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# **The Future of Pleiotropic Therapy in Heart Failure.**

## **Lessons from the Benefits of Exercise Training on Endothelial Function.**

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## **Abstract**

A novel generation of drugs is introduced in the treatment of heart failure (HF). These drugs, including phosphodiesterase-5 inhibitors, guanylate cyclase stimulators and activators, share the feature that their action is either endothelial-mediated or substitutes for endothelial pathways, i.p. the nitric oxide-cyclic guanosine monophosphate pathway, thereby influencing homeostatic balances in virtually each organ system in a pleiotropic fashion. Unfortunately, recent clinical trials with some of these drugs have shown disappointing results, at least in the setting of HF with a preserved ejection fraction. This suggests that their clinical use may require approaches that diverge from traditional pharmacological approaches the latter often titrated on the effects of drugs on hemodynamic parameters or single biomarkers.

In this paper we preconize that heart failure drugs with an endothelial profile should be applied conform principles of endothelial physiology and systems pharmacology. This type of drug therapy should be viewed as a systems physio-pharmacological intervention and its clinical use accustomed to systems pharmacological principles, comparable to the systemic endothelial-mediated benefits induced by exercise training in HF. We will review the actions of these drugs and define criteria to which trials with these drugs should comply in order to increase chances of success.

## **Introduction**

During the past 35 years, landmark randomized trials have demonstrated an improvement in the treatment of patients with chronic heart failure (HF) with reduced ejection fraction (HFrEF), with benefits resulting from agents interfering with the renin–angiotensin–aldosterone as well as the adrenergic nervous system. However, hospital readmissions remain high and there still is no cure<sup>1</sup>. Currently, we witness the introduction of a novel generation of drugs in patients with HF, including serelaxin, sildenafil and other phosphodiesterase-5 (PDE5) inhibitors, neutral endopeptidase inhibitors, guanylate cyclase stimulators and activators, neuregulin-1, natriuretic peptides, myosin activators, mitochondria protectants, and heart rate slowing agents. Many of these novel drugs share the feature that their action is either endothelium-mediated or substitutes for endothelial pathways, and i.p. the nitric oxide (NO)-cyclic guanosine monophosphate (cGMP) pathway<sup>2</sup>. These drugs open a new conceptual avenue in the pharmacological treatment of HF. Unfortunately, recent clinical trials with some of these drugs have already shown disappointing results<sup>3,4</sup>, at least in the setting of HF with a preserved ejection fraction. Although the reasons for these disappointing results are probably multiple, these trials demonstrate that the clinical introduction of drugs whose mode of action is predominantly explained by activation of endothelial pathways may be more challenging than originally anticipated, and may require approaches that differ from traditional pharmacological approaches.

We preconize that drugs with a predominant endothelial profile will improve overall homeostasis and outcomes in HF provided that they are applied conform the basic principles of endothelial physiology, hence allowed to develop pleiotropic actions throughout the body. Perhaps, the basic rules to apply these drugs clinically can be derived from the principles of exercise training therapy. We suggest that exercise is the most genuine way to preserve and

activate the endothelial system and to restore overall body homeostasis and health. In this paper, we will present pharmacological endothelial activation as a systems physiological pharmacological intervention and define some criteria to which clinical trials with such drugs may have to comply in order to increase chances of success. This is not a classical review in which we limit ourselves to discussing scientific facts, but a conceptual paper in which we present novel hypotheses ([Table 1](#)).

## **The Endothelial System**

Because of its unique position at the interface between the individual organs and the circulating blood the endothelial system is, despite some vascular bed-specific heterogeneity, well placed in stabilizing overall homeostasis by simultaneously affecting all organ functions in the body<sup>5</sup>. The key role of the endothelial system has been generally well recognized in macro- and micro-resistance vessels of most organs, where endothelial cells interact with subjacent smooth muscle cells, thereby controlling vasomotricity, hence perfusion to the organs. More important but less well studied however are endothelial cells in capillaries, which constitute with approximately 85% the largest endothelial surface in each organ. In capillaries, endothelial cells directly communicate with subjacent organ and tissue cells<sup>5,6</sup>, e.g. neurons in the brain<sup>7</sup>, alveolar cells in the lungs<sup>8</sup>, cardiomyocytes in the heart<sup>5</sup>, tubular and glomerular cells in the kidney<sup>9,10</sup>, hepatocytes in the liver<sup>11</sup>, circulating immune cells<sup>12</sup>, and fat cells in adipose tissue<sup>13-15</sup>.

Although Paulus et al. have proposed a concept indicating that endothelial dysfunction is more important in HFpEF than in HFrEF<sup>16,17</sup>, numerous studies have demonstrated that in HF, regardless of EF, the entire cardiovascular endothelial system has become significantly dysfunctional<sup>18,19</sup>. Accordingly, because of the stabilizing role of the endothelial system at the

interface between plasma and subjacent tissue cells, interventions that improve or replace endothelial function would be expected to act as a major driving force in orchestrating the normalization of cardiovascular homeostasis of all body organs and systems<sup>20</sup>. This will result in an improved organ perfusion and correction of the hemodynamic mismatch between the LV and RV with respectively the systemic and pulmonary arterial systems, and also optimize the endothelial cell-to-cell communication with subjacent renal, muscular, liver, gastro-intestinal, cerebral organ cells.

### **Exercise Training Activates the Endothelium and Acts as a Systems Physio-pharmacological Intervention**

Probably the most genuine way to activate or restore endothelial pathways is exercise training<sup>21,22</sup>, a therapeutic intervention with benefits on prognosis, quality of life, and hospitalization frequency. In the HF-ACTION trial, after adjustment for highly prognostic predictors, exercise training was associated with modest significant reductions for both all-cause mortality or hospitalization and cardiovascular mortality or heart failure hospitalization<sup>23</sup>. In a recent Cochrane systemic review, including 33 trials with 4740 patients predominantly with HFREF and New York Heart Association classes II and III, there was no difference in pooled mortality between exercise-based rehabilitation versus no exercise control, at least in trials with up to one-year follow-up<sup>24</sup>. However, there was a trend towards a reduction in mortality with exercise in trials with more than one year of follow-up. Compared with control, exercise training reduced the rate of overall and HF specific hospitalization and exercise also resulted in a clinically important improvement in the Minnesota Living with Heart Failure questionnaire<sup>24</sup>.

Features of exercise training therapy in HF may serve as a strategic guide for testing some of the novel generation of drugs in clinical trials. Exercise training therapy has become a well-

established recommendation in the management of patients with HF<sup>25-29</sup>. Best tolerated and most successful are 3-4 weekly exercise training therapeutic programs with a very slow and gentle onset, gradually increasing in duration and intensity under strict medical monitoring over a period of about 3-4 months, and continued thereafter with 3-4 weekly one-hour training sessions. Wisloff and co-workers experimented with intensified training programs in HF patients (“aerobic interval training, 3 times per week upto 95% of peak heart rate instead of 70% of peak heart rate) and showed that effects on VO<sub>2</sub> max and LV remodeling became larger<sup>30</sup>. However, this trial was small (n=27 patients) and effects on mortality have not been tested.

Intriguingly, principles of above intermittent and appropriately gauged exercise training therapy profoundly differ from principles of contemporary pharmacology, the latter aiming at rapid up-titration of uninterrupted dosing regimens and stable drug plasma concentrations. The beneficial effects of exercise training therapy have been ascribed to an improved physiological homeostasis of all organ functions, resulting from a balanced activation of the various body systems acting in parallel, i.e. the cardiovascular, the neuro-hormonal<sup>31</sup>, the endocrine, the immunological, and more recently the endothelial system<sup>5</sup>. (Fig. 1).

Improvement of endothelial function has been shown to play a central coordinating role in integrating and coordinating the exercise-induced beneficial effects, i.p. in re-establishing organ homeostasis. Factors that may contribute to this improvement are shear stress-activation of endothelial cells as well as various exercise-induced plasma constituents which may affect endothelial function, e.g. metabolites, hormones, stressors, free radicals, miRNAs.

Furthermore, exercise training improves endothelial progenitor cell number and function<sup>32</sup>.

Improvement of capillary endothelial function will guide normalization of overall homeostasis (i) by normalizing the complex cell-to-cell communication between endothelial

cells and subjacent organ and tissue cells (Fig. 2), involving different interacting and interdependent paracrine pathways acting in parallel, i.p. the NO pathway, the endothelin-1 pathway, the neuregulin-1 pathway<sup>33</sup>, the prostacyclin pathway, the vascular endothelial growth factor pathway and the NOX pathway, (ii) by optimizing some vital endothelial (enzymatic) activities, i.p. those dependent of angiotensin converting enzyme, carbonic anhydrase-4, Von Willebrand factor, cyclo-oxygenase 1 and 2, estrogen receptor signaling<sup>34</sup>, neutral endopeptidase, natriuretic peptides, platelet endothelial cell adhesion molecule (PECAM-1) and dipeptidyl peptidase IV<sup>35</sup>, (iii) by normalizing, clearing and metabolic processing by the endothelial cells of various plasma constituents<sup>36</sup> and (iv) by counteracting the endothelial inflammasome as well as optimizing the plasma oxidant-antioxidant balance.

Accordingly from a pharmacological point of view, exercise training therapy in HF seems to resemble the ideal endothelium-mediated drug with pleiotropic activity, i.e. by orchestrating the improvement of the homeostasis of all body organ functions. Alternatively from a physiological point of view, the beneficial effects of exercise training on organ function could be viewed as a systems biological or systems pharmacological intervention. Being complementary to one another, both the pleiotropic and the systems pharmacological views may equally contribute to the re-establishment of overall body homeostasis during exercise training in patients with HF. Indeed, the HF syndrome is the clinical manifestation of failing complexity involving most body organs and systems. It is not the mere result of a failing single organ, cell, molecule or gene. The connotation systems physio-pharmacology is, therefore, most appropriate to describe the therapeutic effect of exercise training at all hierarchical scales in each one of the body systems from intra-cellular, cellular, tissue, organ, overall organism, patient's pathophenotype and disease<sup>37</sup>.

### **Considerations for Future Clinical Trials in HF**

What can the clinician learn from the beneficial effects of exercise training therapy in patients with HF and the role of the endothelial system? What can be learned with respect to designing novel drugs and launching appropriate clinical trials for the treatment HF? By taking into account lessons learned from the beneficial pleiotropic effects of exercise training in HF, hence by gently and progressively targeting homeostasis rather than focusing on the pharmacological effects on single biomarkers<sup>38</sup>, future clinical trials in HF may acquire additional physiological elegance and credibility. A gentle and well dosed systems physio-pharmacological approach, similarly as used during exercise training therapy may, therefore, be a fresh concept for designing future HF clinical trials with the novel drugs acting primarily on the endothelium and the NO-cGMP pathway. The negative results observed in some recent clinical trials with some of these drugs, following significant initial successes in the earlier trials, can perhaps be explained by the fact that these principles have been insufficiently venerated<sup>39, 40</sup>.

In the 80-90ies, clinical trials in HF were successfully launched based on the adagio one-target-one-response of contemporary pharmacology leading to the successes with ACE inhibitors, beta-1 receptor antagonist and aldosterone receptors antagonists. It is currently believed, however, that the success of this triade of drugs in the treatment of HF results from their pleiotropic actions as much as from inhibiting the original single enzyme or receptor target or biomarker. Management of HF has been designed to target almost exclusively one mechanism, and historically cardiomyocytes, inotropy and ejection fraction, were the most popular low hanging fruit targets. The heart is, however, a pluricellular organ consisting of interacting and interdependent<sup>41, 42</sup> cells, i.e. cardiomyocytes, fibroblasts<sup>43, 44</sup>, cardiac (endocardial and myocardial capillary) endothelial cells, and inflammatory cells. It is not surprising then that many of the subsequently described pleiotropic beneficial effects of ACE inhibitors, beta-1 receptor antagonist and aldosterone receptors antagonists are now to a large

extent attributed to actions on non-cardiomyocytes, i.e. fibroblasts, inflammatory cells and most importantly endothelial cells<sup>5, 45, 46</sup>. Moreover, besides eliciting unanticipated beneficial effects in non-cardiomyocytes in the heart, these drugs also elicit effects in other organs such as the kidney and the brain, thereby resembling typical network behavior. Hence, the connotation systems physio-pharmacology was launched by some as a more appropriate term in describing the beneficial effects of these drugs, and as the optimal way of treating complex diseases such as HF.

### **New Ideas for Designing Future Clinical Trials in Heart Failure.**

The pivotal role of a dysfunctional endothelial system in the pathogenesis of HF has been demonstrated in several studies<sup>19, 47</sup>. Preservation of endothelial function has been recognized as a potential therapeutic target during the very early phases of HF<sup>48-52</sup>. We have recently witnessed the introduction of novel molecules which could either normalize endothelial function or substitute for some of the endothelial-mediated pathways, i.p. the NO-cGMP pathway. Based on the pivotal role of the endothelial system in orchestrating the systems physio-pharmacological benefits of exercise training in patients with HF, designing future clinical trials with a new generation of drugs with a predominant endothelial activity may benefit from a systems physio-pharmacological strategy while taking into account endothelial physiology. From this, a couple general physiological principles lead to some practical recommendations, summarized in Table [21](#). These recommendations relate to the action of the drug, the characteristics of the patients to be treated with the drug, the dosing regimen and the trial design including the choice of trial end-points, intermediate analyses beyond mortality and exercise tolerance, and identification of responders and non-responders.

We have recently hypothesized that drugs with a predominant endothelial activity may require prolonged exposure to very low dosages, even if at first no measurable changes are observed<sup>2</sup>. As various signaling events between the endothelium and subjacent organ cells are interdependent and balanced, one may expect a mismatch in these events in patients with HF. This mismatch may be further hampered if only one of these pathways, e.g. either the NO-cGMP pathway or the brain natriuretic peptide-cGMP pathway, or the NRG-1/ErbB pathway, or the endothelin-1/ET-A receptor pathway would be targeted by high dosages of such a drug. By contrast, a low dosage is expected to re-establish a balanced crosstalk between the ventricles and respectively the pulmonary and systemic circulation.

For example, in HF patients with concomitant pulmonary hypertension and right ventricle-pulmonary artery mismatch, chronic exposure to lower dosages of drugs that activate the endothelial NO-sGMP pathway would allow for a gentle, auto-amplifying shear stress-induced and endothelial-mediated hemodynamic normalization to a low impedance/high capacitive pulmonary vascular system, while avoiding sudden unwanted drop in left atrial preload. Similarly in the resistive systemic circulation, as most of these drugs are vasodilators, focus in HF patients should rather be on modulating systemic arterial impedance while avoiding an unwanted arterial pressure drop. Therefore, both in the systemic and pulmonary circulations, a prolonged exposure (in terms of months) at low dosages of these “endothelial drugs” should aim at correcting any left ventricular-arterial mismatch while maintaining arterial perfusion pressure within physiological limits.

Furthermore, prolonged exposure to low dosages of drugs with a predominant endothelial and NO-cGMP stimulating activity are expected to re-establish a balanced ventricular-arterial coupling by modulating timing and rate of LV relaxation, both directly through endothelium-

mediated effects on onset and rate of relaxation of the cardiomyocytes and indirectly by subtle changes in arterial impedance resulting in shifts in timing of incoming reflected waves, hence of the loading sequence during LV systole. As a result, a dosed reverse cardiac and vascular remodeling will ensue. Even in the absence of any immediate and manifest peak hemodynamic effect, progressive and subtle drug-induced changes at low dosages may be estimated and gauged by measuring ventricular time intervals, i.p. shifts in the time sequence of the ventricular loading conditions due to time shifts of the returning reflected waves from early to late systole. Neglecting these basic physiological rules by applying high, “pharmacologically active” dosages and measuring only static peak hemodynamic variables will provide misleading information.<sup>53-55</sup> It is like neglecting the lessons learned from the above exercise training programs, and abruptly imposing an exercise training program consisting of ever-repeating 100 meter runs to a patient with HF.

Also in peripheral organs, as e.g. the kidneys, liver, gastro-intestinal tract, skeletal muscle and brain, drugs with a predominant endothelial activity will affect organ function in two ways, (i) indirectly, by increasing organ perfusion through the relaxing effects of endothelium on vascular smooth muscle, and (ii) much more importantly however, through direct cell-to-cell communication between capillary endothelial cells and subjacent organ cells, as e.g. alveolar epithelial cells, glomerular and tubular epithelial cells, hepatocytes or neurons, resulting in improved renal function, gauging and optimizing the cardiac-renal axis, exercise tolerance, brain cognitive function and organ homeostasis in general.

### **Clinical assessment of endothelial dysfunction**

Given its pathophysiological importance and the emerging pharmacological tools to target endothelial dysfunction, clinical assessment of endothelial function would be useful (Table

32). Monitoring endothelial function may be of great value during staging disease, establishing the direct endothelial efficacy of a drug, and for individualized patient selection. Endothelial functional tests should be safe, non-invasive, reproducible, cheap and standardized. In addition, the results should allow a broad appreciation of the numerous functions of the endothelium, not merely reflecting the integrity of one pathway (e.g. the NO-cGMP pathway) or of one process (e.g. the regulation of vascular tone). Ideally, endothelial tests should discriminate between endothelial function in different organ beds, e.g. to reflect endothelial abnormalities in myocardial capillaries, glomerular capillaries, blood-brain-barrier, or resistance vessels. Indeed, it is to be expected that endothelial dysfunction does not uniformly deteriorates throughout the body during the natural history of a disease, and may show inter-individual variability. Unfortunately, no single test fulfills these requirements and endothelial tests still have a limited role in individual clinical decision-making; endothelial testing has been seldom used in clinical trials. We refer to a number of excellent publications, reviewing the currently available tests of endothelial function<sup>56-59</sup>.

Unfortunately, clinical endothelial function tests currently have no role in the assessment of individual patients and in individual clinical decision-making, and have been seldom used in clinical trials mostly due to biological and assay variability of the results. While endothelial tests are still too difficult, expensive and variable for clinical use, endothelial testing should nevertheless be further explored with the purpose to (i) stratify populations with regard to the importance of endothelial dysfunction in the pathophysiology of disease, (ii) help selecting drugs in early proof-of-concept trials and dose finding studies, and (iii) help predicting therapeutic response.

### **Endothelial effects of drugs used in HF**

A substantial number of drugs, applied as a treatment of HF, has been shown to influence endothelial function or to mimic the function of one or more endothelial factors. Some of these drugs have been designed and/or developed based on the knowledge of endothelial physiology (e.g. endothelin receptor antagonists, phosphodiesterase type 5 (PDE5) inhibitors, NO-donors, neuregulin-1 and direct soluble guanylate cyclase stimulators). Table [43](#) summarizes the most interesting clinical trials that have been performed with such drugs.

Other drugs were originally not designed to modify or restore endothelial function, but appeared to have a direct or indirect influence on the endothelium during subsequent analysis.

In table [45](#) we summarize evidence for indirect endothelial activity of mineralocorticoid receptor antagonists, ACE inhibitors, angiotensin receptor antagonists, statins and xanthine oxidase inhibitors. This table also informs on a number of dietary interventions in HF for which effects on endothelial function are available.

Critically looking at the trials in table [43](#), it is obvious that there has been little or no effort to relate the pharmacological effects of the investigated therapeutic candidates on the endothelium to the clinical benefits of these agents. Appropriate dose finding studies, ideally based on assessment of endothelial function, have been most frequently skipped. Some HF trialists advocate skipping dose finding studies altogether and moving toward large phase III outcome trials with minimal dose finding studies. This strategy has been occasionally successful, such as the case with ACE inhibitors and angiotensin receptor blockers, or more recently, with LCZ 696. However, if skipping dose-finding studies would have been applied to mineralocorticoid receptor antagonists, we would have totally missed the benefits of this important HFrEF therapy. Indeed, it was only after careful selection of the “lowest” effective dose (on atrial natriuretic factor) that the RALES trial was designed, avoiding the higher doses used in hypertension or for diuresis in congestive patients at that time <sup>60</sup>.

## **Looking into the future: Ongoing trials with new drugs that stimulate the NO-cGMP pathway.**

Direct soluble guanylate cyclase (sGC) stimulators offer a novel approach to address the endothelial dysfunction-induced cGMP deficit in HF. To our knowledge and surprisingly, these agents have not been clinically investigated as to their effect on endothelial function. Dose selection for future HF outcome trials was based only on the effects on NTproBNP<sup>61</sup>. In the SOLuble guanylate Cyclase stimulator in heArT failure Studies (SOCRATES-REDUCED) compared with placebo in HFrEF patients, the pooled vericiguat group (daily target doses of oral vericiguat 1.25 mg, 2.5 mg, 5 mg, 10 mg) did not have a statistically significant effect on change in NT-proBNP level at 12 weeks<sup>62</sup>. Nevertheless, based on the results of an exploratory secondary analysis, suggesting a dose-response relationship whereby higher vericiguat doses were associated with greater reductions in NT-proBNP level, the investigators called for further trials, probably with the highest tested dose of 10 mg which was well tolerated. Following the reasoning in this paper, we would recommend low dosages, too low to affect hemodynamic parameters acutely, sustained for prolonged periods allowing homeostatic adaptations in all organs conform the principles of physical rehabilitation and exercise programs. Furthermore, we would recommend including measurements of endothelial function (Table 32) instead of relying only on measurements of circulating biomarkers.

### **Conclusion**

Pharmacologically enhancing or protecting endothelial activity is a fascinating and likely powerful new tool in the treatment of HF. It has the potential to orchestrate the normalization of cardiovascular homeostasis of all body organs and systems, in a similar way as exercise therapy-induced effects on endothelial function do. As such, we hypothesize that introducing

drugs with a predominant endothelial activity in clinical practice should be based on basic principles of endothelial physiology and its use accustomed to principles of systems pharmacology to increase chances of success. This paper is meant to summarize these principles. We hope it may serve as a guide for further clinical development of endothelial drug therapy, and for pleiotropic therapy in HF in general.

## Tables

**Table 1.**

<b><u>SCIENTIFIC BACKGROUND</u></b>	<b><u>NOVEL HYPOTHESES</u></b>
<p><b><u>The health benefits of exercise therapy extend across a wide range of organ systems, and are particularly evident in heart diseases</u></b></p>	<p><b><u>Trying to mimic such a wide approach pharmacologically remains a major challenge in the management of heart failure</u></b></p>
<ul style="list-style-type: none"> <li>• <u>Exercise therapy improves the function of virtually every organ</u></li> <li>• <u>Exercise therapy improves a wide range of chronic diseases</u></li> <li>• <u>Principles of exercise therapy profoundly differ from the principles of contemporary pharmacology</u></li> <li>• <u>Exercise therapy improves endothelial function</u></li> <li>• <u>The endothelium communicates with virtually every tissue cell in the body</u></li> <li>• <u>Paracrine endothelial function results from many interdependent pathways, not just from nitric oxide alone</u></li> <li>• <u>A new class of heart failure drugs improves or mimics endothelial function, but usually changes only one of the endothelial pathways</u></li> <li>• <u>Introduction of these drugs into clinical practice has been more difficult than anticipated, with several failed clinical trials</u></li> </ul>	<ul style="list-style-type: none"> <li>• <u>Benefits of exercise therapy in heart failure result from convergent pleiotropic amplification of all organ functions mediated and coordinated through improved endothelial function</u></li> <li>• <u>Drug therapy can be designed according to principles of exercise therapy (gentle, intermittent and slowly up titrated)</u></li> <li>• <u>Future pharmacological heart failure trials should not target one pathway or biomarker, but be designed to allow slow improvements of homeostasis</u></li> <li>• <u>Clinical use of the new class of drugs with endothelial action should take into account basic principles of endothelial physiology and of exercise therapy</u></li> </ul>

**Table 12: criteria for successful clinical trials with endothelial drugs in heart failure**

### **1. Compound**

#### **Establish effects on endothelial pathways**

- Preclinical in vitro research on endothelial cell cultures
- Preclinical animal models of endothelial dysfunction

- Clinical tests

**Define pre-clinical effects on cardiac function in relevant animal models**

**Establish pleiotropic action on:**

- Multiple organ, cell types and signaling pathways

## **2. Patient characteristics**

**Include endothelial dysfunction as inclusion criterium**

- Based on quantitative measurements of endothelial function

**Include a homogenous population**

- Initial disease mechanisms, stage of disease, comorbidities, EF, ...

**Include stringent criteria for the diagnosis of heart failure, especially in HFPEF**

## **3. Adapt dosing regimen to principles of exercise training and endothelial physiology**

**Physiological doses, avoiding acute drops in blood pressure**

- If uptitration is preferred, go VERY slow
- Consider intermittent or cyclical therapy

## **4. Trial design and end points**

**Establish effect beyond exercise tolerance and mortality**

**Include effect on LV diastolic and endothelial function as an endpoint**

**Identify responders and non-responders to allow for concept validation and follow-up trials**

- Based on measurements of endothelial function
- Based on clinical endpoints (e.g. exercise tolerance, symptoms)

**Table 32: clinical and experimental endothelial function tests.** NO : nitric oxide ; MRI: magnetic resonance imaging; US: ultrasound; EPC: endothelial progenitor cell, miRNA: microRNA.

Test	Location	Method	Advantage	Disadvantage	Predicts outcome in HF	Ref
Coronary vasodilation	Coronary arteries	Angiography	Reversible	Invasive, limited to NO-pathway	Yes	56, 63

MRI isometric handgrip exercise	Coronary arteries	MRI of coronary arteries	Non-invasive, repeatable	Limited experience		64, 65
Venous occlusion plethysmography	Forearm resistance vessels	Forearm volume changes to venous occlusion	Reversible	Invasive, limited to NO-pathway	Yes	66, 67
US flow-mediated dilation	Brachial	US of brachial hyperemic response	Gold standard, non-invasive, reversible	Limited to NO-pathway	Yes	56, 68, 69
Pulse wave analysis	Radial	Applanated vessel tonometry	Non-invasive, simple technique	Needs validation		70
Flow mediated slowing	Brachial	Slowing of pulsed wave velocity	Non-invasive, repeatable	Non-validated		71
EPC function	Systemic	Blood sample, EPC isolation & culture	Non-invasive, repeatable	Non-validated, labor intensive		72
Proteomics	Systemic	Plasma sample, mass spectrometry	Non-invasive, repeatable	Non-validated, complex analysis		73
miRNAs	Systemic	Plasma sample, qPCR, sequencing	Non-invasive, repeatable	Non-validated		74

**Table 34. Clinical studies with drugs with an endothelial profile.** NO: nitric oxide ; PDE-phosphodiesterase 5, cGMP: cyclic guanosine monophosphate; HT: hypertension, PAPs: pulmonary arterial pressure in systole. PAWP: pulmonary arterial wedge pressure; ET: endothelin

Drug	Action	Trial	Design	Outcome	Ref
NO donors	Increase of NO bioavailability	A-Heft  NEAT-HFpEF	<ul style="list-style-type: none"> <li>• NYHA III/IV, HREF &lt;45%</li> <li>• Hydralazine/ISDN fixed dose (35,5/20 mg TID)</li> <li>• HFPEF</li> <li>• ISMN 6 week therapy, rapid escalation regime (30-&gt;60-&gt;120mg/d)</li> </ul>	RR 0.61 all cause mortality RR 0.67 first hospitalization  Adverse effect on daily activity	75  3
PDE-5 inhibitors	Prevention of cGMP catabolism	Guazzi et al.  RELAX  Hoendermis et al.	<ul style="list-style-type: none"> <li>• HFPEF + pulmonary HT</li> <li>• Sildenafil 50 mg TID</li> <li>• HFPEF + or - pulmonary HT</li> <li>• Sildenafil 20mg -&gt; 60 mg TID</li> <li>• HFPEF, PAPs &gt;25mmHg, PAWP &gt;15mmHg (post-capillary PHT)</li> <li>• Sildenafil 60 mg TID</li> </ul>	Improvement of RV function and pulmonary hemodynamics No effect on symptoms or clinical status  No effect on clinical or invasive parameters	76  4  40
ET-receptor antagonist	Antagonism of ET-A receptor and/or ET-B receptor	REACH  ENABLE  Kaluski et al.	<ul style="list-style-type: none"> <li>• NYHA III/IV, HFREF&lt;35%</li> <li>• Bosentan (500 mg BID, slow or fast uptitration)</li> <li>• NYHA III/IV, HFREF&lt;35%</li> <li>• Bosentan (125 mg BID)</li> <li>• NYHA III/IV, HFREF&lt;35%, sPAP&gt;40 mmHg</li> <li>• Bosentan (125 mg BID)</li> </ul>	Early termination of study because of safety concerns  Early termination of study because worsening HF Worsening HF	77  78  79
Neuregulin-1	Activation of ErbB signaling	Gao et al.	<ul style="list-style-type: none"> <li>• HFREF, 10 hrs infusion for 10 days (0.3 -1.2 mg/kg)</li> </ul>	Reverse LV remodeling at 30 and 90 days after infusion	80
Serelaxin	Activation of relaxin receptor and ETB receptor	RELAX-AHF	<ul style="list-style-type: none"> <li>• AHF, 48 hrs infusion within 16 hrs after admission (30 µg/kg per day)</li> </ul>	Reduction in HF symptoms	81

**Table 54. Indirect endothelial effects of heart failure drugs or dietary interventions in heart failure.** FMD: flow mediated dilation, ACE: angiotensin converting enzyme; CCB: calcium channel blockers; NO: nitric oxide.

**Mineralocorticoid.** Spironolactone improve endothelial function (brachial FMD) in HF patient on conventional HF therapy <sup>82</sup>.

**ACE inhibitors and angiotensin receptor blockers (ARB):** ACEIs and ARBs improve endothelial function (brachial FMD) in patients with endothelial dysfunction caused by various conditions and are superior to CCBs and  $\beta$ -blockers <sup>83</sup>.

**Statins.** Atorvastatin and rosuvastatin mobilize EPCs and improve endothelial function in HFREF patients beyond their lipid lowering effect <sup>84, 85</sup>.

**Xanthine oxidase inhibition.** Allopurinol improves endothelial function and reduces markers of oxidative stress in HFREF patients <sup>86, 87</sup>.

**L-arginine and L-citrulline.** L-arginine and L-citrulline supplementation improved endothelial function in HFREF patients <sup>88, 89</sup>.

**Cocoa.** Flavanol-rich chocolate improves endothelial function in HFREF patients <sup>90</sup>.

**Dietary Nitrate.** Acute intake of beetroot juice increases NO bioavailability, and increased exercise capacity in HFPEF patients <sup>91-93</sup>.

## Figure legends

**Figure 1: The pleiotropy of exercise.** Exercise training is the most potent medicine known to man. Beneficial effects of physical activity have been demonstrated in virtually all organ systems, including the cardiovascular system, the renal system, the liver, skeletal muscle, metabolism, immune system, respiratory system and neuronal system, orchestrating the homeostasis of all organ functions. Beneficial effects of physical activity in clinical medicine have been documented both in primary prevention and in numerous chronic diseases including HF, arterial hypertension, atherosclerosis, metabolic syndromes such as diabetes, chronic kidney failure, fatty liver disease, chronic obstructive lung disease (COPD) and pulmonary hypertension, and mental and neurodegenerative diseases<sup>23, 26-29, 66, 94-101</sup>. Exercise training also improves the function of the endothelial system. Accordingly, the endothelial actions may be vital to integrate the exercise-induced beneficial effects. Especially in the capillaries, endothelial cells directly influence tissue cells through the release of interdependent paracrine substances.

**Figure 2. The endothelium is a sensor for various biological stimuli and acts as an effector on neighboring organ cells.** Endothelial cells have a sensing function to detect changes in hemodynamic, chemical, neurohormonal, and mechanical stimuli. Shear stress is an important stimulus in arteries and larger arterioles. Endothelial cells in specific microcirculations such as the heart or skeletal muscle are subjected to mechanical stress such as cyclical stretching and compression, and load dependent strain. Furthermore, all endothelial cells have receptors for metabolites, neurohormonal factors, cytokines, and growth factors. Secreted angiocrine substances constitute the endothelial effector function of the myocardium, these include small molecules, peptides, proteins, microvesicles, and microRNAs. ADP: adenosine diphosphate; ANP: atrial natriuretic peptide; CGRP: calcitonin gene-related peptide; CNS: central nervous system; CTGF: connective tissue growth factor;

DKK3: dickkopf related protein 3; FGF-2: fibroblast growth factor-2; FST: follistatin; IL-1: interleukin-1; NO: nitric oxide; NOX: NADPH-oxidase family<sup>102, 103</sup>; PDGF: platelet derived growth factor; PECAM: platelet endothelial cell adhesion molecule; TGFβ: transforming growth factor beta; TNFα: tumor necrosis factor alpha; TSP-1: thrombospondin-1; VEGF: vascular endothelial growth factor; vWF: vonWillebrand factor.

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