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**Effect of cardiac resynchronization therapy on endothelium-dependent vasodilatation
in the cutaneous microvasculature**

Erik (E.J.) Tesselaar, Ph.D.^{1,4}, Angélique (A.A.J.J.) Schiffer, Ph.D.³, Jos (J.W.M.G)

5 *Widdershoven, Ph.D., M.D.², Herman (H.) Broers², Eric (E.) Hendriks², Kees (C.) Luijten²,
John (J.H.) Creusen¹*

¹ Department of Clinical Physics, ² Department of Cardiology, ³ Department of Medical
Psychology, TweeSteden Ziekenhuis, Tilburg, The Netherlands

⁴ Department of Clinical and Experimental Medicine, Faculty of Health Sciences, University
10 Hospital, Linköping, Sweden

Running title

CRT and endothelial function

Correspondence

15 Erik Tesselaar, Ph.D., Department of Clinical and Experimental Medicine, University
Hospital of Linköping, SE-581 85 Linköping, Sweden, Phone: + 46 10 103 37 56, Fax: + 46
10 103 28 36, E-mail: erik.tesselaar@liu.se

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Psychology, Tilburg University, the Netherlands.

Conflict of Interest

This work was financially supported by Medtronic NL B.V., Heerlen, The Netherlands.

Abstract

25 **Aims** Cardiac resynchronization therapy (CRT) improves hemodynamic parameters,
exercise capacity, symptoms, functional status and prognosis among patients with chronic
heart failure (CHF). The role of the vascular endothelium in these improvements is largely
unknown. In this study, we aimed to investigate whether the endothelium-dependent
30 reactivity of the peripheral microcirculation improves in CHF patients during the first 2
months of CRT.

Methods We used local heating and iontophoresis of acetylcholine (ACh) and
sodium nitroprusside (SNP) to measure endothelial function and smooth muscle function in
the cutaneous microvasculature of 11 CHF patients before and 2 months after CRT.

Results We found that the perfusion response in the skin to local heating was
35 increased 2 months post-CRT compared with baseline, both in terms of maximum perfusion
(baseline: 113 [90-137] versus. 2-months post-CRT: 137 [98-175], $p = 0.037$) and area under
curve (baseline: 1601 [935 to 2268] versus 2-months CRT: 2205 [1654 to 2757], $p = 0.047$).
Also, the perfusion response to iontophoresis of ACh was improved (E_{\max} : 23.9 [20.6 to 26.2]
vs at 2-months CRT: 31.2 [29.3 to 33.4], $p = 0.005$). No difference was found between the
40 responses to SNP before and after CRT.

Conclusion These results show that CRT improves endothelium-dependent
vasodilatory capacity in the peripheral microcirculation within 2 months of therapy. The
improvement in functional capacity that is seen in patients treated with CRT may therefore be
in part mediated by an improvement of endothelium-dependent vasodilatory capacity.

45 **Key words: heart failure, biventricular pacing, endothelium, microcirculation**

Introduction

In chronic heart failure (CHF) patients, the function of major and intermediate vessels in the systemic circulation and microvascular function are compromised (2; 3). In recent years, the role of a failing endothelium in CHF has been recognised and investigated (4; 5). Both myocardial and peripheral perfusion are impaired, partly due to a reduction in endothelium-dependent vasodilatory capacity. It has been suggested that the severity of endothelial dysfunction can be used as a prognostic factor for the long term outcome in CHF and coronary artery disease (5; 6). Andreassen and colleagues (7) found that peripheral endothelial –dependent vasodilatation is attenuated in candidates for heart transplantation, but improves within the first year after the transplantation (8). Also, peripheral endothelial function one month after the transplantation may be used to identify the risk of cardiac allograft vasculopathy (9).

In a subgroup of CHF patients, cardiac output is reduced due to asynchronous contraction of the ventricles. These patients can be treated with cardiac synchronisation therapy (CRT) by implanting a biventricular pacemaker. In large-scale clinical trials, CRT has been shown to improve haemodynamic parameters, exercise capacity, symptoms, and functional status, and to reduce mortality and hospitalisation (10-12). However, the mechanisms responsible for these improvements are not yet understood.

A recent study by Akar and colleagues (13) indicated that a dysfunctioning endothelium might identify responders to CRT (as defined using the Clinical Composite Score), but they were not able to find a significant improvement in endothelial function in these responders. In another study it is shown that biventricular pacing leads to an acute improvement in microvascular perfusion (14), but it is unknown whether CRT restores the impaired endothelium-dependent vasodilatory capacity on the longer term.

70 We hypothesised that the improvement in functional capacity that is seen in CHF patients treated with CRT may be mediated by an improvement of endothelial function. The aim of this study was therefore to investigate whether the endothelium-dependent reactivity of the peripheral microcirculation improves in CHF patients during the first 2 months of CRT.

Methods

75 Study Population

Between April 2008 and November 2009, all CHF patients eligible for CRT at the Cardiology Department of the TweeSteden teaching Hospital in Tilburg, the Netherlands, were approached for participation in the current study. Twelve patients were asked for participation and 11 (92%) agreed and completed the baseline assessment and follow-up after 80 2 months (5 women, mean age 70.5 ± 7.4 years, range 62 to 83 years).

All patients were treated according to the most recent ACC/AHA guidelines (15; 16). They all had symptomatic CHF (New York Heart Association (NYHA) classes III or IV), delayed ventricular conduction (QRS duration 195 ± 5 ms), and were on optimal medical therapy. Nine patients were implanted with a biventricular pacemaker, 2 with a biventricular 85 pacemaker/defibrillator. Inclusion criteria for CRT, and thereby for this study, were (1) diagnosis of systolic CHF, (2) being on optimal medical therapy, (3) NYHA functional class III or IV, with a QRS duration ≥ 120 ms, and (4) left ventricular ejection fraction (LVEF) $\leq 40\%$. In addition, (5) at least one of the following echocardiographic criteria had to be fulfilled: an aortic pre-ejection delay > 140 ms, an interventricular mechanical delay > 40 ms, 90 or delayed activation of the posterolateral left ventricular wall. Patients were asked to refrain from ingesting alcohol, caffeine and vitamin C for 6 hours before the start of the measurements. Also, they were asked not to take any vasoactive medication in the morning prior to the measurement.

The study conforms with the principles outlined in the Declaration of Helsinki and was
95 approved by the hospital medical ethics committee. Every patient received verbal and written
information about the study and provided written informed consent. Participation was
voluntary and patients were free to withdraw at any time during the study without further
explanation or consequences for their treatment.

100 **Study design**

After patients enrolled in the study, echocardiographic and microvascular measurements were
performed within 3 days before pacemaker implantation (baseline) and were repeated 60 (\pm
7) days after CRT. Echocardiographic parameters included LV ejection fraction, dP/dt and
inter- and intraventricular delays. Endothelial function was assessed by measuring the
105 microvascular perfusion response in the forearm skin upon local heating to 44 °C and by
measuring the response to acetylcholine (ACh) given transcutaneously by iontophoresis.
Also, sodium nitroprusside (SNP) was given by iontophoresis to investigate endothelium-
independent reactivity. Within one day after the device had been implanted, atrioventricular
and interventricular timings of the pacemaker were optimised by tissue velocity imaging in
110 all patients.

Echocardiography

Measurements were made according to the criteria published by the American Society of
Echocardiography (17). Patients were examined in the left lateral decubitus position using a
commercially available system (Vivid 7, GE Healthcare, Horten, Norway). A 3.5-MHz
115 transducer was used to obtain images and was focused at a depth of 16 cm in the parasternal
and apical views (standard long-axis and two- and four-chamber images).

End-systolic and end-diastolic left ventricular diameters were measured in the parasternal long axis view using the M-mode. Left ventricular ejection fraction was calculated using the modified Simpson's rule from apical four- and two-chamber views. dP/dt was measured from
120 the continuous-wave Doppler spectrum of the mitral regurgitation jet.

Intraventricular delays were measured using Tissue Doppler imaging. The sample volume was placed in the basal portions of the septum and the lateral wall. The septal-to-lateral delay in peak velocity was calculated as an indicator of ventricular dyssynchrony. Interventricular time delay was defined as the time difference between the onset of the velocity time integral
125 at the left ventricular outflow tract and the right ventricular outflow tract, using the R-wave on the ECG channel as a reference point.

During follow-up measurements, CRT was optimised prior to the echocardiographic examinations by adjusting the A-V and V-V interval, such that stroke volume and left-ventricular dP/dt were maximised. Data were analysed using commercial software (Echopac
130 6.1, General Electric-Vingmed). Echocardiographic measurements were performed by the same experienced sonographer.

Microvascular measurements

Microvascular function was assessed by iontophoresis of vasoactive drugs and by local heating, both in combination with laser-Doppler flowmetry. Iontophoresis is a method of
135 controlled drug delivery, in which drugs are delivered from an electrode chamber into the skin in a by means of a small, constant electric current. The effects of the drugs on the local microcirculation can be assessed at the same time by a fiberoptic laser-Doppler probe, which is integrated in the electrode chamber (18; 19).

A laser-Doppler Perfusion Monitoring unit (PeriFlux 5000, Perimed AB, Järfälla, Sweden)
140 with a laser Doppler probe for iontophoresis (PF481-1, Perimed AB, Järfälla, Sweden) was

used for the simultaneous delivery of drugs and measurement of local skin perfusion.

Furthermore, a thermoregulated laser-Doppler probe (PF457, Perimed AB, Järfälla, Sweden) was used for simultaneous heating of the skin and measurement of perfusion.

Disposable drug delivery electrodes (PF 383, Perimed AB, Järfälla, Sweden) were mounted
145 to the laser Doppler probe to deliver drug solutions to the skin using iontophoresis. A battery-
powered iontophoresis controller was used to deliver a constant electric current of 0.02 mA
(PeriIont 382, Perimed AB, Järfälla, Sweden).

Acetylcholine chloride (Miochol®-E 10 mg/ml, Novartis Healthcare, Denmark), and sodium
nitroprusside (25 mg/ml, prepared by hospital pharmacy) were used without further dilution.

150 Drug concentrations were the same throughout the experiments. All drugs were prepared
immediately before being used and SNP was kept in the dark.

All experiments were done in a temperature-controlled room (22-23°C). Patients were seated
comfortably in a half upright position during the measurements with one of the forearms
rested on an arm support slightly below heart level. After the flexor side of the forearm had
155 been gently cleaned with an ethanol wipe, the iontophoresis probe and accompanying
iontophoresis electrode was filled with either ACh or SNP and attached to the skin by double
adhesive tape. An indifferent electrode was applied to the wrist and both electrodes were
connected to the current controller. Patients rested for 15 minutes before any data were
collected. In 1 patient, the response to ACh and SNP was not measured due to technical
160 difficulties.

In previous studies we have optimised our iontophoresis protocols to reduce the vasodilatory
effect of the current alone, and to maximise the response to the drugs, with most responses
ending as plateaus (18; 20). After a current-free baseline period of 1 minute, a constant direct
electrical current of 0.02 mA was applied during 10 minutes, while the perfusion in the area

165 of the skin under the probe was continuously recorded with a sampling rate of 1 Hz. This procedure was performed for both drugs, and a different area of the skin were used for each drug. ACh was given by anodal iontophoresis, SNP was given by cathodal iontophoresis. Drugs were given in a randomised order.

The thermoregulated probe was attached to the skin by double adhesive tape on a separate
170 area of the same forearm. After a baseline period of 1 minute, the probe was heated to 44 °C. This temperature was reached within a few seconds and was sustained for 20 minutes, while the perfusion in the area of the skin under the probe was measured continuously, also with a sampling rate of 1 Hz.

Each area of measurement on the skin was selected to avoid visible veins and pigmented
175 nevi. The subjects were told to hold their arms still during the experiment.

Data analysis and statistics

Perfusion responses were calculated as change from baseline, where baseline was defined as the mean perfusion value during 1 minute before starting the local heating or iontophoresis.

The data obtained with the local heating experiments were analysed by calculating the area
180 under the curve from 5 to 20 minutes after the temperature was set to 44 °C, because the perfusion response during the first 5 minutes contains a neurally mediated component. The maximum response to local heating was calculated as the mean response during the last 5 minutes of the measurement.

The data obtained with the iontophoresis experiments were analysed by fitting an E_{\max} model
185 to the perfusion data as described previously (18).

The maximum response (E_{\max}) and the respective confidence intervals (95% CI) were estimated for each response by fitting the model to the perfusion data. Since baseline flow was subtracted, E_{\min} was constrained to zero.

The significance of the difference in best-fit parameters between baseline and at 2 months
190 CRT was tested using an F-test. Probabilities of less than 0.05 were accepted as significant.
Data in the figures are presented as means \pm SEM. For clarity, a limited number of data
points are shown in the figures. Data were analysed using GraphPad Prism version 5.00 for
Windows (GraphPad Software, San Diego, California, USA).

Results

195 Patient characteristics

Fifty-five percent of the patients were male. All patients had impaired left ventricular function (LVEF 28 ± 8 ; dP/dt 645 ± 161 ; LV end-systolic diameter 56 ± 6 ; LV end-diastolic diameter 65 ± 8) and all were in NYHA class 3 when they enrolled in the study. Forty-five percent of the patients did not receive ACE-inhibitors because of contraindications. There
200 were no changes in the use of any of the medications between baseline and 2 months of CRT (McNemar test, $p > 0.24$). A detailed summary of the demographic data is given in Table 1. Clinical measures at baseline and follow-up are given in Table 2.

Responses to local heating

A strong increase in skin perfusion was observed in all patients immediately after the
205 temperature was increased to $44\text{ }^{\circ}\text{C}$. The perfusion continued to increase gradually during the 20 minutes of local heating. The maximum perfusion and the area under the curve (AUC) were significantly higher 2 months post-CRT compared with the AUC at baseline ($p = 0.037$ and $p = 0.047$, respectively). An overview of the results is provided in Table 3 and Figure 1.

Responses to ACh and SNP

210 The endothelium-dependent increase in perfusion seen during iontophoresis of ACh was significantly higher 2 months post-CRT compared with baseline (E_{max} : 31.2 ± 0.9 A.U. versus 23.9 ± 1.7 A.U, $p = 0.005$), whereas there was no change in the maximum response to SNP (2 months CRT, E_{max} : 21.7 ± 1.1 A.U. versus baseline, 21.8 ± 1.6 A.U.; $p = 0.94$). The results are summarised in Table 3 and Figures 2 (ACh) and 3 (SNP).

215

Discussion

The objective of the current study was to examine whether the endothelium-dependent reactivity of the peripheral microcirculation improves in CHF patients during the first months of resynchronisation therapy. We found that endothelium-dependent vasodilatation, as
220 assessed by local heating and iontophoresis of acetylcholine, is increased in CHF patients within two months of resynchronisation therapy, whereas the total vasodilatory capacity of smooth muscle, measured as the vascular response to sodium nitroprusside, is unchanged.

During recent years, it has been recognised that endothelial dysfunction has a crucial role in the development of symptoms in CHF, such as decreased exercise capacity (2-4; 8). At least
225 part of the reduction in ventricular function is the result of an impaired endothelium-dependent vasodilatory capacity of the coronary vessels. Also, decreased peripheral vasodilatory capacity leads to higher systemic vascular resistance, which further increases cardiac workload and worsens symptoms (21).

One of the most important contributing factors to endothelial dysfunction is the reduction in
230 the availability of NO. Possible mechanisms are the reduction in flow, which leads to less shear stress in conductance and resistance arteries, and the release of increased amounts of reactive oxygen species (ROS), which rapidly inactivate NO. Although CRT does not have a direct effect on peripheral vascular function, it induces favourable changes in peripheral vascular function. A previous study found that CRT acutely improved microcirculatory
235 perfusion, as measured by orthogonal polarisation spectral imaging in the sublingual microvascular bed (14). These acute changes are considered to eventually lead to an improvement in peripheral function, through increased peripheral shear stress, better cardiac loading conditions, and neurohormonal activation. All these may lead to the increase in the production of endothelium-derived NO that we observed in the current study. Another

240 possible mediator is the effect of CRT on physical activity. At baseline, only one patient was physically active. After 2 months of CRT, 5 patients reported physical activity. Although we were unable to find any difference in the change in microvascular reactivity between patients who became more physically active and patients who did not (Student's t-test, $p > 0.12$), we can not rule out the possibility of an effect of physical activity, due to the limited statistical
245 power of the study.

The results of the current study also agree with the results from the recent study by Akar et al. (13), who found that responders to CRT could be identified by their endothelial function before implantation, as measured by flow-mediated vasodilatation. They also observed an increase in endothelial function, as assessed by flow-mediated vasodilatation, after 90 days of
250 CRT, although this did not reach statistical significance. Another study showed that after 3 months, CRT improved the post-occlusive hyperaemia response in the forearm (22). Post-occlusive reactive hyperaemia is considered to be NO-dependent and the test is often used to assess endothelial function.

There are several methods to quantify endothelium function in the peripheral circulation. The
255 most common is venous occlusion plethysmography after occlusion of the brachial artery or after a challenge with endothelium-dependent and -independent dilators. In the present study, we used local heating and iontophoresis of acetylcholine on the forearm, which are both well established, non-invasive techniques that have been used in a number of previous studies to measure endothelial function. Andersson et al. (2) used the same techniques to compare
260 microvascular reactivity between healthy young adults, healthy elderly adults and elderly adults with heart failure. A clear reduction was found of the vasorelaxant responses in the heart failure group compared to the elderly group without heart failure and compared to the young adults. In a later study (23) it was found that the blood flow responses to local heating were reduced in heart failure patients compared to age- and gender-matched controls. Similar

265 studies in heart transplantation (7), coronary heart disease (24), diabetes (25) and essential hypertension (1; 26) have indicated that impaired microvascular reactivity in the skin, as measured by the techniques used in our study, is related to endothelial dysfunction.

The vasodilatory response seen with local heating is known to be biphasic, with an initial neurally mediated peak and a late plateau which is almost completely dependent on NO
270 production from the endothelium (27; 28). Interestingly, although we have often observed the biphasic response to local heating using our protocol in healthy volunteers, we did not observe an initial peak in the blood flow response to local heating in our current study.

Whether the absence of an initial peak is the result of an impaired neurogenic response in our patient group remains to be elucidated. The vasodilation induced by exogenous ACh in
275 human skin is mainly mediated by the release of NO from the endothelium through activation of NOS, although part of the response is mediated by prostaglandins, and possibly other factors (29).

This study has a number of limitations. First, the number of included patients is quite small. Also, the number of mechanical responder was high (72-91% of the patients had improved
280 end-systolic diameter, QRS duration and intraventricular delay). For these reasons, we consider it impossible to relate changes in endothelial function to other known markers that are important in predicting prognosis after CRT. However, even with the limited number of patients, the results indicate that the techniques used in this study are valuable in assessing changes in endothelial function in patients that are treated with CRT, not least since these are
285 established techniques that are noninvasive and relatively easy to perform. We therefore recommend larger longitudinal studies to relate individual improvements in endothelial function to response to therapy.

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Author's contributions

	Concept / Design	Data collection	Data Analysis / interpretation	Drafting article	Critical revision	Approval	Statistics	Funding
ET	x	x	x	x	x	x	x	
AS	x	x	x	x	x	x	x	
JW	x		x	x	x	x		x
HB		x			x	x		
EC		x			x	x		
CL		x			x	x		
JC					x	x		x

Tables

295 *Table 1. Characteristics of the patients included in the study.*

Patient Characteristics (N = 11)

Demographics

sex (male) 55%

Medication

ACE 55%

digoxin 9%

beta blockers 91%

aldosterone antagonists 36%

statines 27%

aspirin 64%

loop diuretics 91%

Comorbidities

hypertension 64%

lipid disorders 64%

diabetes mellitus type II 27%

COPD 9%

Cardiac History

Myocardial infarction 50%

Arrhythmia 17%

PCI 33%

CABG 25%

Table 2. Clinical and echocardiographic measures at baseline and after 2 months of CRT. * $p < 0.05$; ** $p < 0.01$.

Clinical measures	Baseline		2 months CRT		p-value
	mean	SD	mean	SD	
Age (years)	70.5	(7.4)			
NYHA class	3	(0)	2.9	(0.3)	0.34
Systolic blood pressure (mmHg)	133	(28)	125	(22)	0.37
Diastolic blood pressure (mmHg)	76	(8.0)	75	(8.1)	0.91
Left ventricular ejection fraction (%)	28	(7.6)	32	(8.3)	0.68
dP/dt (mmHg)	645	(161)	886	(280)	0.07
LV end-systolic diameter (mm)	56	(6.1)	50	(9.2)	0.04*
LV end-diastolic diameter (mm)	65	(8.4)	61	(9.9)	0.012*
LV intraventricular delay (ms)	49	(30)	24	(18)	0.005**
Heart rate (min ⁻¹)	70	(17)	69	(8.3)	0.76
QRS duration (ms)	154	(32)	112	(21)	0.0012**

300 *Table 3. Skin blood flow responses to local heating and iontophoresis of acetylcholine (endothelium-dependent) and nitroprusside (endothelium-independent).*

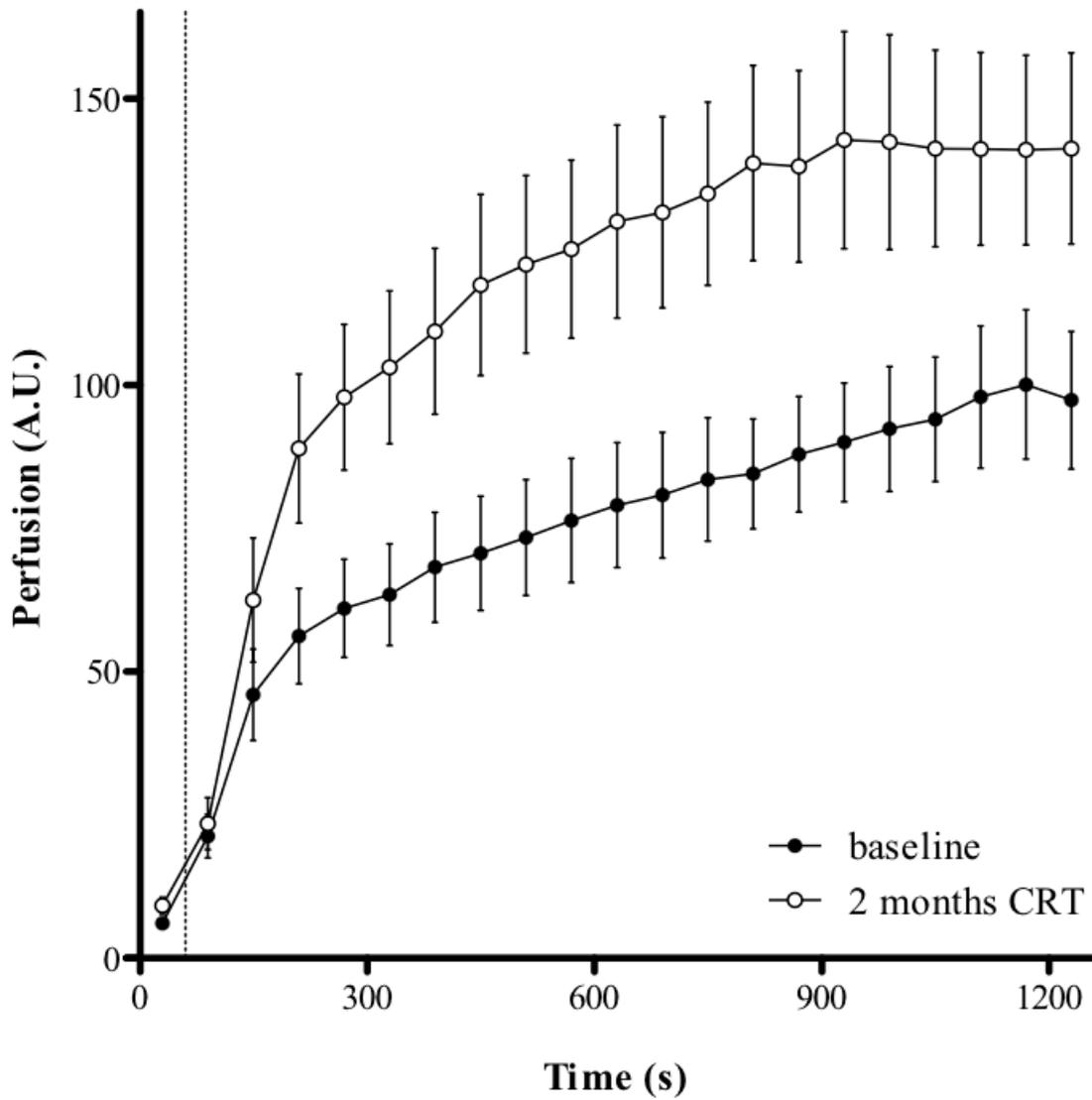
	Local heating				
	Baseline		2 months CRT		p-value
	Mean	SD	Mean	SD	
Baseline perfusion	7.9	(3.5)	9.1	(4.6)	0.2
Maximum response	97	(42)	137	(53)	0.037*
AUC	1466	(991)	2205	(770)	0.047*

	Acetylcholine iontophoresis				
	Baseline		2 months CRT		p-value
	Mean	SD	Mean	SD	
Baseline perfusion	3.1	(1.3)	3.3	(2.3)	0.85
E _{max}	23.9	(1.7)	31.2	(0.9)	0.005**

	Sodium Nitroprusside iontophoresis				
	Baseline		2 months CRT		p-value
	Mean	SD	Mean	SD	
Baseline perfusion	2.9	(0.8)	2.5	(1.3)	0.84
E _{max}	21.8	(1.6)	21.7	(1.1)	0.94

Figures

Figure 1. Skin hyperemic blood flow response to local heating to 44°C before and after 2 months of CRT. Maximum vasodilatation and area under the curve were significantly increased after 2 months of CRT.



305

Figure 2. Endothelium-dependent blood flow response to acetylcholine delivered by iontophoresis. The maximum vasodilatory response was significantly increased after 2 months of CRT.

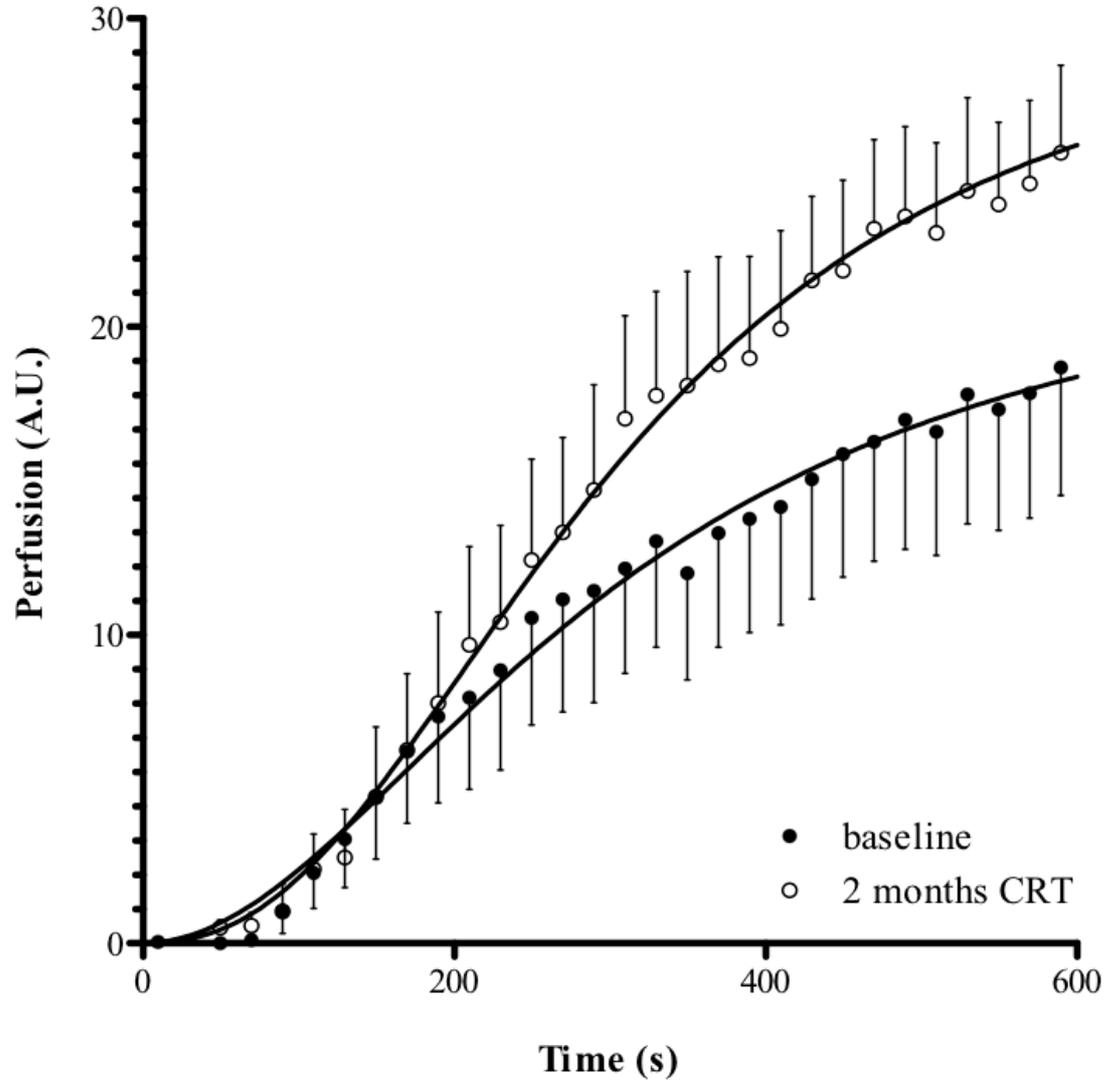
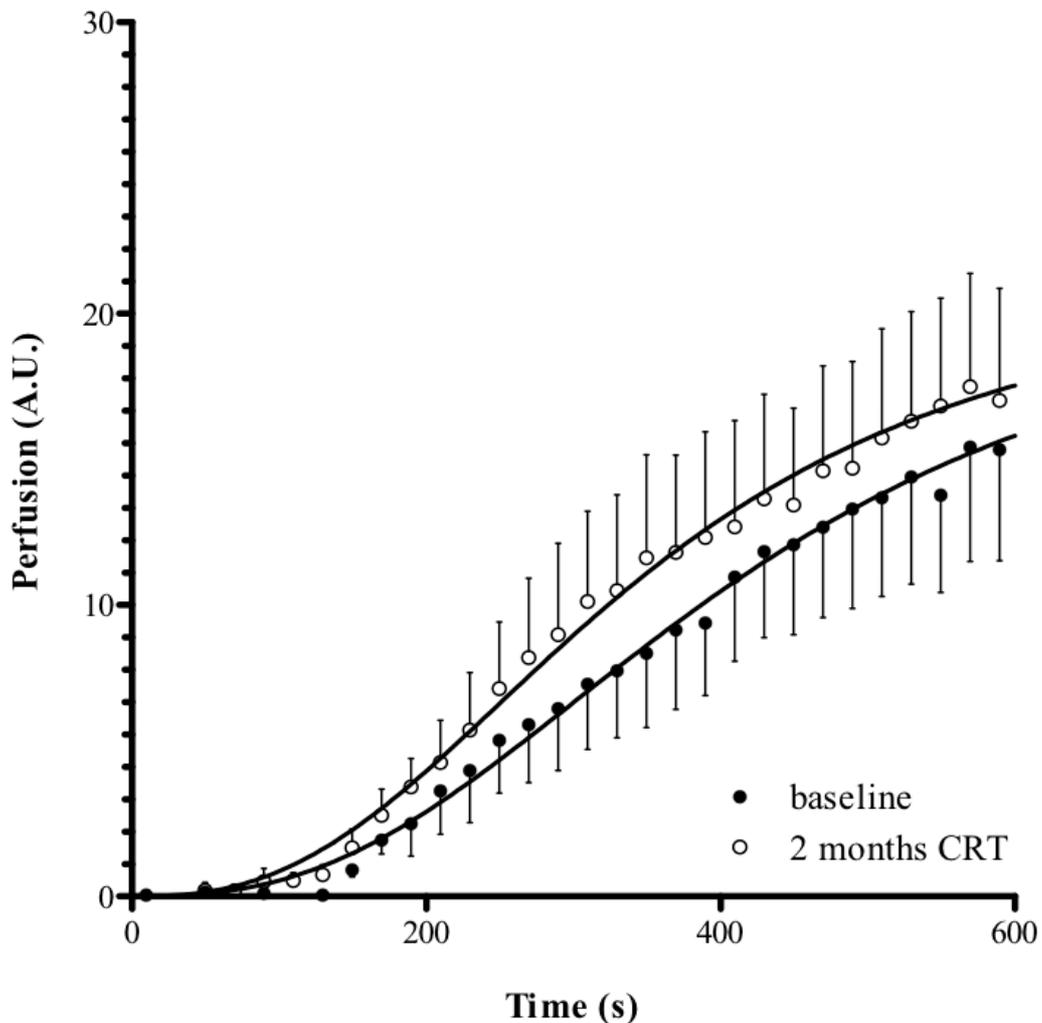


Figure 3. Total vasodilatory capacity as measured by the skin blood flow response to sodium
 310 nitroprusside delivered by iontophoresis. There was no difference between responses at baseline
 and after 2 months of CRT.



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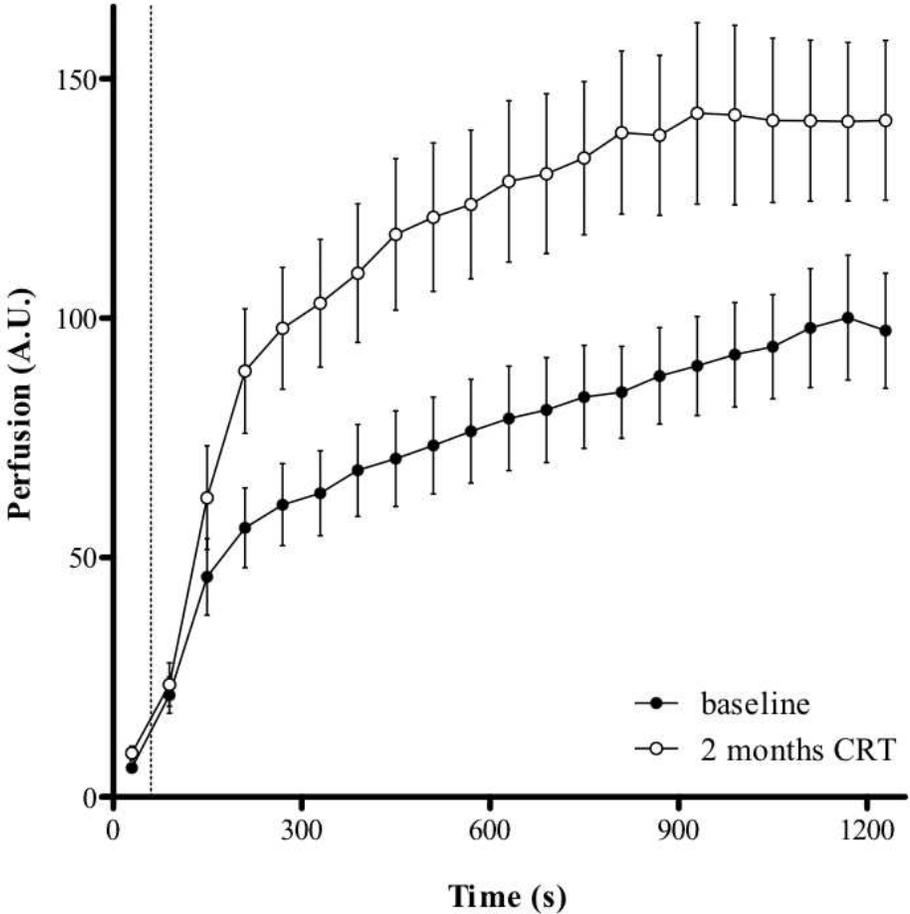


Figure 1. Skin hyperemic blood flow response to local heating to 44°C before and after 2 months of CRT. Maximum vasodilatation and area under the curve were significantly increased after 2 months of CRT.

127x130mm (150 x 150 DPI)

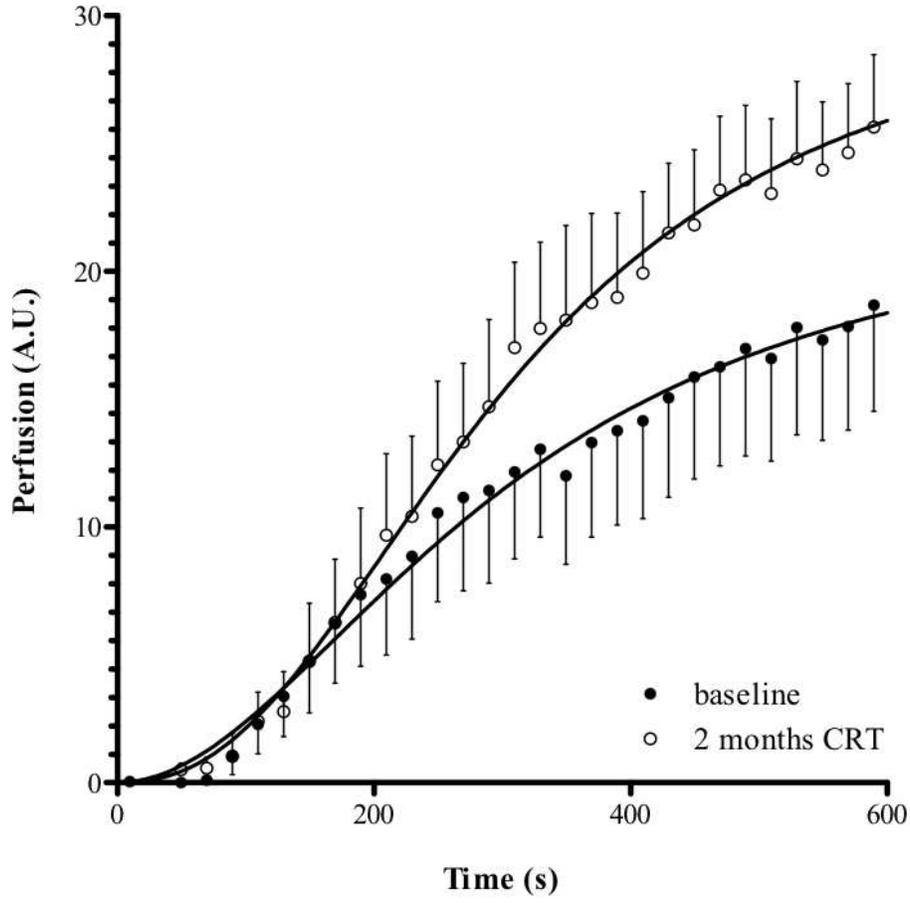


Figure 2. Endothelium-dependent blood flow response to acetylcholine delivered by iontophoresis. The maximum vasodilatory response was significantly increased after 2 months of CRT.
131x133mm (150 x 150 DPI)

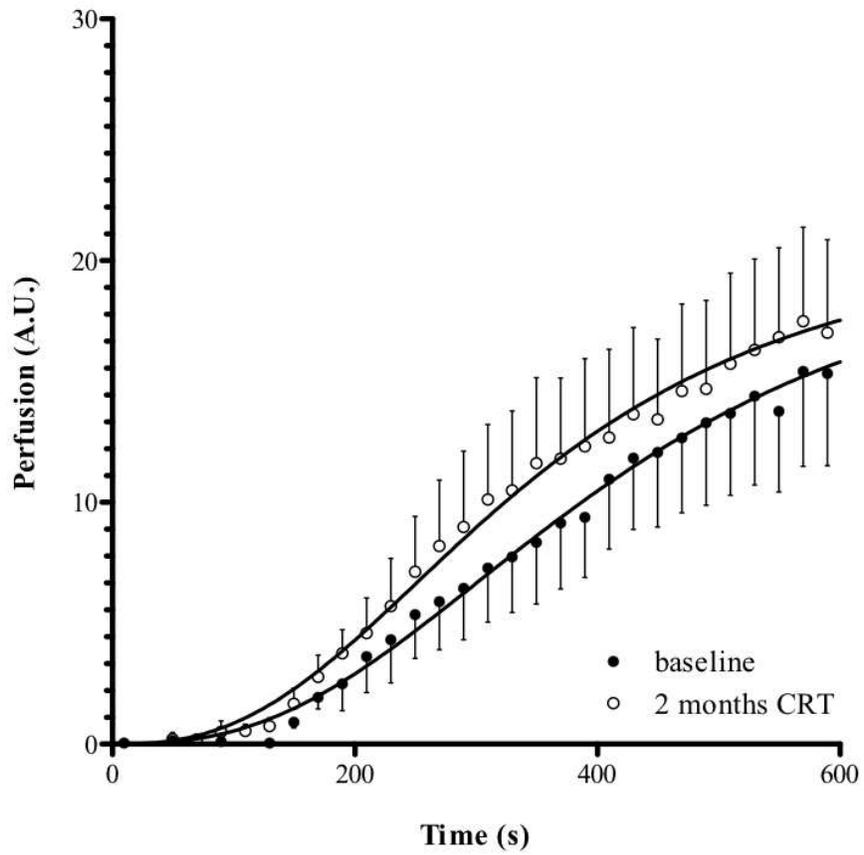


Figure 3. Total vasodilatory capacity as measured by the skin blood flow response to sodium nitroprusside delivered by iontophoresis. There was no difference between responses at baseline and after 2 months of CRT.
139x129mm (150 x 150 DPI)