Cardiac resynchronization therapy improves minute ventilation/carbon dioxide production slope and skeletal muscle capillary density without reversal of skeletal muscle pathology or inflammation

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Aims
We evaluated the effects of cardiac resynchronization therapy (CRT) on skeletal muscle pathology and inflammation in patients with heart failure.

Methods and results
Stable patients (n = 21, 14 males, mean age 70 ± 7 years) with symptomatic heart failure (mean left ventricular ejection fraction 24 ± 6%) and an indication for CRT were included. Ergospirometry, skeletal muscle open biopsy, and blood sampling were performed prior to implantation and after 6 months of CRT. After CRT there was a reduction in both left ventricular end-diastolic diameter (LVEDD; 6.8 ± 0.8 vs. 6.3 ± 0.7 cm, P < 0.001) and native QRS duration (D) minus biventricular paced QRSD (172.9 ± 23 vs. 136.3 ± 23 ms, P ≤ 0.001). These changes were associated with an increase in peak slope oxygen uptake (consumption) (VO2) (13.3 ± 2.2 vs. 14.5 ± 2.6 mL/kg/min, P = 0.07) and an improvement in the minute ventilation/carbon dioxide production slope (VE/VCO2) slope (41.6 ± 7.4 vs. 39.1 ± 5.6, P = 0.012). There were no statistically significant changes in levels of pro-inflammatory cytokines, in mediators of mitochondrial biosynthesis or skeletal muscle pathology, except for an increase in skeletal muscle capillary density (4.5 ± 2.4 vs. 7.7 ± 3.3%, P = 0.002). Both the reduction of QRS duration and the increase in peak VO2 correlated significantly with the change in mitochondrial density (r = 0.57, P = 0.008 and r = 0.54, P = 0.027, respectively).

Conclusion
Cardiac resynchronization therapy, with improved functional status and reduced LVEDD resulted in increased peak VO2, improvement in VE/VCO2 slope and capillary density in skeletal muscle, with no reduction in systemic pro-inflammatory cytokines, increase in intramuscular levels of mediators of mitochondrial biosynthesis or improvement in skeletal muscle ultrastructure per se.

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Keywords
Chronic heart failure • CRT • Skeletal muscle • Inflammation • Cytokines • Peak VO2 • VE/VCO2 slope

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What’s new?

- Following cardiac resynchronization therapy (CRT) implantation, reductions in both left ventricular end-diastolic diameter and QRS duration were associated with a modest increase in peak VO₂ and an improvement in the VE/VCO₂ slope. There was an increase in skeletal muscle capillary density, but no statistically significant changes in the levels of pro-inflammatory cytokines, in mediators of mitochondrial biosynthesis, or skeletal muscle pathology. The effect of CRT on symptoms is likely related to the effect on central haemodynamics with no observed improvement in skeletal muscle ultrastructure or reduction in systemic pro-inflammatory cytokines per se.
- Deconditioning and inactivity may be important factors aggravating the underlying pathophysiology, and exercise training has been shown to improve exercise performance by increasing the oxidative capacity of skeletal muscle often accompanied by an improvement in symptoms. Improved central haemodynamic performance after CRT might be a pre-requisite for initiating exercise training in these highly symptomatic heart failure patients.

Introduction

Both the neurohumoral and cytokine model assist in our understanding of the histopathology of heart failure (HF). More recently, the muscle hypothesis supports the assumption that abnormalities in peripheral muscle might be a source for the stimulus of symptoms and for the ergo-receptor reflex abnormalities associated with increased ventilation and fatigue in chronic HF (CHF). The pathological changes in the muscles related to this hypothesis include a shift from aerobic type I fibres to anaerobic type II fibres, changes in mitochondrial volume and density, a reduced capillary density as well as reduced cytochrome oxidase activity reflecting a decreased oxidative capacity. Deconditioning and inactivity may be important factors aggravating the underlying pathophysiology, and exercise training has been shown to improve exercise performance by increasing the oxidative capacity of skeletal muscle which is accompanied by an improvement in symptoms.

In patients with HF and left bundle branch block (LBBB), the associated dyssynchrony of the left ventricle (LV) and the frequent mitral insufficiency further compromise cardiac function. Reductions of QRS duration and improvements in central haemodynamics with cardiac resynchronization therapy (CRT) are associated with improved functional status and prognosis. However, still a large proportion (~30%) of eligible patients does not respond clinically to CRT. Whether this response is dependent on reversal of peripheral skeletal muscle pathology and reduction in levels of pro-inflammatory cytokines, which again may influence muscle performance, are less well documented. Indeed, there are as far as we know no data on the effect on skeletal muscle pathology after CRT.

Most interventions that improve functional capacity in patients with HF have a direct effect also on skeletal muscle vasculature, metabolism, and inflammation. In contrast, CRT does not target any direct skeletal muscle pathology. The relationship between the improvement of central haemodynamics and improved exercise capacity following CRT and the structural and functional changes of the skeletal muscle has not been adequately investigated. We therefore sought to explore the effects of CRT on skeletal muscle ultrastructure, mitochondrial biosynthesis, and related inflammatory mediators.

Methods

Patients

Patients were recruited consecutively from the outpatient HF clinic at Stavanger University Hospital. Eligible patients were candidates for CRT due to symptomatic HF with systolic dysfunction and LBBB. All patients were on stable medication. The study was approved by the local ethics committee and conducted according to the Declaration of Helsinki. Written informed consent for participation was received from all individuals.

Pacemaker implantation

A bi-polar lead was placed with conventional technique to the apex or the mid-septal wall of the right ventricle and a second lead was placed in an epicardial coronary vein. In patients with sinus rhythm, a third lead was placed into the right atrium. We used visual assessment with four-chamber echocardiography, tissue Doppler, experimental atrioventricular timing, and titration of the posterior wall/septum delay. Inter-ventricular delay was programmed to optimize inter- and intra-ventricular synchrony which subsequently most often was associated with the shortest QRS-width. Echocardiography was also used to avoid fusion of the E and A waves, pre-systolic mitral insufficiency or truncating of the A-wave. Estimates for left ventricular end-diastolic diameter (LVEDD) and dyssynchrony were performed using standard two-dimensional echocardiography (GE, Vivid-7). We verified that the postero-lateral myocardial segment was viable using CMRI with gadolinium uptake and echocardiography prior to CRT implantation.

Cardiopulmonary exercise test

The patients were evaluated on an upright, electrically braked ergometer bicycle (Model KEM III, Mijnhardt) using a 15 W/min ramp protocol as previously described. Briefly, gas exchange data were collected continuously with an automated breath-by-breath system 2001 (Medical Graphics Corporation). The actual ventilation values are plotted every 20 s on the graph. The test was performed prior to inclusion and after ~6 months of CRT. The VE/VCO₂ slope was calculated from the linear regression of the VE vs. VCO₂ relationship. Peak VO₂ was estimated based on 3–4 measurements at the plateau at the end of the test. The subject rested on the bicycle for 1 min while respiratory gases were collected. Pedaling was started at a constant rate of 60 rpm. Respiratory gases were collected for the duration of the test. Peak VO₂ at 6 months could only be satisfactory assessed in 17 patients due to skeletomuscular problems or unwillingness to perform the test.

Blood sampling protocol

Sampling was performed prior to exercise testing at baseline and after 6 months. A catheter was placed in the antecubital vein with the
patient resting in a supine position for 30 min. Blood was drawn into pyrogen-free blood collection tubes with (plasma) or without (serum) EDTA as anticoagulant. Plasma tubes were immediately immersed in melting ice, and centrifuged at 2500 g for 10 min at 4 °C within 20 min. Serum was collected after allowing blood to clot at room temperature for 60 min before centrifugation at 2000 g for 15 min at 4 °C. All samples were stored at −80 °C and thawed only once.

Cytokine analysis in plasma
Plasma levels of tumor necrosis factor (TNF)-α, interleukin (IL)-6, IL-8, and macrophage chemoattractant protein (MCP)-1/CCL2 were measured using a multiplex cytokine immunoassay (Bio-Plex Human Cytoke Plex Panel, Bio-Rad Laboratories). The samples were analysed on a Multiplex Analyzer (Bio-Rad Laboratories).

Muscle biopsies
To obtain high-quality samples available for electron microscopy, biopsies from the quadriceps muscle were performed with an open surgical technique using local subcutaneous anaesthetic and avoiding injection into the muscle tissue.16 The specimens for electron microscopy were mounted on a small stick to ensure the quality of the samples and adequate orientation of fibres. The second biopsy (at 6 months) was harvested from the contralateral quadriceps muscle to avoid scar artefacts from the first biopsy.

Light microscopy
The muscle specimens were flash-frozen in isopentane chilled to −150 °C and then stored in liquid nitrogen. Measurements of fibre diameter, interstitial fibrosis, vascular density, and inflammation were done using morphometric technique (computer-aided microscopy, quamtmet; Leica QWIN V3; Leica Cambridgew Ltd).

Haematoxylin-stained sections were used for estimating capillary density. Extracellular nuclei account for the nuclei in capillary endothelial cells, and may thus be used to estimate the degree of capillarization. Mean muscle fibre area, number of muscle fibres, and number of extracellular nuclei were counted in artefact-free areas of 0.15 mm². Capillary density data were expressed as number of extracellular nuclei per muscle fibre, and muscle fibre area (mm²) per extracellular nucleus.

Electron microscopy
Biopsy specimens from muscle tissue were immediately fixed for a minimum of 24 h in McDowell’s fixative. Post-fixation was performed in 1% aqueous OsO₄. The specimens were dehydrated in series of graded ethanol and thereafter embedded in Epoxy resin. Semi-thin sections (2 μm) of four blocks from each biopsy were stained with 1% toluidine blue. After verifying representative material by light microscopy, one block from each biopsy was randomly selected for further stereology analysis of transmission electron microscopy. Ultra-thin sections (70 nm) from the selected blocks were placed on single-hole copper grids covered with formvar film. The ultra-thin sections were contrasted with 5% uranylacetat and subsequently with Reynolds lead citrate. The sections were examined in a JEOL 1010 electron microscope.

Stereology
A stereological method was used to quantify the relations of volume of the different tissue components and the volumes of the different cell organelles. Ten random pictures of each section were taken on ×10 000 magnification. Point-counting stereology on the electron micrographs was used for morphometric registration. The electron micrographs were covered by a 1 × 1 cm² lattice. Grid: grid points lying in interstitum (non-myocyte), myocyte, mitochondria, and lipids were counted. For each section, the number of counted grid points from 10 micrographs were summarized and expressed as absolute values.

Real-time quantitative reverse transcriptase-polymerase chain reaction skeletal muscle
Real-time quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) was only possible to perform in 17 of the 21 patients due to technical reasons. Total RNA was extracted from skeletal muscle using TRIzol (Invitrogen), DNase treated, cleaned up using RNeasy Mini Columns (Qiagen), and stored at −80 °C. cDNA was synthesized using High Capacity cDNA Archive Kit (Applied Biosystems). Quantification of gene expression was performed using the ABI Prism 7500 (Applied Biosystems). Power SYBR Green Master Mix (Applied Biosystems), and sequence-specific PCR primers designed using the Primer Express software, version 3.0 (Applied Biosystems). Gene expression of the housekeeping gene GAPDH was used for normalization. We also analysed the expression 185 rsRNA as an alternative housekeeping gene. GAPDH and 185 levels correlated well and similar findings were found when relating the expression of target mRNAs to 185.

Statistics
The technicians performing the muscle and plasma analyses were blinded for both patient identity and sequence. All data were analysed using SPSS 17. All quantitative measurements were reported as means ± 1 SD and changes from baseline to final visit were calculated as differences or ratios (the value at follow-up minus or divided by the baseline value, respectively). Paired t-test was used to test for significant differences from baseline to follow-up for normally distributed data and Wilcoxon sign rank test for non-normal data. The Pearson’s bivariate correlation-test and Spearman’s rho were employed for evaluating the relationship between changes in exercise performance, skeletal muscle, and cytokine levels as appropriate. Normal plots were used to assess normal distributions. A 5% significance level was used.

<table>
<thead>
<tr>
<th>Table 1 Baseline characteristics of the heart failure patients in the study</th>
</tr>
</thead>
<tbody>
<tr>
<td>HF patients (n = 21)</td>
</tr>
<tr>
<td>Age (years ± SD)</td>
</tr>
<tr>
<td>Gender (male/female)</td>
</tr>
<tr>
<td>Etiology (IHD/DCM)</td>
</tr>
<tr>
<td>NYHA class (III/IV)</td>
</tr>
<tr>
<td>Sinus rhythm (n)</td>
</tr>
<tr>
<td>Medication (%)</td>
</tr>
<tr>
<td>ACE inhibitor/ARB</td>
</tr>
<tr>
<td>β-Blocker</td>
</tr>
<tr>
<td>Diuretics</td>
</tr>
<tr>
<td>Aldosterone antagonist</td>
</tr>
</tbody>
</table>

HF, heart failure; IHD, ischaemic heart disease; DCM, dilated cardiomyopathy; NYHA, New York Heart Association; LBBB, left bundle branch block; ACE, angiotensin-converting enzyme; ARB, angiotensin II receptor blocker.
Results

Cardiac resynchronization therapy implantation was successful in all 21 patients without complications

Left ventricular remodelling, QRS width, and functional capacity

Baseline data are presented in Table 1. Three patients were in New York Heart Association (NYHA) functional class II and 18 in NYHA functional class III. The mean QRS duration was 173 ± 23 ms and the mean LVEF was 24 ± 6%. After 6 months of CRT, there was a significant decrease in LVEDD and QRS duration, and a decrease in QRS duration was seen in all but three patients (Table 2). These changes were accompanied by a modest but statistically significant improvement of VE/VCO₂ slope and a borderline increase in peak VO₂. Twelve patients improved one NYHA class and one patient reported a worsening of symptoms. The remainder of the patients reported a minor improvement of less than 1 NYHA class (Table 2).

Skeletal muscle morphology

Adequate samples were harvested from all 21 patients. Capillary density increased, while there was no increase in skeletal muscle fibre diameter (Table 3). There was a borderline statistically significant increase in proportion of both fibrosis and inflammatory cells (Table 3). We found no statistical significant changes in skeletal muscle ultra structure assessed with electron microscopy (density of mitochondria, myocytes, non-myocyte, or lipid droplets) (Table 3).

Cytokine levels

Assessment of plasma levels of cytokines were performed in all 21 patients, whereas homogenization of skeletal muscle for analysis of skeletal muscle mRNA of TNF-α, IL-6, and mediators of mitochondrial biosynthesis was performed in 17 patients only, due to technical reasons (thawed samples). We found no statistically significant changes, neither in plasma levels nor in muscular mRNA levels of pro-inflammatory cytokines after CRT, although there was a trend for reduction of mRNA IL-6 (Table 4).

Mitochondrial stimulating enzymes

There were no significant changes in mRNA levels of peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α), transcription factor A, mitochondrial (TFAM), vascular endothelial growth factor (VEGF), or nicotinamide phosphoribosyltransferase (NAMPT) in homogenized skeletal muscle after CRT (Table 5). However, these values of mRNAs correlated with each other both at baseline and after 6 months of CRT suggesting some significant interactions (Figure 1).

Table 2 Effect of CRT for 6 months on clinical variables

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Follow-up</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVEDD (cm)</td>
<td>6.8 ± 0.8</td>
<td>6.3 ± 0.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>QRS duration (ms)</td>
<td>172.9 ± 23.1</td>
<td>136.3 ± 23.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VE/VCO₂ slope</td>
<td>41.6 ± 7.4</td>
<td>39.1 ± 5.6</td>
<td>0.012</td>
</tr>
<tr>
<td>Peak VO₂ (mL/kg/min)</td>
<td>13.3 ± 2.2</td>
<td>14.5 ± 2.6</td>
<td>0.07</td>
</tr>
<tr>
<td>RER max</td>
<td>1.2 ± 0.1</td>
<td>1.2 ± 0.1</td>
<td>0.38</td>
</tr>
</tbody>
</table>

n = 21 heart failure patients. n = 17 for ergospirometry. Data are mean ± SD. NYHA, New York Heart Association; LVEF, left ventricular ejection fraction; LVEDD, LV end-diastolic diameter; RER max, respiratory exchange ratio at max exercise.

Table 3 Effect of CRT for 6 months on morphometric characteristics of skeletal muscle (N = 21)

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Follow-up</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light microscopy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibre diameter (μm)</td>
<td>61.8 ± 10.4</td>
<td>57.8 ± 12.4</td>
<td>0.29</td>
</tr>
<tr>
<td>Fibrosis (%)</td>
<td>15.2 ± 6.1</td>
<td>18.8 ± 4.6</td>
<td>0.047</td>
</tr>
<tr>
<td>Microvascular fraction (%)</td>
<td>4.5 ± 2.4</td>
<td>7.7 ± 3.3</td>
<td>0.002</td>
</tr>
<tr>
<td>Inflammatory cells (%)</td>
<td>0.7 ± 0.4</td>
<td>1.1 ± 0.67</td>
<td>0.040</td>
</tr>
<tr>
<td>Electron microscopy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mitochondria (grid points)</td>
<td>189 ± 72</td>
<td>205 ± 72</td>
<td>0.35</td>
</tr>
<tr>
<td>Myocyte (grid points)</td>
<td>2603 ± 120</td>
<td>2610 ± 113</td>
<td>0.75</td>
</tr>
<tr>
<td>Non-myocyte (grid points)</td>
<td>184 ± 120</td>
<td>152 ± 85</td>
<td>0.38</td>
</tr>
<tr>
<td>Lipid droplets (grid points)</td>
<td>37 ± 27</td>
<td>33 ± 21</td>
<td>0.94</td>
</tr>
</tbody>
</table>

n = 21 heart failure patients. Data are presented as mean ± SD.

Table 4 Effect of cardiac resynchronization therapy for 6 months on levels of inflammatory cytokines

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Follow-up</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma levels, N = 21</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNF-α (pg/mL)</td>
<td>46.3 ± 30.7</td>
<td>43.2 ± 26.6</td>
<td>NS</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>15.6 ± 9.4</td>
<td>15.8 ± 11.0</td>
<td>NS</td>
</tr>
<tr>
<td>IL-8 (pg/mL)</td>
<td>21.9 ± 13.6</td>
<td>21.7 ± 17.1</td>
<td>NS</td>
</tr>
<tr>
<td>MCP-1 (pg/mL)</td>
<td>63.8 ± 31.4</td>
<td>62.1 ± 33.8</td>
<td>NS</td>
</tr>
<tr>
<td>Muscular gene expression, N = 17</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNF-α mRNA</td>
<td>4.6 ± 2.7</td>
<td>4.5 ± 2.0</td>
<td>NS</td>
</tr>
<tr>
<td>IL-6 mRNA</td>
<td>4.7 ± 3.1</td>
<td>3.8 ± 1.6</td>
<td>NS</td>
</tr>
</tbody>
</table>

n = 21 heart failure patients for plasma levels. N = 17 for muscular gene expression. Data are presented as mean ± SD. mRNA levels are arbitrary and presented relative to the expression of GAPDH. TNF, tumor necrosis factor; IL, interleukin; MCP, monocyte chemoattractant protein.
Correlation between changes in QRS duration, ventilation, and skeletal muscle

Changes in mitochondrial density correlated with both changes in QRS duration ($r = 0.57$, $P = 0.008$) and changes in peak VO$_2$ ($r = 0.54$, $P = 0.03$; Figure 2). In patients with increased mitochondrial density after CRT the difference between baseline values and 6 month values for QRS duration and peak VO$_2$ were highly significant (Table 6). Changes in VE/VCO$_2$ were statistically significant correlated with PGC-1a ($r = 0.569$, $P = 0.034$; Figure 3).

Responders

Response to CRT is a major, unresolved issue related to the fact that evolution of HF is still unpredictable in the single patient. The effort to improve patient selection in order to maximize human and financial resource utilization has fallen through so far. Instead of using a cut-off value for some certain parameters, risk stratification employing determination of LV volumes, aetiology, QRS duration, and morphology, etc. may better define the non-responder. With this in mind we explored if there were any significant changes in cytokine levels, mitochondrial mediators or skeletal muscle ultra-structure in patients with some kind of response to CRT.

Peak VO$_2$

Eleven of 17 patients increased their peak VO$_2$. At baseline there were no differences between the groups except for a borderline significant lower number of previous myocardial infarctions in the responder group. After CRT for 6 months there were no differences between responders and non-responders except for the change in LVEDD which was reduced from 7.0 to 6.2 ($P = 0.002$) in responders (difference between groups $P = 0.022$). There were no consistent improvements in inflammation or skeletal muscle ultra-structure.

Left ventricular end-diastolic diameter

Five patients did not show a reduction in LVEDD after CRT. Independent sample testing showed a significant difference between groups (based on LVEDD reduction or not) with regard to QRS reduction ($P < 0.05$) and a borderline difference with regard to increase in peak VO$_2$ ($P = 0.049$). There were no consistent improvements in inflammation or skeletal muscle ultra-structure.

**NYHA class**

Twelve patients improved their NYHA class with 1 level. There were no additional statistical significant changes in any parameter compared to the findings in the whole group. There were no consistent improvements in inflammation or skeletal muscle ultra-structure.

**Discussion**

In the present study we show that after at least 6 months of CRT, the increased exercise capacity was accompanied by increased

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**Table 5** Effect of CRT for 6 months mitochondrial stimulating enzymes

<table>
<thead>
<tr>
<th>Muscular gene expression, $N = 17$</th>
<th>Baseline</th>
<th>Follow-up</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGC-1α</td>
<td>2.05 ± 1.43</td>
<td>2.37 ± 2.58</td>
<td>NS</td>
</tr>
<tr>
<td>TFAM</td>
<td>2.93 ± 1.72</td>
<td>2.53 ± 1.39</td>
<td>NS</td>
</tr>
<tr>
<td>VEGF</td>
<td>0.68 ± 0.24</td>
<td>0.73 ± 0.44</td>
<td>NS</td>
</tr>
<tr>
<td>NAMPT</td>
<td>0.69 ± 0.36</td>
<td>0.61 ± 0.22</td>
<td>NS</td>
</tr>
</tbody>
</table>

$N = 17$ for muscular gene expression. Data are presented as mean ± SD. mRNA levels are arbitrary and presented relative to the expression of GAPDH.

PGC-1α, peroxisome proliferator-activated receptor (PPAR)γ coactivator-1α; TFAM, transcription factor A, mitochondrial; VEGF, vascular endothelial growth factor; NAMPT, nicotinamide phosphoribosyltransferase.
capillary density and improved VE/VCO₂ slope. In contrast, there were no statistically significant reductions in pro-inflammatory cytokines and no increase in mediators of mitochondrial biosynthesis or improvement in skeletal muscle pathology except for the increased capillary density.

Exercise capacity, VE/VCO₂, and capillary density

In the current study we found a significant reduction of LVEDD and improvement of electrical dyssynchrony with decreased QRS duration, confirming the well-known effect of CRT on central haemodynamics which in other studies also includes an increased cardiac index and reduced PCWP. This improvement was associated with an improved functional status as assessed by an overall statistically borderline increase in peak VO₂, statistically enforced in patients with decreased LVEDD, underlining the importance of improved cardiac function as a mediator for improved exercise capacity after CRT. These findings were supported by a statistically significant difference in LVEDD between VO₂max responders and non-responders.

CRT was associated with an improved VE/VCO₂ slope, in accordance with a recent publication on CRT effects in 27 patients with severe HF22 and this parameter might be more sensitive for changes in functional status in this population. In addition, VE/VCO₂ slope is superior to peak VO₂ as a prognostic indicator in that VE/VCO₂ slope is less related to patients’ effort than peak VO₂. The latter parameter is also more influenced by skeletal muscle mass and function than VE/VCO₂ slope.23 Cardiac resynchronization therapy improves cardiac function with improvements in pulmonary pressures and cardiac output, thus improving VE/VCO₂ due to these central-mediated effects. In addition to its relationship to pulmonary perfusion and central haemodynamics, VE/VCO₂ slope has also been associated with the ergo-receptor reflex in HF.24 This ergo-reflex is a neural over signalling sensitive to muscle by-products which causes exercise hyperventilation. In the current study we found a statistically significant correlation between improvement in VE/VCO₂ slope and changes in PGC-1α, a critical factor coordinating the activation of metabolic genes required for substrate utilization and mitochondrial biogenesis in skeletal as well as in cardiac muscle.

Improved cardiac synchronization was also associated with an increased capillary density. This finding is in accordance with the results from a recent study demonstrating an increased functional capillary density by use of orthogonal polarization spectral imaging sublingually 6 months after CRT implant.25 Although we found no correlation between VEGF and this phenomenon, the involvement...
of other angiogenesis-stimulating factors cannot be excluded. Increased physical activity after CRT implantation inducing increased capillary density may be operative, but we did not encourage the patients to be more active at this point of time.

The concept of capillary density is controversial. Capillary density may vary with fibre type, and fibre-type switching may be a dynamic process in patients with HF. Using cell-specific antibodies to measure vascular density, a decreased number of endothelial cells per fibre, implying reduced capillary density has been documented. However, other investigators have reported a normal skeletal muscle capillary density in patients with HF, but this may be influenced by the actual NYHA class. We have previously compared capillary count obtained by haematoxylin staining and staining with antibodies to factor VIII. The counts obtained by the two different staining methods varied by <15%. Due to this and because of less staining-related artefacts the haematoxylin staining was preferred in the present study.

**Skeletal muscle and inflammation**

Changes in peak VO$_2$ and the shortening of the QRS complex after CRT significantly correlated with changes in mitochondrial density, but we found no statistically significant increase in mitochondrial density or reversal of skeletal muscle pathology after CRT. This indicates that increased physical activity might be a prerequisite to improve skeletal muscle function in HF. Another explanation might merely be that HF is a dynamic and progressive syndrome and that this process is more or less slowed down in the different HF patients after CRT. Mitochondrial volume and density are increased in the initial and intermediate phase of HF as a compensatory mechanism, whereas there is a decrease in mitochondrial density in advanced HF. The biogenesis of mitochondria requires the expression of a large number of genes encoded by both nuclear and mitochondrial genetic systems. TFAM has been shown to be one of the mechanisms regulating mitochondrial biogenesis in regenerating skeletal muscle. TFAM is imported into mitochondria and controls the expression of mitochondrial DNA which contributes to protein products that are vital for electron transport and ATP synthesis. Activation by PGC-1α and TFAM has been shown to be one of the mechanisms regulating mitochondrial biogenesis in regenerating skeletal muscle. Exercise induces the activation of PGC-1α. In addition, exercise-induced NAMPT activity, which indirectly activates PGC-1α, correlates with mitochondrial content.

The effect of CRT on functional capacity seems to be mostly related to the improvement of central haemodynamics and increased capillary density without an improvement in skeletal muscle pathology per se or inflammation. A normalization of peripheral pathology may thus be dependent on increased physical activity and exercise training to reverse the pathological effect of de-conditioning. The increase in vascular density may be the first sign of increased physical activity which may be due to improved central haemodynamics. Exercise training exerts antioxidative effects in the skeletal muscle in CHF, in particular, due to an augmentation in activity of radical scavenger enzymes. In addition, exercise training significantly reduces the local expression of TNF-α, IL-1β, IL-6, and inducible NOS in the skeletal muscle of CHF patients. This is in accordance with a recently published study on 50 patients treated with CRT who were randomized to exercise training or not after CRT. The authors found that exercise training leads to further improvements in exercise capacity, haemodynamic measures, and QOL in addition to the improvements seen after CRT.

A reduction of inflammatory status after CRT has previously been documented, but only in patients with reverse remodelling as well as in patients with better clinical prognosis. The lack of effect in the current study on TNF is in accordance with a small study on eight patients in which the authors did not find changes in the levels of IL-1β, TNF-α, IL-10, but only in IL-6 which also tended to decrease in the present study. Our findings are also partially supported by the findings in a study on 20 symptomatic HF patients in whom there was only a borderline decrease in levels of TNF-α and its soluble receptors after 3 months of pacing.

**Limitations**

This study is limited by its open design and the limited number of patients. However, the scientists and technicians who performed the analyses of skeletal muscle, enzymes, and cytokines were ‘blinded’ with no knowledge of ID number of the individual patient or sample sequences. The analysis after CRT was performed ~6–10 months post-CRT insertion. We cannot exclude the possibility that increased aerobic activity may have a major role in increasing the capillary density in skeletal muscle. However, the patients were not instructed or encouraged to perform exercise training during the study period. Also, a learning effect from previous tests may influence the results of the peak VO$_2$-test. On the other hand, most of these patients have performed bicycle ergospirometry in research and for clinical assessment many times before. The effect on functional capacity might have been better assessed with endurance or sub-maximal tests.

**Conclusions**

Reversed remodelling after CRT was associated with improved functional capacity and increased capillary density which may be the first sign of increased physical activity secondary to improved cardiac function and improved skeletal muscle perfusion. An improvement of VE/VCO$_2$ slope was correlated to changes in PGC1α, a marker of mitochondrial biosynthesis and oxidative metabolism. We found no reduction of pro-inflammatory cytokines, and no increase in skeletal muscle mitochondrial biosynthesis or fibre diameter after 6 months of CRT. Thus, the improved cardiac function is not directly reversing skeletal muscle pathology or inflammation, but may be the prerequisite for increased physical activity necessary to improve the actual peripheral pathology.

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