, CFTR (cystic fibrosis transmembrane conductance regulator). Impairment to this channel leads to inflammation and infection in the lung causing the majority of morbidity and mortality. However, CF is a multiorgan disease affecting many tissues, including vascular smooth muscle. Studies have revealed young people with cystic fibrosis lacking inflammation and infection still demonstrate vascular endothelial dysfunction, measured per flow-mediated dilation (FMD). In other disease cohorts, i.e. diabetic and obese, endurance exercise interventions have been shown improve or taper this impairment. However, long-term exercise interventions are risky, as well as costly in terms of time and resources. Nevertheless, emerging research has correlated the acute effects of exercise with its long-term benefits and advocates the study of acute exercise effects on FMD prior to longitudinal studies. The acute effects of exercise on FMD have previously not been examined in young people with CF, but could yield insights on the potential benefits of long-term exercise interventions.

The aims of these studies were to 1) develop and test the reliability of the FMD method and its applicability to study acute exercise effects; 2) compare baseline FMD and the acute exercise effect on FMD between young people with and without CF; and 3) explore associations between the acute effects of exercise on FMD and demographic characteristics, physical activity levels, lung function, maximal exercise capacity or inflammatory hsCRP levels.

Methods: Thirty young volunteers (10 people with CF, 10 non-CF and 10 non-CF active matched controls) between the ages of 10 and 30 years old completed blood draws, pulmonary function tests, maximal exercise capacity tests and baseline FMD measurements,

before returning approximately 1 week later and performing a 30-min constant load training at 75% HR_{max}. FMD measurements were taken prior, immediately after, 30 minutes after and 1 hour after constant load training. ANOVAs and repeated measures ANOVAs were employed to explore differences between groups and timepoints, respectively. Linear regression was implemented and evaluated to assess correlations between FMD and demographic characteristics, physical activity levels, lung function, maximal exercise capacity or inflammatory hsCRP levels. For all comparisons, statistical significance was set at a *p*-value of $\alpha < 0.05$.

Results: Young people with CF presented with decreased lung function and maximal exercise capacity compared to matched controls. Baseline FMD was also significantly decreased in the CF group (CF: 5.23% v non-CF: 8.27% v non-CF active: 9.12%). Immediately post-training, FMD was significantly attenuated (approximately 40%) in all groups with CF still demonstrating the most minimal FMD. Follow-up measurements of FMD revealed a slow recovery towards baseline values 30 min post-training and improvements in the CF and non-CF active groups 60 min post-training. Linear regression exposed significant correlations between maximal exercise capacity (VO_2 peak), BMI and FMD immediately post-training.

Conclusion: These new findings confirm that CF vascular endothelial dysfunction can be acutely modified by exercise and will aid in underlining the importance of exercise in CF populations. The potential benefits of long-term exercise interventions on vascular endothelial dysfunction in young people with CF warrants further investigation.

Abstrakt

Einleitung: Mukoviszidose (CF) ist eine genetische Erkrankung, die die Funktion eines Epithelien Oberflächenanionenkanals, CFTR (cystic fibrosis transmembrane conductance regulator), stört. Eine Beeinträchtigung dieses Kanals führt zu Entzündungen und Infektionen in der Lunge, die den Großteil der Morbidität und Mortalität verursachen. CF ist jedoch eine Multiorganerkrankung, die viele Gewebe einschließlich vaskulärer glatter Muskeln betrifft. Studien haben gezeigt, dass junge Menschen mit Mukoviszidose, die keine Entzündung und Infektion aufweisen, immer noch eine vaskuläre Dysfunktion aufweisen, gemessen anhand der durchflussbedingten Dilatation (FMD). In anderen Krankheitskohorten, u.a. Diabetes und Fettleibigkeit, wurde gezeigt, dass Ausdauersporteingriffe diese Beeinträchtigungen verbessern oder reduzieren. Langfristige Bewegungseingriffe sind jedoch riskant und kostenintensiv in Bezug auf Zeit und Ressourcen. Nichtsdestotrotz hat die aufkommende Forschung die akuten Auswirkungen von körperlicher Bewegung mit ihren langfristigen Vorteilen korreliert und befürwortet die Untersuchung akuter Bewegungseffekte auf FMD vor longitudinalen Studien. Die akuten Auswirkungen von körperlicher Bewegung auf FMD wurden bisher bei jungen Menschen mit Mukoviszidose nicht untersucht, konnten jedoch Erkenntnisse über die potenziellen Vorteile langfristiger Bewegungseingriffe liefern.

Die Ziele dieser Studien waren, 1) die Zuverlässigkeit der FMD-Methode und ihre Anwendbarkeit zu entwickeln, um akute Übungseffekte zu untersuchen; 2) Vergleich der Grundlinien-FMD und der Akutübungswirkung bei FMD zwischen Jugendlichen mit und ohne CF; und 3) Zusammenhänge zwischen den akuten Auswirkungen von körperlicher Bewegung auf FMD und demographischen Merkmalen, der körperlichen Aktivität, der Lungenfunktion, der maximalen körperlichen Belastbarkeit oder den entzündlichen hsCRP-Spiegeln zu untersuchen.

Methoden: Dreißig junge Freiwillige (10 CF-Patienten, 10 gesunde und 10 aktive, gesunde Kontrollpersonen) im Alter von 10 bis 30 Jahren führten zuvor Blutabnahmen, Lungenfunktionstests, maximale Belastungstests und Grundlinien-FMD-Messungen durch Rückkehr etwa 1 Woche später und Durchführung eines 30-minütigen Dauerlasttrainings bei 75% HF_{max} durch. FMD-Messungen wurden vor, unmittelbar nach, 30 Minuten nach und 1 Stunde nach konstantem Belastungstraining durchgeführt. ANOVAs und ANOVAs mit wiederholten Messungen wurden verwendet, um Unterschiede zwischen Gruppen bzw. Zeitpunkten zu untersuchen. Die lineare Regression wurde implementiert und evaluiert, um Korrelationen zwischen FMD und demographischen Merkmalen, körperlichen Aktivitätsniveaus, Lungenfunktion, maximaler Belastungskapazität oder inflammatorischen hsCRP-Spiegeln zu bestimmen. Für alle Vergleiche wurde die statistische Signifikanz auf einen *p*-Wert von $\alpha < 0{,}05$ eingestellt.

Ergebnisse: Jugendliche mit Mukoviszidose zeigten eine verminderte Lungenfunktion und maximale Belastbarkeit im Vergleich zu Kontrollpersonen. Baseline FMD (%) war auch in der CF-Gruppe (CF: 5.23% v nicht-CF: 8.27% v nicht-CF-aktive: 9.12%) signifikant verringert. Unmittelbar nach dem Training war die FMD in allen Gruppen mit CF, die immer noch die minimalste FMD aufwiesen, signifikant abgeschwächt (~40%). Follow-up-Messungen von FMD zeigte eine langsame Erholung in Richtung Baseline-Werte 30 Minuten nach dem Training und Verbesserungen in der CF-und nicht-CF-aktive Gruppen 60 Minuten nach dem Training. Die lineare Regression zeigte signifikante Korrelationen zwischen maximaler Belastungsfähigkeit (VO_2-Peak), BMI und FMD unmittelbar nach dem Training.

Feststellung: Diese neuen Ergebnisse bestätigen, dass die vaskuläre Dysfunktion der CF durch sportliche Betätigung akut verändert werden kann und dazu beitragen wird, die Bedeutung von Bewegung in CF-Populationen zu unterstreichen. Die potenziellen Vorteile von

Langzeitübungsinterventionen bei vaskulärer Dysfunktion bei jungen CF-Patienten rechtfertigen weitere Untersuchungen.

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Chapter 1 - Introduction

1.0 Cystic Fibrosis (CF)

Epidemiology

Cystic fibrosis (CF) was initially diagnosed and documented by Dr. Dorothy Andersen in 1938 as she described mucous plugging of the pancreas's glandular ducts in infants dying of malnutrition, leading her to characterize this disease as a 'cystic fibrosis of the pancreas' (1). Later, fat and protein malabsorption, failure to thrive, pulmonary disease and a thick, viscous mucus would be identified to better define the disease, hence, the term "mucoviscidosis" (2). Soon after, simple Mendelian autosomal recessive gene inheritance was determined to be how the disease was acquired (3). Years later with the aid of positional cloning, the exact defect would be localized chromosome 7 and the specific gene in question would be identified, and appropriately named the cystic fibrosis transmembrane conductance regulator or CFTR (4, 5).

Through decades of further research and with advances in technology, understanding of the basic CF gene defect, microbiology, physiology, pathology and the genetic, environmental, and therapeutic factors, which influence them, have enabled us to better manage disease burden and to prolong the lives of people with CF. Only 30 years after the CFTR gene's discovery, the median survival age of a person with CF has increase from 25 years to 44 years (6). By this age, half of the CF patient population would be expected to have died. Increased survival age has provided greater opportunities for data collection in the form of longitudinal studies and patient registries, which in turn have yielded overwhelming amounts of information regarding the genetic, environmental and therapeutic influences on CF disease and survival placing CF at the forefront of genetic disease epidemiology (7).

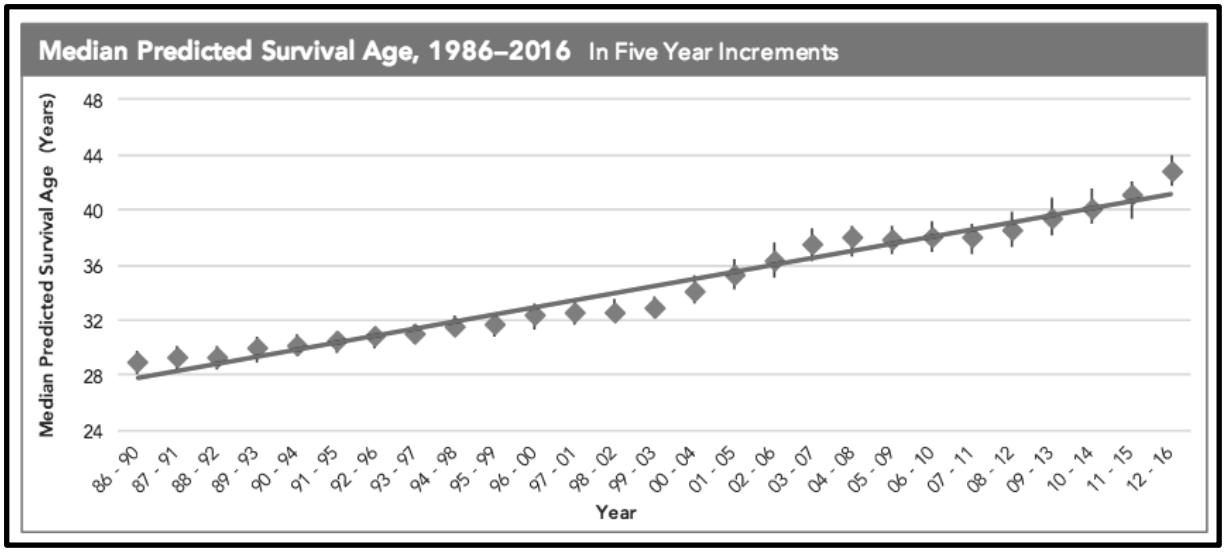


Figure 1. Median predicted survival age, 1986-2016. (From CFF Annual Data Report 2016 (6))

Among peoples of European ancestry, CF is one of the most common lethal genetic diseases. In the United States, it is estimated that there are approximately 30,000 people living with CF. Although CF has been reported in all races and ethnicities, incidence rates do vary between studies and countries, ranging between 1:1,353 births (Ireland) to 1:25,000 births (Finland) in Europe (8). Incidence rates in Germany are estimated to be 1:3,300 births, whereas incidence rates in the United States have been reported to be somewhere between 1:3,200 births for Caucasians, 1:15,000 births for African Americans and 1:31,000 births for Asian Americans (6, 9). While still acknowledging the improvements in life span expectancy, the majority of people living with CF are very young, 62% being under 20 years of age (10). This trend will however change in the near future with the current predicted median survival approaching the 40s to 50s for those born in the 2000s (11, 12). This increase in life expectancy can be attributed to several factors, including earlier diagnosis with newborn screening, more aggressive nutritional management strategies, intensified techniques of chest physiotherapy, comprehensive care in multidisciplinary CF centers, and better

molecular understanding of CF pathobiology along with more specifically targeted therapies (13, 14). In 2012, the first medications were approved and made available to address CF's underlying genetic defect. This treatment however is only beneficial for CF patients with the specific gating mutations of CFTR (i.e., G551D) (14) and although there has been a surge in research trying to correct the protein trafficking defects of CFTR or to lengthen the opening time of CFTR, these therapies are not expected to correct all disease manifestations in adults with mild to severe CF (14). These circumstances combined with the brutal nature of the disease's pathology make CF research all the more important, as improved health in younger populations will lead to more patients entering adulthood in better health, thus improving survival in adults.

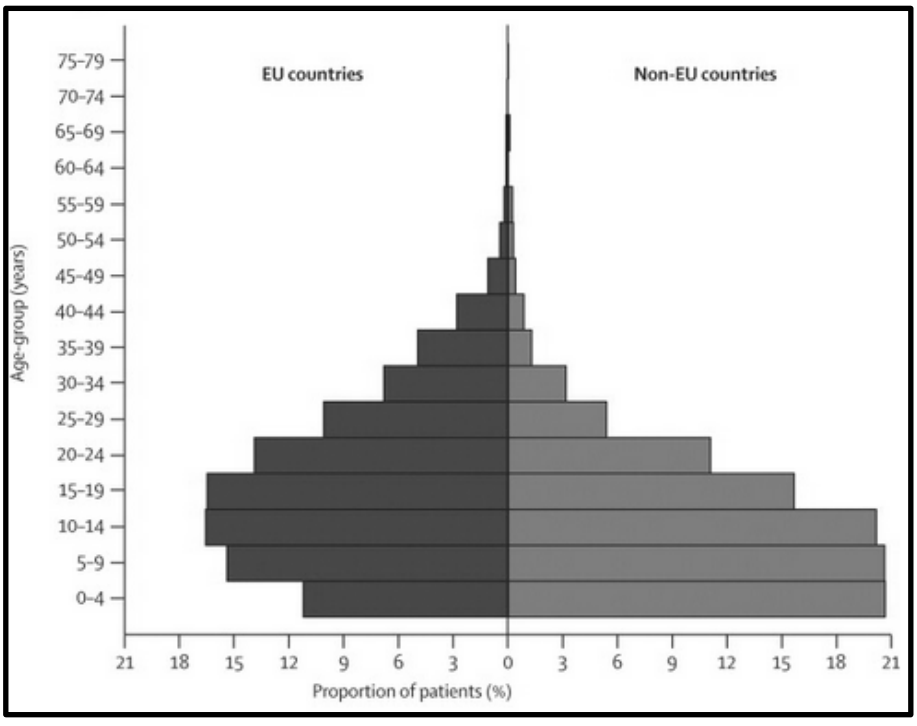


Figure 2. Population pyramid of mean age of people with CF in EU. (From McCormick et al. 2010 (15))

Molecular Biology and Physiology of CF

As previously stated, CF is an autosomal recessive genetic disease affecting the CFTR gene, which produces CFTR protein (4, 16, 17). However, over 1,600 different mutations of the CFTR gene have been described, but the most common one, ΔF508, is found in nearly 70% of people with CF (18).

The CFTR protein functions principally as an ion channel (19, 20), regulating liquid volume, content and pH on epithelial surfaces through chloride secretion and inhibition of sodium absorption (21, 22). In CF, this ion channel is dysfunctional, leading to a dysregulation of epithelial secretions and absorptions. Still today, sweat chloride testing using the quantitative pilocarpine iontophoresis sweat method is the gold standard test for diagnosis of CF. A sweat chloride concentration of > 60 mmol/L is considered consistent with the diagnosis of CF. Genotyping assays are also useful diagnostic tests, as depending on the exact genetic mutation, the disruption of CFTR function will occur at different levels of protein production, which may influence disease severity.

Normally, the CFTR gene is tightly packed into a supercoil of DNA that forms the chromosome. The chromosome is then instructed to reveal the CFTR gene for transcription, in which an mRNA copy of the gene is made. This mRNA strand passes through the nuclear pore into cytoplasm to begin the process of translation. The mRNA sequence is read by ribosomes and translated into a polypeptide chain of amino acids (i.e. the immature CFTR protein). Upon completion, the fully extended CFTR polypeptide is released, which then folds to form an immature CFTR channel. The immature CFTR protein further undergoes post-translational modification in Golgi bodies preparing for transport to the cell surface. CFTR is then transported in vesicles to the cell surface in a process called protein trafficking. Finally, the

vesicle fuses with the cell membrane, allowing for surface expression and CFTR can immediately begin functioning as an ion channel.

Currently, there are 6 classes into which CF mutations are grouped: protein production mutations (class I), protein processing mutations (class II), gating mutations (class III), conduction mutations (class IV), insufficient protein mutations (class V) and instable protein mutations (class 6) (23). Please see Figure 3 below for illustrations of these different classes.

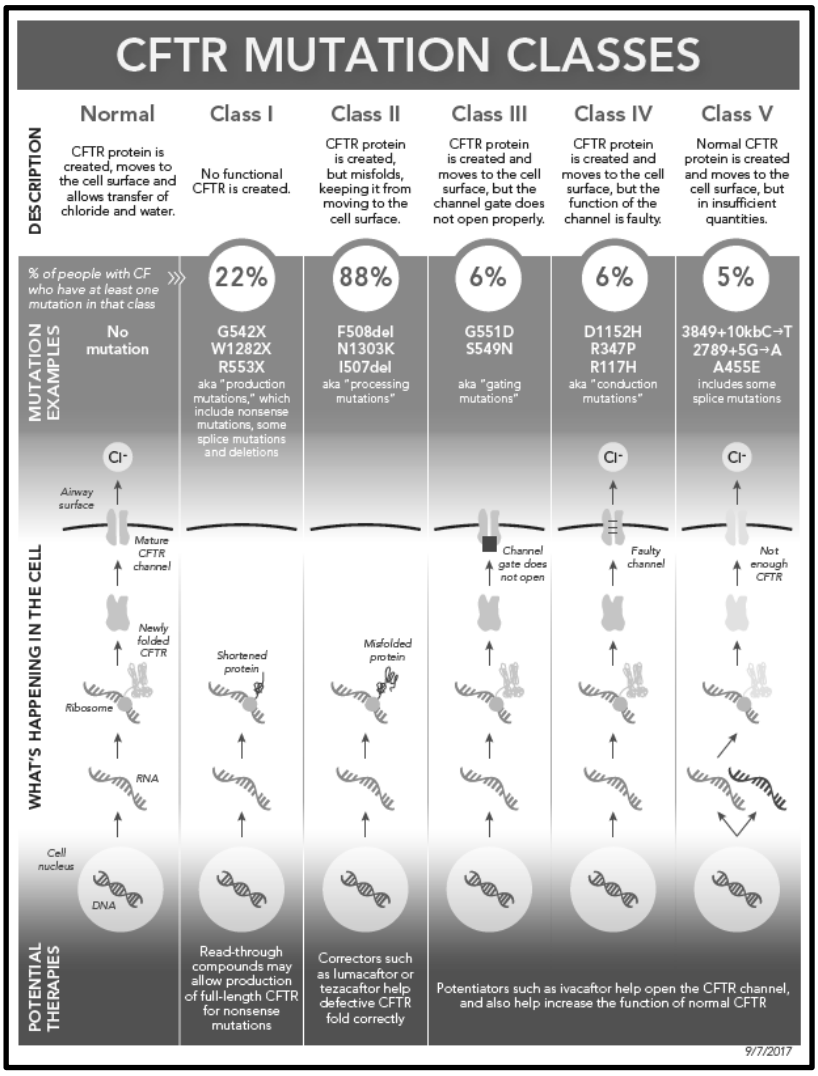


Figure 3. CFTR mutation classification. (From CFF Annual Data Report 2016 (6))

283 Regardless the mutation, these abnormalities especially plague the airways of the lungs and
284 the ducts of the pancreas (24, 25), where viscous secretions cause obstructions leading to
285 inflammation, infection, impairment and ultimately death. Still, while most of the morbidity
286 and mortality (80%) comes from the lung disease in CF (Cystic Fibrosis Foundation Patient
287 Registry Annual Data Report 2011. Cystic Fibrosis Foundation, 2012), CF is in fact a multi-organ
288 disease altering other organ systems containing epithelia, such as the sinuses (26), skin (27),
289 liver (28), pancreas (24), intestines (29), and reproductive organs (30). Further research has
290 shown us however that CFTR is not limited to epithelia cells. CFTR has also been localized in
291 skeletal (31) and smooth muscle (32), bone (33) and nerve cells (34). Continuing research is
292 revealing just how ubiquitous CFTR is, but the implications and whether certain pathologies
293 are primary or secondary effects of CF is still not well understood.
294
295 *CF pathology*
296 CF lung disease, which causes the majority of morbidity and mortality, is marked by chronic
297 bacterial airway infections, prominent neutrophilic inflammation, mucus-obstructed airways
298 and progressive bronchiectasis (5). Many of these symptoms present themselves very early
299 in children with CF. Yet even before symptom manifestation, pulmonary inflammation and
300 infection are often present, although it is still unknown which precedes (35-37). This
301 information, knowing that disease precedes symptom manifestation, has led to earlier and
302 more aggressive interventions, which has yielded promising results.
303 Through animal models, the progression of lung disease has been more thoroughly mapped.
304 CF mice, rats, ferrets and pigs have all been developed to avoid some of the limitations of
305 studying humans. Pigs, because of their anatomy, physiology, biochemistry, size, life span and
306 genetics, may be the most comparable model to date (38). These CF pigs present with many

307 of the typical features seen in people with CF, including the lung disease. CF pigs develop
308 lower and upper airway disease consistent with that of humans (infection, inflammation,
309 tissue remodeling, mucus accumulation and obstruction of the airways) spontaneously within
310 weeks or months after birth (39-41).
311 However, as stated earlier, CF is a multi-organ disease and in addition to the prominent
312 respiratory disease, the liver is defined by biliary cirrhosis, portal hypertension, gall stones
313 and bile duct stricture (28), while the CF gastrointestinal tract is also complicated by severe
314 conditions such as constipation, distal intestinal obstruction syndrome, Crohn's disease,
315 coeliac disease, milk protein intolerance and an increased incidence of malignancy. The
316 pancreas is one of the earliest and most severely affected organs in CF. These changes begin
317 in utero and are consist of small and large duct obstruction, which eventually results
318 inflammation, continued obstruction of ducts by mucus, the destruction of acini and
319 generalized fibrosis. This ultimately leads to the destruction of the pancreas and in
320 combination with the aforementioned gastrointestinal complications leads to maldigestion
321 and malnutrition (42). Other affected organ systems include the skeletal system. Bone disease
322 in CF appears to be multifactorial influenced by primary and secondary factors, which include
323 malabsorption of vitamin D and K, reduced calcium deposition, poor nutritional status,
324 physical inactivity, glucocorticoid therapy and delayed pubertal maturation or early
325 hypogonadism. Moreover, the inflammation common to CF increases serum cytokine levels,
326 in turn, stimulating increased bone resorption and decreased bone formation. This decreased
327 quantity and quality of bone can lead to pathological fractures and kyphosis decades earlier
328 than expected (43). In combination, these liver, pancreas and bone irregularities are primarily
329 responsible for the diminutive stature of most people with CF (underweight and short). Other
330 organ systems disturbed in CF include the genito-urinary system and the sweat glands. The

genito-urinary system is distinguished by infertility due to a bilateral absence of the vas deferens, stress incontinence and vaginal candidiasis, while sweat glands are known to produce sweat depleted of electrolytes. The one system however not yet mention, the cardiovascular system, is likewise affected in CF.

CF cardiovascular abnormalities

CFTR protein is present in the heart, and the cardiac isoform of the CFTR chloride channel is the same found in the respiratory epithelium (44). In fact, the CF gene encodes a cAMP-dependent chloride channel in the heart that shortens the action potential duration and is potentially arrhythmogenic (45). However, there are no studies linking the presence of cardiac CFTR chloride channels to heart disease. The role of the cardiac CFTR has still yet to be defined and may have little direct clinical significance in the development of heart disease. Yet, abnormalities to the heart and cardiovascular system in CF have long been documented and as CF patients continue living on to increasingly older ages, these non-pulmonary CF complications have become all the more common (46). While receiving more aggressive therapies and better care, CF patients are living with severe lung disease for longer periods of time. This however entails the subsequent development of secondary symptoms such as pulmonary hypertension, right ventricular dysfunction and cor pulmonale. Cor pulmonale was first reported as a consequence of CF in 1946 (47) and shortly thereafter studies would determine that 45% of CF patients over the age of 15 present with cor pulmonale for at least 2 weeks before death (48).

Cor pulmonale in CF is thought to be caused by hypoventilation due to the obstruction of the airways by mucus plugging. As alveolar air trapping progresses, a local retention of carbon dioxide and decreased delivery of oxygen occurs. Abnormalities in ventilation-perfusion

properties develop, which lead to hypoxia, a potent factor in the development of pulmonary hypertension (49). In healthy individuals, acute hypoxia elicits a vasoconstrictive response adjusting capillary perfusion to alveolar ventilation. The site of vasoconstriction is located in the small pulmonary arteries associated with terminal and respiratory bronchioles (50). Chronic hypoxia will however cause structural remodeling in these vessels. The hypertension then induces a muscularization of the arterial media in sites that are normally non-muscular. Over time, increasing muscular hypertrophy of the pulmonary arteries, engorgement of the pulmonary vascular bed, and destruction of the peripheral pulmonary vasculature develop. The right heart initially compensates for the elevated pulmonary pressures by increasing output with ventricular hypertrophy and dilation, but progression leads to cor pulmonale and eventual right heart failure.

Left ventricular dysfunction is less common in CF, but is possible in patients with secondary amyloidosis or in older people with CF and atherosclerotic coronary artery disease. There is also evidence that left ventricular function may be mechanically impaired by the enlargement of the right ventricular in chronic cor pulmonale (51, 52), or by expiratory airflow limitation (53). In a review of 65 CF patients being evaluated for lung transplantation, significant left ventricular dysfunction occurred in only 2% (54).

In addition to the heart, other aberrations have been documented in the aorta, bronchial arteries and systemic capillaries of people with CF (55). The bulk of these abnormalities are thought however to be secondary effects caused by pulmonary hypertension and increased vascular resistance due to lung disease, however, in CF, chronic inflammation and oxidative stress could as well potentially mediate cardiovascular disease.

As mentioned earlier, the main cause of morbidity and mortality in CF is the lung disease, where chronic respiratory infections are common. These infections then activate a chronic

inflammatory-immune response dominated by massive infiltration of polymorphonuclear neutrophil leukocytes into both the airways and the alveoli (56). People with cystic fibrosis develop 10 times more inflammation at a given bacterial load compared to a person without the disease and this trend is similar for other challenges such as viruses, airborne particulate matter and pollutants (57). This response produces an array of proinflammatory cytokines in addition to excessive amounts of reactive oxygen species and nitrogen species, which are believed to further influence CF lung disease (58-61). There are several encompassing reviews discussing inflammation, oxidative stress and potential repercussions to the cardiovascular system in CF (62, 63), unfortunately a complete understanding of the inflammatory process in CF is lacking and therefore its full impact, specifically on the cardiovascular system, is unknown (64). However, it is known from other chronic inflammatory diseases, such as diabetes and COPD, that there is a direct connection between inflammatory marker levels and cardiovascular disease (65, 66).

Nevertheless, the impact of cardiovascular disease on people without CF was first recognized and scientifically confronted nearly 70 years ago by the now famous long-term, and still ongoing, cohort study: The Framingham Heart Study. The study originally began in 1948 with 5,209 subjects from the small town of Framingham, Massachusetts, USA, and is presently monitoring its third generation of participants (67). Although there is still so much to learn when it comes to CVD risk factors and the interplay between them, prior to the Framingham Heart Study practically nothing was established concerning the "epidemiology of hypertensive and arteriosclerotic cardiovascular disease" (68), and much of what is accepted now concerning CVDs, such as the effects of diet, exercise and common medications, like aspirin, come from the Framingham Heart Study’s findings.

The impact and burden of cardiovascular diseases on modern developed societies cannot be understated. The incidence of cardiovascular disease death has been rapidly rising since the early 1990s (69) and using the most recent data from 2015, the World Health Organization estimated 17.7 million people died from cardiovascular diseases, representing 31% of all global deaths. In terms of economic burden, cardiovascular diseases are also devastating with medical costs in the USA currently totaling $318 billion. The cost in terms of loss of productivity is also immense, $237 billion, and cannot be forgotten or neglected. These numbers are expected only to double in the following 20 years (70). Similar figures and trends are found throughout the developing and developed world. According to the 2008 Federal Health Report of Germany, the costs of cardiovascular diseases amounted to approximately €35.5 billion, 1/6 of Germany's healthcare budget (71). In China, data from 2003 estimated the country's direct economic cost due to cardiovascular diseases to be ¥209 billion ($26 billion) and these numbers are certain to be even greater now (72).

In CF, many traditional cardiovascular risk factors are indeed present, while others are completely absent or minimal. For example, due to decreased lipid absorption, total cholesterol and LDL cholesterol levels, both known to be risk factors when elevated, are consistently low or within the optimal ranges in people with CF. In contrast, the chronic inflammation, oxidative stress, high fat diet, relative physical inactivity and endothelial dysfunction observed in CF all increase the risk of cardiovascular disease in this population. This balancing act between a lack of certain risk factors, but an abundance and intensity of others makes their cumulative effects on the cardiovascular system difficult to predict.

Yet, studies investigating CF cardiovascular abnormalities in humans are rare, even rarer in younger ''healthy'' people with CF, but there is evidence of functional cardiovascular differences in young, seemingly ''healthy'' people with cystic fibrosis at as early as 18 years of

age (73-75). The abnormalities seen in the previously mentioned studies included decreased right ventricular function, decreased endothelial function measured by flow-mediated dilation (FMD), microvascular dysfunction, decreased HR_{max} and increased levels of C-reactive protein, which in the general population predict a higher risk of cardiovascular disease and cardiovascular events (76, 77). These findings in younger, "healthy" people with CF entertain the possibility of an additional (more controversial) factor contributing to cardiovascular disease with recent studies questioning the inherent physiological role of CFTR in smooth muscle function. To reiterate, CFTR is present in human smooth muscle cells and is thought to modulate the release of Ca^{2+} in response to contractile stimuli (32, 78).

Through the animal models of CF, smooth muscle morphology and function have been investigated immediately after birth, prior to CF lung disease and inflammation. Major smooth muscle differences, in the pulmonary (79, 80), gastrointestinal (81) and cardiovascular system (82), have been observed even prior to disease and inflammation. Additionally, one study administering CFTR corrector for the G551D-CFTR mutation, ivacaftor, found rapid restoration of CFTR function followed by increased airway distensibility and decreased vascular tone measured by pulse wave velocity further supporting the idea of a congenital smooth muscle defect in CF (83).

Earlier Detection

Based on retrospective data from people without CF obtained through the Framingham Heart Study, algorithms have been developed to estimate the 10-year cardiovascular risk of an individual, known as Framingham Risk Scores (84). Primary predictors include hypertension, hyperlipidemia, smoking status, the presence of diabetes, gender and age. Age being the single most predictive variable, it becomes obvious how important early detection and

intervention is. Studies are continuing to reveal that the incidence of cardiovascular disease and their risk factors in younger people are on the rise. These statistics are mainly contributed to an increased frequencies in obesity, physical inactivity, poor diets and substance abuse (85). These trends do cause major concerns; hence physicians and scientists have been searching for better ways to detect cardiovascular disease earlier, albeit with earlier screenings (86, 87) or more accurate and reliable methods for detection of such diseases and at earlier stages of their pathogenesis.

1.1 Flow-mediated Dilation (FMD)

Atherosclerosis, one of the earliest processes in cardiovascular disease development, begins as early as childhood. Fatty streaks are seen in the aortas of children already at the age of 3 years and in the coronary arteries by adolescence (88). Endothelial dysfunction is one of the earliest physiological steps in the advancement of atherosclerosis (89). In-vitro studies have demonstrated the endothelium begins functioning abnormally even before plaques exist, actually predisposing the arterial wall to thrombosis, leucocyte adhesion and proliferation of smooth muscle cells. Therefore, for the screening of atherosclerosis, a clinical non-invasive method for the assessment of endothelial dysfunction was developed and successfully tested in children and adults at risk of atherosclerosis (90). Today, flow-mediated dilation (FMD) is recognized as biomarker of endothelial function and an important non-traditional prognostic of cardiovascular risk (91). This approach measures endothelial function per B-mode ultrasound in the conduit arteries, most frequently the brachial artery. The diameter of the artery is observed in response to an increase in blood flow during induced reactive hyperemia. Hyperemia is induced by inflating a pressure cuff below the artery of interest causing ischemia then subsequently deflating the cuff. This increase in blood flow and corresponding shear-stress forces lead to endothelium-dependent dilatation. Specifically, the changes in artery diameter are caused by the release of endothelial derived vasoactive mediators after the stimulation of shear-stress sensing mechanoreceptors on the arterial wall surface. This response to mechanostimulation can be blocked with pretreatment of nitric oxide synthase inhibitors, suggesting the endothelial release of nitric oxide as a key contributor (92, 93).

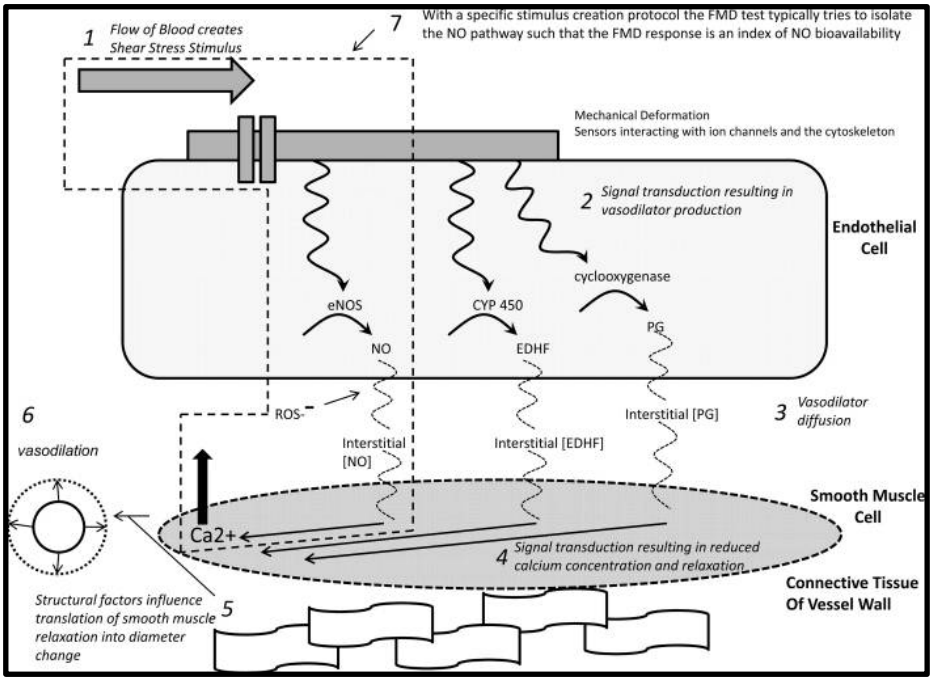


Figure 4. Flow-mediated dilation (FMD) mechanism. (From Thijssen et al. 2010 (94))

Factors influencing FMD

Through investigating the underlying mechanisms and clinical implications of FMD, many influencing factors have been identified. For example, intrinsic characteristics including age, sex and fitness have significant impacts on FMD. Age is known to be inversely related to FMD and this phenomenon may actually be even more profound in women. Contrastingly, fitness seems to be directly correlated with FMD (95). In women, the factors influencing FMD are even more complex as FMD has been found to vary depending on the different stages of the menstrual cycle (96), to be enhanced during pregnancy after the 10^{th} week of gestation (97), but impaired across all stages of menopause (98). Interestingly, a diurnal variation in FMD has been demonstrated, however there is no evidence of a diurnal variation in nitric oxide, which is thought to be the main mediator of FMD (99). Additionally, FMD is known to be defective in several diseases and conditions including, but not limited to, obesity (100), hypertension (101), type 1 and 2 diabetes (102, 103), coronary artery disease (104) and interestingly for

517 this thesis, CF (74). Likewise, many extrinsic factors influencing flow mediated dilation or its
518 measurement have been described. For example, sympathetic stimulators, such as caffeine
519 and nicotine, both acutely reduce FMD values after ingestion and inhalation, respectively
520 (105, 106). In fact, an ingested meal can induce depletion of FMD magnitude and the
521 magnitude of change is dependent on the composition meal (107). Many medications, for
522 example anti-inflammatories, antioxidants, b_2-adrenergic agonists and local
523 vasoconstrictors/vasodilators also influence FMD. Other studies have found associations
524 between sleep quality and FMD (108). Finally, and perhaps of most interest in this thesis, is
525 the well-established effect that physical activity or exercise interventions have on FMD in
526 healthy as well as diseased cohorts(109, 110). The effects of habitual exercise can already be
527 seen very early in healthy children between the ages of 5 and 10 years old. In this study, the
528 physical activity levels of these children were strongly associated, more strongly associated
529 than any other variable in their analysis, with FMD emphasizing the importance of exercise
530 even at such a young age (111). This relationship is also observed in healthy adolescents as
531 well as children and adolescents with type 1 diabetes or obesity (112, 113). Similar findings
532 are reported in adults at various ages with and without disease (114-117). Controversy
533 remains on whether FMD is elevated in athletes; however, a recent meta-analysis indicates
534 that experienced athletes, but not young athletes present with greater FMD compared to age-
535 matched non-CF controls suggesting this relationship could be age dependent.
536 Importantly, not only is endothelial function measured by FMD modifiable, endothelial
537 dysfunction measured by FMD is reversible (118) and in the context of exercise and physical
538 activity, which would be considered modifiable lifestyle behaviors, much effort has been
539 made to understand how exercise improves endothelial function and to further optimize
540 interventions unveiling the true potential of such interventions. Recently and exceptionally

reviewed by Early et al 2017, many studies in diverse cohorts, young and old, healthy and diseased, have validated the direct benefits of exercise interventions (110). The exact mechanisms that govern this effect are not completely understood, but evidence indicates that exercise improves vascular structure, oxidative stress status and NO bioavailability through intermittent increases of laminar shear stress associated with increased cardiac output during physical exertion (119).

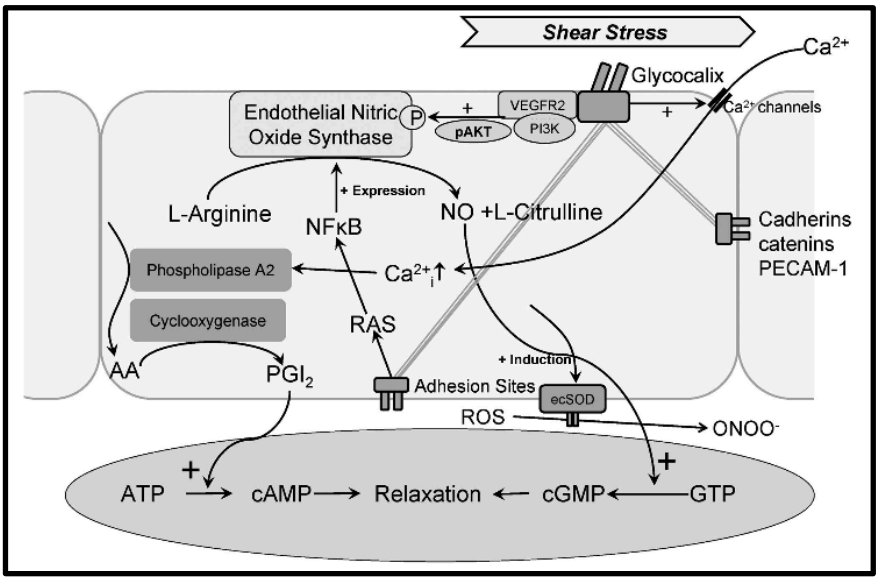


Figure 5. Effects of exercise on the vascular endothelial function are mediated by increases of laminar shear stress associated with increased cardiac output during physical exertion. Akt indicates protein kinase B; PECAM-1, platelet endothelial cell adhesion molecule-1; Ras, small GTPase; ONOO, peroxynitrite; PGI2, prostaglandin I2; VEGFR2, VEGF receptor 2; NFkB, nuclear factor-kB; ecSOD, extracellular SOD; and AA, Arachidonic acid. (Reprinted from Gielen et al 2010 (119). Originally from Davies et al. 2008 (120))

Why look at acute effects of exercise?

Despite the comprehensive use of FMD to assess interventions effect (medicinal or lifestyle), sport scientists have only more recently began using FMD to explore the effects of a single, acute bout of exercise on vascular endothelial function. Thompson et al. argues that the acute effects of exercise could possibly help predict the effects of a long-term exercise intervention, as observed in several other variables, for example blood pressure (121). The acute exercise model has many advantages regarding the control of confounding variables and additionally has the potential to ask interesting questions concerning exercise variables (i.e. mode, intensity, duration, etc.). The acute exercise model also allows for a comparison between the acute and chronic effects of exercise, which may be useful in deciphering the mechanisms behind exercise-induced changes in FMD.

Unfortunately, at present, the exact importance acute exercise effects have in relation to long-term adaptations is still unknown, but this in part could be due to the wide variation in methods. The acute effect of exercise has however been confirmed and well described as an immediate decrease in FMD after exercise followed by normalization and sometimes followed by a further increase in FMD above baseline levels (122). The notion that acute exercise poses a challenge to the cardiovascular system that when repeatedly sustained ultimately promotes adaptation is epitomized in the “hormesis” hypothesis. The “hormesis” theory is a physiological concept concluding that improvement to physiological parameters can be induced through repeated stimuli, if these stimuli challenge and temporarily impair the physiological system (123). The nature, strength and direction of this biphasic pattern seem to be determined by several aspects like the type, duration and intensity of the exercise, the investigated population (age, diseased, trained vs. untrained) and other methodological factors including the timing of FMD measurement (124). These components most likely

collaborate in modifying the stimuli, which generates the acute FMD response to exercise, through changes in shear and oxidative stress, changes in arterial diameter and antioxidant status. A schematic representation of FMD's acute exercise response as well as potential factors influencing the nature of this response are found in Figure 6, from Dawson et al. 2013 (124).

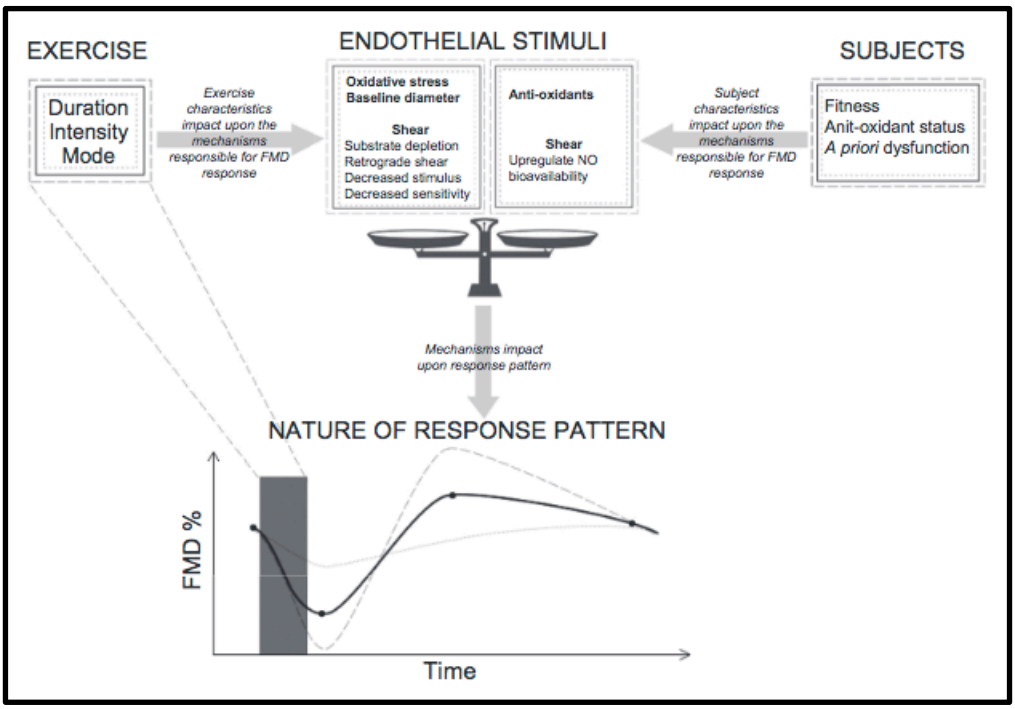


Figure 6. The biphasic response in flow-mediated dilatation (FMD) after an acute bout of exercise. (From Dawson et al. 2013 (124))

To the author's knowledge, the therapeutic effects of exercise in regard to vascular endothelial dysfunction have yet to be investigated in people with CF. Yet before investing investigator resources and participant time and energy in longitudinal exercise intervention studies, one could imagine first investigating the effects of acute exercise in this CF cohort. Considering again the "hormesis" hypothesis, the potential benefits of a longitudinal exercise

intervention in people with CF could possibly be estimated through looking at the acute effects of exercise. One would suspect if FMD can be modulated acutely through exercise, further investigations concerning the potential therapeutic effects of a long-term exercise training intervention.

1.2 CF and Exercise

Exercise capacity is an important prognostic in CF, as well as being a major determinant of quality of life (125, 126), and although physical activity is recommended and numerous studies have shown the valuable effects of physical activity in terms of exercise capacity, lung function and quality of life (127), there still exists a certain negative stigma concerning vigorous exercise and exercise testing in CF. In a survey of nearly 200 CF clinics in the United Kingdom, exercise testing was only performed on approximately 30% of the patients, while only 25% of the clinics were equipped to offer exercise testing and training leaving the authors of the study to conclude that despite the importance given to exercise testing and training by healthcare providers, exercise was still underused as both an assessment tool and a therapeutic intervention in people with CF in the United Kingdom (128). This underuse can be attributed to many things including lack of resources and expertise, as well as a precaution due to the known exercise intolerance in CF. Exercise intolerance in CF is multifaceted with detrimental contributions from the respiratory, cardiovascular and musculoskeletal systems (129). In the respiratory system, expiratory airflow limitation, hypoxemia, respiratory muscle weakness and increased work to breathe all contribute to exercise intolerance. Cardiovascular factors such as left ventricular dysfunction, right ventricular dysfunction, low stroke volume and pulmonary hypertension are also partially responsible for exercise intolerance in CF. Low muscle mass, deconditioning, hypoxia, use of certain medications, andropause and decreased moderate to vigorous activity combine to limit the musculoskeletal system's ability to tolerate exercise. Despite intolerance, exercise in CF has repeatedly been proven to be safe and exercise testing to be reproducible (130-132).

Exercise actually poses a therapeutic opportunity to correct several symptoms occurring in CF, also which contribute to the exercise intolerance, by improving lung function, increasing

levels of hemoglobin and plasma volume, improving heart function, increasing myoglobin concentrations, hyperplasia and hypertrophy of mitochondria, enhancing enzyme activity, increasing aerobic power, augmenting glucose uptake and glycogen stores and adapting specific muscle fibers to training (133). Additionally, regular moderate exercise may produce anti-inflammatory effects as well as decrease infection susceptibility in people with CF, however this area of research needs more clarification (134). Exercise is also suspected of playing a role in the stimulation of airway hydration and improvement of cilia beating in CF airways, thereby delaying or preventing the development of mucus plugs, inflammation and infection, and thus the progression in lung parenchyma degradation (135, 136).

With the positive effects seemingly outweighing the negative, the effectiveness and adherence of many exercise interventions have been tested in cystic fibrosis. Researchers have found supervision of an individualized training that incorporates activities enjoyed by the individual leads to improved levels of adherence and acceptability. Whereas, regimented training programs utilizing only a single activity, for example cycle ergometry, are tedious and time-consuming (137). In terms of effectiveness, evidence is more limited. Due to mythological differences, studies concerning the effects of exercise in CF are difficult to compare, but thus introducing other interesting questions. For example, short-term training interventions did not show any effects, however long-term training interventions did improve some physiological and psychological outcomes. Yet even within long-term studies, some inconsistency was observed, which could be credited to differences in exercise training type, intensity and single session duration (138). Despite the lack of definite evidence, no negative side effects have been reported due to exercise interventions, so there is no reason to discourage it, but certainly high-quality randomized control trials are warranted to better

assess the benefits of exercise training in people with cystic fibrosis and additionally to compare aerobic, anaerobic or a combination of both towards their care.

1.3 Research Questions and Hypotheses

The acute effect of exercise on endothelial vascular function and its relevance in predicting possible long-term effects of a training regime are relatively new ideas and concepts in the field of sports medicine. Though the first studies investigated the long-term effects of training on FMD in the late 1990s (139), and the first studies to consider and explore the acute effects of exercise were published in the early 2000s (140), the first studies to connect and acknowledge a possible interaction or relationship between the two are not seen for another 15 years (141), after which, the field has continued to grow as methodologies and protocols are shared and consolidated. This heterogeneity in the literature has produced problems when trying to compare studies and results, but also in the applicability of results to certain populations (124). A great majority of the original studies looking into the effects of exercise on endothelial vascular function were performed in young, healthy cohorts or aged cohorts with preexisting cardiovascular disease. However, soon these principles were applied to younger cohorts known to have abnormally functioning vascular endothelia, for example children with obesity or diabetes (142-145). Through these studies in healthy and diseased populations, the research community has benefited and gained insight into the physiology and mechanisms behind endothelial vascular function. To expand, much has been learned how different exercise modes, local or systemic, and their intensity are important variables that will influence outcome parameters. The innate characteristics such as age, sex, physical fitness level and disease-state are also beginning to be teased out. Young people with CF were only recently observed to possess abnormal endothelial vascular function, but many questions still remained as to what exactly could and would cause this particular phenotype in young people with CF (74). These defects could possibly be congenital to CF itself or secondary effects of the chronic inflammation and infection known to plague people with CF.

The other obvious question raised from this study is if the endothelial vascular dysfunction in CF can be impeded or if it is even reversible. One treatment known to have beneficial implications on vascular health, and specifically acute endothelial vascular function, is exercise. Namely 6-12 weeks of endurance exercise training has been used as the gold standard exercise therapy in these trials. However, current recommendations advocate for the investigation of the acute exercise effects on FMD, as these investigations should yield useful information to be transferred later into long-term training studies. As it is unknown how exercise will affect endothelial vascular dysfunction in young people with CF acutely or chronically, the first logical step was to explore the acute effects of exercise in CF. Recent literature implicates an innate smooth muscle defect in CF and that this defect may be caused by a dysregulation of Ca^{2+} in the sarcoplasmic reticulum (146). Ca^{2+} also plays an integral role in the vasodilation of vascular smooth muscle as well as the effect of exercise on vascular endothelial function (see Figure 5). Thus, by using these recent findings from other patient populations and implementing them into a new study using the most current and recommended methodology, a vast deal of information and knowledge can be generated from and for young people with CF. Particularly, can endothelial vascular function be manipulated with acute exercise and do these acute changes have anything to do with the demographic, lung function or fitness level characteristics of the participants.

Before answering this question, the development and validation of these methods needed testing and optimization for this particular study. Although these have been derived in other research centers investigating unique cohorts, the reliability of a single FMD measurement, the optimal acute exercise training intensity, as well as the reliability concerning the acute effect of exercise training on FMD were addressed in three pilot studies:

Pilot study 1: Reliability of single FMD measurement

Pilot study 2: Optimal intensity of submaximal exercise training

Pilot study 3: Reliability of acute exercise training effect on FMD

Finally, application of the newly developed and validated methods will be transferred to investigate three core research questions (RQs). Initially, a replication study will be performed to confirm baseline differences in FMD between young people with CF and non-CF controls (normal and active groups to investigate the influence of physical activity levels on FMD). The second core RQ will compare the effects of acute exercise on FMD over time in young people with CF and non-CF controls (normal and active to investigate the influence of physical activity levels on the acute effect of exercise training on FMD). Finally, baseline FMD, post-training FMD and the acute effect of training on FMD will be compared to lung function, physical activity levels, maximal exercise capacity and inflammation levels to examine the for associations.

RQ1: Is baseline FMD different between groups (CF, Non-CF and Non-CF Active)?

RQ2: Does acute exercise affect FMD differently between groups (CF, Non-CF and Non-CF active)?

RQ3: Are baseline FMD or post-training FMDs associated with demographics, physical activity levels, lung function, maximal exercise capacity or inflammatory hsCRP levels?

Chapter 2 – Materials and Methods

Participants

Thirty young volunteers (10 people with CF, 10 non-CF and 10 non-CF active matched controls) between the ages of 10 and 30 years old were recruited for this study. CF was defined as a clinical diagnosis based on positive sweat tests and genotype analysis by a physician with over 20 years of CF-specific experience. Current CF disease status and history were documented. Matching criteria were dependent upon age, sex and physical activity level. Participant exclusion criteria included FEV_1 <50% predicted, resting oxygen saturation <85%, smoking, hypertension, other cardiovascular or metabolic diseases, sleeping disorders and current illness or infection. Participants on anti-inflammatory, b_2-adrenergic agonistic and local vasoconstriction medications were excluded from the study. Women receiving hormonal or contraceptive therapy were also excluded. Additionally, women were only studied during the follicular phase. Finally, participants were instructed to refrain from foods or beverages containing antioxidants 1 week prior to FMD examinations. On testing days, participants were asked to fast, especially avoiding caffeine, while people with CF were informed to maintain the timing of their daily treatments and come to the clinic following their normal morning airway clearance therapy and inhaled medications. Exercise abstinence of at least 12 hr prior to testing was also requested. All study protocols were approved by the University of Potsdam Ethics Committee (No. 13/2016) and written/verbal consent was obtained from all subjects or parents prior to their participation.

Groups (CF and non-CF) were matched according to age, sex, BMI, and physical activity levels, while a third control group (non-CF active) was only matched for age, sex and BMI. Inclusion criteria for this group ensured an increased average physical activity level of more than 5 hours per week. Yet, at baseline major differences were still observed between all groups.

Group characteristics and clinical values for people with CF, non-CF and non-CF active controls are summarized in Table 1. The average age of the groups was similar across all groups, approximately 18 years of age. Equal proportions of males and females were recruited and studied in each group. In regard to age, height, weight, BMI and blood pressure, no differences were found between groups. All reported BMIs and blood pressures were well within the range of normal healthy values taken from national registries of children and young adults of comparable ages. Self-reported physical activity levels were significantly greater in the active control group, 7.5 ± 1.9 hr/wk in comparison to people with CF (2.3 ± 1.3 hr/wk) and the control group (2.3 ± 0.7 hr/wk). No differences in self-reported physical activity levels were found between people with CF and the control group. People with CF presented with slightly lower resting oxygen saturations compared to the other two groups, however these findings (≈98%) are all within the normal clinical range. Finally, the general blood marker for inflammation, hsCRP, was found to be significantly elevated in people with CF (1.2 ± 0.8 mg/L) compared to non-CF and non-CF active controls (0.2 ± 0.2 and 0.6 ± 0.6 mg/L). Therefore, Individuals with CF presented with lower resting oxygen levels and increased levels of hsCRP, indicative of mild lung disease and inflammation known to occur in CF. Again, however, the hsCRP levels found in all three groups all fall well with-in the normal range.

809 Table 1. Group characteristics. (mean ± SD)

Variable	CF	Non-CF	Non-CF Active	*p*-value
No.	10	10	10	
Sex, M/F	4/6	4/6	4/6	
Age, yr	17.9 ± 6.2	17.3 ± 5.1	18.2 ± 5.8	0.93
Height, cm	160.7 ± 11.8	168.4 ± 13.5	164.4 ± 12.4	0.40
Weight, kg	51.9 ±11.6	60.3 ± 10.2	60.2 ± 12.5	0.19
BMI, kg/m^2	19.9 ± 2.6	21.1 ± 0.7	22.0 ± 2.2	0.07
SBP, mm Hg	107 ± 8	114 ± 7	107 ± 9	0.09
DBP, mm Hg	76 ± 15	71 ± 7	71 ± 13	0.54
Resting O_2 Sat %	97.4 ± 1.3	98.9 ± 0.03	98.9 ± 0.3	0.0001*
Physical Activity, hr/wk	2.3 ± 1.3	2.3 ± 0.7	7.5 ± 1.9	< 0.0001**
hsCRP, mg/L	1.2 ± 0.8	0.2 ± 0.2	0.6 ± 0.6	0.005*

810 * CF significantly different compared to Non-CF and Non-CF Active.

811 ** Non-CF Active significantly different compared to CF and Non-CF.

812

813 *Design*

814 Study participants reported to the University of Potsdam Outpatient Clinic on two separate
815 testing days separated approximately by 1 week. The first day began with a study
816 consultation, the acquiring of written/verbal consent and a medical examination
817 guaranteeing the ability to perform the required exercise tests and training. These
818 assessments were performed by a sports medicine physician and contained a basic
819 orthopedic/cardiopulmonary examination, as well as a resting ECG. Next, oxygen saturation

levels and baseline pulmonary function were assessed before concluding day 1 with a maximal exercise capacity test.

Day 2 of testing began with a standard venipuncture blood draw, followed by a baseline FMD measurement. Participants then performed an individualized 30 min constant load training at 75% HR_{max} (determined by their initial maximal exercise capacity test) succeeded by three additional FMD measurements (immediately post training, 30 min post training and 60 min post training) to examine the acute effects of exercise on FMD.

Participant characteristics and laboratory values

Standard anthropometric data (height, weight, calculated BMI and blood pressure) were assessed and documented by clinical staff. Physical activity levels were obtained through discussions with the participants and noted. Concentrations of high sensitivity C-reactive protein (hsCRP) were obtained through standard venipuncture blood draws and analyzed.

Pulmonary function test (PFTs)

Pulmonary function tests were performed using the ZAN 100 Spirometer (nSpire Health, Inc., Longmont, CO, USA) in accordance to the standards of the American Thoracic Society (147). Outcome measures included functional vital capacity (FVC), forced expiratory volume (FEV_1, FEV_1 % predicted, FEV_1/FVC % and forced expiratory flow, FEF_{25-75}). % predicted values were determined using spirometry reference standards from the National Health and Nutrition Examination Survey (NHANES) III.

844 *Maximal exercise capacity test*

845 Participants began with a 3 min unloaded peddling warm-up after which a maximal exercise
846 capacity test was performed on a LODE Excalibur Sport cycle ergometer using the Godfrey
847 protocol. The Godfrey protocol is a continuous incremental cycle protocol to volitional fatigue
848 and is recommended for use in cystic fibrosis (148). Depending on the height of the individual
849 performing the exercise test, work rate starts with 10 (< 120 cm), 15 (120-150 cm) or 20 W (>
850 150 cm). Work rate is then increased by 10, 15 or 20 W/min, respectively. Participants were
851 asked to maintain a cadence of 80-90 rotations per min on the cycle. A declining deviation in
852 the rotations per min or any physical complaints signified the end of the test. The termination
853 guidelines of (149) were adhered to. Respiratory values were assessed using the wireless
854 portable breath-by-breath Metamax 3B system (Cortex, Leipzig, Germany).

855 Prior to using, the Metamax 3B system was allowed to warm up for at least 20 min, then
856 calibrated prior to every test according to manufacturer recommendations. This required
857 calibrating the gas analyzers with a reference gas (14.97% O_2, 4.96% CO_2 and balanced with
858 N_2, followed by verifying the calibration against ambient air. Next, a volume calibration was
859 performed using a standardized 3-L syringe. All facemasks and their fit were inspected before
860 and throughout testing to avoid potential gas leakages. The standard error of the Metamax
861 3B system ranges from ± 2% (150).

862 Using the analysis software Metasoft v. 3.9.5, the respiratory inspiration and expiration data
863 (volume, O_2 [ ] and CO_2 [ ]) were calculated. VO_2 and VCO_2 were calculated using standard
864 metabolic algorithms employing the Haldane transformation corrected for changes in
865 ambient conditions (151).

866 Heart rate was monitored throughout testing using a 12-lead ECG. For nutrition
867 standardization, participants were asked to document their nutritional intake of the 24 hr

prior to the test and instructed not to change their nutritional habits before the next measurement. Relative outcome measures from this test included VO_2 peak, relative VO_2 peak, final workload, final relative workload (W) and maximum heart rate (HR_{max}). VO_2 peak and HR_{max} were defined as the averaged value of the final 30 s. Relative VO_2 max was calculated by normalization to bodyweight. Final workload was reported as the load level at test termination. Again, relative final workload was calculated by normalization to bodyweight in kg.

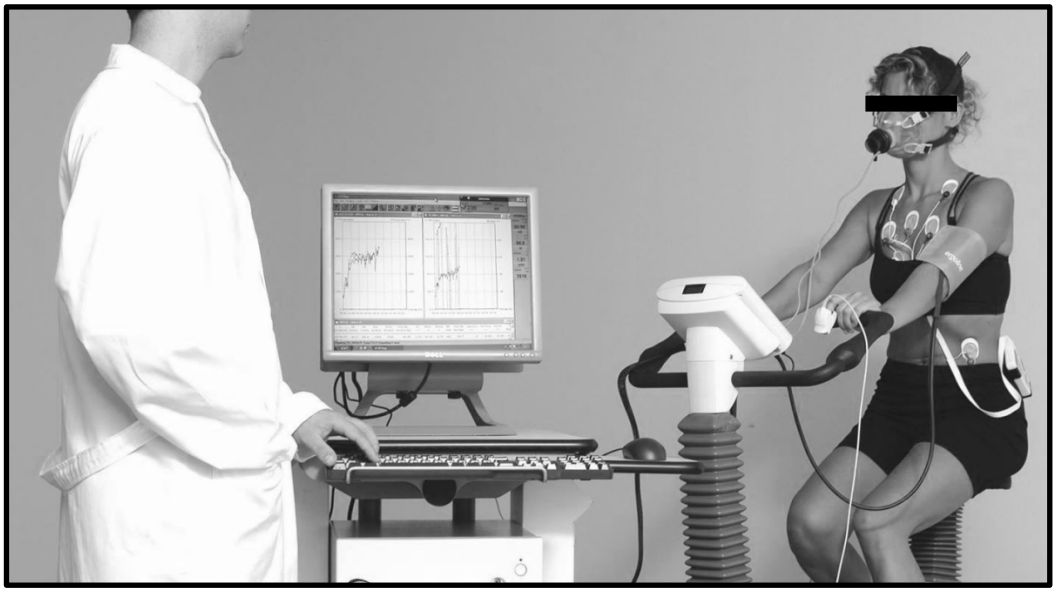


Figure 7. Example photo of maximal exercise capacity test with gas exchange analysis and HR monitoring with 12-lead ECG.

Flow Mediated Dilation (FMD)

All FMD measurements were performed in the morning. Participants first rested in the supine position for 15 min in a quiet air-conditioned room (approx. 22-24°C). Endothelial dependent vasodilation was then assessed as dilation of the brachial artery in response to increased blood flow in accordance with the current guidelines (94, 152).

In detail, participants stayed supinely positioned while a sphygmomanometer blood pressure (BP) cuff was positioned on the right forearm, 2 cm below the elbow. A 3-lead ECG was then placed to allow for ECG gaiting in later analysis. The right brachial artery was then located, superficially marked and scanned longitudinally between 5 and 10 cm above the elbow using B-mode and Doppler (duplex mode) of an ultrasound linear array transducer (13 MHz, GE Vivid q, General Electric Company, Boston, USA) and insonation angle was corrected to 60°. The transducer was held in this position throughout the scan by the study investigator to ensure greater image stability. This method, which allows for subtle tracking of the artery, was preferred due to the inevitable movement of younger participants. Baseline imaging of arterial diameter and blood velocity continued for 1 min until the blood pressure cuff was then inflated to 250 mmHg for 5 min after which it was deflated to induce reactive hyperemia. Following deflation, imaging continued for a further 2 minutes to ensure the capture of peak reactive hyperemia. Peak reactive hyperemia was defined as the maximum percentage increase in brachial artery flow after cuff release as compared to baseline flow.

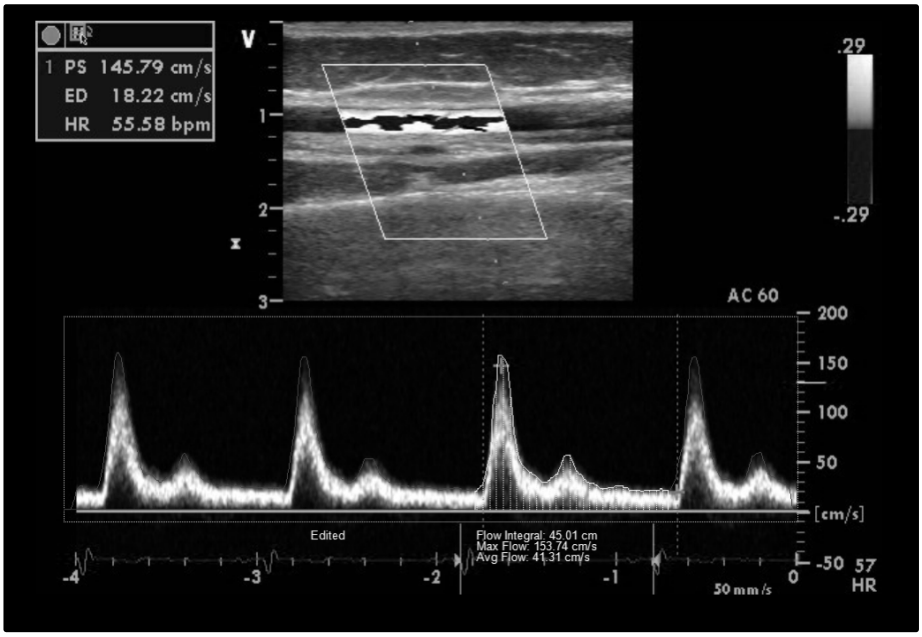
Ultrasound images from the 1 min baseline assessment, the final 30 s of ischemia and the 2 min following cuff release were later semi-automatically analyzed using the edge detection software, Brachial Analyzer (Medical Imaging Applications LLC, Iowa City, IA, USA). Baseline diameter, peak reactive hyperemia diameter, time to peak reactive hyperemia diameter and shear rate (AUC) were documented. FMD was calculated as the percent change in baseline diameter to peak diameter in response to reactive hyperemia in relation to baseline diameter.

$$FMD\ (\%) = \frac{\text{peak diameter} - \text{baseline diameter}}{\text{baseline diamter}} * 100$$

907 Finally, to eliminate the potential influencing factor of differing shear profiles and to allow for
908 better cross study comparisons, FMD was normalized to shear rate (AUC) by dividing the
909 percentage of FMD by shear rate (AUC) (153, 154).
910 In this laboratory, the intra-observer reliability for FMD analysis (coefficient of variations) for
911 baseline diameter, FMD, and FMD/shear are 3%, 16%, and 20%, respectively. Please see the
912 supplementary materials for more information regarding the methods, statistical tests used
913 and the results.
914


915

916 Figure 8. Picture of FMD assessment in progress. (Image from Areas et al. 2018)



917

Figure 9. Example doppler ultrasound image of the brachial artery for the analysis of blood flow velocity and shear stress rates.

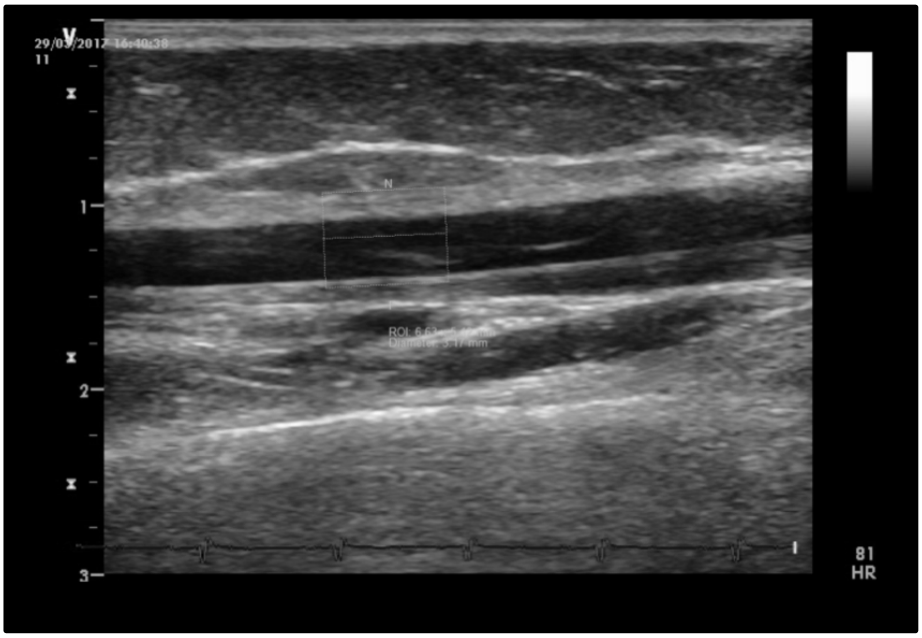


Figure 10. Example B-mode ultrasound image of the brachial artery for the analysis of diameter and subsequently FMD %.

Submaximal exercise test

Using data from the previously explained maximal exercise capacity test, individualized exercise intensities were calculated (75% HR_{max}). Approximately 1 week after maximal exercise capacity tests, participants performed a similar 3 min warm up followed by a 30-min constant load training at 75% HR_{max}. This exercise duration, mode and intensity have been shown in literature and in pilot studies to elicit a significant, immediate reduction in FMD (155). Please refer to the supplementary methods sections for more information regarding the determination of the optimal submaximal exercise intensity. Again, heart rate was

monitored throughout testing using a 12-lead ECG to confirm and maintain exercise intensity targets.

Statistics

Sample sizes were estimated using Gpower Statistical Software (Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany) according to effect sizes of similar studies, one investigating baseline FMD differences between young people with CF and non-CF controls, while the others investigated the acute effects of exercise on vascular structure and endothelial function in young diseased populations (142, 156, 157).

All data were analyzed, graphed and presented using Statistical Package for Social Sciences (SPSS Statistics 21, IBM, Armonk, New York, USA), Prism (GraphPad Software Inc., La Jolla, CA, USA) and Excel (Microsoft Office V.10, Redmond, WA, USA). After collection, data was transferred to a database and checked for plausibility using range checks. Implausible values and outliers were double-checked and corrected or excluded accordingly. Descriptive data are presented as means ± standard deviations (SD). Before statistical comparisons means, data were tested for normal distribution (Shapiro-Wilk). To investigate differences between people with CF, non-CF and non-CF active controls at baseline and post-intervention, ANOVAs were employed. To investigate differences between baseline and post-intervention values of people with CF, non-CF and non-CF active controls, repeated measures ANOVAs were employed. For all comparisons, statistical significance was set at a p-value of $\alpha < 0.05$.

Furthermore, to assess correlations between FMD, physical activity levels, fitness levels, lung function and hsCRP values, linear regression was performed and evaluated.

Chapter 3 – Results

3.0 Group clinical values.

Pulmonary function test results are presented in Table 2. All tests were performed without complication and were deemed reliable. People with CF exhibited significantly decreased values in all PFT parameters, again at levels suggestive of mild lung disease. No differences were found between non-CF and non-CF active controls. For retrospect, FEV1 % predicted was approximately 85% in people with CF, where as non-CF and non-CF active controls displayed values over 100% (104.5 ± 13.7 and 109.7 ± 9.2, respectively). FEV1 % predicted values are based on large population registries and are calculated relative to those norm values. Therefore, a FEV1 % value of 84.8% would infer 15.2% less pulmonary function compared to that of a normal healthy control without CF. In this CF cohort, a predicted FEV1 of 84.8% would suggest a mild to moderate lung phenotype at their age. Due to the heterogenous nature of lung disease in CF, a large standard of deviation was foreseeable and, as seen below, documented in the CF group.

Table 2. Pulmonary function test parameters. (mean ± SD)

Variable	CF	Non-CF	Non-CF Active	*p*-value
FVC, L	3.22 ± 0.85	4.18 ± 1.12	4.19 ± 0.89	0.04*
FEV_1, L	2.57 ± 0.74	3.76 ± 0.91	3.74 ± 0.80	0.004*
FEV_1, % predicted	84.8 ± 20.5	104.5 ± 13.7	109.7 ± 9.2	0.0005*
FEV_1/FVC, %	79.6 ± 7.8	90.3 ± 3.9	89.3 ± 5.7	0.0007*
FEF_{25-75}, L/s	2.51 ± 1.07	4.05 ± 0.72	4.1 ± 0.63	0.0002*

* CF significantly different compared to Non-CF and Non-CF Active.

In Table 3, maximal exercise test outcomes for all three groups are illustrated. Again, all tests were performed to exhaustion without complication or premature test termination. Heart rate peak during maximal exercise was deemed to be roughly 188 bpm in the non-CF active group, 187 bpm in the non-CF group and 181 bpm in the CF group, yet no statistical differences in peak heart rate were observed between groups; however, VO_2 peak (absolute, relative and predicted) were significantly lower in people with CF. On average, relative VO_2 peak was reduced 25% in people with CF. CF achieved approximately 77% of their predicted VO_2 peak, non-CF controls 91% and non-CF active controls 104%. Peak work capacity (W) was also determined to be lowest in the patient group (-25% compared to control) and greatest in the active group (+15% compared to control). All three groups were indeed significantly different in terms of peak work capacity. These differences in peak work capacity were only exaggerated when normalized to body weight in kg. In summary, excluding HR_{max}, all maximal exercise capacity outcome parameters were decreased in CF. VO_2 peak values were reduced roughly 25% and 30% compared to control groups, non-CF and non-CF active, respectively. Percentile comparisons between relative maximum work outputs yielded very similar results with CF exhibiting approximately 25% - 35% less compared to control groups.

Table 3. Maximal exercise test parameters. (mean ± SD)

Variable	CF	Non-CF	Non-CF Active	*p*-value
VO_2 peak, L/min	2.01 ± 0.44	2.62 ± 0.36	2.82 ± 0.52	0.001*
VO_2 peak, mL/kg/min	30.7 ± 6.4	40.0 ± 6.3	44.0 ± 9.0	0.001*
VO_2 peak, % predicted	77 ± 20	91 ± 12	104 ± 21	0.008*
Heart rate peak, bpm	181 ± 8	187 ± 6	188 ± 9	0.10
Work peak, W	160 ± 50	214 ± 53	246 ± 64	0.007***
Work peak, W/kg	3.1 ± 0.6	3.5 ± 0.4	4.1 ± 0.6	0.002**

* CF significantly different compared to Non-CF and Non-CF Active.

** CF significantly different compared to Non-CF Active.

*** All groups significantly different.

3.1 Baseline FMD is decreased in young people with CF

Baseline FMD parameters are reported in Table 4 below. Although non-CF active participants tended to have larger baseline brachial artery diameters than CF participants, who tended to have larger arteries compared to non-CF controls, the three groups showed no differences in baseline diameter and peak diameter (0.348 ± 0.057 cm v 0.321 ± 0.025 cm v 0.317 ± 0.067 cm, $p = 0.07$), yet absolute change in brachial-arterial diameter was found to be less in people with CF compared to the other groups, non-CF and non-CF active (CF: 0.017 ± 0.005 cm v non-CF: 0.027 ± 0.011 cm v non-CF active: 0.032 ± 0.008 cm, $p = 0.001$). FMD was also significantly less in the patient group. Time-to-peak vasodilation occurred in less than 60 seconds for all groups. No differences in time-to-peak vasodilation, as well as shear rate, were observed between groups. Even after normalization of FMD to shear rate, FMD was still significantly decreased in CF compared to controls. Please refer to Figure 1.

1017 Table 4. Baseline FMD parameters. (mean ± SD)

Variable	CF	Non-CF	Non-CF Active	*p*-value
Baseline diameter, cm	0.321 ± 0.025	0.317 ± 0.067	0.348 ± 0.057	0.07
Peak diameter, cm	0.337 ± 0.024	0.344 ± 0.077	0.379 ± 0.062	0.22
FMD absolute change, cm	0.017 ± 0.005	0.027 ± 0.011	0.032 ± 0.008	0.001*
Time to peak, s	53 ± 16	48 ± 21	47 ± 19	0.99

1018 * CF significantly different compared to Non-CF and Non-CF Active.

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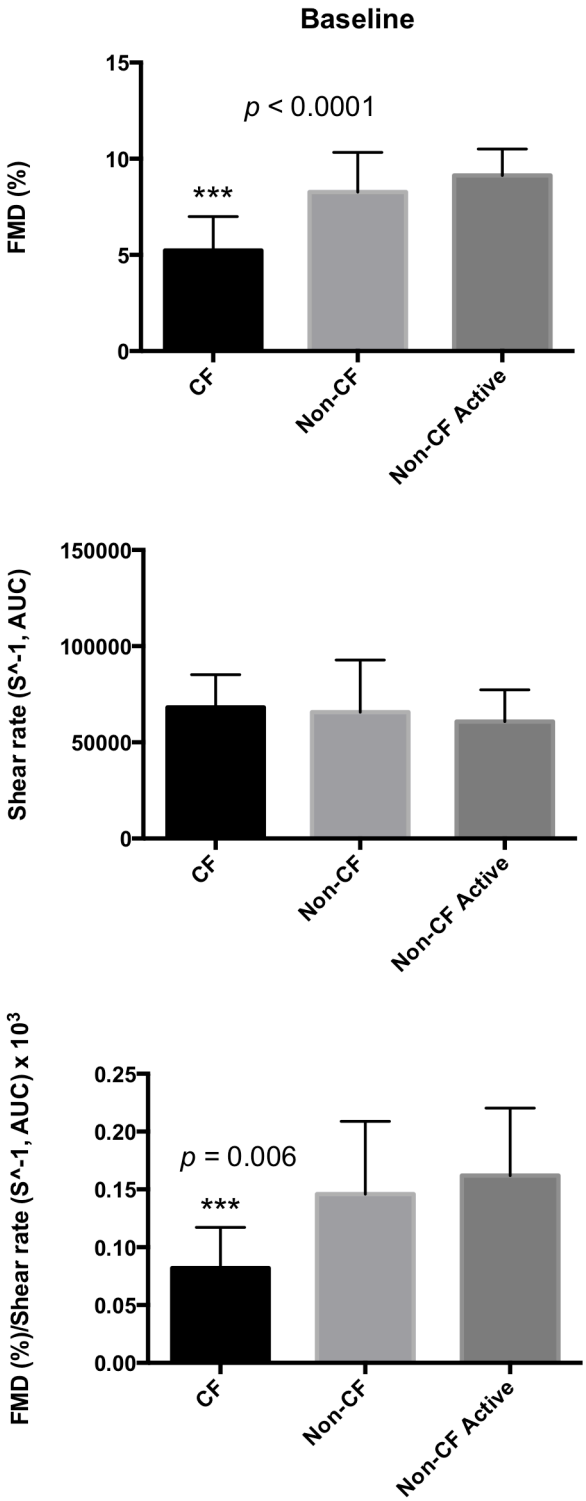

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1024 Figure 11. Baseline FMD, shear rate AUC, and FMD normalized for shear. Values are presented

1025 as mean ± SD. *** Indicates significant differences. AUC = area under the curve; FMD = flow-

1026 mediated dilation.

1027

3.2 Acute exercise modulates FMD in young people with CF similarly to non-CF controls

The next question posed was whether the vascular endothelial dysfunction observed in young people with CF could be modified by an acute 30-minute endurance exercise training at 75% HR_{max}.

Immediately post-training, brachial artery diameters were significantly increased in CF and non-CF groups compared to baseline, but no significant changes were seen in non-CF active individuals. Still, baseline and peak diameters were comparable between all groups immediately post-training. Significant reductions in FMD absolute change (cm), as well as FMD, were observed immediately post-training within all groups. When FMD absolute change and FMD change were compared between groups immediately post-training, CF values were significantly different only to non-CF active group. No differences in time to peak vasodilation were recorded between baseline and immediately post-training, nor between groups immediately post-training. No differences in shear rate were observed immediately post-training or between groups. FMD normalized to shear rate was still significantly decreased in CF compared to both non-CF groups immediately post-training. The exercise training protocol applied in this study acutely narrowed the gap in FMD% between CF and non-CF, as non-CF and non-CF active had similar absolute decreases in FMD% immediately post-training.

30 minutes post-training, brachial diameters had returned to pre-training sizes and were not different between groups. FMD absolute change and FMD% change also returned to pre-training levels with CF once again exhibiting significantly less function compared to the non-CF groups, non-CF and non-CF active, respectively (CF: 0.015 ± 0.006 cm v non-CF: 0.026 ± 0.010 cm v non-CF active: 0.030 ± 0.010 cm). Peak artery diameters, time-to-peak vasodilation, shear rates were similar between groups, but after FMD% normalization to shear rate, the differences in FMD% change between groups 30 minutes post-training lost

significance. This is most likely due to the larger standard deviations seen in some shear rate measurements.

FMD and FMD parameters were measured for the last time 60 minutes post-training. At this time point, baseline and peak brachial artery diameters were again analogous to pre-training diameters amongst all groups. However, FMD absolute change and FMD% change were significantly elevated in the CF and non-CF active groups compared to baseline findings. These parameters were unchanged in non-CF when compared to baseline. Group comparisons 60 minutes post-training still revealed a relatively limited FMD absolute change (CF: 0.021 ± 0.005 cm v non-CF: 0.030 ± 0.010 cm v non-CF active: 0.036 ± 0.006 cm) and FMD% change in CF. Time to peak vasodilation and shear stress were similar to pre-training values and no differences between groups could be established 60 minutes post-training. Summarizing, the final follow-up FMD measurement occurring 60 minutes post-training, revealed further recovery of FMD% towards levels surpassing those at baseline in all groups, however this increase was only deemed significant for the CF and non-CF active groups. Despite this augmentation of vascular endothelial function, CF group values were still significantly less than both control groups, although after normalization to shear stress, this difference was only significant between CF and non-CF active. FMD time-course findings pre- and post-training indicated that an acute bout of exercise could induce a biphasic CF FMD response, but could not correct FMD to normal control values. A time course group comparison of FMD% change is illustrated in Figure 15.

Table 5. FMD parameters immediately post-training. (mean ± SD)

Variable	CF	Non-CF	Non-CF Active	*p*-value
Baseline diameter, cm	0.339 ± 0.023	0.347 ± 0.066	0.367 ± 0.050	0.40
Peak diameter, cm	0.347 ± 0.022	0.362 ± 0.072	0.386 ± 0.053	0.24
FMD absolute change, cm	0.008 ± 0.005	0.015 ± 0.007	0.019 ± 0.009	0.005**
Time to peak, s	56 ± 11	54 ± 9	53 ± 14	0.76

Table 6. FMD parameters 30 minutes post-training. (mean ± SD)

Variable	CF	Non-CF	Non-CF Active	*p*-value
Baseline diameter, cm	0.327 ± 0.022	0.335 ± 0.072	0.350 ± 0.048	0.57
Peak diameter, cm	0.341 ± 0.018	0.362 ± 0.080	0.380 ± 0.054	0.29
FMD absolute change, cm	0.015 ± 0.006	0.026 ± 0.010	0.030 ± 0.010	0.001*
Time to peak, s	52 ± 17	51 ± 11	49 + 19	0.91

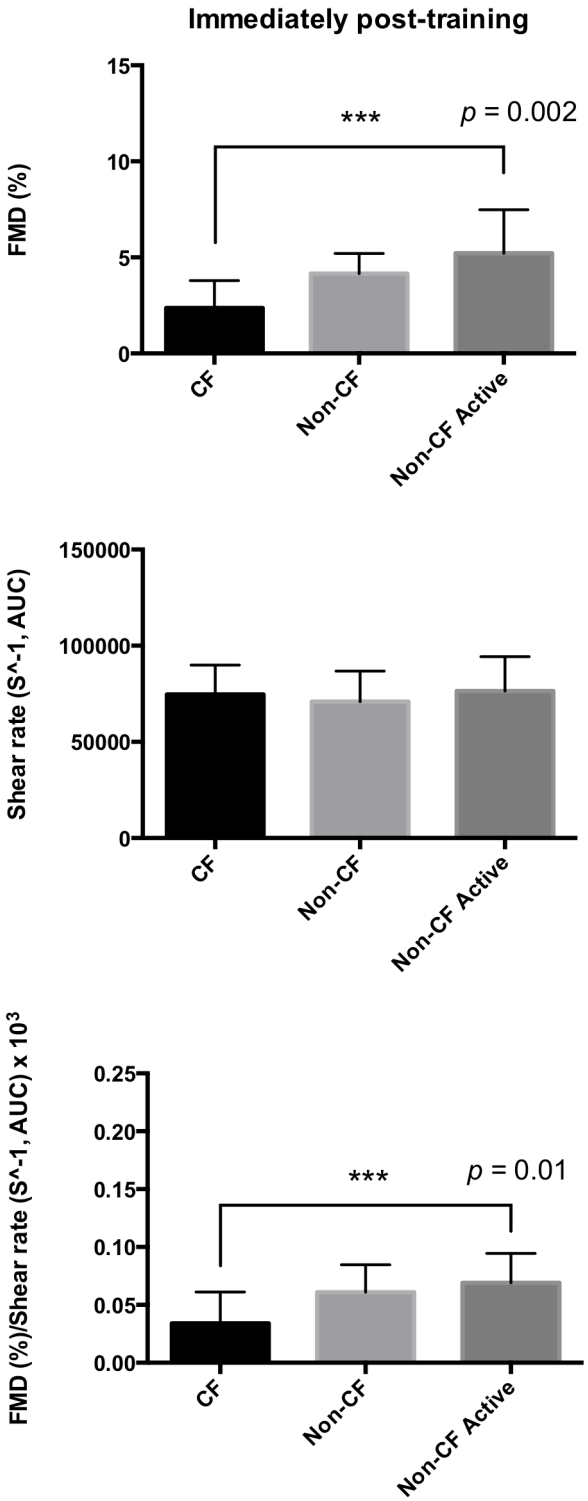
Table 7. FMD parameters 60 minutes post-training. (mean ± SD)

Variable	CF	Non-CF	Non-CF Active	*p*-value
Baseline diameter, cm	0.328 ± 0.021	0.324 ± 0.067	0.352 ± 0.058	0.42
Peak diameter, cm	0.349 ± 0.019	0.354 ± 0.074	0.388 ± 0.061	0.24
FMD absolute change, cm	0.021 ± 0.005	0.030 ± 0.010	0.036 ± 0.006	0.0002*
Time to peak, s	51 ± 13	51 ± 19	49 ± 11	0.92

Tables 5-7:

* CF significantly different compared to Non-CF and Non-CF Active.

** CF significantly different only compared to Non-CF Active.



1084

1085 Figure 12. Immediate post-training FMD%, shear rate AUC, and FMD% normalized for shear.

1086 Values are presented as mean ± SD. *** Indicates significant differences. AUC = area under

1087 the curve; FMD = flow-mediated dilation.

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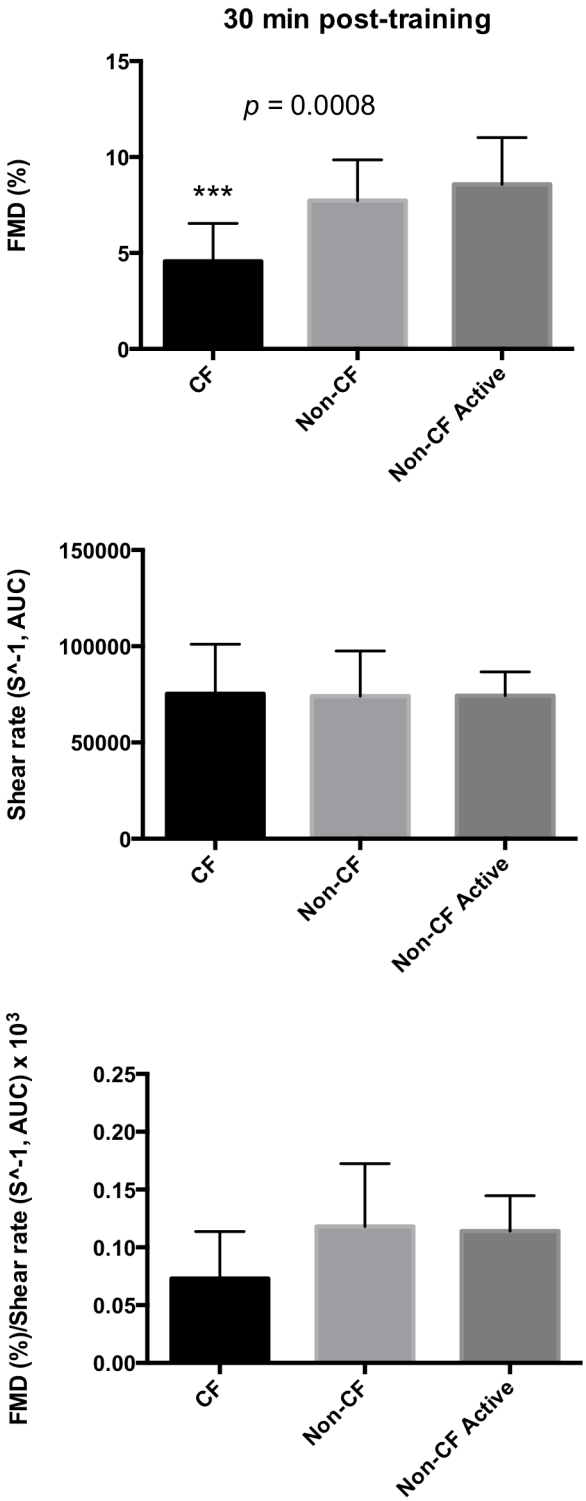

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1090 Figure 13. 30 min post-training FMD%, shear rate AUC, and FMD% normalized for shear.

1091 Values are presented as mean ± SD. *** Indicates significant differences. AUC = area under

1092 the curve; FMD = flow-mediated dilation.

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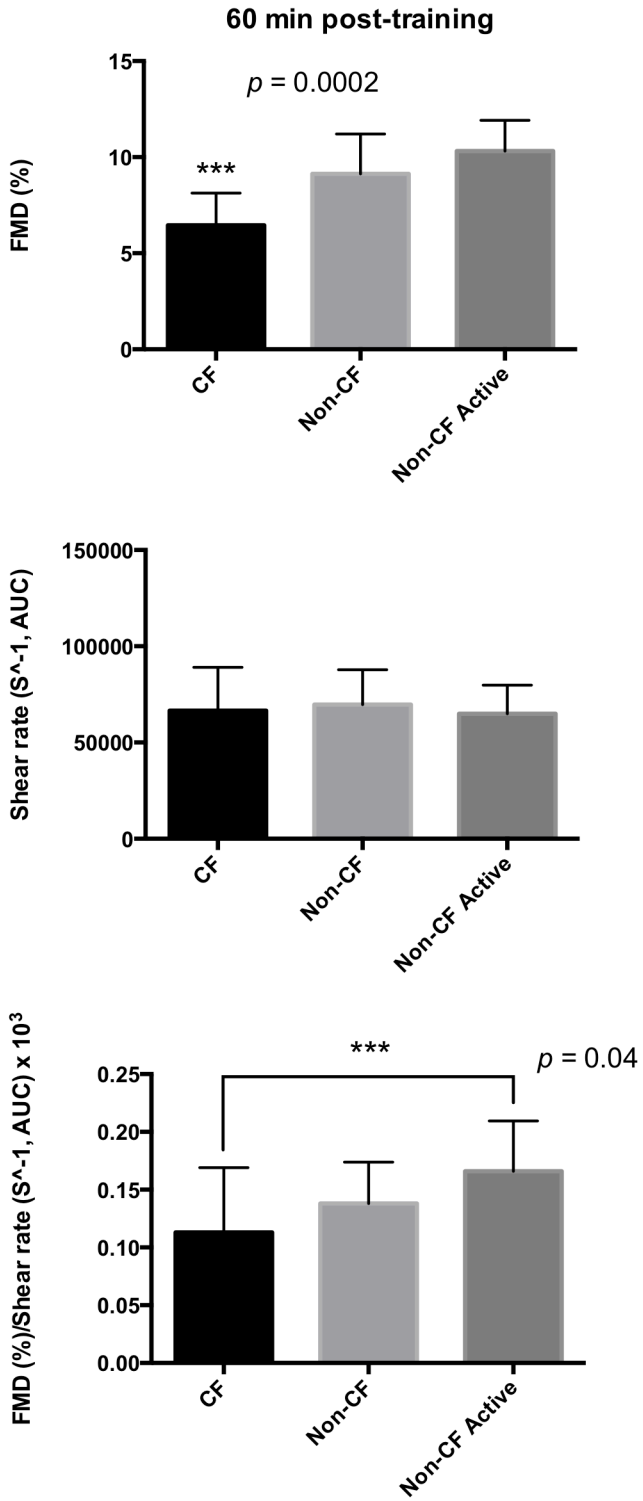

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1095 Figure 14. 60 min post-training FMD%, shear rate AUC, and FMD% normalized for shear.

1096 Values are presented as mean ± SD. *** Indicates significant differences. AUC = area under

1097 the curve; FMD = flow-mediated dilation.

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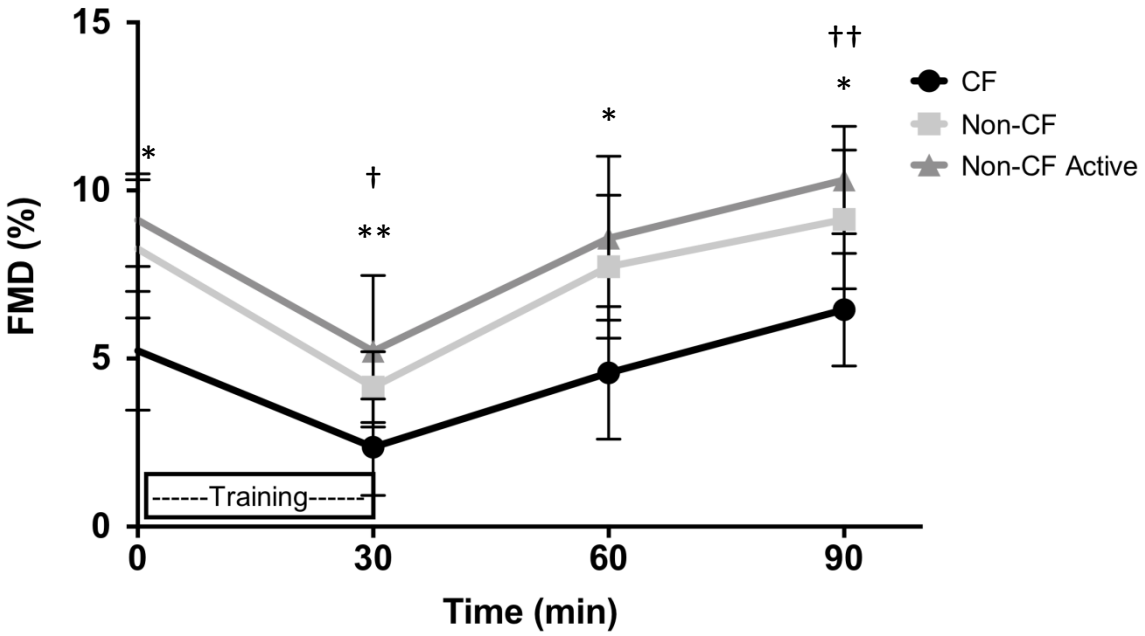

Figure 15. FMD% time-course (pre- and post-training). Values are presented as mean ± SD. AUC = area under the curve; FMD = flow-mediated dilation. * CF significantly different compared to Non-CF and Non-CF Active. ** CF significantly different only compared to Non-CF Active. † All groups significantly different compared to baseline. †† CF and Non-CF Active groups significantly different compared to baseline.

3.3 Baseline and post-training FMDs are associated with BMI and maximal exercise capacity

Baseline, post-training FMD% and differences were compared to age (yr), BMI (kg/m^2), physical activity levels (hr/wk), lung function measured by FEV1 (L), maximal exercise capacity measure as VO_2 peak (mL/kg/min) and the inflammation marker, hsCRP (mg/L), using multiple linear analysis methods. Group analysis revealed significantly different trends within the CF group between FMD% immediately post-training and both FEV1 (L) and lung function measured by FVC (L), $p < 0.05$. For FEV1, the equation and R^2 value describing the relationship between FMD immediately post-training and FEV1 in young people with CF were: $\mathrm{Y} = -1.239 * \mathrm{X} + 5.54$, $R^2 = 0.41$, $p = 0.04$. This indicated a significantly unique elevation or intercept for young people with CF, but concluded that the slopes of the lines were not statistically significant from one another. Although the associations were not statistically significant, the line equation and R^2 value describing the linear relationship between FMD immediately post-training in young controls were: $\mathrm{Y} = 0.624 * \mathrm{X} + 1.80$, $R^2 = 0.29$, $p = 0.11$ for non-CF controls and $\mathrm{Y} = 0.527 * \mathrm{X} + 3.25$, $R^2 = 0.03$, $p = 0.61$ for non-CF active controls. and Concerning FVC, the equation and R^2 value describing the relationship between FMD immediately post-training in young people with CF were: $\mathrm{Y} = -1.086 * \mathrm{X} + 5.85$, $R^2 = 0.42$, $p = 0.04$. This indicated a significantly different elevation or intercept for the CF relationship, but concluded that the slopes of the lines were not statistically significant from one another. Non-CF controls also displayed a statistically unique relationship between FVC and FMD immediately post-training. The equation and R^2 value describing the relationship between FMD immediately post-training in young non-CF controls were: $\mathrm{Y} = 0.613 * \mathrm{X} + 1.58$, $R^2 = 0.43$, $p = 0.42$. In non-CF controls, the linear relationship between FVC and FMD immediately post-training not found to be significant: $\mathrm{Y} = 0.423 * \mathrm{X} + 3.45$, $R^2 = 0.42$, $p = 0.65$.

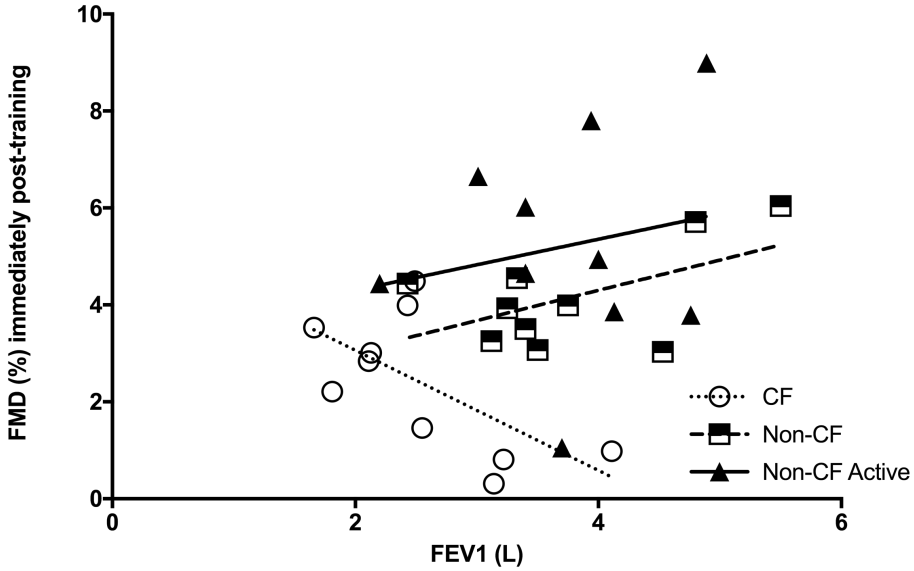

Figure 16. Scatter plot illustrating the relationship between FMD% immediately post-training and baseline lung function measured by FEV1 (L). Linear regression was performed on all three groups (n = 10 per group) independently. A significant difference between CF and both non-CF groups was observed, ($p < 0.05$).

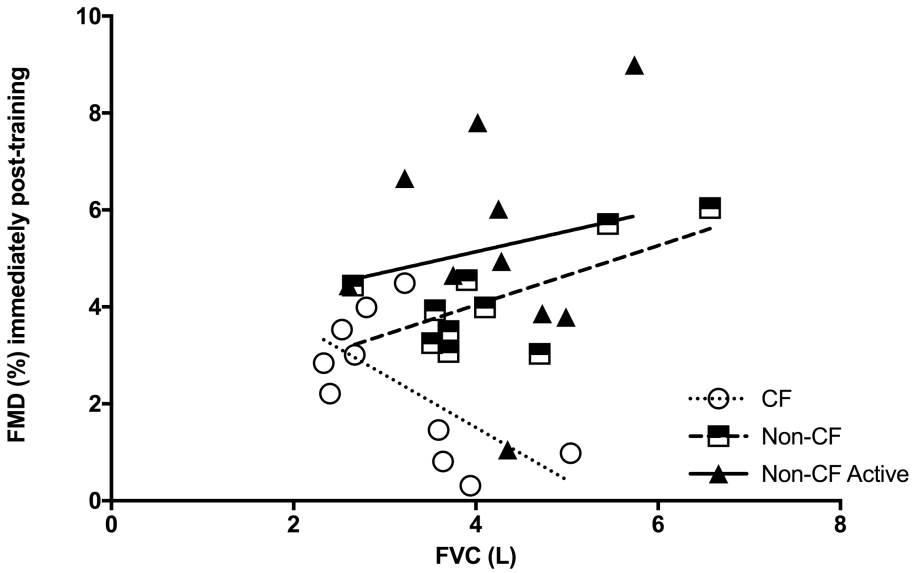

Figure 17. Scatter plot illustrating the relationship between FMD% immediately post-training and baseline lung function measured by FEV1 (L). Linear regression was performed on all three groups (n = 10 per group) independently. A significant difference between CF and both non-CF groups was observed, ($p < 0.05$).

No other significant findings were reported during within-group analysis. Subsequently, all three groups were analyzed together as a sample of n = 30. This analysis exposed multiple significant correlations between participant characteristics and FMD values.

Age was however not significantly correlated to FMD at any timepoint during investigations. There were also no significant correlations between BMI and FMD at any timepoint, although there was a slight positive trend (p = 0.07) at baseline between the two. Physical activity levels were significantly positively correlated to FMD values at all four timepoints, but the best associations were seen at baseline (p = 0.008; R^2 = 0.22) and 60 min post-training (p = 0.005 ; R^2 = 0.25). FEV1 also correlated positively with FMD at several timepoints, namely baseline and 30 min post-training, (p = 0.04; R^2 = 0.15) and (p = 0.01; R^2 = 0.21), respectively. A comparable trend was found between FEV1 and FMD immediately post-training (p = 0.06; R^2

= 0.12). Similarly, FEV1 % predicted also associated significantly and positively with FMD measurements at all timepoints. Again, these associations remained quite similar across all measurement points (range p = 0.001 – 0.03; R^2 = 0.16 - 0.39). Interestingly, FVC was not found to be significantly correlated with FMD at any timepoint after linear regression was performed on the sample as a whole, however in the single group analysis, the opposite was seen. Maximal exercise capacity, measure as VO_2 peak (mL/kg/min), associated positively and significantly with FMD values at all timepoints and these results were most significant at baseline (p = 0.009; R^2 = 0.22) and 60 min post-training (p = 0.002; R^2 = 0.29). Finally, the inflammation marker, hsCRP (mg/L), was examined and linear regression revealed significant negative correlations at baseline (p = 0.01; R^2 = 0.21), 30 min post-training (p = 0.04; R^2 = 0.14) and 60 min post-training (p = 0.003; R^2 = 0.27). For more details, please see the supplementary materials section)

Chapter 4 – Discussion & Conclusions

The main purpose of this thesis was to reinvestigate previous reports of endothelial dysfunction, measured by FMD, in young people with CF and to examine if these findings were replicable. Acute modification of endothelial function in young people with CF with exercise training was attempted as a proof of principle to warrant further follow-up studies exploring the long-term effects of exercise training on endothelial function in this cohort. Finally, patient demographics, lung function, physical activity levels, fitness levels and inflammation levels were compared to baseline and post-training FMD values to probe for associations, which may be helpful in predicting the effects of exercise on FMD in these young people with CF.

In order to pursue these research questions, it was necessary to establish and validate the FMD methods, then optimize and validate the acute exercise intervention. The same healthy cohort, average age of 22 years, was used for all pilot studies. No FMD baseline parameters were significantly different between test and retest. All FMD baseline parameters, besides time to peak dilation, displayed good to excellent reliability in terms of ICC values. Specifically, the standard error of the FMD% measurement, SEM = 1.7 %, was comparable to published results from research groups specialized in these measurements (158). This review scrutinized 27 studies involving 48 study groups and a total of 1,537 participants to determine the relationship between FMD reproducibility and adherence to current expert guidelines of FMD measurements. After analysis of Bland-Altman plots, a systematic bias of 0.32% was found, but deemed insignificant, whilst random bias was also minimal with all data points falling within the 95% limits of agreement. Using the requirements of Bland & Altman 1986, the limits of agreement are small enough to be confident that the method is reproducible and can be used for clinical purposes.

After demonstrating FMD baseline measurements were reproducible, establishment and optimization the acute exercise training protocol were addressed. Due to previous findings in other chronic inflammatory diseases showing improvements to FMD after long-term endurance training, this form of exercise training was adopted and applied to the CF study (156, 159). In these other diseased populations, 12 weeks of moderate endurance training was shown to augment FMD to magnitudes roughly 2 times greater than those observed at baseline. Previous studies in healthy subjects have shown a certain training intensity and training duration is required to observe acute effects on the FMD% (155), yet people with CF also have exercise intolerance. Therefore, the minimum effective dose (intensity and duration) of endurance training in which acute FMD effects could be recorded was tested. Based on previous findings, 30 minutes of endurance training on a bicycle at 60% HR_{max} was compared to the same training at 75% HR_{max}. Again, the same participants as previously described completed both protocols on separate days. The 30-minute training protocol at 60% HR_{max} had no significant effect on any post-training FMD parameters ($p > 0.05$). The 75% HR_{max} protocol for 30 minutes however did change FMD parameters post-training. Immediately post-training brachial artery diameter was significantly larger, shear rate was increased, FMD% was reduced by approximately 40% and time to peak vasodilation occurred later after the 75% HR_{max} training protocol compared to the 60% HR_{max} training protocol. Results 30 minutes after training are more difficult to interpret. Brachial artery diameter was still increased and time to peak vasodilation still delayed, shear rate had normalized, yet FMD% was now increased. This finding although statistically significant, may not be relevant. Finally, 60 minutes after training, differences were still observed in FMD parameters between protocols. The 75% HR_{max} protocol produced an increase in baseline diameter, peak FMD diameter, FMD absolute change and FMD%. Knowing the 75% HR_{max} training protocol would

induce the bi-phasic reaction typical of FMD after acute exercise, this protocol was chosen to be adapted in later studies investigating these effects in young people with CF.

Next, the reliability of FMD parameters after such an acute training protocol needed further assessment. Therefore, pilot study participants were invited for one further measurement of FMD parameters before and after the 75% HR_{max} training protocol. Good to excellent ICC values (range = 0.73 – 0.98) were observed in all FMD parameters throughout all measurements pre- and post-training. To the authors' knowledge, only one published study has investigated the reliability of FMD parameters after an acute bout of training (160). Although the studies' goals and methods differed greatly in comparison, as their study investigated overweight men performing a walking protocol on a treadmill, reliability outcomes such as ICCs and CVs were quite comparable. This study employed very similar assessments of reproducibility compared to the current study: (1) a two-way analysis of variance (ANOVA), (2) Intraclass correlation coefficients (ICC), (3) Pearson correlations (r), and (4) coefficient of variation (CV %) at each time-period. Interestingly, when comparing ICC values with the current study, both studies reported FMD% to be most unreliable when measured at baseline before training. After ascertaining the reliability of the method, the main hypotheses could now be tested confidently knowing any and all differences were due to conditions or interventions.

Is baseline FMD different between groups?

Groups (CF and non-CF) were matched according to age, sex, BMI, and physical activity levels, while a third control group (non-CF active) was only matched for age, sex and BMI. Inclusion criteria for this group ensured an increased average physical activity level of more than 5 hours per week. Yet, at baseline major differences were still observed between all groups.

Individuals with CF presented with lower resting oxygen levels and increased levels of hsCRP, indicative of mild lung disease and inflammation known to occur in CF. All pulmonary function test results were diminished in CF, again at levels suggestive of mild lung disease. Excluding HR_{max}, all maximal exercise capacity outcome parameters were decreased in CF. VO_2 peak values were reduced roughly 25% and 30% compared to control groups, non-CF and non-CF active, respectively. Percentile comparisons between relative maximum work outputs yielded very similar results with CF exhibiting approximately 25% - 35% less compared to control groups. Compared to a recently published review, the maximal exercise capacity results of the CF group fall well within the range of expected values (161). Since this review differentiates maximal exercise capacity between CFTR mutation classes in young people with CF and the young CF cohort in this study all have the same mutation, thus mutation class, valid comparisons can be made. In their study, patients of the same age range with the ΔF508 mutation, a class II mutation, presented with a predicted FEV_1 of 79% and a predicted $VO_{2\ peak}$ of approximately 80%.

Regarding baseline FMD parameters, no differences in brachial artery diameter were measured, however after induced reactive hyperemia; absolute change in artery diameter was significantly less in CF, resulting in a significantly reduced FMD in the CF group even after normalization to shear stress. These findings replicate those of Poore et al. 2013, who were the first to identify evidence of vascular endothelial dysfunction in young people with CF (74). In comparison to this study, which was performed in a slightly younger cohort, values for exercise capacity, as well as baseline FMD parameters were of greater magnitude. Differences in artery diameter are likely due to the age differences between the studies as referred to earlier, however this is less likely to be the case for the slight differences in FMD%, as FMD% should stay quite stable between 6 and 18 years of age (162), after which it should remain

stable until 40 years of age (163). This could be due to slight differences in the method, for example cuff placement, edge-detection software settings or the duration of image recording after cuff release, which has been shown to be of influential (164). Despite these minimal differences to previously reported findings, the broader finding that vascular endothelial function is reduced in CF was corroborated. Although the actual magnitudes may vary across studies, due to differing methods and study populations, when compared to other diseases such as obesity, type 1 and 2 diabetes, in which endothelial vascular dysfunction is also observed, the reduction seen in CF is of similar magnitude. Studies investigating cohorts diagnosed with these aforementioned diseases also reveal approximately a 1/3 reduction in function that is directly due to disease [Endothelial Function and Weight Loss in Obese Humans; Impaired flow-mediated dilation response and carotid intima-media thickness in patients with type 1 diabetes mellitus with a mean disease duration of 4.1 years. ; Type 2 diabetes is associated with impaired endothelium-dependent, flow-mediated dilation, but impaired glucose metabolism is not; The Hoorn Study]. The authors further stipulate that these impairments in FMD may in part explain the increased cardiovascular disease risks observed in these cohorts.

Does acute exercise affect FMD differently between groups?

The next question posed was whether the vascular endothelial dysfunction observed in young people with CF could be modified by an acute 30-minute endurance exercise training at 75% HR_{max}. Immediately post-training, significant increases in brachial artery diameter and decreases in FMD% were measured in all groups when compared to baseline values and these FMD differences remained significant after normalization to shear stress These reductions in

function (~40%) are similar to those reported from other groups using analogous designs and methods (155).

Previous studies have reported increases, decreases or no change in FMD following acute exercise training(165-167), but interpretation of this literature is challenging due to differences in exercise intensities and modes, the timing of FMD measurements after exercise, technical differences related to artery diameter measures and FMD techniques. These factors, independently or in unison, may directly influence FMD making it difficult to pinpiont the exact effect of acute exercise on endothelial vascular function.

The reasoning behind the assessment of acute exercise effects on FMD concerns the impact of repeated exercise on arterial adaptation. It may seem intuitive that a single bout of exercise would acutely enhance endothelial vascular function, as longterm training promotes beneficial adaptations, however this assumption is oversimplistic. Reduced FMD due to acute exercise is not necessarily associated with down-regulation as an adaptive response (168) and this hypothesis was again underlined by Padilla et al., who suggests there are many examples of up-regulation in response to stimuli, which acutely challenge pathways in integrative human physiology (123). This concept is epitomized by the concept of “hormesis”.

Immediately post-training group differences in FMD% were only observed between CF and the non-CF active groups. The exercise training protocol applied in this study had acutely narrowed the gap in FMD% between CF and non-CF, and this seemed dependent upon pre-training FMD levels, as non-CF and non-CF active had similar absolute decreases in FMD% immediately post-training. These decreases in FMD were expected, however whether the magnitude of change between the group would be similar was unknown. The literature, although vague as to exactly when the FMD measurement was taken post-training, suggests a greater reduction in vascular endothelial function immediately post-training for females and

for individuals who do not regularly exercise (169). The authors did speculate their results may be due to differences in exercise habits between genders, as most regularly exercising subjects were male and ΔFMD showed significant correlation with exercise habit. This significant correlation between ΔFMD and exercise habit persisted even when the effect of gender was adjusted, suggesting exercise habit as an important factor in the process. The authors also observed a greater pre-exercise FMD in their female cohort, which they suggested was due to a smaller baseline artery diameter and speculated that these differences could account for baseline gender difference in endogenous vasodilation and also differences in hormonal status.

Due to small group sizes, a comparison between males and females within groups was not possible, but this phenomenon is intriguing and warrants further investigation.

Looking at FMD parameters 30 minutes post-training, FMD had recovered back to baseline values, but defects in FMD could still be identified in the young CF group, although these differences negated after normalization to shear stress. No other differences between any of the groups were seen at this time-point post-training. The final follow-up FMD measurement occurring 60 minutes post-training, revealed further recovery of FMD towards levels surpassing those at baseline in all groups, however this increase was only deemed significant for the CF and non-CF active groups. Despite this augmentation of vascular endothelial function, CF group values were still significantly less than both control groups, although after normalization to shear stress, this difference was only significant between CF and non-CF active. These findings are complementary to the only other known study in which the acute FMD response to exercise was investigated in a patient population also known for chronic inflammation, obesity. Harris et al. 2012 demonstrated that the FMD of active overweight men increased acutely 60 minutes post-exercise, however not of inactive overweight men,

but admittingly they could not provide insight as to the mechanisms whilst examining interactions between IL-6 and TNF-α (165). Admittingly, these findings were attained from an older patient population with a different underlying disease, but nonetheless we find it noteworthy that all groups investigated in this study possessed greater physical activity levels than those studied by Harris et al. This result substantiates the findings of Harris et al. and generalizes the finding further applying it to younger people as well as people with CF. However, studies from Birk et al. 2013 reported FMD to have returned to baseline levels 1 hr after acute exercise. In this study they investigated several exercise intensities and modes, as well as performing their measurements in young healthy adults, and found that under all conditions FMD had returned to baseline after 1 hr. FMD time-course findings pre- and post-training indicated that an acute bout of exercise could induce a biphasic CF FMD response, but could not correct FMD to normal control values.

Are baseline FMD or post-training FMDs associated with demographics, physical activity levels, lung function, maximal exercise capacity or inflammatory hsCRP levels?

Upon inspection for pre- and post-training FMD associations with demographics, physical activity levels, lung function, maximal exercise capacity or inflammatory hsCRP levels, very little insight with relevance was found. When examining the three groups independently, only two significant associations were revealed. After interpretation of the correlations between VO_2 peak, BMI and FMD immediately post-training, one could again claim base fitness levels and a healthy body mass as prerequisites for an optimal acute exercise training effect. After finding little relation between variables within groups, all three groups were examined collectively to search for additional insight. This analysis revealed several significant differences, which help interpret the complicated underlying mechanisms influencing the

effect of acute exercise on FMD. No correlations between inflammation, measured as hsCRP, and FMD parameters could be shown. Despite the well-characterized immune response post-exercise, researchers have found it difficult to link inflammatory markers to acute FMD effects post-training (170). Others have had better luck describing the associations between the hemodynamic characteristics, rather than participant characteristics, during bouts of acute exercise (122). They arrive at these conclusions by assessing FMD after slow and high speed muscle contraction exercises. Slow contractions induced higher blood pressures and subgroup analysis revealed this high blood pressure to associate with the observed changes in FMD. For example, participants with blood pressures >100 mm Hg during exercise displayed greater decreases in FMD than those with lower exercise blood pressures. In reality, both participant characteristics and exercise mode most likely play an important role in determining the acute FMD response.

Limitations

This study is unique in the fact that baseline FMD was measured before an exercise training session and at three timepoints after training: immediately after, 30 minutes after and 60 minutes after. The study protocol was designed specifically to incorporated the biphasic nature of the FMD response post-acute exercise. Many studies only measure once, either immediately post-training or 60 minutes post. These design decisions are perhaps based on different research questions, but nonetheless greatly influence the results and outlooks of the studies. Had only one timepoint been measured post-training in this study, completely opposite results would have been obtained and other interpretations made. For this reason, the current guidelines for recommend the measurement of FMD at several timepoints post exercise (124). Some would still argue that the actual measurement of FMD biases the next

measurement; however, this was tested and debunked. FMD was measured every 30 minutes for 2 hours (5 FMD measurements in total) and the results across timepoints compared. FMD was remained unchanged, ICC of all measurements = 0.62 and a CV = ~10%, throughout the 2 hours leaving the authors to conclude repetitive reactive hyperemia over 2 hours had no effect on FMD (171). Though the FMD method has been validated and widely used, some disagreement still exists as to analysis and interpretation, specifically the allometric scaling of artery diameter and the normalization of FMD to shear rate. These two methods have previously been used to address the baseline diameter dependency of the method and the large variability in reactive hyperemia-induced shear stress between subjects, respectively, but no consensus on their use is currently available (154, 172). Therefore, as the current FMD guidelines state, all FMD parameter values for each FMD measurement are disclosed, so that readers may interpret these findings with all of the available knowledge and without bias.

Others might argue that the acute exercise effects are interesting, but perhaps not relevant. Thankfully, recent research has empirically proven the hormesis hypothesis true in its application to the acute effects of exercise on FMD. Dawson et al. 2018 measure the acute effects of a 30-minute endurance cycling training session at 80% HR_{max} in healthy young men, then followed up after a 2-week training intervention (five 30-minute cycle exercise sessions at 80% HR_{max}). Their findings indicated that acute post-exercise changes in FMD were associated with changes in resting FMD after 2 weeks of the endurance exercise training. They speculated this effect could be related to exercise-induced increases in antegrade shear rate (141). Specifically, larger increases in shear stress during exercise was proportional to increases in post-exercise FMD, whilst the latter response associated better with improved baseline FMD after 2 weeks of exercise training. However, this study only investigated cycling exercises and their two-week training intervention is relatively short, therefore these results

may not be applicable to other modes of exercises and it remains unknown whether longer training interventions would provide more substantial improvements in vascular endothelial function.

Admittingly, the small group sizes in the current study are a limitation and may influence the impact of findings, but CF is a rare genetic disease with an estimated incidence of 1:3,300 in Germany and finding 10 young people with CF healthy enough to participate in such a study should be considered a success. However, due to small group sizes, comparisons between genders and associations between FMD and participant characteristics proved difficult if not impossible.

Further, only the effects of one exercise type, endurance cycling, were investigated, but emerging studies are showing even more improvement to FMD through use of other training forms, for example high-intensity interval training (173-176). However, few studies to date have applied high-intensity interval training to CF populations and with exercise intolerance in this cohort, the gold standard form of endurance exercise might be more favorable (177, 178). Currently, two other clinical trials are pursuing ways to improve exercise intolerance in CF by correcting or enhancing vascular endothelial function with the use of antioxidants and drugs, such as phosphodiesterase type 5 inhibitors (179, 180).

These future directions and approaches are fascinating, but believe a clinical trial investigating the long-term effects of an endurance exercise training intervention on FMD in a CF population would be most beneficial towards conceptually proving vascular endothelial dysfunction is modifiable by exercise in CF. These types of studies however are extremely difficult and would be extra complicated in CF patient groups, but nonetheless would contribute greatly to the understanding of vascular endothelial dysfunction in CF. These new findings that vascular endothelial dysfunction in CF can be acutely modified by exercise should

spur more time and resources into this area of research to improve the daily lives of people with CF.

Summary

Cystic fibrosis (CF) is a genetic disease causing dysregulation and dysfunction in multiple organ systems. As the expected life span of people with CF continues to increase due to modern medicines and therapies, complications other than the common lung morbidity and mortality of CF, specifically endothelial dysfunction, begin to become of greater importance. Reports from other diseased cohorts have proved endurance exercise as therapy to halt or reverse endothelial dysfunction, but investigations of acute exercise effects haven been suggested prior to implementation and testing of long-term exercise interventions. This thesis began by reinvestigating previous reports of endothelial dysfunction, measured by FMD, in young people with CF to examine if these published findings were replicable. Young people with CF possessed decreased lung function and maximal exercise capacity compared to matched controls and baseline FMD was confirmed to be significantly decreased in the CF. Next, the acute effects of endurance exercise on endothelial function in young people with CF with was attempted as a proof of principle to warrant further follow-up studies exploring the long-term effects of exercise training on endothelial function in this cohort. Immediately post-training, FMD was significantly attenuated in all groups with CF still demonstrating the most minimal FMD. Follow-up measurements of FMD revealed a slow recovery towards baseline values 30 min post-training and improvements to FMD in the CF and non-CF active groups 60 min post-training. Finally, patient demographics, lung function, physical activity levels, fitness levels and inflammation levels were compared to baseline and post-training FMD values to probe for associations, which could help in the prediction of exercise effects on FMD in these young

people with CF. Linear regression indeed revealed significant correlations between maximal exercise capacity (VO_2 peak), BMI and FMD immediately post-training. These new findings confirm CF vascular endothelial dysfunction and confirm that this dysfunction can be acutely modified by exercise. These results should further aid in underlining the importance of exercise in CF populations. However, the potential benefits of long-term exercise interventions on vascular endothelial dysfunction in young people with CF warrants further investigation.

Appendix

Abbreviations

AA – Arachidonic acid

Akt – protein kinase B

BMI – body-mass index

CF – cystic fibrosis

CFF – cystic fibrosis foundation

CFTR – cystic fibrosis transmembrane regulator

CVD – cardiovascular disease

ecSOD – extracellular SOD

FEV1 – forced expiratory volume in one second

FMD – flow-mediated dilation

FVC – forced vital capacity

hsCRP – high sensitivity C-reactive protein

LoA – limits of agreement

NFkB – nuclear factor-kB;

ONOO – peroxynitrite

PECAM-1 – platelet endothelial cell adhesion molecule-1

PGI2 – prostaglandin I2

Ras – small GTPase

RQ – research question

VEGFR2 – VEGF receptor 2

VO_2 peak – peak rate of oxygen consumption measured during incremental exercise

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Figure 12. Immediate post-training FMD, shear rate AUC, and FMD normalized for shear. Values are presented as mean ± SD. *** Indicates significant differences. AUC = area under the curve; FMD = flow-mediated dilation.

Figure 13. 30 min post-training FMD, shear rate AUC, and FMD normalized for shear. Values are presented as mean ± SD. *** Indicates significant differences. AUC = area under the curve; FMD = flow-mediated dilation.

Figure 14. 60 min post-training FMD, shear rate AUC, and FMD normalized for shear. Values are presented as mean ± SD. *** Indicates significant differences. AUC = area under the curve; FMD = flow-mediated dilation.

Figure 15. FMD time-course (pre- and post-training). Values are presented as mean ± SD. AUC = area under the curve; FMD = flow-mediated dilation. * CF significantly different compared to Non-CF and Non-CF Active. ** CF significantly different only compared to Non-CF Active. † All groups significantly different compared to baseline. †† CF and Non-CF Active groups significantly different compared to baseline.

Figure 16. Scatter plot illustrating the relationship between FMD% immediately post-training and baseline lung function measured by FEV1 (L). Linear regression was performed on all three groups (*n* = 10 per group) independently. A significant difference between CF and both non-CF groups was observed.

Figure 17. Scatter plot illustrating the relationship between FMD% immediately post-training and baseline lung function measured by FEV1 (L). Linear regression was performed on all three groups (*n* = 10 per group) independently. A significant difference between CF and both non-CF groups was observed.

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Supplementary materials

Reliability & Training Intensity Pilot Study Methods

Participants

Twenty healthy volunteers between the ages of 10 and 30 years old were recruited at the University of Potsdam and its Outpatient Clinic by word of mouth for these studies (Table S1). Participant exclusion criteria included smoking, cardiovascular or metabolic diseases, sleeping disorders and current illness or infection. Participants on anti-inflammatory, b_2-adrenergic agonistic and local vasoconstriction medications were excluded from the study. Women receiving hormonal or contraceptive therapy were also excluded. Additionally, women were only studied during the follicular phase. Finally, participants were instructed to refrain from foods or beverages containing antioxidants 1 week prior to FMD examinations. On testing days, participants were asked to fast, especially avoiding caffeine. Exercise abstinence of at least 12 hr prior to testing was also requested. All study protocols were approved by the University of Potsdam Ethics Committee (No. 13/2016) and written/verbal consent was obtained from all subjects or parents prior to their participation.

Design

Study volunteers participated in measurements on 4 separate days over several weeks: one maximal exercise capacity test, one 30 min training session at 60% HR_{max} on a stationary bicycle with FMD measurements before, immediately post-training, 30 min post-training and 60 min post-training and two 30 min training sessions at 75% HR_{max} on the stationary bicycle with FMD measurements before, immediately post-training, 30 min post-training and 60 min post-training. Reliability of baseline FMD measurements (Table S2 and Figure S1) were assessed simultaneously as the optimal training intensities were tested (Tables S3-5 and

Figures S2-5). All exercise test, training and FMD methods used in these pilot studies were identical to those previously described in Chapter 2.

The two intensity levels (60% and 75% HR_{max}) were chosen based on previous studies investigating the acute effects of exercise on FMD (155), who determine the effect of a 30 min leg cycling exercise performed at 3 exercise intensities (50, 70 and 85%) on brachial artery FMD immediately after cycle exercise. They found that FMD decreased to a greater degree immediately after exercise performed at higher exercise intensities. Here, we considered and hoped to find the minimal intensity needed to induce an FMD effect, as the safety and comfort of the people with CF in the following study was desired. After analysis, it was quickly determined that 75% HR_{max} would be used as the optimal training intensity, therefore participants were asked to perform a second 30 min training session at 75% HR_{max}, so the reliability of this training's acute effects on FMD could be analyzed (Tables S6-9 and Figures S2-5).

Statistics

All data were analyzed, graphed and presented using Statistical Package for Social Sciences (SPSS Statistics 21, IBM, Armonk, New York, USA), Prism (GraphPad Software Inc., La Jolla, CA, USA) and Excel (Microsoft Office V.10, Redmond, WA, USA). After collection, data was transferred to a database and checked for plausibility using range checks. Implausible values and outliers were double-checked and corrected or excluded accordingly. Descriptive data are presented as means ± standard deviations (SD). Before statistical comparisons means, data were tested for normal distribution (Shapiro-Wilk).

Baseline and post-training FMD parameters and outcomes between tests and retests were compared using:

1) Intraclass correlation coefficient (ICC, 2.1) with 95% confidence interval (181)
2) SEM (SEM = SD * √(1 - ICC)) (182)
3) Coefficient of variation (CV (%) = SD / mean) (183)
4) Limits of agreement analysis [bias ± 1.96 * SD = 95% - absolute limits of agreement, LoA], for FMD%, shear rate (AUC) and FMD/shear data (183-185)

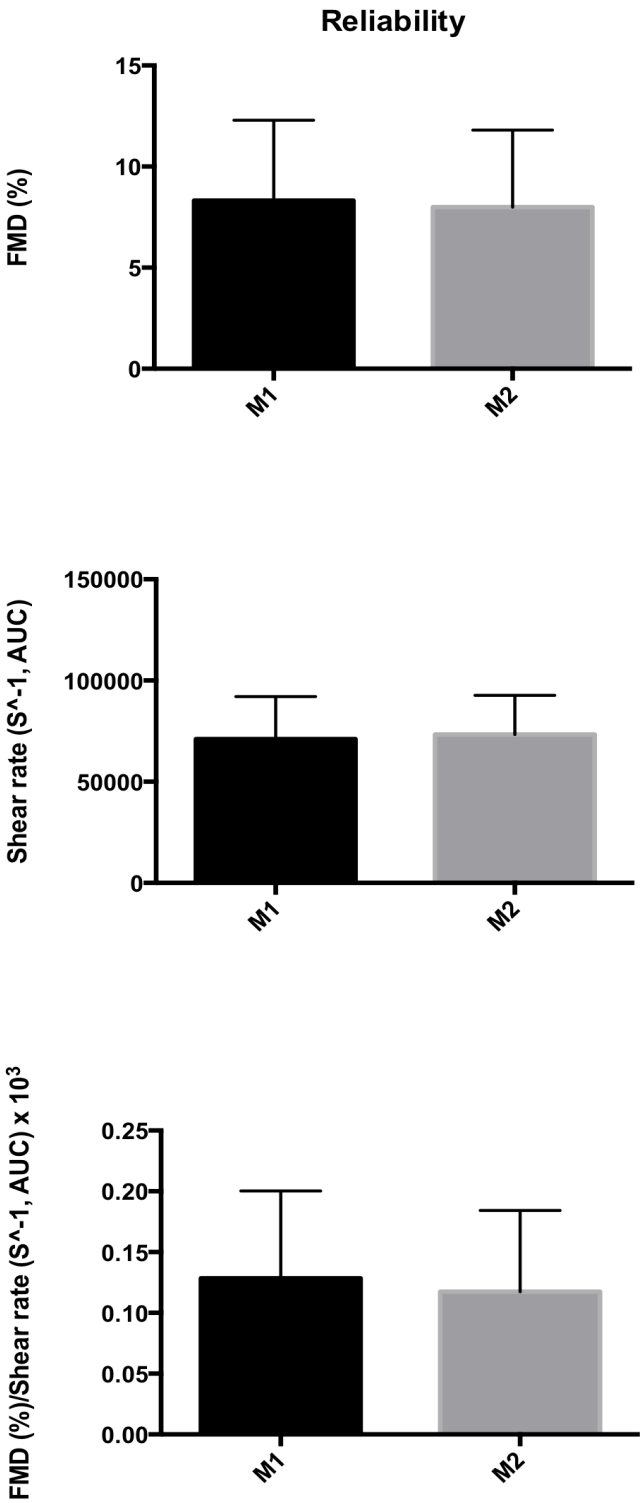
To investigate FMD and parameters pre- and post-training (30 min at 60% HR_{max} or 75% HR_{max}) dependent t-tests were used. For all comparisons, statistical significance was set at a *p*-value of $\alpha < 0.05$.

Table S1. Group characteristics. (mean ± SD)

Variable	
n, M/F	10/10
Age, **yr**	21.9 ± 6.2
Height, cm	171.7 ± 8.9
Weight, kg	65.1 ± 10.5
BMI, kg/m^2	22.1 ± 3.4
SBP, mm Hg	117 ± 10
DBP, mm Hg	79 ± 12
Resting O_2 Sat %	99.4 ± 1.3
Physical Activity, **hr**/wk	3.3 ± 1.3
hsCRP, mg/L	0.4 ± 0.8
VO_2 peak, mL/kg/min	46.3 ± 7.2
Heart rate peak, bpm	191 ± 13
Work peak, W	265 ± 40
Work peak, W/kg	4.07 ± 0.67

Table S2. Reliability of baseline FMD parameters. (mean ± SD)

Variable	M1	M2	Difference	*ICC*	*SEM*	*CV*
Baseline diameter, cm	0.371 ± 0.058	0.370 ± 0.056	0.016 ± 0.020	0.90	0.018	3%
Peak diameter, cm	0.402 ± 0.066	0.398 ± 0.054	0.023 ± 0.024	0.85	0.023	4%
FMD absolute change, cm	0.031 ± 0.016	0.029 ± 0.012	0.008 ± 0.007	0.70	0.008	18%
FMD%	8.32 ± 3.98	7.99 ± 3.81	1.84 ± 1.61	0.81	1.71	16%
Shear rate (S^-1, AUC)	71,077 ± 21,026	73,340 ± 19,404	8,203 ± 5,333	0.88	6,866	8%
FMD/Shear (S^-1, AUC) x 10^3	0.128 ± 0.072	0.117 ± 0.067	0.034 ± 0.023	0.83	0.029	20%
Time to peak, s	48 ± 10	49 ± 7	6 ± 4	0.64	5.2	9%



1700

1701 Figure S1. Test (M1) and retest (M2) results of baseline FMD, shear rate AUC, and FMD

1702 normalized for shear. Values are presented as mean ± SD. AUC = area under the curve; FMD

1703 = flow-mediated dilation.

1704

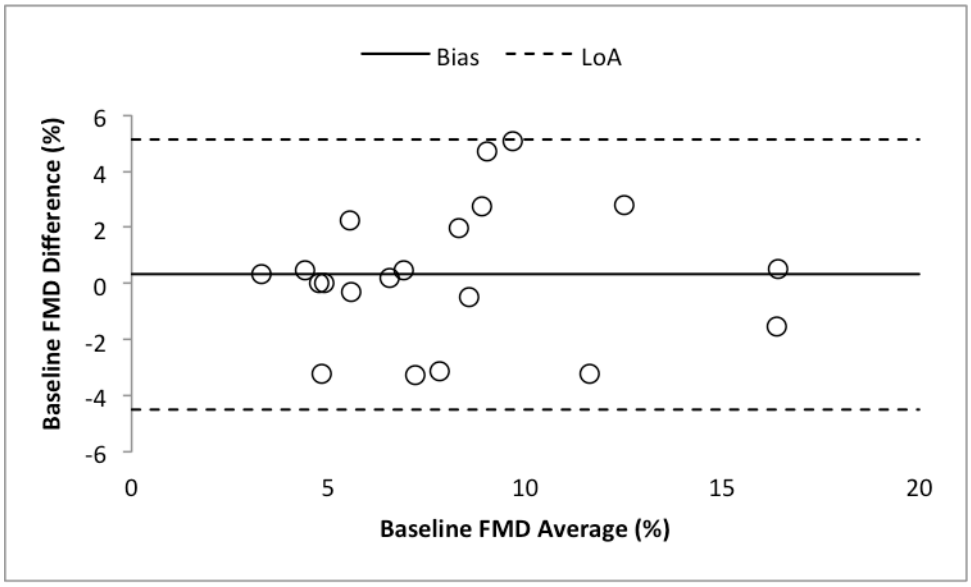


Figure S2. Bland-Altman plot of baseline FMD%. Bias (mean difference between measurements) = 0.32%; LoA (95% limits of agreement, bias ± 1.96*SD) = 5.13% (high) and -4.49% (low).

Table S3. Baseline FMD parameters. (mean ± SD)

Variable	60% HR_{max}	75% HR_{max}	*p*-value
Baseline diameter, cm	0.371 ± 0.058	0.370 ± 0.056	0.87
Peak diameter, cm	0.402 ± 0.066	0.399 ± 0.054	0.64
FMD absolute change, cm	0.031 ± 0.016	0.029 ± 0.011	0.25
FMD%	8.32 ± 3.98	8.00 ± 3.81	0.57
Shear rate (S^-1, AUC)	71,077 ± 21,026	73,340 ± 19,404	0.31
FMD/Shear (S^-1, AUC) x 10^3	0.128 ± 0.072	0.117 ± 0.067	0.23
Time to peak, s	49 ± 11	48 ± 7	0.85

Table S4. FMD parameters immediately post-training. (mean ± SD)

Variable	60% HR_{max}	75% HR_{max}	*p*-value
Baseline diameter, cm	0.390 ± 0.057	0.403 ± 0.053	0.02*
Peak diameter, cm	0.420 ± 0.060	0.421 ± 0.054	0.83
FMD absolute change, cm	0.030 ± 0.014	0.018 ± 0.011	< 0.001*
FMD%	7.89 ± 3.75	4.57 ± 2.88	< 0.0001*
Shear rate (S^-1, AUC)	75,957 ± 22,836	84,205 ± 18,656	0.001*
FMD/Shear (S^-1, AUC) x 10^3	0.113 ± 0.064	0.057 ± 0.039	0.0001*
Time to peak, s	53 ± 9	58 ± 7	0.002*

Table S5. FMD parameters 30 minutes post-training. (mean ± SD)

Variable	60% HR_{max}	75% HR_{max}	*p*-value
Baseline diameter, cm	0.383 ± 0.057	0.394 ± 0.053	0.01*
Peak diameter, cm	0.412 ± 0.059	0.423 ± 0.053	0.03*
FMD absolute change, cm	0.029 ± 0.011	0.029 ± 0.010	0.75
FMD%	7.72 ± 3.14	7.92 ± 3.29	0.04*
Shear rate (S^-1, AUC)	70,657 ± 19,711	71,955 ± 20,273	0.65
FMD/Shear (S^-1, AUC) x 10^3	0.120 ± 0.066	0.117 ± 0.055	0.006*
Time to peak, s	50 ± 9	53 ± 7	0.04*

Table S6. FMD parameters 60 minutes post-training. (mean ± SD)

Variable	60% HR_{max}	75% HR_{max}	*p*-value
Baseline diameter, cm	0.391 ± 0.053	0.407 ± 0.053	< 0.001*
Peak diameter, cm	0.422 ± 0.057	0.446 ± 0.054	< 0.001*
FMD absolute change, cm	0.032 ± 0.012	0.039 ± 0.012	< 0.001*
FMD%	8.19 ± 3.32	9.80 ± 3.40	< 0.0001*
Shear rate (S^-1, AUC)	63,140 ± 15,124	62,040 ± 13,842	0.60
FMD/Shear (S^-1, AUC) x 10^3	0.140 ± 0.075	0.168 ± 0.072	0.003*
Time to peak, s	48 ± 7	48 ± 5	0.81

Tables S3-S5:

* Significant difference between 60% HR_{max} and 75% HR_{max}

Table S7. Reliability of baseline FMD parameters. (mean ± SD)

Variable	75% HR_{max} M1	75% HR_{max} M2	Difference	*ICC*	*SEM*	*CV*
Baseline diameter, cm	0.370 ± 0.056	0.372 ± 0.053	0.017 ± 0.013	0.92	0.015	3%
Peak diameter, cm	0.398 ± 0.054	0.401 ± 0.057	0.020 ± 0.017	0.89	0.018	3%
FMD absolute change, cm	0.029 ± 0.012	0.030 ± 0.012	0.008 ± 0.004	0.73	0.006	19%
FMD%	8.00 ± 3.81	8.03 ± 3.24	2.02 ± 0.94	0.79	1.57	18%
Shear rate (S^-1, AUC)	73,340 ± 19,404	77,430 ± 21,393	9,480 ± 5,374	0.87	7,658	9%
FMD/Shear (S^-1, AUC) x 10^3	0.117 ± 0.067	0.136 ± 0.105	0.051 ± 0.102	0.81	0.079	23%
Time to peak, s	48 ± 7	49 ± 8	4 ± 2	0.79	3	6%

Table S8. Reliability of FMD parameters immediately post-training. (mean ± SD)

Variable	75% HR_{max} M1	75% HR_{max} M2	Difference	*ICC*	*SEM*	*CV*
Baseline diameter, cm	0.390 ± 0.057	0.401 ± 0.054	0.022 ± 0.020	0.97	0.009	2%
Peak diameter, cm	0.418 ± 0.054	0.424 ± 0.055	0.012 ± 0.009	0.97	0.010	2%
FMD absolute change, cm	0.018 ± 0.010	0.018 ± 0.012	0.004 ± 0.002	0.89	0.004	22%
FMD%	4.57 ± 2.88	4.50 ± 2.95	1.13 ± 0.52	0.91	0.88	23%
Shear rate (S^-1, AUC)	84,205 ± 18,657	85,605 ± 21,144	6,260 ± 3,854	0.93	5,162	6%
FMD/Shear (S^-1, AUC) x 10^3	0.057 ± 0.039	0.057 ± 0.041	0.014 ± 0.011	0.90	0.012	23%
Time to peak, s	57 ± 7	60 ± 8	5 ± 3	0.75	4	6%

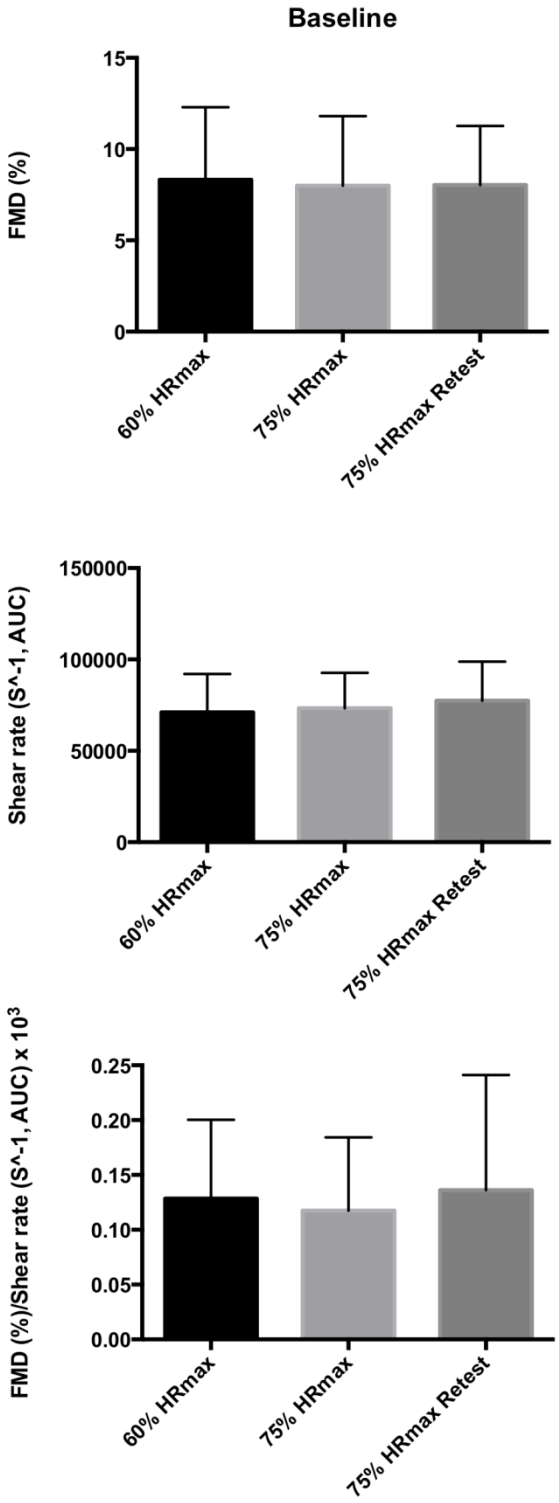
Table S9. Reliability of FMD parameters 30 minutes post-training. (mean ± SD)

Variable	75% HR_{max} M1	75% HR_{max} M2	Difference	*ICC*	*SEM*	*CV*
Baseline diameter, cm	0.392 ± 0.054	0.396 ± 0.053	0.015 ± 0.011	0.94	0.013	3%
Peak diameter, cm	0.422 ± 0.054	0.423 ± 0.054	0.015 ± 0.012	0.93	0.014	3%
FMD absolute change, cm	0.030 ± 0.012	0.027 ± 0.010	0.006 ± 0.004	0.81	0.005	17%
FMD%	7.92 ± 3.29	6.95 ± 2.78	1.78 ± 1.01	0.81	1.44	17%
Shear rate (S^-1, AUC)	71,955 ± 20,273	74,965 ± 17,375	8,390 ± 3,734	0.89	6,467	8%
FMD/Shear (S^-1, AUC) x 10^3	0.117 ± 0.055	0.099 ± 0.047	0.024 ± 0.023	0.86	0.023	16%
Time to peak, s	51 ± 7	54 ± 7	4 ± 3	0.75	4	6%

Table S10. Reliability of FMD parameters 60 minutes post-training. (mean ± SD)

Variable	75% HR_{max} M1	75% HR_{max} M2	Difference	*ICC*	*SEM*	*CV*
Baseline diameter, cm	0.406 ± 0.054	0.409 ± 0.053	0.011 ± 0.005	0.98	0.008	2%
Peak diameter, cm	0.445 ± 0.056	0.447 ± 0.054	0.012 ± 0.007	0.97	0.010	2%
FMD absolute change, cm	0.039 ± 0.013	0.038 ± 0.011	0.004 ± 0.002	0.82	0.004	9%
FMD%	9.80 ± 3.40	9.49 ± 3.11	1.03 ± 0.60	0.83	0.835	18%
Shear rate (S^-1, AUC)	62,040 ± 13,842	62,760 ± 17,410	6,200 ± 5,574	0.86	5,829	7%
FMD/Shear (S^-1, AUC) x 10^3	0.168 ± 0.072	0.161 ± 0.067	0.017 ± 0.010	0.86	0.014	19%
Time to peak, s	47 ± 6	48 ± 5	4 ± 3	0.75	4	6%

Test (M1) and retest (M2)



1754

1755 Figure S3. Baseline FMD, shear rate AUC, and FMD normalized for shear. Values are presented

1756 as mean ± SD. *** Indicates significant differences. AUC = area under the curve; FMD = flow-

1757 mediated dilation.

1758

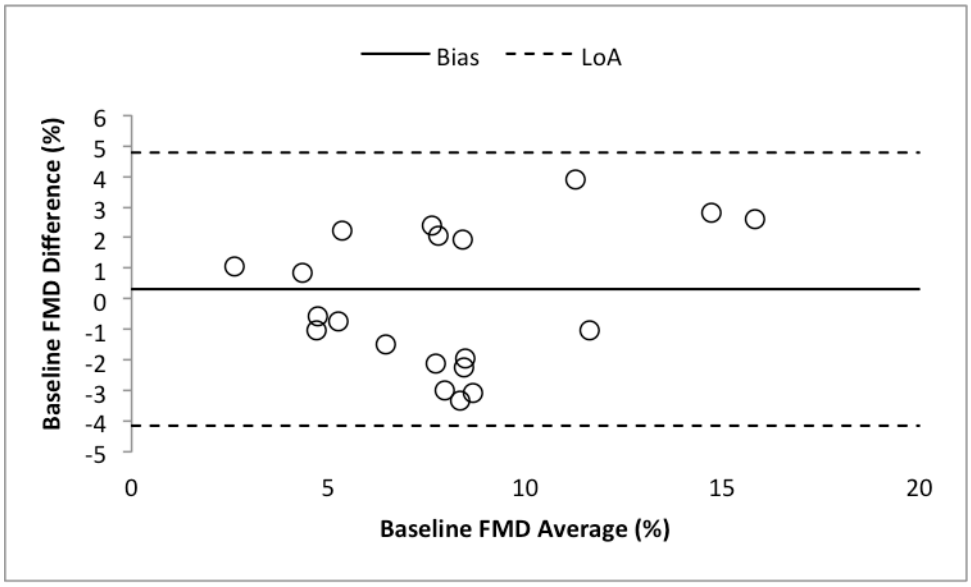
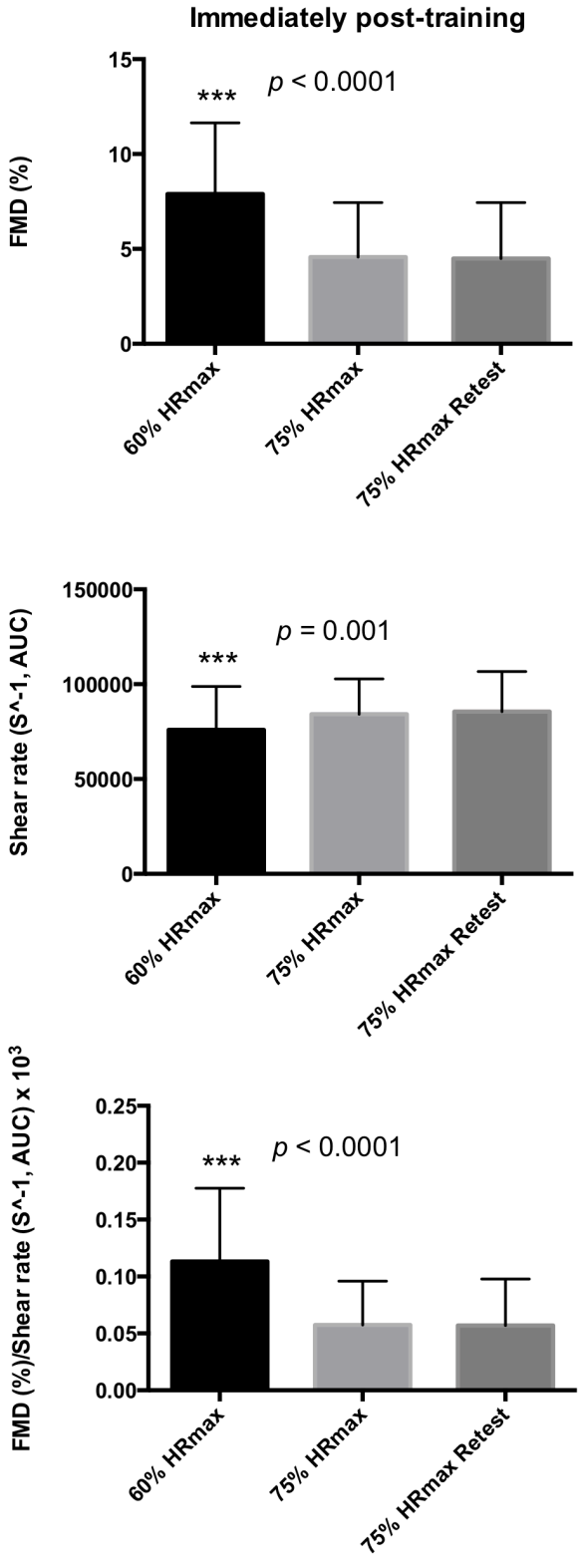


Figure S4. Bland-Altman plot of baseline FMD%. Bias (mean difference between measurements) = 0.32%; LoA (95% limits of agreement, bias ± 1.96*SD) = 4.78% (high) and -4.14% (low).



1773

1774 Figure S5. Immediate post-training FMD, shear rate AUC, and FMD normalized for shear.

1775 Values are presented as mean ± SD. *** Indicates significant differences. AUC = area under

1776 the curve; FMD = flow-mediated dilation.

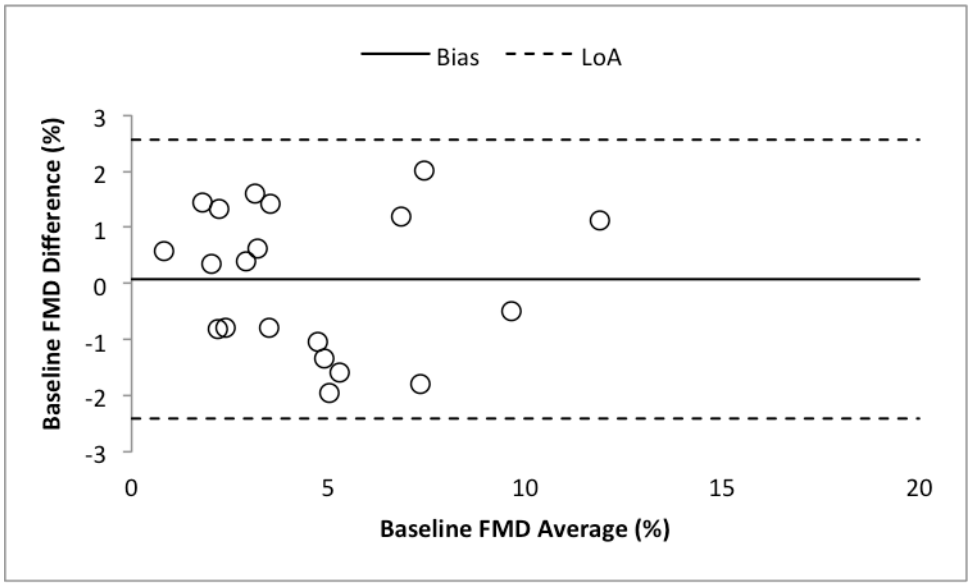
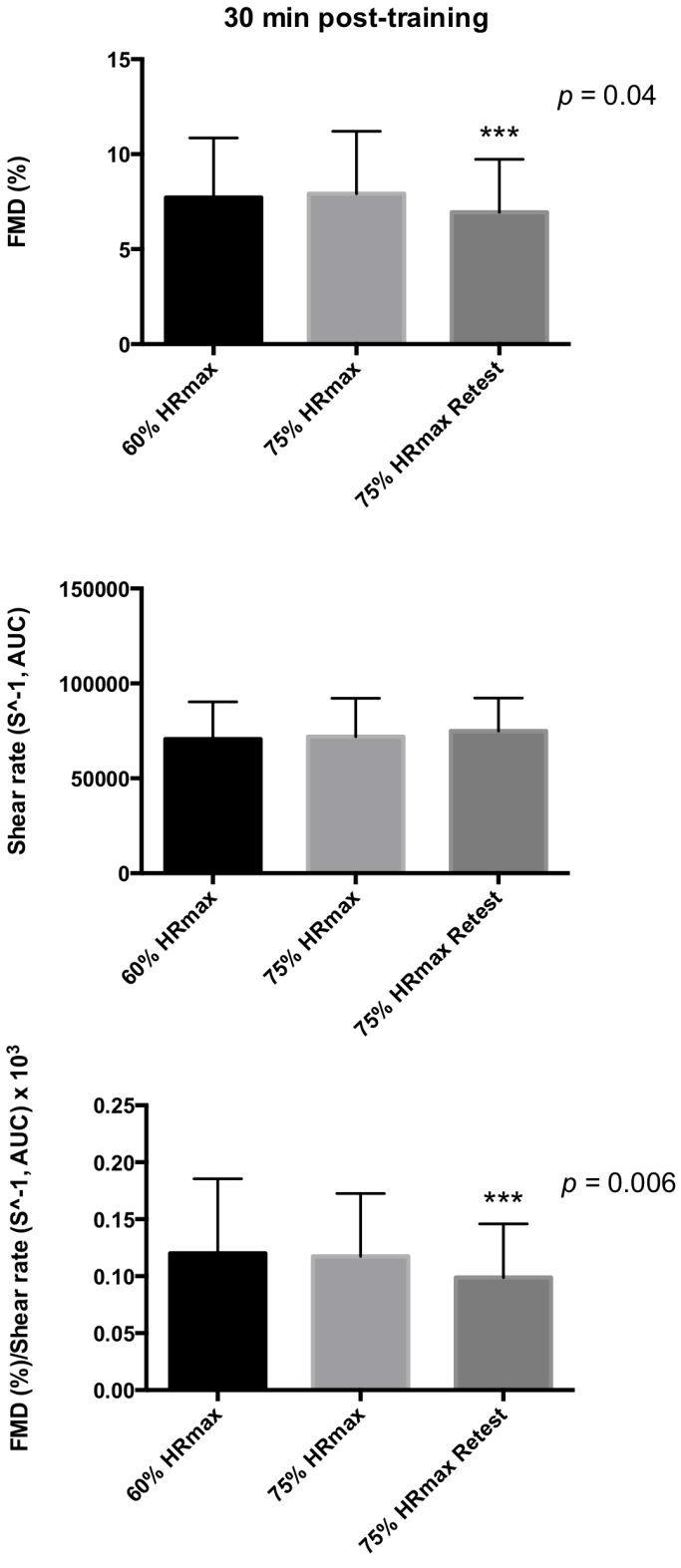


Figure S6. Bland-Altman plot of FMD% immediately post-training. Bias (mean difference between measurements) = 0.08%; LoA (95% limits of agreement, bias ± 1.96*SD) = 2.56% (high) and -2.41% (low).



1793

1794 Figure S7. 30 min post-training FMD, shear rate AUC, and FMD normalized for shear. Values

1795 are presented as mean ± SD. *** Indicates significant differences. AUC = area under the curve;

1796 FMD = flow-mediated dilation.

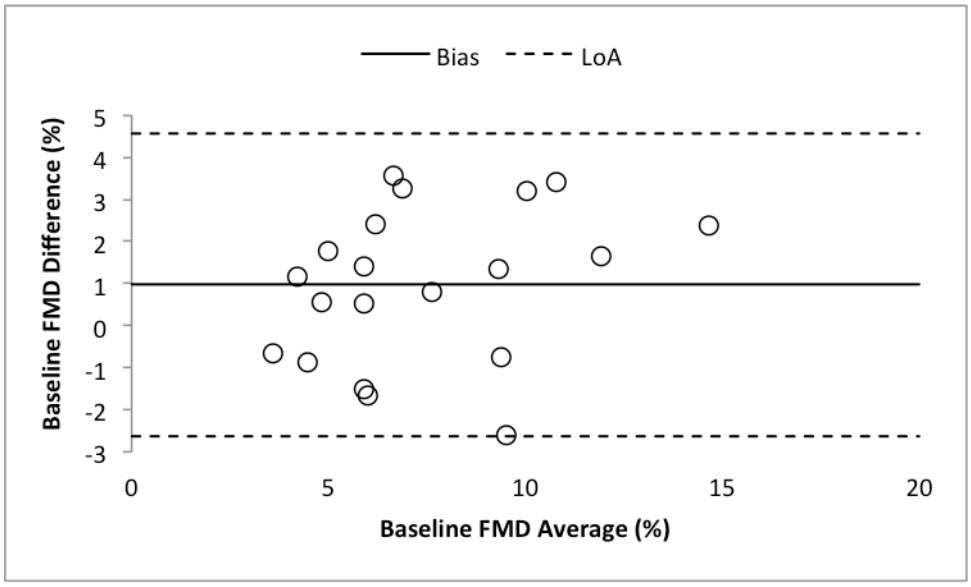
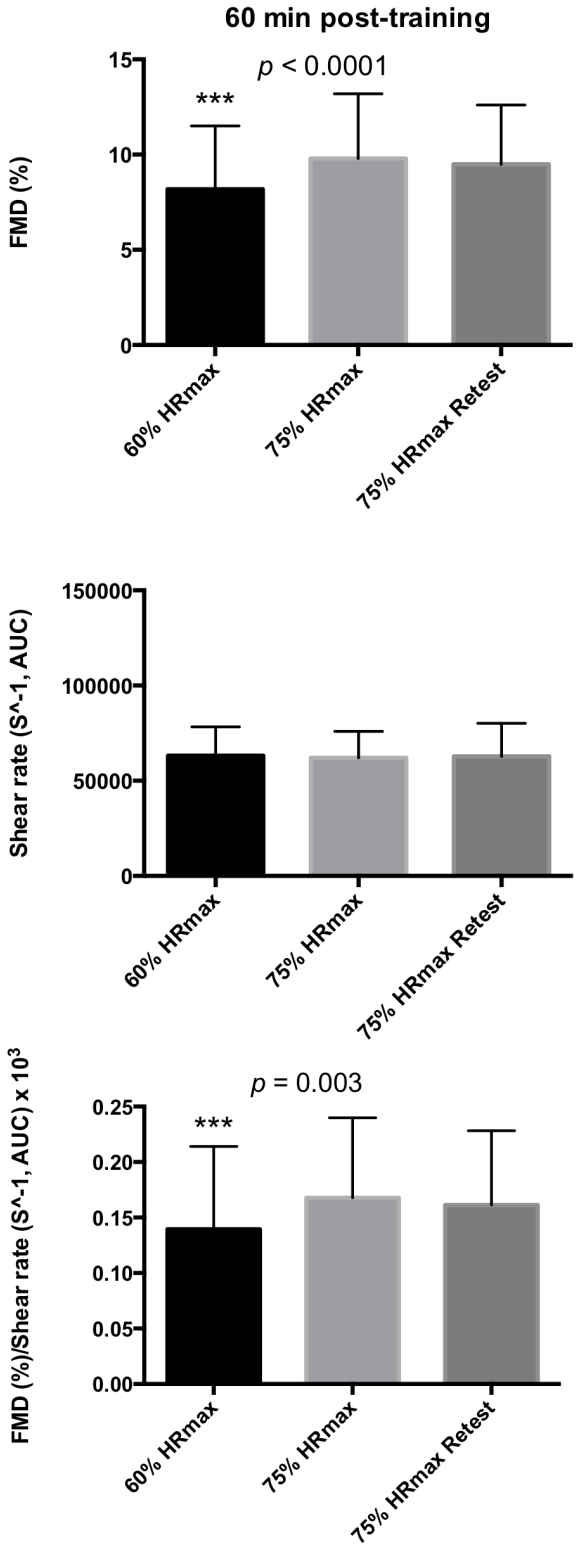


Figure S8. Bland-Altman plot of FMD% 30 minutes post-training. Bias (mean difference between measurements) = 0.97%; LoA (95% limits of agreement, bias ± 1.96*SD) = 4.56% (high) and -2.62% (low).



1809

1810 Figure S9. 60 min post-training FMD, shear rate AUC, and FMD normalized for shear. Values

1811 are presented as mean ± SD. *** Indicates significant differences. AUC = area under the curve;

1812 FMD = flow-mediated dilation.

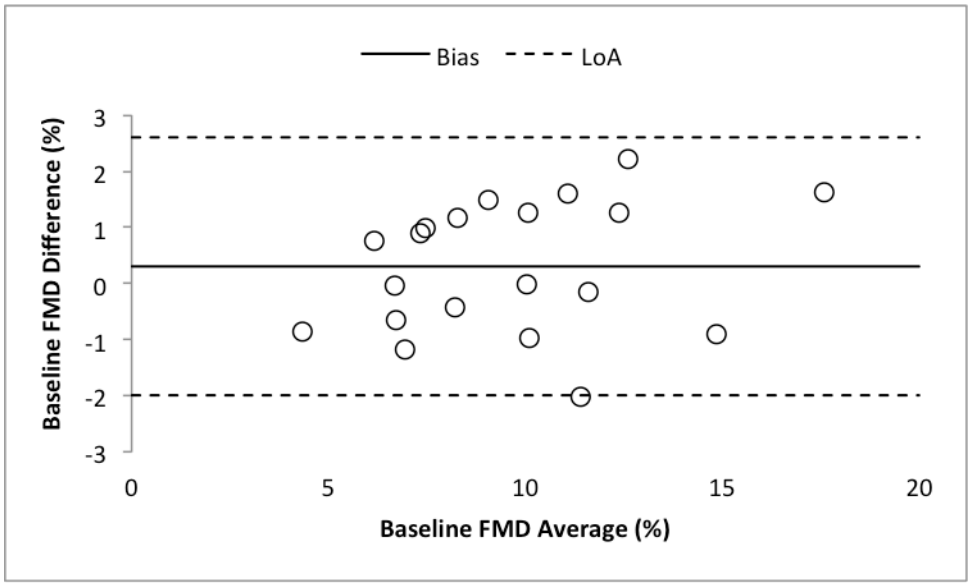


Figure S10. Bland-Altman plot of FMD% 60 minutes post-training. Bias (mean difference between measurements) = 0.31%; LoA (95% limits of agreement, bias ± 1.96*SD) = 2.60% (high) and -1.99% (low).

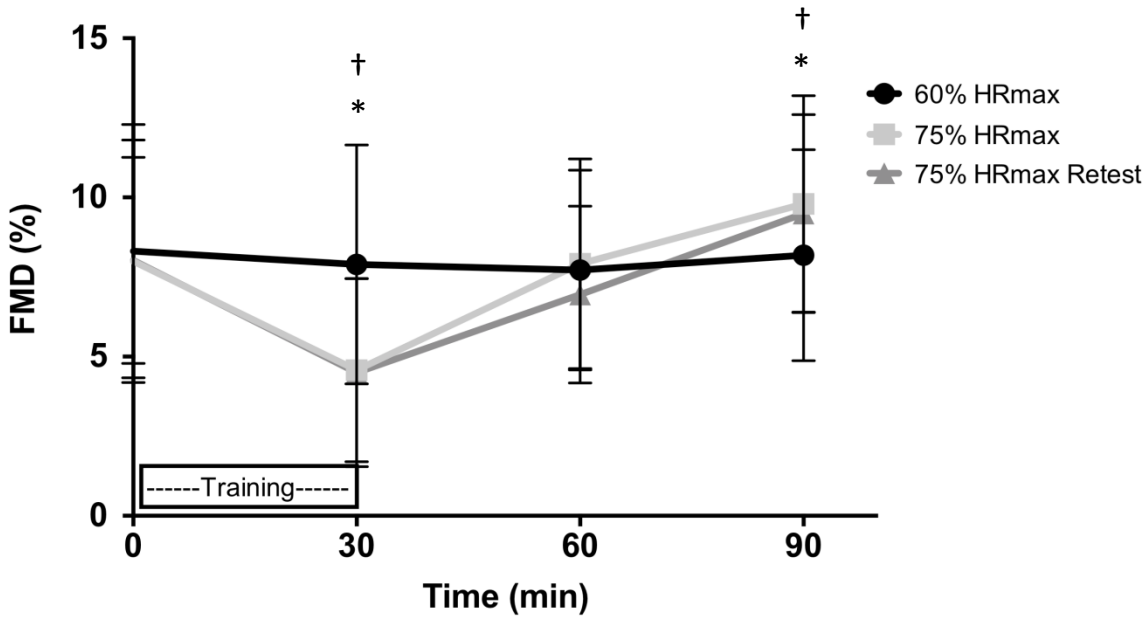
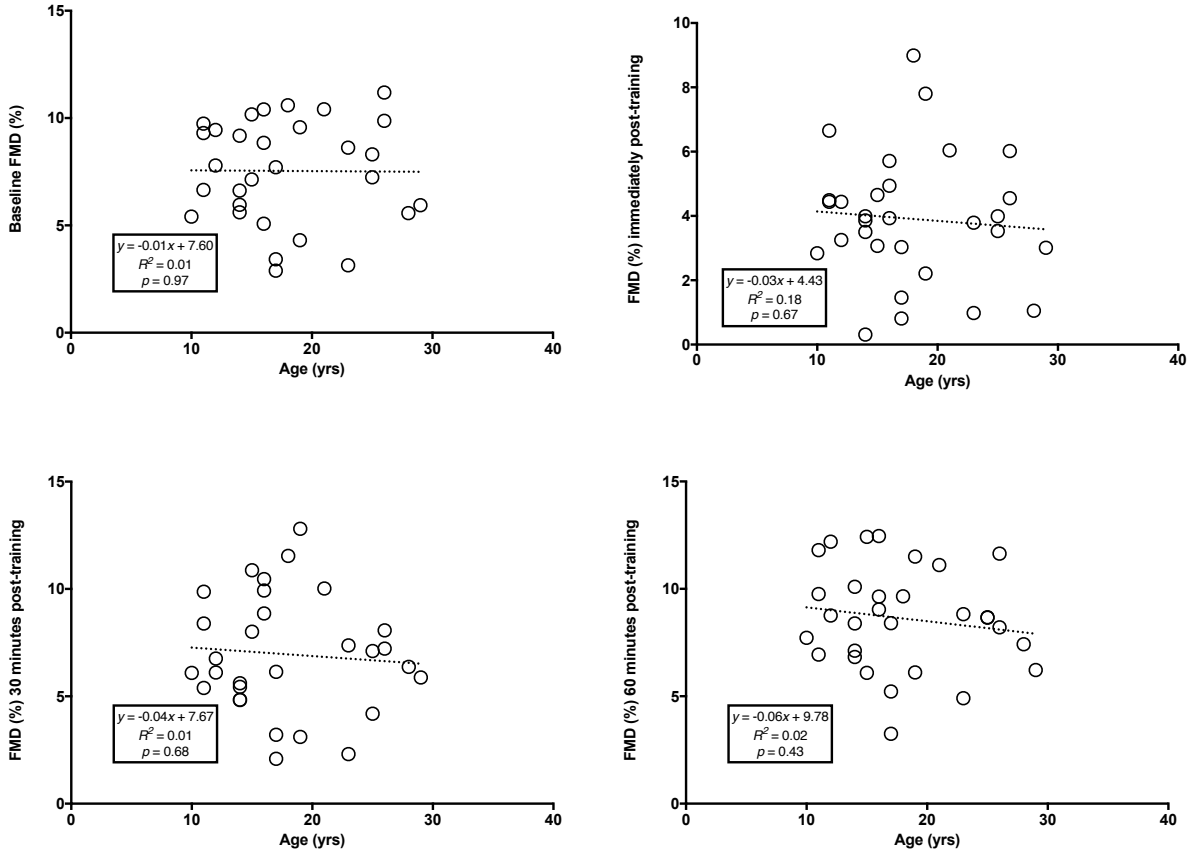
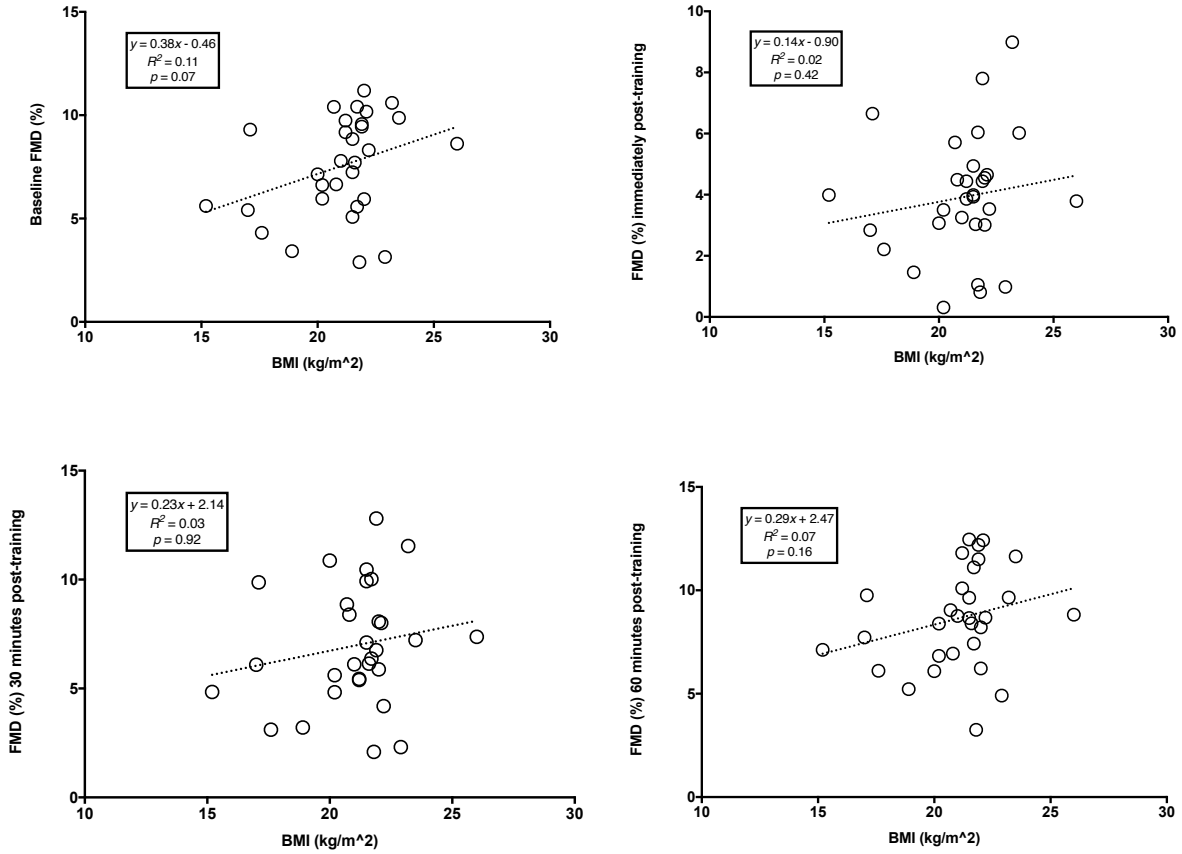


Figure S11. FMD time-course (pre- and post-training). Values are presented as mean ± SD. AUC = area under the curve; FMD = flow-mediated dilation. * 75% HR_{max} significantly different compared 60% HR_{max}. † 75% HR_{max} and 75% HR_{max} retest significantly different compared to baseline.



1847

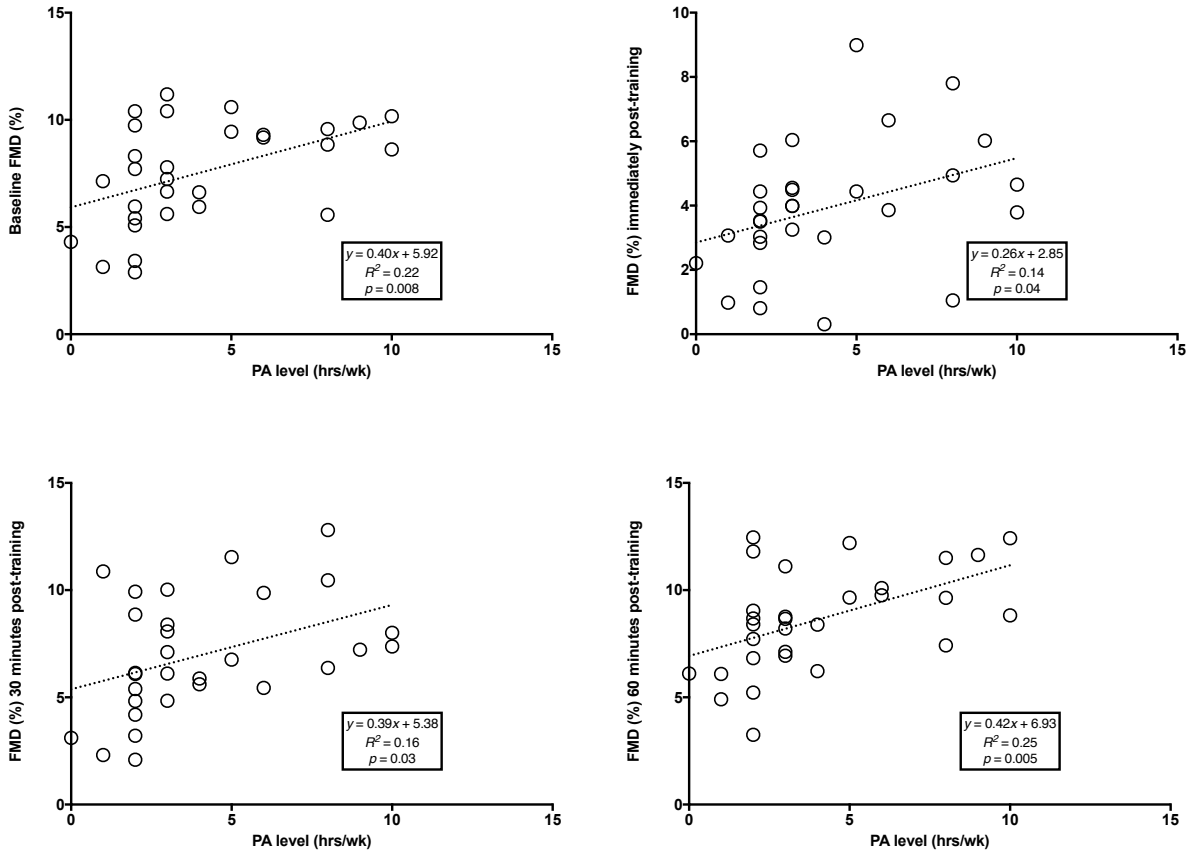
1848 Figure S12. Linear regression analysis of FMD% pre- and post-training related to age in years.



1849

1850 Figure S13. Linear regression analysis of FMD% pre- and post-training related to body-mass

1851 index (BMI, kg/m^2).

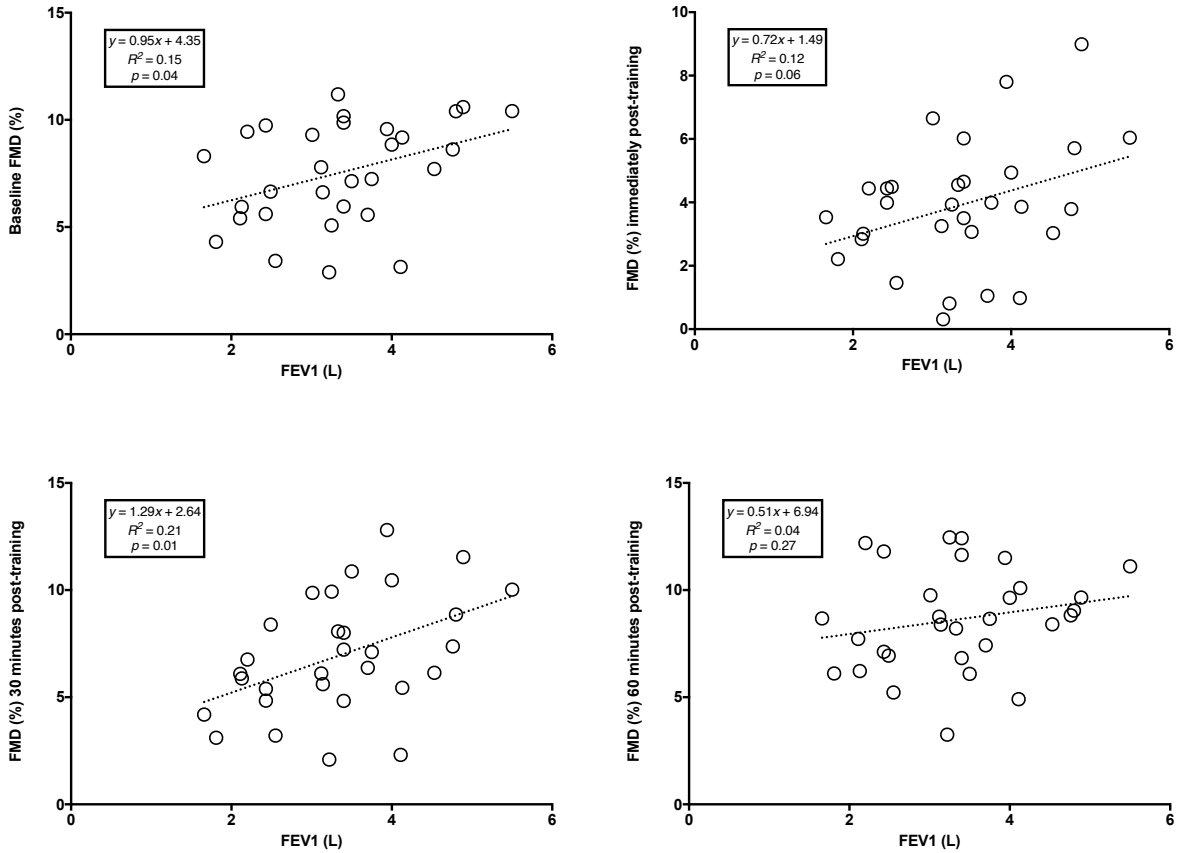


1852

1853 Figure S14. Linear regression analysis of FMD% pre- and post-training related to physical

1854 activity levels (PA level, hr/wk).

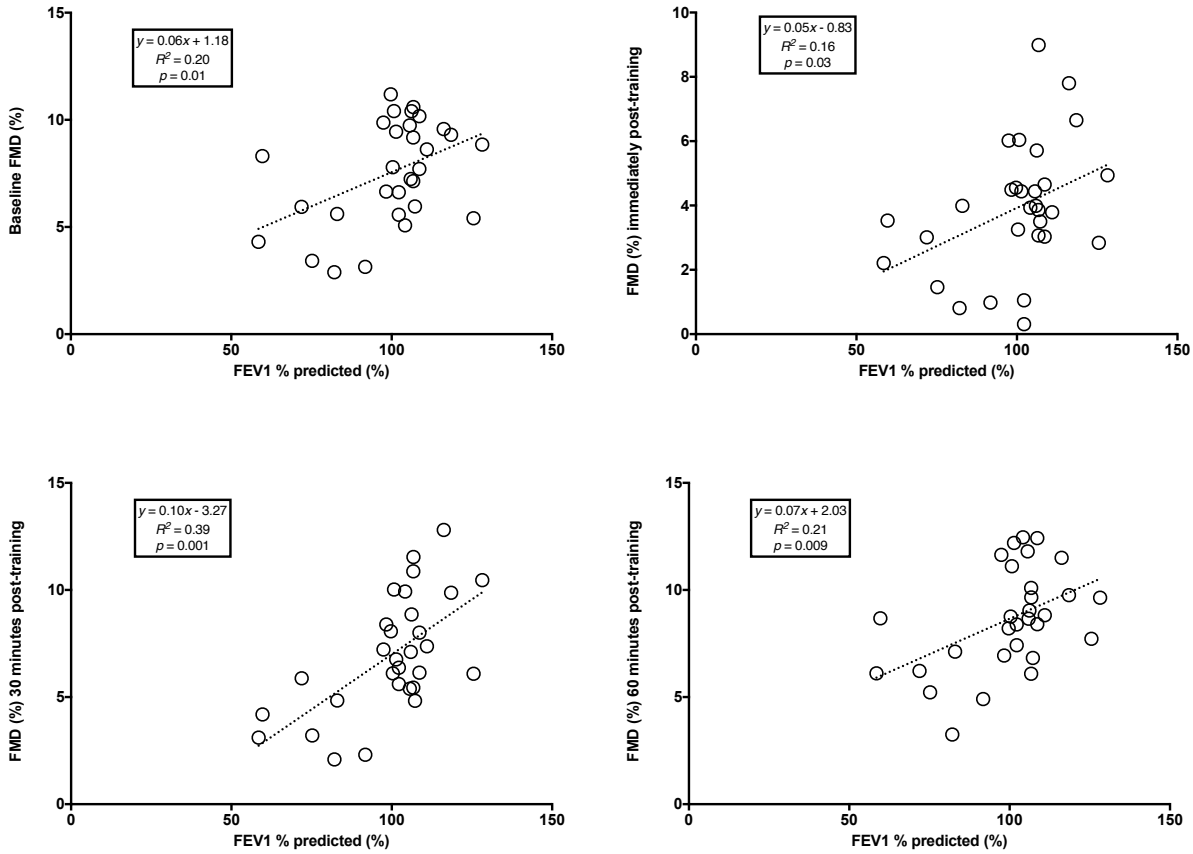
1855



1856

1857 Figure S15. Linear regression analysis of FMD% pre- and post-training related to forced

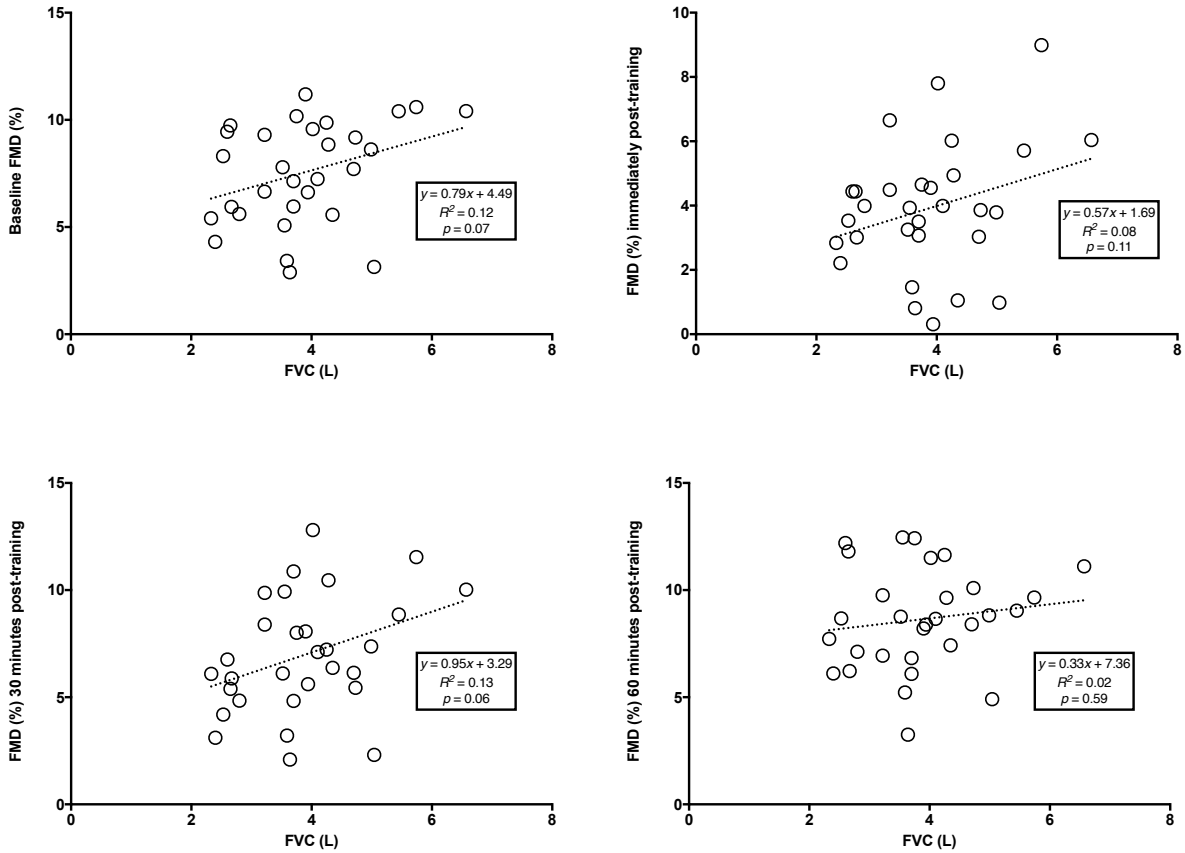
1858 expiratory volume in one second (FEV1, L).



1859

1860 Figure S16. Linear regression analysis of FMD% pre- and post-training related to percent of

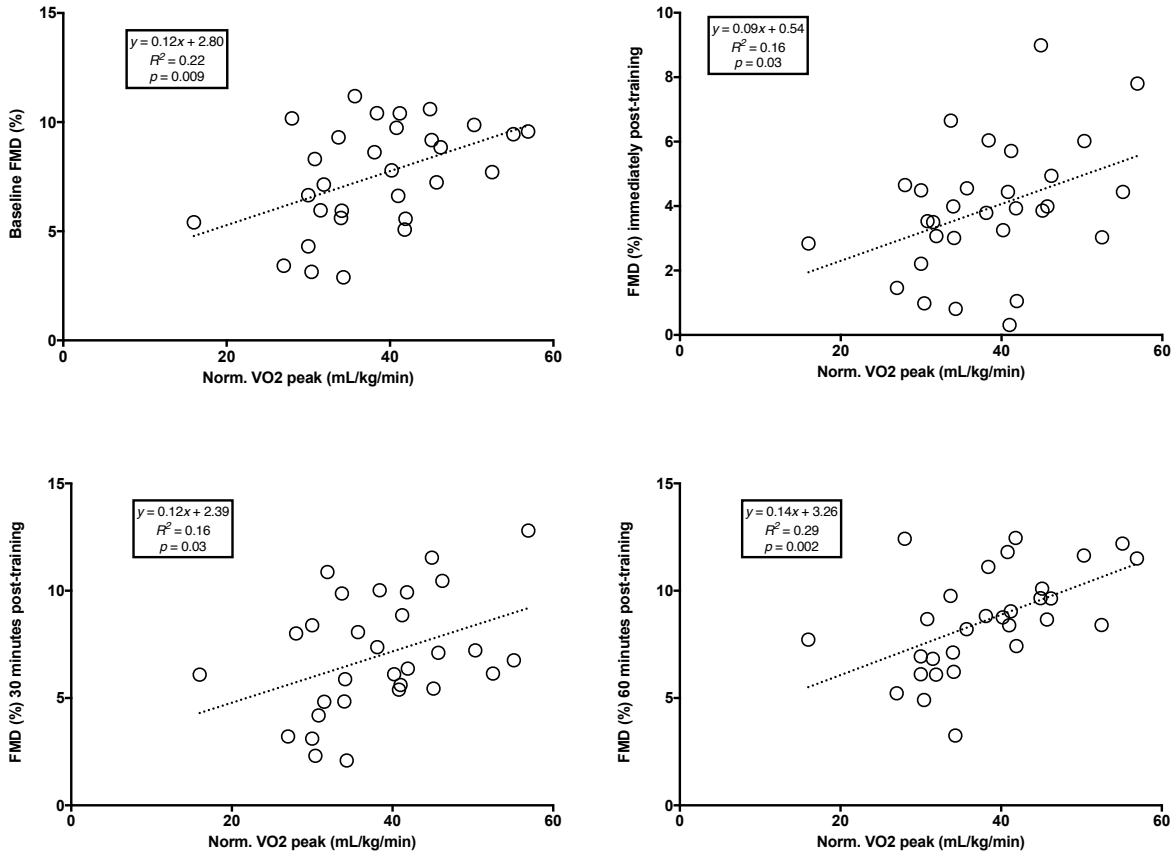
1861 predicted FEV1 (%).



1862

1863 Figure S17. Linear regression analysis of FMD% pre- and post-training related to forced vital

1864 capacity (FVC, L).



1865

1866 Figure S18. Linear regression analysis of FMD% pre- and post-training related to peak rate of

1867 oxygen consumption measured during incremental exercise (VO_2 peak, mL/kg/min).

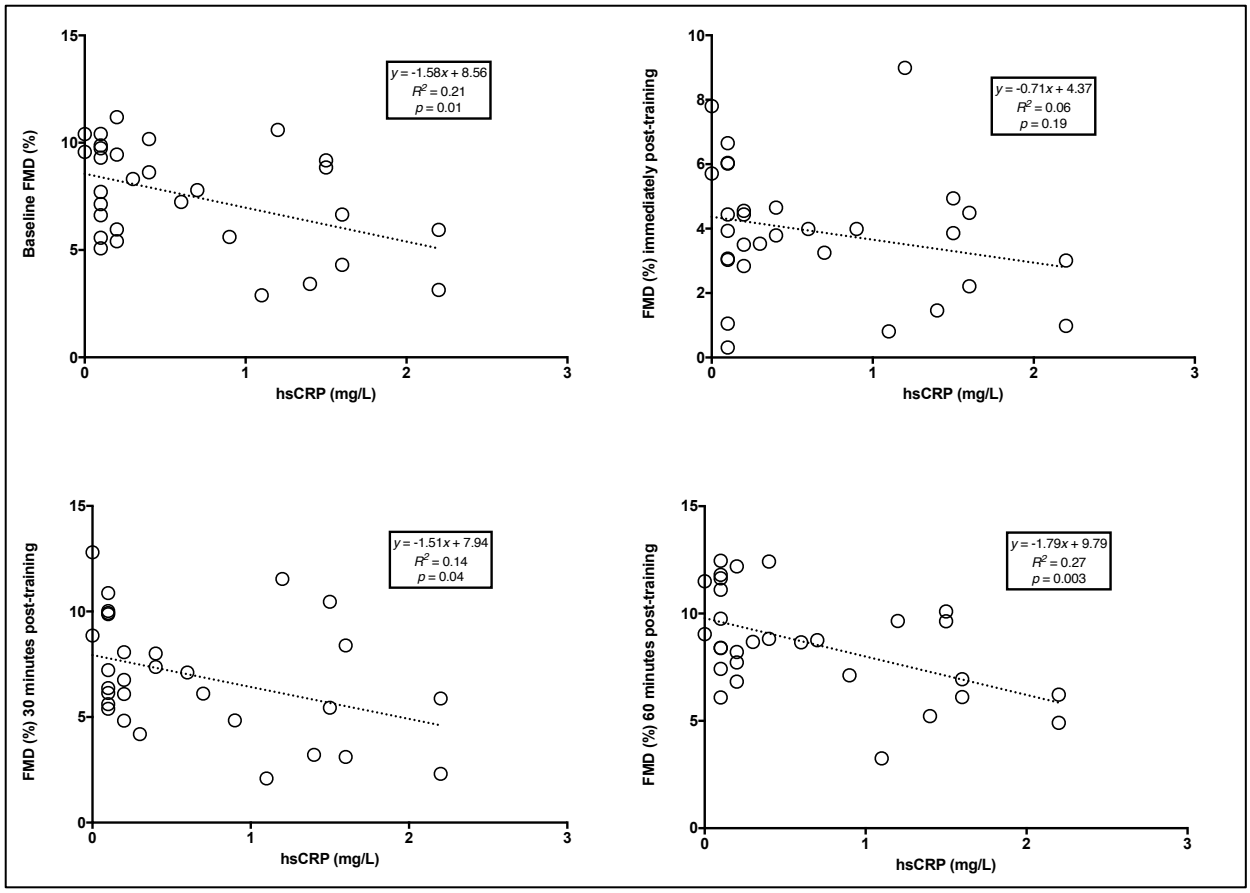


Figure S19. Linear regression analysis of FMD% pre- and post-training related to high sensitivity C-reactive protein (hsCRP, mg/L).

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compared 60% HR_{max}. † 75% HR_{max} and 75% HR_{max} retest significantly different compared to baseline.

Figure S12. Linear regression analysis of FMD% pre- and post-training related to age in years.

Figure S13. Linear regression analysis of FMD% pre- and post-training related to body-mass index (BMI, kg/m^2).

Figure S14. Linear regression analysis of FMD% pre- and post-training related to physical activity levels (PA level, hr/wk).

Figure S15. Linear regression analysis of FMD% pre- and post-training related to forced expiratory volume in one second (FEV1, L).

Figure S16. Linear regression analysis of FMD% pre- and post-training related to percent of predicted FEV1 (%).

Figure S17. Linear regression analysis of FMD% pre- and post-training related to forced vital capacity (FVC, L).

Figure S18. Linear regression analysis of FMD% pre- and post-training related to peak rate of oxygen consumption measured during incremental exercise (VO_2 peak, mL/kg/min).

Figure S19. Linear regression analysis of FMD% pre- and post-training related to high sensitivity C-reactive protein (hsCRP, mg/L).

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Affidavits

According to the doctoral degree regulations (§ 4 (2), sentences No. 4 and 7) of the Faculty of Human Sciences, University of Potsdam: I hereby declare that this thesis entitled “The acute effect of exercise on flow-mediated dilation in young people with cystic fibrosis” is the original work of the author. I did not receive any help or support from commercial consultants. All sources and/or materials applied are listed and specified in the thesis. All parts or single sentences which have been taken analogously or literally from other sources are identified as citations. Furthermore, I declare that this thesis or parts thereof have not yet been submitted for a doctoral degree to this or any other institution neither in identical nor in similar form.

Potsdam, 05.11.2018 _____ *Michael Rector*

Place, Date Michael Rector

References

1. D A. Cystic fibrosis of the pancreas and its relation to celiac disease Am J Dis Child. 1938;56:pp. 344-99.
2. Farber S, Shwachman H, Maddock CL. Pancreatic Function and Disease in Early Life. I. Pancreatic Enzyme Activity and the Celiac Syndrome. J Clin Invest. 1943;22(6):827-38.
3. Andersen DH, Hodges RG. Celiac syndrome; genetics of cystic fibrosis of the pancreas, with a consideration of etiology. Am J Dis Child. 1946;72:62-80.
4. Riordan JR, Rommens JM, Kerem B, Alon N, Rozmahel R, Grzelczak Z, et al. Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. Science. 1989;245(4922):1066-73.
5. Welsh M, Ramsey B, Accurso F, Cutting G. Cystic Fibrosis. In: Scriver CR ea, editors., editor. New York: McGraw-Hill; 2001.
6. Registry CFFP. 2011 Annual Data Report. Bethesda, Maryland Cystic Fibrosis Foundation; 2012.
7. Goss CH, Rosenfeld M. Update on cystic fibrosis epidemiology. Curr Opin Pulm Med. 2004;10(6):510-4.
8. Farrell PM. The prevalence of cystic fibrosis in the European Union. J Cyst Fibros. 2008;7(5):450-3.
9. Orenstein D, Rosenstein, B, Stern, R. Cystic fibrosis: medical care. Philadelphia, PA: Lippincott Williams & Wilkins; 2000.
10. Mehta G, Macek M, Jr., Mehta A, European Registry Working G. Cystic fibrosis across Europe: EuroCareCF analysis of demographic data from 35 countries. J Cyst Fibros. 2010;9 Suppl 2:S5-S21.
11. Dodge JA, Lewis PA, Stanton M, Wilsher J. Cystic fibrosis mortality and survival in the UK: 1947-2003. Eur Respir J. 2007;29(3):522-6.
12. Hurley MN, McKeever TM, Prayle AP, Fogarty AW, Smyth AR. Rate of improvement of CF life expectancy exceeds that of general population--observational death registration study. J Cyst Fibros. 2014;13(4):410-5.
13. Parkins MD, Parkins VM, Rendall JC, Elborn S. Changing epidemiology and clinical issues arising in an ageing cystic fibrosis population. Ther Adv Respir Dis. 2011;5(2):105-19.
14. Rowe SM, Heltshe SL, Gonska T, Donaldson SH, Borowitz D, Gelfond D, et al. Clinical mechanism of the cystic fibrosis transmembrane conductance regulator potentiator ivacaftor in G551D-mediated cystic fibrosis. Am J Respir Crit Care Med. 2014;190(2):175-84.
15. McCormick J, Mehta G, Olesen HV, Viviani L, Macek M, Jr., Mehta A, et al. Comparative demographics of the European cystic fibrosis population: a cross-sectional database analysis. Lancet. 2010;375(9719):1007-13.
16. Rommens JM, Iannuzzi MC, Kerem B, Drumm ML, Melmer G, Dean M, et al. Identification of the cystic fibrosis gene: chromosome walking and jumping. Science. 1989;245(4922):1059-65.
17. Kerem B, Rommens JM, Buchanan JA, Markiewicz D, Cox TK, Chakravarti A, et al. Identification of the cystic fibrosis gene: genetic analysis. Science. 1989;245(4922):1073-80.
18. Institute NHGR. International Consortium Completes Human Genome Project. 2015.
19. Boucher RC, Stutts MJ, Knowles MR, Cantley L, Gatzy JT. Na+ transport in cystic fibrosis respiratory epithelia. Abnormal basal rate and response to adenylate cyclase activation. J Clin Invest. 1986;78(5):1245-52.
20. Winpenny JP, McAlroy HL, Gray MA, Argent BE. Protein kinase C regulates the magnitude and stability of CFTR currents in pancreatic duct cells. Am J Physiol. 1995;268(4 Pt 1):C823-8.
21. Pezzulo AA, Tang XX, Hoegger MJ, Abou Alaiwa MH, Ramachandran S, Moninger TO, et al. Reduced airway surface pH impairs bacterial killing in the porcine cystic fibrosis lung. Nature. 2012;487(7405):109-13.
22. Tang XX, Ostedgaard LS, Hoegger MJ, Moninger TO, Karp PH, McMenimen JD, et al. Acidic pH increases airway surface liquid viscosity in cystic fibrosis. J Clin Invest. 2016;126(3):879-91.

23. Rogan MP, Stoltz DA, Hornick DB. Cystic fibrosis transmembrane conductance regulator intracellular processing, trafficking, and opportunities for mutation-specific treatment. Chest. 2011;139(6):1480-90.
24. Gibson-Corley KN, Meyerholz DK, Engelhardt JF. Pancreatic pathophysiology in cystic fibrosis. J Pathol. 2016;238(2):311-20.
25. Stoltz DA, Meyerholz DK, Welsh MJ. Origins of cystic fibrosis lung disease. N Engl J Med. 2015;372(16):1574-5.
26. Le C, McCrary HC, Chang E. Cystic Fibrosis Sinusitis. Adv Otorhinolaryngol. 2016;79:29-37.
27. Bernstein ML, McCusker MM, Grant-Kels JM. Cutaneous manifestations of cystic fibrosis. Pediatr Dermatol. 2008;25(2):150-7.
28. Kobelska-Dubiel N, Klincewicz B, Cichy W. Liver disease in cystic fibrosis. Prz Gastroenterol. 2014;9(3):136-41.
29. De Lisle RC, Borowitz D. The cystic fibrosis intestine. Cold Spring Harb Perspect Med. 2013;3(9):a009753.
30. Seale TW, Flux M, Rennert OM. Reproductive defects in patients of both sexes with cystic fibrosis: a review. Ann Clin Lab Sci. 1985;15(2):152-8.
31. Lamhonwah AM, Bear CE, Huan LJ, Kim Chiaw P, Ackerley CA, Tein I. Cystic fibrosis transmembrane conductance regulator in human muscle: Dysfunction causes abnormal metabolic recovery in exercise. Ann Neurol. 2010;67(6):802-8.
32. Michoud MC, Robert R, Hassan M, Moynihan B, Haston C, Govindaraju V, et al. Role of the cystic fibrosis transmembrane conductance channel in human airway smooth muscle. Am J Respir Cell Mol Biol. 2009;40(2):217-22.
33. Shead EF, Haworth CS, Condliffe AM, McKeon DJ, Scott MA, Compston JE. Cystic fibrosis transmembrane conductance regulator (CFTR) is expressed in human bone. Thorax. 2007;62(7):650-1.
34. Guo Y, Su M, McNutt MA, Gu J. Expression and distribution of cystic fibrosis transmembrane conductance regulator in neurons of the human brain. J Histochem Cytochem. 2009;57(12):1113-20.
35. Armstrong DS, Grimwood K, Carlin JB, Carzino R, Gutierrez JP, Hull J, et al. Lower airway inflammation in infants and young children with cystic fibrosis. Am J Respir Crit Care Med. 1997;156(4 Pt 1):1197-204.
36. Balough K, McCubbin M, Weinberger M, Smits W, Ahrens R, Fick R. The relationship between infection and inflammation in the early stages of lung disease from cystic fibrosis. Pediatr Pulmonol. 1995;20(2):63-70.
37. Khan TZ, Wagener JS, Bost T, Martinez J, Accurso FJ, Riches DW. Early pulmonary inflammation in infants with cystic fibrosis. Am J Respir Crit Care Med. 1995;151(4):1075-82.
38. Rogers CS, Abraham WM, Brogden KA, Engelhardt JF, Fisher JT, McCray PB, Jr., et al. The porcine lung as a potential model for cystic fibrosis. Am J Physiol Lung Cell Mol Physiol. 2008;295(2):L240-63.
39. Chang EH, Pezzulo AA, Meyerholz DK, Potash AE, Wallen TJ, Reznikov LR, et al. Sinus hypoplasia precedes sinus infection in a porcine model of cystic fibrosis. Laryngoscope. 2012;122(9):1898-905.
40. Ostedgaard LS, Meyerholz DK, Chen JH, Pezzulo AA, Karp PH, Rokhlina T, et al. The DeltaF508 mutation causes CFTR misprocessing and cystic fibrosis-like disease in pigs. Sci Transl Med. 2011;3(74):74ra24.
41. Stoltz DA, Meyerholz DK, Pezzulo AA, Ramachandran S, Rogan MP, Davis GJ, et al. Cystic fibrosis pigs develop lung disease and exhibit defective bacterial eradication at birth. Sci Transl Med. 2010;2(29):29ra31.
42. Wilschanski M, Novak I. The cystic fibrosis of exocrine pancreas. Cold Spring Harb Perspect Med. 2013;3(5):a009746.
43. Stalvey MS, Clines GA. Cystic fibrosis-related bone disease: insights into a growing problem. Curr Opin Endocrinol Diabetes Obes. 2013;20(6):547-52.

44. Levesque PC, Hart PJ, Hume JR, Kenyon JL, Horowitz B. Expression of cystic fibrosis transmembrane regulator Cl- channels in heart. Circ Res. 1992;71(4):1002-7.
45. Hart P, Warth JD, Levesque PC, Collier ML, Geary Y, Horowitz B, et al. Cystic fibrosis gene encodes a cAMP-dependent chloride channel in heart. Proc Natl Acad Sci U S A. 1996;93(13):6343-8.
46. Cross CE, Reverri EJ, Morrissey BM. Joining the crowd: cystic fibrosis and cardiovascular disease risk factors. Chest. 2013;143(4):882-4.
47. Wiglesworth FW. Fibrocystic disease of the pancreas. Am J Med Sci. 1946;212(3):351-65.
48. Stern RC, Borkat G, Hirschfeld SS, Boat TF, Matthews LW, Liebman J, et al. Heart failure in cystic fibrosis. Treatment and prognosis of cor pulmonale with failure of the right side of the heart. Am J Dis Child. 1980;134(3):267-72.
49. Goldring RM, Fishman AP, Turino GM, Cohen HI, Denning CR, Andersen DH. Pulmonary Hypertension and Cor Pulmonale in Cystic Fibrosis of the Pancreas. J Pediatr. 1964;65:501-24.
50. Symchych PS. Pulmonary hypertension in cystic fibrosis. A description and morphometric analysis of the pulmonary vasculature. Arch Pathol. 1971;92(6):409-14.
51. Jacobstein MD, Hirschfeld SS, Winnie G, Doershuk C, Liebman J. Ventricular interdependence in severe cystic fibrosis. A two-dimensional echocardiographic study. Chest. 1981;80(4):399-404.
52. Jessup M, Sutton MS, Weber KT, Janicki JS. The effect of chronic pulmonary hypertension on left ventricular size, function, and interventricular septal motion. Am Heart J. 1987;113(5):1114-22.
53. Vizza CD, Lynch JP, Ochoa LL, Richardson G, Trulock EP. Right and left ventricular dysfunction in patients with severe pulmonary disease. Chest. 1998;113(3):576-83.
54. Hortop J, Desmond KJ, Coates AL. The mechanical effects of expiratory airflow limitation on cardiac performance in cystic fibrosis. Am Rev Respir Dis. 1988;137(1):132-7.
55. Moss AJ. The cardiovascular system in cystic fibrosis. Pediatrics. 1982;70(5):728-41.
56. Ulrich M, Worlitzsch D, Viglio S, Siegmann N, Iadarola P, Shute JK, et al. Alveolar inflammation in cystic fibrosis. J Cyst Fibros. 2010;9(3):217-27.
57. Davies JC, Alton EW, Bush A. Cystic fibrosis. BMJ. 2007;335(7632):1255-9.
58. Cantin AM, White TB, Cross CE, Forman HJ, Sokol RJ, Borowitz D. Antioxidants in cystic fibrosis. Conclusions from the CF antioxidant workshop, Bethesda, Maryland, November 11-12, 2003. Free Radic Biol Med. 2007;42(1):15-31.
59. Hull J, Vervaart P, Grimwood K, Phelan P. Pulmonary oxidative stress response in young children with cystic fibrosis. Thorax. 1997;52(6):557-60.
60. van der Vliet A, Eiserich JP, Marelich GP, Halliwell B, Cross CE. Oxidative stress in cystic fibrosis: does it occur and does it matter? Adv Pharmacol. 1997;38:491-513.
61. Ziady AG, Hansen J. Redox balance in cystic fibrosis. Int J Biochem Cell Biol. 2014;52:113-23.
62. Cohen-Cymberknoh M, Kerem E, Ferkol T, Elizur A. Airway inflammation in cystic fibrosis: molecular mechanisms and clinical implications. Thorax. 2013;68(12):1157-62.
63. Reverri EJ, Morrissey BM, Cross CE, Steinberg FM. Inflammation, oxidative stress, and cardiovascular disease risk factors in adults with cystic fibrosis. Free Radic Biol Med. 2014;76:261-77.
64. Elston C, Geddes D. Inflammation in cystic fibrosis--when and why? Friend or foe? Semin Respir Crit Care Med. 2007;28(3):286-94.
65. Berg AH, Scherer PE. Adipose tissue, inflammation, and cardiovascular disease. Circ Res. 2005;96(9):939-49.
66. Maclay JD, MacNee W. Cardiovascular disease in COPD: mechanisms. Chest. 2013;143(3):798-807.
67. Mahmood SS, Levy D, Vasan RS, Wang TJ. The Framingham Heart Study and the epidemiology of cardiovascular disease: a historical perspective. Lancet. 2014;383(9921):999-1008.
68. Dawber TR, Meadors GF, Moore FE, Jr. Epidemiological approaches to heart disease: the Framingham Study. Am J Public Health Nations Health. 1951;41(3):279-81.
69. Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet. 2012;380(9859):2095-128.

70. A. KOPDL. Projections of Cardiovascular Disease Prevalence and Costs: 2015–2035 Technical Report. 2016 Nov. Report No.: RTI Project Number 0214680.003.001.001.
71. (Publisher) RKI. Health in Germany. Federal Health Reporting. Berlin; 2008.
72. Yang L, Wu M, Cui B, Xu J. Economic burden of cardiovascular diseases in China. Expert Rev Pharmacoecon Outcomes Res. 2008;8(4):349-56.
73. Ozcelik N, Shell R, Holtzlander M, Cua C. Decreased right ventricular function in healthy pediatric cystic fibrosis patients versus non-cystic fibrosis patients. Pediatr Cardiol. 2013;34(1):159-64.
74. Poore S, Berry B, Eidson D, McKie KT, Harris RA. Evidence of vascular endothelial dysfunction in young patients with cystic fibrosis. Chest. 2013;143(4):939-45.
75. Rodriguez-Miguelez P, Thomas J, Seigler N, Crandall R, McKie KT, Forseen C, et al. Evidence of microvascular dysfunction in patients with cystic fibrosis. Am J Physiol Heart Circ Physiol. 2016;310(11):H1479-85.
76. Green DJ, Jones H, Thijssen D, Cable NT, Atkinson G. Flow-mediated dilation and cardiovascular event prediction: does nitric oxide matter? Hypertension. 2011;57(3):363-9.
77. Pearson TA, Mensah GA, Alexander RW, Anderson JL, Cannon RO, 3rd, Criqui M, et al. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. Circulation. 2003;107(3):499-511.
78. Hays SR, Ferrando RE, Carter R, Wong HH, Woodruff PG. Structural changes to airway smooth muscle in cystic fibrosis. Thorax. 2005;60(3):226-8.
79. Cook DP, Rector MV, Bouzek DC, Michalski AS, Gansemer ND, Reznikov LR, et al. Cystic Fibrosis Transmembrane Conductance Regulator in Sarcoplasmic Reticulum of Airway Smooth Muscle. Implications for Airway Contractility. Am J Respir Crit Care Med. 2016;193(4):417-26.
80. Meyerholz DK, Stoltz DA, Namati E, Ramachandran S, Pezzulo AA, Smith AR, et al. Loss of cystic fibrosis transmembrane conductance regulator function produces abnormalities in tracheal development in neonatal pigs and young children. Am J Respir Crit Care Med. 2010;182(10):1251-61.
81. De Lisle RC, Meldi L, Mueller R. Intestinal smooth muscle dysfunction develops postnatally in cystic fibrosis mice. J Pediatr Gastroenterol Nutr. 2012;55(6):689-94.
82. Sellers ZM, Kovacs A, Weinheimer CJ, Best PM. Left ventricular and aortic dysfunction in cystic fibrosis mice. J Cyst Fibros. 2013;12(5):517-24.
83. Adam RJ, Hisert KB, Dodd JD, Grogan B, Launspach JL, Barnes JK, et al. Acute administration of ivacaftor to people with cystic fibrosis and a G551D-CFTR mutation reveals smooth muscle abnormalities. JCI Insight. 2016;1(4):e86183.
84. D'Agostino RB, Sr., Vasan RS, Pencina MJ, Wolf PA, Cobain M, Massaro JM, et al. General cardiovascular risk profile for use in primary care: the Framingham Heart Study. Circulation. 2008;117(6):743-53.
85. Andersson C, Vasan RS. Epidemiology of cardiovascular disease in young individuals. Nat Rev Cardiol. 2018;15(4):230-40.
86. Cohn JN, Hoke L, Whitwam W, Sommers PA, Taylor AL, Duprez D, et al. Screening for early detection of cardiovascular disease in asymptomatic individuals. Am Heart J. 2003;146(4):679-85.
87. National Heart L, and Blood Institute; National Institutes of Health; U.S. Department of Health and Human Services. Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents: Summary Report. Oct 2012.
88. Stary HC. Evolution and Progression of Atherosclerotic Lesions in Coronary-Arteries of Children and Young-Adults. Arteriosclerosis. 1989;9(1):I19-I32.
89. Healy B. Endothelial-Cell Dysfunction - an Emerging Endocrinopathy Linked to Coronary-Disease. J Am Coll Cardiol. 1990;16(2):357-8.
90. Celermajer DS, Sorensen KE, Gooch VM, Spiegelhalter DJ, Miller OI, Sullivan ID, et al. Noninvasive Detection of Endothelial Dysfunction in Children and Adults at Risk of Atherosclerosis. Lancet. 1992;340(8828):1111-5.

91. Ras RT, Streppel MT, Draijer R, Zock PL. Flow-mediated dilation and cardiovascular risk prediction: A systematic review with meta-analysis. Int J Cardiol. 2013;168(1):344-51.
92. Joannides R, Haefeli WE, Linder L, Richard V, Bakkali EH, Thuillez C, et al. Nitric-Oxide Is Responsible for Flow-Dependent Dilatation of Human Peripheral Conduit Arteries in-Vivo. Circulation. 1995;91(5):1314-9.
93. Green DJ, Dawson EA, Groenewoud HM, Jones H, Thijssen DH. Is flow-mediated dilation nitric oxide mediated?: A meta-analysis. Hypertension. 2014;63(2):376-82.
94. Thijssen DH, Black MA, Pyke KE, Padilla J, Atkinson G, Harris RA, et al. Assessment of flow-mediated dilation in humans: a methodological and physiological guideline. Am J Physiol Heart Circ Physiol. 2011;300(1):H2-12.
95. Black MA, Cable NT, Thijssen DH, Green DJ. Impact of age, sex, and exercise on brachial artery flow-mediated dilatation. Am J Physiol Heart Circ Physiol. 2009;297(3):H1109-16.
96. Hashimoto M, Akishita M, Eto M, Ishikawa M, Kozaki K, Toba K, et al. Modulation of endothelium-dependent flow-mediated dilatation of the brachial artery by sex and menstrual cycle. Circulation. 1995;92(12):3431-5.
97. Savvidou MD, Kametas NA, Donald AE, Nicolaides KH. Non-invasive assessment of endothelial function in normal pregnancy. Ultrasound Obstet Gynecol. 2000;15(6):502-7.
98. Moreau KL, Hildreth KL, Meditz AL, Deane KD, Kohrt WM. Endothelial function is impaired across the stages of the menopause transition in healthy women. J Clin Endocrinol Metab. 2012;97(12):4692-700.
99. Bau PF, Bau CH, Naujorks AA, Rosito GA, Fuchs FD. Diurnal variation of vascular diameter and reactivity in healthy young men. Braz J Med Biol Res. 2008;41(6):500-3.
100. Bruyndonckx L, Hoymans VY, Van Craenenbroeck AH, Vissers DK, Vrints CJ, Ramet J, et al. Assessment of endothelial dysfunction in childhood obesity and clinical use. Oxid Med Cell Longev. 2013;2013:174782.
101. Gokce N, Holbrook M, Duffy SJ, Demissie S, Cupples LA, Biegelsen E, et al. Effects of race and hypertension on flow-mediated and nitroglycerin-mediated dilation of the brachial artery. Hypertension. 2001;38(6):1349-54.
102. Henry RMA, Ferreira I, Kostense PJ, Dekker JM, Nijpels G, Heine RJ, et al. Type 2 diabetes is associated with impaired endothelium-dependent, flow-mediated dilation, but impaired glucose metabolism is not - The Hoorn study. Atherosclerosis. 2004;174(1):49-56.
103. Shivalkar B, Dhondt D, Goovaerts I, Van Gaal L, Bartunek J, Van Crombrugge P, et al. Flow mediated dilatation and cardiac function in type 1 diabetes mellitus. Am J Cardiol. 2006;97(1):77-82.
104. Gori T, Muxel S, Damaske A, Radmacher MC, Fasola F, Schaefer S, et al. Endothelial function assessment: flow-mediated dilation and constriction provide different and complementary information on the presence of coronary artery disease. Eur Heart J. 2012;33(3):363-71.
105. Buscemi S, Verga S, Batsis JA, Donatelli M, Tranchina MR, Belmonte S, et al. Acute effects of coffee on endothelial function in healthy subjects. Eur J Clin Nutr. 2010;64(5):483-9.
106. Neunteufl T, Heher S, Kostner K, Mitulovic G, Lehr S, Khoschsorur G, et al. Contribution of nicotine to acute endothelial dysfunction in long-term smokers. J Am Coll Cardiol. 2002;39(2):251-6.
107. Thom NJ, Early AR, Hunt BE, Harris RA, Herring MP. Eating and arterial endothelial function: a meta-analysis of the acute effects of meal consumption on flow-mediated dilation. Obes Rev. 2016;17(11):1080-90.
108. Cooper DC, Ziegler MG, Milic MS, Ancoli-Israel S, Mills PJ, Loredo JS, et al. Endothelial function and sleep: associations of flow-mediated dilation with perceived sleep quality and rapid eye movement (REM) sleep. J Sleep Res. 2014;23(1):84-93.
109. Clarkson P, Montgomery HE, Mullen MJ, Donald AE, Powe AJ, Bull T, et al. Exercise training enhances endothelial function in young men. J Am Coll Cardiol. 1999;33(5):1379-85.
110. Early KS, Stewart A, Johannsen N, Lavie CJ, Thomas JR, Welsch M. The Effects of Exercise Training on Brachial Artery Flow-Mediated Dilation: A Meta-analysis. J Cardiopulm Rehabil Prev. 2017;37(2):77-89.

111. Abbott RA, Harkness MA, Davies PS. Correlation of habitual physical activity levels with flow-mediated dilation of the brachial artery in 5-10 year old children. Atherosclerosis. 2002;160(1):233-9.
112. Farpour-Lambert NJ, Aggoun Y, Marchand LM, Martin XE, Herrmann FR, Beghetti M. Physical activity reduces systemic blood pressure and improves early markers of atherosclerosis in pre-pubertal obese children. J Am Coll Cardiol. 2009;54(25):2396-406.
113. Trigona B, Aggoun Y, Maggio A, Martin XE, Marchand LM, Beghetti M, et al. Preclinical noninvasive markers of atherosclerosis in children and adolescents with type 1 diabetes are influenced by physical activity. J Pediatr. 2010;157(4):533-9.
114. Franzoni F, Ghiadoni L, Galetta F, Plantinga Y, Lubrano V, Huang Y, et al. Physical activity, plasma antioxidant capacity, and endothelium-dependent vasodilation in young and older men. Am J Hypertens. 2005;18(4 Pt 1):510-6.
115. Luk TH, Dai YL, Siu CW, Yiu KH, Chan HT, Fong DYT, et al. Habitual physical activity is associated with endothelial function and endothelial progenitor cells in patients with stable coronary artery disease. Eur J Cardiov Prev R. 2009;16(4):464-71.
116. Payvandi L, Dyer A, McPherson D, Ades P, Stein J, Liu K, et al. Physical activity during daily life and brachial artery flow-mediated dilation in peripheral arterial disease. Vasc Med. 2009;14(3):193-201.
117. Suboc TB, Strath SJ, Dharmashankar K, Coulliard A, Miller N, Wang J, et al. Relative importance of step count, intensity, and duration on physical activity's impact on vascular structure and function in previously sedentary older adults. J Am Heart Assoc. 2014;3(1):e000702.
118. Celermajer DS. Endothelial dysfunction: does it matter? Is it reversible? J Am Coll Cardiol. 1997;30(2):325-33.
119. Gielen S, Schuler G, Adams V. Cardiovascular effects of exercise training: molecular mechanisms. Circulation. 2010;122(12):1221-38.
120. Davies PF. Hemodynamic shear stress and the endothelium in cardiovascular pathophysiology. Nat Clin Pract Cardiovasc Med. 2009;6(1):16-26.
121. Thompson PD, Crouse SF, Goodpaster B, Kelley D, Moyna N, Pescatello L. The acute versus the chronic response to exercise. Med Sci Sports Exerc. 2001;33(6 Suppl):S438-45; discussion S52-3.
122. Gonzales JU, Thompson BC, Thistlethwaite JR, Scheuermann BW. Association between exercise hemodynamics and changes in local vascular function following acute exercise. Appl Physiol Nutr Me. 2011;36(1):137-44.
123. Padilla J, Simmons GH, Bender SB, Arce-Esquivel AA, Whyte JJ, Laughlin MH. Vascular effects of exercise: endothelial adaptations beyond active muscle beds. Physiology (Bethesda). 2011;26(3):132-45.
124. Dawson EA, Green DJ, Cable NT, Thijssen DH. Effects of acute exercise on flow-mediated dilatation in healthy humans. J Appl Physiol (1985). 2013;115(11):1589-98.
125. de Jong W, Kaptein AA, van der Schans CP, Mannes GP, van Aalderen WM, Grevink RG, et al. Quality of life in patients with cystic fibrosis. Pediatr Pulmonol. 1997;23(2):95-100.
126. Nixon PA, Orenstein DM, Kelsey SF, Doershuk CF. The prognostic value of exercise testing in patients with cystic fibrosis. N Engl J Med. 1992;327(25):1785-8.
127. Hebestreit H, Kieser S, Rudiger S, Schenk T, Junge S, Hebestreit A, et al. Physical activity is independently related to aerobic capacity in cystic fibrosis. European Respiratory Journal. 2006;28(4):734-9.
128. Stevens D, Oades PJ, Armstrong N, Williams CA. A survey of exercise testing and training in UK cystic fibrosis clinics. J Cyst Fibros. 2010;9(5):302-6.
129. Almajed A, Lands LC. The evolution of exercise capacity and its limiting factors in cystic fibrosis. Paediatr Respir Rev. 2012;13(4):195-9.
130. Ruf K, Winkler B, Hebestreit A, Gruber W, Hebestreit H. Risks associated with exercise testing and sports participation in cystic fibrosis. Journal of Cystic Fibrosis. 2010;9(5):339-45.
131. Stanghelle JK. Physical Exercise for Patients with Cystic-Fibrosis - a Review. Int J Sports Med. 1988;9:6-18.

132. McKone EF, Barry SC, FitzGerald MX, Gallagher CG. Reproducibility of maximal exercise ergometer testing in patients with cystic fibrosis. Chest. 1999;116(2):363-8.
133. Wilkes DL, Schneiderman JE, Nguyen T, Heale L, Moola F, Ratjen F, et al. Exercise and physical activity in children with cystic fibrosis. Paediatr Respir Rev. 2009;10(3):105-9.
134. van de Weert-van Leeuwen PB, Arets HG, van der Ent CK, Beekman JM. Infection, inflammation and exercise in cystic fibrosis. Respir Res. 2013;14:32.
135. Cholewa JM, Paolone VJ. Influence of Exercise on Airway Epithelia in Cystic Fibrosis: A Review. Med Sci Sports Exerc. 2012;44(7):1219-26.
136. Salh W, Bilton D, Dodd M, Webb AK. Effect of Exercise and Physiotherapy in Aiding Sputum Expectoration in Adults with Cystic-Fibrosis. Thorax. 1989;44(12):1006-8.
137. Prasad SA, Cerny FJ. Factors that influence adherence to exercise and their effectiveness: Application to cystic fibrosis. Pediatr Pulmonol. 2002;34(1):66-72.
138. Radtke T, Nevitt SJ, Hebestreit H, Kriemler S. Physical exercise training for cystic fibrosis. Cochrane Db Syst Rev. 2017(11).
139. Hornig B, Maier V, Drexler H. Physical training improves endothelial function in patients with chronic heart failure. Circulation. 1996;93(2):210-4.
140. Silvestro A, Scopacasa F, Oliva G, de Cristofaro T, Iuliano L, Brevetti G. Vitamin C prevents endothelial dysfunction induced by acute exercise in patients with intermittent claudication. Atherosclerosis. 2002;165(2):277-83.
141. Dawson EA, Cable NT, Green DJ, Thijssen DHJ. Do acute effects of exercise on vascular function predict adaptation to training? Eur J Appl Physiol. 2018;118(3):523-30.
142. Fuchsjager-Mayrl G, Pleiner J, Wiesinger GF, Sieder AE, Quittan M, Nuhr MJ, et al. Exercise training improves vascular endothelial function in patients with type 1 diabetes. Diabetes Care. 2002;25(10):1795-801.
143. Okada S, Hiuge A, Makino H, Nagumo A, Takaki H, Konishi H, et al. Effect of exercise intervention on endothelial function and incidence of cardiovascular disease in patients with type 2 diabetes. J Atheroscler Thromb. 2010;17(8):828-33.
144. Seeger JP, Thijssen DH, Noordam K, Cranen ME, Hopman MT, Nijhuis-van der Sanden MW. Exercise training improves physical fitness and vascular function in children with type 1 diabetes. Diabetes Obes Metab. 2011;13(4):382-4.
145. Watts K, Beye P, Siafarikas A, O'Driscoll G, Jones TW, Davis EA, et al. Effects of exercise training on vascular function in obese children. J Pediatr. 2004;144(5):620-5.
146. Cook DP, Rector MV, Bouzek DC, Michalski AS, Gansemer ND, Reznikov LR, et al. Cystic Fibrosis Transmembrane Conductance Regulator in Sarcoplasmic Reticulum of Airway Smooth Muscle Implications for Airway Contractility. Am J Respir Crit Care Med. 2016;193(4):417-26.
147. Standardization of Spirometry, 1994 Update. American Thoracic Society. Am J Respir Crit Care Med. 1995;152(3):1107-36.
148. Hebestreit H, Arets HG, Aurora P, Boas S, Cerny F, Hulzebos EH, et al. Statement on Exercise Testing in Cystic Fibrosis. Respiration. 2015;90(4):332-51.
149. Fletcher GF, Balady GJ, Amsterdam EA, Chaitman B, Eckel R, Fleg J, et al. Exercise standards for testing and training: a statement for healthcare professionals from the American Heart Association. Circulation. 2001;104(14):1694-740.
150. Macfarlane DJ, Wong P. Validity, reliability and stability of the portable Cortex Metamax 3B gas analysis system. Eur J Appl Physiol. 2012;112(7):2539-47.
151. Wasserman KH, J.E.; Sue, D.Y.; Casaburi, R.; Whipp, B.J.; Verheugt, F.W.A. Principles of exercise testing and interpretation. Including pathophysiology and clinical applications. Philadelphia: Lippincott Williams & Wilkins; 1999.
152. Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. J Am Coll Cardiol. 2002;39(2):257-65.

153. Nishiyama SK, Walter Wray D, Berkstresser K, Ramaswamy M, Richardson RS. Limb-specific differences in flow-mediated dilation: the role of shear rate. J Appl Physiol (1985). 2007;103(3):843-51.
154. Padilla J, Johnson BD, Newcomer SC, Wilhite DP, Mickleborough TD, Fly AD, et al. Normalization of flow-mediated dilation to shear stress area under the curve eliminates the impact of variable hyperemic stimulus. Cardiovasc Ultrasound. 2008;6:44.
155. Birk GK, Dawson EA, Batterham AM, Atkinson G, Cable T, Thijssen DH, et al. Effects of exercise intensity on flow mediated dilation in healthy humans. Int J Sports Med. 2013;34(5):409-14.
156. Maiorana A, O'Driscoll G, Cheetham C, Dembo L, Stanton K, Goodman C, et al. The effect of combined aerobic and resistance exercise training on vascular function in type 2 diabetes. J Am Coll Cardiol. 2001;38(3):860-6.
157. Woo KS, Chook P, Yu CW, Sung RY, Qiao M, Leung SS, et al. Effects of diet and exercise on obesity-related vascular dysfunction in children. Circulation. 2004;109(16):1981-6.
158. Greyling A, van Mil AC, Zock PL, Green DJ, Ghiadoni L, Thijssen DH, et al. Adherence to guidelines strongly improves reproducibility of brachial artery flow-mediated dilation. Atherosclerosis. 2016;248:196-202.
159. Schjerve IE, Tyldum GA, Tjonna AE, Stolen T, Loennechen JP, Hansen HEM, et al. Both aerobic endurance and strength training programmes improve cardiovascular health in obese adults. Clin Sci (Lond). 2008;115(9-10):283-93.
160. Harris RA, Padilla J, Hanlon KP, Rink LD, Wallace JP. Reproducibility of the flow-mediated dilation response to acute exercise in overweight men. Ultrasound in Medicine and Biology. 2007;33(10):1579-85.
161. Radtke T, Hebestreit H, Gallati S, Schneiderman JE, Braun J, Stevens D, et al. CFTR Genotype and Maximal Exercise Capacity in Cystic Fibrosis A Cross-Sectional Study. Ann Am Thorac Soc. 2018;15(2):209-16.
162. Black MA, Cable NT, Thijssen DH, Green DJ. Impact of age, sex, and exercise on brachial artery flow-mediated dilatation. Am J Physiol Heart Circ Physiol. 2009;297(3):H1109-16.
163. Celermajer DS, Sorensen KE, Spiegelhalter DJ, Georgakopoulos D, Robinson J, Deanfield JE. Aging Is Associated with Endothelial Dysfunction in Healthy-Men Years before the Age-Related Decline in Women. J Am Coll Cardiol. 1994;24(2):471-6.
164. Black MA, Cable NT, Thijssen DHJ, Green DJ. Importance of measuring the time course of flow-mediated dilatation in humans. Hypertension. 2008;51(2):203-10.
165. Harris RA, Padilla J, Hanlon KP, Rink LD, Wallace JP. The flow-mediated dilation response to acute exercise in overweight active and inactive men. Obesity (Silver Spring). 2008;16(3):578-84.
166. Johnson BD, Padilla J, Wallace JP. The exercise dose affects oxidative stress and brachial artery flow-mediated dilation in trained men. Eur J Appl Physiol. 2012;112(1):33-42.
167. Jones H, Green DJ, George K, Atkinson G. Intermittent exercise abolishes the diurnal variation in endothelial-dependent flow-mediated dilation in humans. Am J Physiol Regul Integr Comp Physiol. 2010;298(2):R427-32.
168. Suvorava T, Kojda G. Prevention of transient endothelial dysfunction in acute exercise: a friendly fire? Thromb Haemost. 2007;97(3):331-3.
169. Hwang IC, Kim KH, Choi WS, Kim HJ, Im MS, Kim YJ, et al. Impact of acute exercise on brachial artery flow-mediated dilatation in young healthy people. Cardiovasc Ultrasound. 2012;10.
170. Brown WM, Davison GW, McClean CM, Murphy MH. A Systematic Review of the Acute Effects of Exercise on Immune and Inflammatory Indices in Untrained Adults. Sports Med Open. 2015;1(1):35.
171. Harris RA, Padilla J, Rink LD, Wallace JP. Variability of flow-mediated dilation measurements with repetitive reactive hyperemia. Vasc Med. 2006;11(1):1-6.
172. Atkinson G, Batterham AM. Allometric scaling of diameter change in the original flow-mediated dilation protocol. Atherosclerosis. 2013;226(2):425-7.
173. Bond B, Hind S, Williams CA, Barker AR. The Acute Effect of Exercise Intensity on Vascular Function in Adolescents. Med Sci Sports Exerc. 2015;47(12):2628-35.

174. Choi Y, Akazawa N, Zempo-Miyaki A, Ra SG, Shiraki H, Ajisaka R, et al. Acute Effect of High-Intensity Eccentric Exercise on Vascular Endothelial Function in Young Men. J Strength Cond Res. 2016;30(8):2279-85.
175. Kolmos M, Krawcyk RS, Kruuse C. Effect of high-intensity training on endothelial function in patients with cardiovascular and cerebrovascular disease: A systematic review. SAGE Open Med. 2016;4:2050312116682253.
176. Siasos G, Athanasiou D, Terzis G, Stasinaki A, Oikonomou E, Tsitkanou S, et al. Acute effects of different types of aerobic exercise on endothelial function and arterial stiffness. Eur J Prev Cardiol. 2016;23(14):1565-72.
177. Effects of high intensity interval training on exercise capacity in people with cystic fibrosis: a randomised controlled trial [Internet]. 2017 Aug 29 [cited 2018 Aug 20]. Available from: https://www.anzctr.org.au/Trial/Registration/TrialReview.aspx?id=372185.
178. Hulzebos HJ, Snieder H, van der Et J, Helders PJ, Takken T. High-intensity interval training in an adolescent with cystic fibrosis: a physiological perspective. Physiother Theory Pract. 2011;27(3):231-7.
179. Blood Flow and Vascular Function in Cystic Fibrosis (CF-FLOW) [Internet]. 2014 Feb 7 [cited 2018 Aug 20]. Available from: https://clinicaltrials.gov/ct2/show/NCT02057458.
180. Mechanisms for Vascular Dysfunction and Exercise Tolerance in CF (CF-AOX) [Internet]. 2016 Feb 24 [cited 2018 Aug 20]. Available from: https://clinicaltrials.gov/ct2/show/NCT02690064.
181. Shrout PE, Fleiss JL. Intraclass correlations: uses in assessing rater reliability. Psychol Bull. 1979;86(2):420-8.
182. Harvill LM. Standard Error of Measurement. Educational Measurement. 1991;10(2):33-41.
183. Atkinson G, Nevill AM. Statistical methods for assessing measurement error (reliability) in variables relevant to sports medicine. Sports Med. 1998;26(4):217-38.
184. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet. 1986;1(8476):307-10.
185. Hopkins WG. Measures of reliability in sports medicine and science. Sports Med. 2000;30(1):1-15.