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Title page

Full title of paper:

Selective β_1-blockade improves endothelial function in patients with congestive heart failure. Novel detection using low frequency blood flow oscillations

Running title:

Blood flow oscillations in heart failure

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Abstract

Laser Doppler flowmetry (LDF) of forearm skin blood flow, combined with iontophoretically-administered acetylcholine and sodium nitroprusside and wavelet spectral analysis, was used for noninvasive evaluation of endothelial function in 17 patients newly diagnosed with New York Heart Association class II-III congestive heart failure (CHF). After 20±10 weeks' treatment with a β_1-blocker (Bisoprolol), the measurements were repeated. Measurements were also made on an age- and sex-matched group of healthy controls (HC). In each case data were recorded for 30 minutes. In HC, the difference in absolute spectral amplitude of LDF oscillations between the two vasodilators manifests in the frequency interval 0.005-0.0095 Hz ($p<0.01$); this difference is initially absent in patients with CHF, but appears following the β_1-blocker treatment ($p<0.01$). For HC, the difference between the two vasodilators also manifests in normalised spectral amplitude in 0.0095-0.021 Hz ($p<0.05$). This latter difference is absent in CHF patients and is unchanged by treatment with β_1-blockers. It is concluded that there are two oscillatory skin blood flow components associated with endothelial function. Both are reduced in CHF. Activity in the lower frequency interval is restored by β_1-blocker treatment, confirming the association between CHF and endothelial dysfunction but suggesting the involvement of two distinct mechanisms.

Key Words iontophoresis laser Doppler flowmetry wavelet transform dynamics

Introduction

Congestive heart failure (CHF) is a complex clinical condition, in which the history of its management reflects the evolving understanding of its pathophysiology. CHF is associated with a typical neurohormonal response involving activation of both the renin–angiotensin system and the sympathetic nervous system. This activation is deleterious and current therapeutic strategies to block the effects of this activation eg with angiotensin converting enzyme inhibitors [1] and β-adrenergic blocking agents [2, 3] have been clearly demonstrated to benefit favourably the poor outcome in all grades of severity of the condition. The results seen with β-blockade are particularly effective in producing a long-term reduction in mortality from both sudden death, and progressive cardiac failure, and improving symptomatology. Despite 30 years of research the mode of action of β-blockers is still incompletely understood. It is likely that they act through multiple mechanisms including reducing tendencies to malignant dysrhythmias, and favourable effects on reverse remodeling [4, 5].

The endothelium plays a pivotal role in regulating blood flow by releasing relaxing and constricting factors, a role that has been shown to be impaired in CHF [6, 7, 8]. One of the mechanisms by which this occurs is through decreased peripheral production of endothelium-derived nitric oxide (NO) [9, 10], possibly because of reduced shear stress due to reduced peripheral perfusion [11]. Isolated cellular models have shown direct effects of β-blockers on endothelial function. [12, 13]. Endothelial dysfunction in CHF has recently been shown to be associated with an increased mortality risk [14].

Various techniques are available to evaluate endothelial function, including brachial arterial imaging [15], or plethysmography and laser-Doppler flowmetry (LDF) [16] that monitor vasodilatory responses to the administration of an endothelial-dependent vasodilator such as acetylcholine (ACh). A decreased response to ACh but not to the endothelial-independent vasodilator sodium nitroprusside (SNP) is considered evidence of endothelial dysfunction. Besides basal differences in responses to these two substances, LDF enables study of the oscillatory components in the blood flow [17-20]. Using LDF it was intended to examine, in particular, those blood flow frequencies that are known to reflect endothelial reactivity.

The study was motivated by the perception of the cardiovascular system in terms of at least 5 distinct coupled oscillatory processes with different frequencies. The state of the system can be characterised by the oscillatory amplitudes, and by the couplings between the oscillators. Heart rate variability arises because lower frequency oscillations (especially, but not only, respiration) are coupled to the cardiac rhythm. Unlike many earlier studies, our interest therefore centres on blood flow *dynamics* [20], rather than on basal values. The main advantage of this approach is that its frequency resolution enables the contributions from different physiological processes to be distinguished. Wavelet analysis of LDF signals has already been applied to other disease states related to the cardiovascular system: type 2 diabetes [21] and post acute myocardial infarction [22]. It was postulated that the difference in endothelial reactivity of the CHF group and an age-matched healthy control (HC) group will be manifested in particular oscillatory components. The mechanism of action of β_1-blockers in CHF is not fully understood, so the final aim of the study was to evaluate the effects that β_1-blockers have on blood flow

dynamics in CHF.

Methods

Subjects

Patients for the CHF group were recruited either from cardiology clinics in the Royal Lancaster Infirmary or from patients referred directly for open access echocardiography. None of them exhibited significant edema. The severity of heart failure was established via echocardiography. Left ventricular diameter and left ventricular ejection fraction (LVEF) were determined. Inclusion criteria were that they had LVEF<35% and symptoms between class II-III of the New York Heart Association (NYHA) classification. Exclusion criteria were recent myocardial infarction, or cerebrovascular accident (within 6 months), fibrillation, other life threatening co-morbidity, advanced frailty, current use of β-blocking drugs, or contra-indications to β-blocker therapy. All but one CHF patients remained on established treatment with angiotensin converting enzyme inhibitors and diuretics throughout the study; the exception was on an angiotensin-receptor antagonist and diuretics.

After the initial set of measurements all CHF patients were then treated with Bisoprolol (a selective β_1-antagonist). This was commenced in a dose of 1.25mg under direct observation, and then increased, at intervals of a minimum of 1 week, through 2.5mg, 3.75mg and 5mg. Finally it was increased at intervals of a minimum of 4 weeks from 7.5mg to 10mg. Upwards titration was stopped if symptomatic hypotension, or pulse rate < 50/minute, or side-effects of the β-blocker, appeared. A second set of measurements was taken after 5 weeks of a stable dose (β–CHF subject group) and 20±10 weeks after the first set of measurements Patients' data are summarized in Table 1.

Healthy age (66±6) and sex (8 F and 13 M) matched control subjects were recruited from the local community. None of them was on medication or had a history of cardiovascular disease or problems related to the cardiovascular system, including hypertension or hypercholesterolemia. A single set of measurements, identical to that recorded for CHF patients, was taken from each of them.

All participants gave their informed consent in writing. The investigation conformed with the principles outlined in the Declaration of Helsinki and was approved by the Local Ethics Committee of the Morecambe Bay Hospitals Trust.

Measurements

Subjects lay supine on a bed and relaxed for 15 minutes prior to the commencement of recording. Peripheral blood flow, heart rate, respiration, and skin temperature were simultaneously recorded for 30 minutes, based respectively on laser Doppler flowmetry (LDF), a conventional 3-lead ECG, a Biopac respiratory effort transducer placed around the thorax, and Thermilinear temperature sensors (YSI Inc, Ohio, USA) placed on the arm and leg. The ECG, respiration and temperature signals were amplified using a specially

designed signal conditioning unit (Cardiosignals, Jozef Stefan Institute, Slovenia). Signals were digitized at 400 Hz with 16-bit resolution by use of a National Instruments PCI-6035E A/D converter, and stored in a personal computer. The temperature of the room was maintained at 21±2 ^{o}C.

Laser Doppler flowmetry

Skin blood flow signals were measured by the laser Doppler perfusion technique using a DRT4 LDF monitor (Moor Instruments Ltd, Axminster, UK). Two MPI–V2 probes were mounted within MIC1–IONlr chambers, where the vasoactive substances were inserted for iontophoretic administration in the same area as that where the blood flow was being recorded. Measurements were made on the volar aspect of the right forearm, choosing areas of skin that were free from blemishes. Both probes were positioned in areas with similar vasculature and without larger vessels in their vicinity. For each set of measurements the two chambers were placed 2 cm apart with random relative orientation. Near-infra-red light (wavelength 780 nm) was delivered to the probes via a nearly loss-free optical fibre. The back-scattered light was collected and returned to the monitor via another optical fibre, where it was converted to an analogue electrical signal. The cut-off frequency of the low-pass filter was 22.5 kHz, and an output time constant of 0.1 s was selected. The LDF probes were calibrated against a flux standard (Moor Instruments) and the blood flow was expressed in arbitrary units (AU).

Iontophoresis

Two vasodilators, ACh, an endothelium-dependent and SNP, an endothelium-independent direct NO donor, were iontophoretically applied. The difference in the blood flow enhancements that they produce in any oscillatory component is related to the responsiveness of the vascular endothelium [17-20]. The 1% ACh and SNP solutions were prepared on the day of the study in each case. They were drawn through the cutaneous barrier at each of the LDF measuring sites by means of a constant electrical current of 100 μA. A Moor Instruments MIC1-e current controller was used. The iontophoresis current was applied in 7 pulses of 20 s, with a separation of 240 s between each, as indicated in Figure 1a. Figure 1b,c shows examples of blood flow responses to ACh and SNP for a healthy subject. They illustrate the large increase of oscillatory activity that typically occurs in addition to the increased average flow.

Data analysis

The time averages of the signals from the LDF probes were calculated, and the signals were then resampled at 10 Hz using a moving average technique. Trends were removed by giving the moving average a window length of 200 s, thereby eliminating frequencies below 0.005 Hz. Following this pre-processing, the wavelet transform (using the Morlet mother wavelet [23]) was computed to analyse the frequency content of the signals. The advantages in comparison to conventional Fourier techniques include the logarithmic frequency resolution, which enables an extremely wide range of frequencies to be accommodated: in the present case, the characteristic frequencies of oscillations in the LDF signals differed by a factor >100. All data analyses including the wavelet transform and data presentation were performed using code written within Matlab (The MathWorks,

Inc.).

Computation of the wavelet transform of the blood flow signal yields the usual 3-dimensional structure above the time-frequency plane, exhibiting clearly resolved spectral peaks whose positions vary in time [18, 20] as shown in Figure 2a, and a time-average as shown in Figure 2b. The positions of the spectral peaks also differ slightly from subject to subject. In earlier work, five frequency intervals were defined [18, 20] such that each of them contains only one peak: 0.0095–0.021, 0.021–0.052, 0.052–0.145, 0.145–0.6, 0.6–1.6 Hz. They are attributed respectively to endothelial [17-19], neurogenic [24, 25], myogenic [17, 26, 27], respiratory and cardiac processes, as summarized in Table 2. In Figure 2, however, an additional spectral peak centred near 0.007 Hz is also evident, recently defined as the sixth, or ultra-low frequency subinterval 0.005–0.0095 Hz [28]. These spectral peaks have recently been confirmed in an independent study [29] based on wavelet transformation of LDF data from healthy subjects. The probable reason why the spectral peak in interval VI was not identified in earlier studies relates to the pre-processing of the LDF raw data (see above) in order to remove trends and very low frequency components. In the previous work, interest centered on the processes involved in blood circulation, and so the low frequency cut-off was set at 0.0095 Hz. Under these conditions, the peak in interval VI would have been very strongly attenuated.

For convenience, these frequency intervals are labeled I–VI as specified in Table 2 and indicated in Figure 2(b). Note that, compared to earlier work [20], the intervals are redesignated, now numbering them starting from the high frequency end, in order to reflect the probable future discovery of oscillatory components at even lower frequencies. In addition, to allow for the increased heart rate in CHF patients, the 1.6 Hz boundary value of the heartbeat-related frequency range (interval I) has been increased to 2 Hz. Detailed description of all the oscillations and their physiological origin is presented in the Appendix.

Two measures are used to quantify the contribution of the oscillations to the total blood flow: their absolute and normalised spectral amplitude. The word ‘spectral’ indicates they are both calculated from the coefficients of the wavelet transform. The absolute spectral amplitude is the mean value of the wavelet transform, calculated within a specific frequency interval. When calculated over the whole frequency interval from 0.005 to 2 Hz it is called average spectral amplitude. The normalised spectral amplitudes are calculated from the absolute spectral amplitudes and are defined as the ratio between the absolute spectral amplitude within a given subinterval and the average spectral amplitude.

R-peaks in each ECG were automatically detected and manually checked, and the corresponding heart rate variability (HRV) time series was generated. The heart rate for each subject and each set of measurements was then calculated as a time-average of the HRV. Maximal values in the respiration signals were also automatically detected, manually checked, and analysed in a similar manner to yield the respiration frequency variability (RFV), and the respiratory frequency (RF) was obtained as a time-average.

The analysis was blinded, in that the relevant researcher had no connection with the experiments, but simply analyzed the anomymized time-series data with which he was provided.

Statistical analysis and presentation

A nonpaired, nonparametric, Wilcoxon rank sum test was used to obtain the probability that two group distributions were equal (data presented in Figs. 4 and 5). A paired, Wilcoxon signed rank test was used to obtain the probability that the effect of two substances, ACh and SNP, was equal for a given group (data presented in Fig. 6). For hemodynamic data (Fig. 3) and skin temperature a parametric t-test was used, a nonpaired in comparing patients with controls and a paired one between patient groups. In all hypothesis tests a value of $p<0.05$ was considered significant. The graphical data in Figure 3, and those that follow below are presented in each case as a box with lines drawn at the 10th percentile, the median and the 90th percentile. Whiskers extend from the ends of the box to the most extreme data value within 1.5×IQR (where IQR is the inter-quartile range of the box). The range $0.01<p<0.05$ is indicated by * and $p<0.01$ by **. The statistical analysis was performed using Matlab Statistics Toolbox (The MathWorks, Inc.).

The demographic data in Table 1 are displayed as mean ± SD.

Results

Hemodynamic data

The treatment with β_1-blockers resulted in a significant reduction in both systolic and diastolic blood pressure. The systolic pressure decreased from 141±16 to 124±18 mm Hg ($p=0.003$). The diastolic pressure decreased from 77±12 to 62±10 mm Hg ($p=0.002$).

The heart rate also decreased significantly from 1.31±0.27 to 0.95±0.15 Hz ($p=0.0003$). Although HRV is not relevant to the main findings of this study, we noted that it decreased very slightly, mainly on account of less ectopy. For the group, the number of ectopic beats dropped from 4.1 to 1.2 per minute. With ectopics included, HRV was significantly greater in the CHF group than in the HCs; when the analysis was repeated with the extra-systoles removed (as shown), there was no significant difference between HRV in the HC and CHF groups. Neither the RF nor RFV were significantly changed by treatment. Figure 3 summarizes the heart and respiration statistics for all three subject groups, including the healthy controls. Note that, in the CHF group, HR, RF and RFV are all significantly higher than for the age-matched HCs. After treatment the difference in RF and RFV is no longer statistically significant.

Skin temperature

There was a small decrease in patients' skin temperature as a result of the β_1-blocker treatment (on arms 35.09±1.49 ºC before, 34.19± 1.18 ºC after). The change is smaller than the variation between individual patients and is statistically insignificant. The hypothesis test was obtained using a paired t-test.

CHF compared to HC

Effect of ACh and SNP on mean value of the LDF signal and average spectral amplitude: A comparison of the HC and CHF groups' responses to ACh and SNP is presented in Figure 4: a shows mean blood flow and b the average spectral amplitude. There are highly significant differences in the mean flows between these two subject groups, in their responses to both ACh and SNP. However, there are no statistically significant differences between them in the average spectral amplitudes of their responses to ACh and SNP.

Effect of ACh and SNP on oscillatory flow components: Figure 5 presents data comparable to those of Figure 4, but with added frequency discrimination, dividing the responses into the frequency intervals I–VI defined above. It is evident that almost all the oscillatory components are affected by CHF, but the effect on the lowest two seems to be different for the two vasodilators.

In more detail, there is a distinction between the CHF and HC groups in their absolute spectral amplitudes in interval VI, which is significant in response to ACh (Figure 5a) but not to SNP (Figure 5c). The same is true of their normalised spectral amplitudes (5b and 5d). Within intervals III and IV, there are highly significant differences between the CHF and HC groups (5a–d), but no distinction between their responses to ACh (5a,b) and SNP (5c,d). There is a significant difference between the normalized amplitudes for the two groups in interval V for ACh (5b).

Effect of β_1-blockers

The effect of Bisoprolol was evaluated in two different ways. First, the responses to ACh before and after the treatment were compared. Similarly, the comparison of responses to SNP before and after the treatment was performed. After the treatment the response to ACh was slightly increased for each oscillatory component, but the increase was not statistically significant. The response to SNP did not change in any of the intervals.

Second, the responses to ACh and SNP were compared. The comparison was performed before the treatment and after the treatment as well as for the HC group. A significant difference between the responses to the two substances was found only within the lowest two frequency intervals and this is shown in Figure 6.

The figure compares absolute and normalized spectral amplitudes in response to ACh and SNP for all three subject groups. Parts a and d illustrate the differences in response to ACh and SNP in the HC group. The difference in normalized amplitude in interval V has been demonstrated in several earlier studies [17-20, 28]. Note that there is, as recently discussed [28], a statistically significant difference also in interval VI.

Parts b and e of Figure 6 illustrate the differences in response to ACh and SNP in the CHF group. There is no statistically significant difference in absolute (6b) and normalised spectral amplitude (6e) in the case of the CHF patients. Following treatment with β□-blockers, however, the CHF-β group exhibits differences in response to ACh and SNP in

interval VI that are statistically significant, both in the absolute (6c) and in the normalised spectral amplitude (6f). The differences in response to ACh and SNP in interval V seen in the HC group is not, however, restored by the β_1-blocker treatment.

Discussion

The major findings in this study are that patients with CHF have significant abnormalities in endothelial function compared with healthy controls, and that treatment with Bisoprolol, a selective β_1 □antagonist, partially reverses the endothelial dysfunction that is found in patients with CHF. Whilst the mechanisms for the improvement in endothelial function are not explored in the present study the differences between intervals V and VI help shed some light on the complex effects seen at the level of endothelial regulation. A more detailed discussion of the origin of the two endothelial related oscillatory components, the changes in CHF compared to healthy controls, and the effect of β_1-blockers on endothelial function in CHF patients, is now presented.

Endothelial dependent oscillatory components

The fact that the oscillatory amplitudes in intervals V and VI are differentially affected by ACh and SNP demonstrates that activity in both intervals is associated with the endothelium. Both intervals are abnormal in CHF compared with healthy controls. Indeed, a number of experimental and clinical studies describe impaired endothelium-dependent vasodilatation and increased plasma concentration of a variety of neurohormones eg endothelin, angiotensin II, natriuretic peptides in CHF [7]. The presence of two distinct spectral peaks strongly suggests the existence of at least two distinct mechanisms of endothelial regulation of the oscillatory dynamics. The probable explanation is that interval V corresponds to NO released from the endothelium [30]; and interval VI relates to a separate local neurohormonal system. Recent research into low frequency oscillations within frequency interval V has clearly shown that it is partially related to NO [19, 31] while frequency interval VI is not related to either NO or prostaglandins [28].

There is an additional reason for using iontophoresis when measuring low frequency oscillations. Vasodilator substances will, by mechanism of their action increase the mean value of the flow as well as all the oscillatory components. This is especially important in facilitating measurements of the low frequency oscillations which make a relatively low contribution to the flow in terms of energy.

CHF cw controls

The results in Figure 5 clearly demonstrate that the responsiveness of the vascular endothelium in CHF patients is reduced, compared to healthy controls. This finding is consistent with a previous study [8] of CHF patients. Note that the latter study [8] investigating elderly patients with CHF using ACh and SNP, involved invasive techniques for the measurement of average flows, without the frequency discrimination possible in studies of oscillations, whereas the current study has derived similar but more extensive information using entirely non-invasive techniques. Abnormalities of endothelial function

in CHF are almost certainly multifactorial. Previous work has implicated NO through either decreased production [32], increased degradation of NO or decreased NO bioavailability [33]. The expression of endothelial NO synthase (eNOS) has been shown to be reduced in both rats [34] and dogs [35] with experimental heart failure.

CHF post β_1-blockade

The differing responses of the CHF and CHF-β groups (Figure 6) found in the present study show clear evidence that treatment with Bisoprolol brings an increase in endothelial responsiveness in frequency interval VI: the ACh/SNP differentiation in absolute and normalized spectral amplitude for untreated CHF patients is statistically insignificant (6b and 6e) whereas, for the CHF-β group it is statistically significant (6c and 6f). Treatment with Bisoprolol evidently ameliorates the impairment of endothelial responsiveness associated with CHF.

The mechanism of action of β_1-blockers in CHF is still not wholly understood. Likely mechanisms involve reduction in sympathetic tone, increase in vagal tone, reduction of subendocardial ischaemia, reduction in rennin and endothelin release, increasing norepinephrine re-uptake, and reducing inflammatory cytokines [4]. We note that β_1-blockers operate in many different ways, and it has yet to be determined which of them is/are responsible for the observed changes in low frequency blood flow spectra.

What the present study has shown is that CHF patients exhibit blood flow abnormalities in frequency interval VI, that iontophoresis and wavelet analysis enable this abnormality to be detected, and that the medication partially restores normal function.

Study limitations

As with all clinical studies involving patient follow up over time, it was not feasible to accommodate for the effects of disease progression. In addition all patients were on medication at the commencement of the study, and alteration of doses of other drugs e.g. diuretics was sometimes necessary at the discretion of the prescribing physician.

The study was not performed in a placebo-controlled fashion because it would have been ethically unacceptable to withhold a life-prolonging treatment from 50% of the patients for 12 weeks.

The main reason for the introduction of normalised spectral amplitudes is to reduce inter-subject variability seen in the case of absolute amplitudes. However, an effect on normalised amplitude within a specific interval can then result from changes in the rest of the spectrum, not only from changes in the interval under consideration. For this reason special care should be taken when interpreting normalised data. In the present study, the main findings are based on comparison of responses to ACh and SNP within frequency intervals V and VI (Figure 6). Average spectral amplitudes for both substances did not differ between any of the subject groups and differences between the effects of the substances were found only in the two lowest frequency intervals. This suggests that oscillations at higher frequencies, such as those related to heartbeat and respiration, did not alter the normalised spectral amplitudes at low frequencies.

Conclusions

The main conclusion is that, compared to healthy controls, CHF patients exhibit abnormally attenuated blood flow oscillations in frequency interval V and VI, which represent different aspects of endothelial function. Treatment with Bisoprolol shifts the abnormality in spectral amplitude back towards the findings in healthy controls.

In more detail, iontophoretic administration of the endothelium-dependent and endothelium-independent vasodilators ACh and SNP has confirmed that the low-frequency spectral peaks in intervals V and VI of the LDF blood flow signal are associated with endothelial reactivity. Both peaks are significantly attenuated in patients with CHF. These spectral peaks are respectively governed by NO, and an as yet unknown factor. Treatment of CHF patients with the β_1-blocker Bisoprolol moves the spectral amplitude of interval VI closer to that of the healthy controls. However, given that no significant change of activity in interval V is detected as a result of the treatment, whereas a highly significant change is observed in interval VI, it can be inferred that the effects of Bisoprolol on the endothelium are at least partly mediated through a non-NO mechanism.

Appendix

Oscillations in the LDF time series and their physiological origin

The dynamics of the microcirculatory flow, measured by LDF, consists of rhythmic variations. It can therefore be analysed using spectral techniques. The strengths of individual oscillating components, revealed by the spectrum, provide information about the dynamics of vascular regulatory mechanisms.

Wavelet analysis of 20-minute LDF recordings using the Morlet mother wavelet resolved five oscillations in the time-frequency domain, the lowest one around 0.01 Hz [36]. By tracing the characteristic frequencies of oscillations in time, the spectrum was divided into five intervals, such that only one oscillation was present in each interval [36, 18, 20]. Recently, in 30-minute recordings, another spectral component was observed at an even lower frequency, around 0.007 Hz [28]. It was shown not to be an artefact related to the measurement or to signal processing technique. The most likely reason why the lower frequency oscillations were not reported before includes the limitations of the Fourier-based and autoregressive methods used, and the fact that they were usually applied to relatively short time series.

The positions of the characteristic frequencies vary slightly, with time and between subjects. They are also changed during physical activity, and are modified in some diseases. An ideal division of the spectrum into frequency intervals is therefore impossible. However, specific physiological processes might dominate in a specific frequency interval and wavelet-based analysis has shown a promising way in these

investigations, especially for low frequency oscillations. Several recent studies, based on wavelet transform, have independently confirmed the presence of the oscillations in the LDF recordings [37, 38, 39, 40, 41, 31, 29] and investigated their physiological origin. However, further work is necessary to fully understand the complex mechanisms involved in the regulation of the microvascular blood flow.

These mechanisms were recently reviewed by Segal [42] who writes "With vasomotor tone reflecting myogenic contraction of smooth muscle cells modulated by shear stress on the endothelium, the initiation of functional vasodilation and its modulation by sympathetic innervation dictate how and where blood flow is distributed in response to metabolic demand". Although metabolic demand can be strongly enhanced by exercise, it is of course continuously present in a living system. Moreover, the oscillatory nature of the flow generated by rhythmic activity of the heart can be expected to be associated with oscillatory mechanisms of regulation. Each of the regulatory mechanisms manifests within a particular frequency interval. In what follows we list the six previously defined [28] frequency intervals and we discuss the rationale of associating them with specific physiological processes.

Frequency interval I (0.6 – 2 Hz), related to the heartbeat and frequency interval II (0.145 – 0.6 Hz), related to the respiratory activity

The oscillation with the highest frequency, around 1 Hz, is related to the activity of the heart. The respiratory activity is around 0.25 Hz. Both oscillatory components appear in the spectrum of the LDF signal and their physiological origin can easily be demonstrated by comparing the frequency content of ECG, respiration and blood pressure signals with that of a simultaneously measured LDF blood flow signal.

Frequency interval III (0.052 – 0.145 Hz), related to intrinsic myogenic activity

In 1964 Folkow suggested that oscillatory changes of arteriolar diameter are resulted from myogenic control [43]. Several years later, Johnson [26] showed that blood vessels respond to transmural pressure elevation with constriction, and to pressure reduction with dilation. He termed this behaviour as myogenic response and suggested that it is inherent to smooth muscle and independent of neural, metabolic, and hormonal influences. It was shown that this response is not constant for all the vessels and that a longitudinal gradient in myogenic responsiveness exists within an arteriolar network [44].

Spontaneous activity recorded in microvascular smooth muscle cells was shown to be in the range of 4-10 events a minute (0.07-0.1 Hz) [45]. Several studies suggested that these waves are of local origin representing intrinsic myogenic activity of smooth muscle cells in resistance vessels [46, 47, 48, 49, 24, 50, 51]. Rhythmical variation in the same frequency interval (6 to 10 cycles a minute) had been observed in blood pressure recordings by Traube in 1865 [52] and confirmed by Hering in 1869 [53]. In 1876 Siegmund Mayer observed similar oscillations [54]. Killip [55] presented evidence that Mayer waves are accompanied by rhythmic fluctuations in vascular resistance.

In a recent study on rat small arteries it was shown that blockade of adrenoceptors, which mediate the effect of the main transmitter in these vessels, does not alter the myogenic

response [56]. Similar findings have been reported after inhibition of nerve action potentials by tetrodotoxin, after blockade of postsynaptic receptor function by receptor antagonists and after chemical vessel denervation in cat cerebral [57], rat cerebral [58], and rat saphenous [59] arteries. These studies provide supporting evidence that the myogenic response is due to an action of transmural pressure on smooth muscle cells only. This is in agreement with the study in humans, where blood flow oscillations from free microvascular flaps deprived of sympathetic nerve activity (SNA) and from intact skin were compared [25]. It was shown that the SNA was not manifested in the frequency interval above 0.05 Hz.

Wavelet analysis of LDF signals was used to trace the myogenic activity related oscillation in time and attribute it to a specific frequency interval. The technique was not used to investigate the physiological origin of this oscillation, which has already been revealed from both blood flow and blood pressure signals as described above.

Applying wavelet analysis to blood flow signals it has been shown that the amplitude of myogenic oscillations can be affected by exercise [60, 61]. It was also shown that the frequency of the myogenic-related oscillation can increase with exercise.

Frequency interval IV (0.021 – 0.052 Hz), related to neurogenic (sympathetic) activity

An oscillation with a characteristic frequency around 0.04 Hz has been observed in blood pressure, blood flow and HRV signals. It was attributed either to metabolic [62] or to neurogenic activity [63], which is superimposed on myogenic activity in the regulation of the blood pressure through adjustment of the vessel's radius.

Kastrup et al. [24], who termed these rhythmic variations as β-oscillations, have in agreement with the study of Golenhofen and Hildebrandt [64] shown that in humans they disappear after local and ganglionic nerve blockade and in chronically sympathectomized tissue. They suggested that β-oscillations are a vascular reaction of purely neurogenic origin. Similarly, in an LDF study of oscillations in rabbit skeletal muscle tissue, slow waves with a frequency of around 1-3 per minute were suggested to be under neurogenic control since they disappeared after pharmacological nerve blockade [65].

Using wavelet analysis with a logarithmic frequency resolution [18] several studies confirmed the involvement of sympathetic nerve activity in this low frequency interval. Analysis of skin blood flows on rats' paws has shown a significant decrease in the relative energy contribution to blood flow oscillations in the low frequency spectrum only in denervated paws [66]. In humans, LDF signals from the surfaces of free microvascular flaps, deprived of sympathetic nerve activity, in comparison with adjacent intact skin showed reduced spectral amplitude only in the 0.02 – 0.05 Hz low frequency interval [25]. Two recently published studies have shown the 0.021 – 0.052 Hz frequency interval to be related to sympathetic activity: after brachial plexus block, a significant decrease was shown in the lower forearm skin compared to controls [67], a result that was confirmed on a similar location as an effect of general anaesthesia [68].

An independent study has confirmed these findings in the same frequency interval by simultaneous measurements of LDF signals on the surfaces of a free latissimus dorsi

myocutaneous flap and on the adjacent intact skin of a healthy limb [39].

Frequency interval V (0.0095 – 0.021 Hz), related to endothelial activity (partly NO-dependent)

It is well accepted that ACh induces vasodilatation through enhancement of the activity of endothelial cells, but the exact mechanism and the mediators involved are still not fully understood. The involvement of endothelium in ACh-induced vasodilatation is the main difference as compared to vasodilatation by SNP. Therefore, iontophoresis with these two substances has been used to examine skin microvascular endothelial cell function [69, 70, 71]. It was suggested [72] that impaired ACh-induced vasodilatation, as compared to the vasodilatation induced by SNP, could be taken as a demonstration of endothelial dysfunction.

Following reports of endothelial and NO involvement in rhythmic activities, the hypothesis was tested that not only the mean value of the blood flow, but also its dynamics is modulated by endothelium [17]. Using wavelet analysis of 20-minute LDF recordings with logarithmic frequency resolution the difference between ACh and SNP responses was found to manifest in only one frequency interval, around 0.01 Hz. The oscillation in this interval has been attributed to endothelium-mediated vasodilatation but its physiological origin was not investigated. These findings were confirmed in a separate study on athletes and healthy control subjects using the same dose-response protocol [73].

Observation of an oscillatory component in the spectrum at an even lower frequency, around 0.007 Hz, has led to 30-minute recordings of LDF signals and an iontophoresis protocol consisting of 7 equal pulses. The protocol was carefully chosen to avoid current induced vasodilation (iontophoretic currents less than 200 μA and total charge less than 8 mC) [69]. Possible effects of the iontophoresis current [74] and transdermal potential difference [75] on the blood flow responses were investigated and were shown not to be involved in the low frequency oscillations.

In further investigation of the low frequency oscillations, five more studies have confirmed the role of endothelium in frequency interval V [19, 28, 67, 68] including the present study on CHF. It was suggested that oscillations in this interval were partly mediated by NO, as they were reduced by infusion of L-NMMA and restored by L-arginine, but not by endogenous prostaglandins [19]. The finding that oscillations within this frequency interval are indeed NO dependent was confirmed in a recent independent study [31].

Oscillations in interval V were significantly reduced following both brachial plexus block [67] and general anaesthesia [68]. Although the difference in responses to ACh and SNP in this interval was abolished after plexus block [67], this was not the case following general anaesthesia [68].

Frequency interval VI (0.005 – 0.0095 Hz), related to endothelial activity (non-NO-dependent)

As within frequency interval V, the proportion of the flow in frequency interval VI was

shown to be significantly higher in response to ACh compared to SNP, indicating an endothelium related origin of the oscillation [28]. However, blocking the endothelial production of NO and prostaglandins did not affect this oscillation, suggesting possible involvement of other endothelial mechanisms.

The probable involvement of endothelium in this frequency interval has also been demonstrated and discussed above in the present CHF study.

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REFERENCES

1. Eichhorn EJ, Bristow MR: Medical therapy can improve the biological properties of the chronically failing heart – A new era in the treatment of heart failure. Circulation 1996; 94:2285–2296.
2. Bristow MR: β–adrenergic receptor blockade in chronic heart failure. Circulation 2000; 101:558–569.
3. López-Sendón J, Swedberg K, McMurray J, Tamargo J, Maggioni AP, Dargie H, Tendera M, Waagstein F, Kjekshus J, Lechat P, Torp-Pederson, C: Expert consensus document on β–adrenergic receptor blockers. Eur Heart J 2004; 25:1341–1362.
4. Silke B: Beta Blockade in CHF: pathophysiological considerations. Eur Heart J 2006 (suppl); 8:C13-C18.
5. Udelson JE: Ventricular remodeling in heart failure and the effects of beta blockade. Am J Cardiol 2004; 93:43B-48B.
6. Drexler H, Hayoz D, Münzel T, Hornig B, Just H, Brunner HR, Zelis R: Endothelial function in chronic congestive heart failure. Am J Cardiol 1992; 69:1596–1601.
7. Vanhoutte PM: Endothelium-dependent responses in congestive heart failure. J Mol Cell Cardiol 1996; 28:2233–2240.
8. Morgan DR, Dixon LJ, Hanratty CG, Hughes SMT, Leahey WJ, Rooney KP, Johnston GD, McVeigh GE: Impaired endothelium-dependent and -independent vasodilation in elderly patients with chronic heart failure. Eur J Heart Fail 2004; 6:901–908.
9. Katz SD, Khan T, Zeballos GA, Matthew L, Potharlanka P, Knecht M, Whelan J: Decreased activity of the L-arginine-nitric oxide metabolic pathway in patients

with congestive heart failure. Circulation 1999; 99:2113-2117.

10. Zhao G, Shen W, Zhang X, Smith CJ, Hintze TH: Loss of nitric oxide production in the coronary circulation after the development of dilated cardiomyopathy: a specific defect in the neural regulation of coronary blood flow. Clin Exp Pharmacol Physiol 1996; 23:715-721.
11. Drexler H: Hypertension, heart failure and endothelial function. Am J Cardiol 1998; 82:20S-22S.
12. Ponikowski P, Chua TP, Piepoli M, Amadi AA, Harrington D, Webb-Peploe K, Volterrani M, Colombo R, Mazzuero G, Giordano A, Coats AJS: Chemoreceptor dependence of very low frequency rhythms in advanced chronic heart failure. Am J Physiol Heart Circ Physiol 1997; 272:J438-H447.
13. Garlichs CD, Zhang H, Mügge A, Daniel WG: Beta-blockers reduce the release and synthesis of endothelin-1 in human endothelial cells. Eur J Clin Invest 1999; 29:12-16.
14. Katz SD, Hryniewicz K, Hriljak I, Balidemaj K, Dimayuga C, Hudaihed A, Yasskiy A: Vascular endothelial dysfunction and mortality risk in patients with chronic heart failure. Circulation 2005; 111:310-314.
15. Kuvin JT, Karas RH: Clinical utility of endothelial function testing. Ready for prime time? Circulation 2003; 107:3243-3247.
16. Cracowski J-L, Minson CT, Salvat-Melis M, Halliwill, JR: Methodological issues in the assessment of skin microvascular function in humans. Trends Pharmacol Sci 2006; 27:503-508.
17. Kvernmo HD, Stefanovska A, Kirkebøen KA, Kvernebo K: Oscillations in the human cutaneous blood perfusion signal modified by endothelium-dependent and endothelium-independent vasodilators. Microvasc Res 1999; 57:298–309.
18. Stefanovska A, Bračič M, Kvernmo HD: Wavelet analysis of oscillations in the peripheral blood circulation measured by laser Doppler technique. IEEE Trans Biomed Eng 1999; 46:1230–1239.
19. Kvandal P, Stefanovska A, Veber M, Kvernmo HD, Kirkebøen KA: Regulation of human cutaneous circulation evaluated by laser Doppler flowmetry, iontophoresis, and spectral analysis: importance of nitric oxide and prostaglandines. Microvasc Res 2003; 65:160–171.
20. Stefanovska A, Bračič M: Physics of the human cardiovascular system. Contemporary Phys 1999; 40:31–55.
21. Urbancic-Rovan V, Stefanovska A, Bernjak A, Azman-Juvan K, Kocijancic A: Skin blood flow in the upper and lower extremities of diabetic patients with and without autonomic neuropathy. J Vasc Res 2004; 41(6):535-545.
22. Ažman-Juvan K, Bernjak A, Urbančič-Rovan V, Stefanovska A, Stajer D: Skin Blood Flow and Its Oscillatory Components in Patients with Acute Myocardial Infarction. J Vasc Res. 2007; 45(2):164-172 [Epub ahead of print]
23. Morlet J: Sampling theory and wave propagation. Issues in acoustic signal/image processing and recognition; NATO ASI series, Berlin, Springer-Verlag, 1983, vol I, 233–261.
24. Kastrup J, Bühlow J, Lassen NA: Vasomotion in human-skin before and after local heating recorded with laser Doppler flowmetry – A method for induction of

vasomotion. Int J Microcirc Clin Exp 1989; 8:205–215.

25. Söderström T, Stefanovska A, Veber M, Svensson H: Involvement of sympathetic nerve activity in skin blood flow oscillations in humans. Am J Physiol Heart Circ Physiol 2003; 284:H1638-H1646.
26. Johnson PC: The myogenic response. News Physiol Sci 1991; 6:41–42.
27. Bertuglia S, Colantuoni A, Intaglietta M: Effects of L-NMMA and indomethacin on arteriolar vasomotion in skeletal-muscle microcirculation of conscious and anesthetized hamsters. Microvasc Res 1994; 48:68–84.
28. Kvandal P, Landsverk SA, Bernjak A, Stefanovska A, Kvernmo HD, Kirkebøen KA: Low frequency oscillations of the laser Doppler perfusion signal in human skin. Microvasc Res 2006; 72:120-127.
29. Hafner HM, Brauer K, Eichner M, Koch I, Heinle H, Rocken M, Strolin A: Wavelet analysis of skin perfusion in healthy volunteers. Microcirculation 2007; 14(2):137-44.
30. Ignarro**,** LJ: Experimental evidences of nitric oxide-dependent vasodilatory activity of Nebivolol, a third generation beta-blocker. Blood Press 2004; 13:2-16, Suppl.
31. Stewart JM, Taneja I, Goligorsky MS, Medow MS: Noninvasive measure of microvascular nitric oxide function in humans using very low-frequency cutaneous laser Doppler flow spectra. Microcirculation 2007; 14(3):169-180.
32. Bernstein RD, Zhang XP, Zhao G, Forfia P, Tuzman J, Ochoa F, Ochoa M, Vogel T, Hintze TH: Mechanisms of nitrate accumulation in plasma during pacing-induced heart failure in conscious dogs. Nitric Oxide 1997; 1:386-396.
33. Belch JJF, Bridges AB, Scott N, Chopra M: Oxygen free radicals and congestive heart failure. Br Heart J 1991; 65:245-248.
34. Comini L, Bachetti T, Gaia G, Pasini E, Agnoletti L, Pepi P, Ceconi C, Curello S, Ferrari R: Aorta and skeletal muscle NO synthase expression in experimental heart failure. J Mol Cell Cardiol 1996; 28:2241-2248.
35. Smith CJ, Sun D, Hoegler C, Roth BS, Zhang X, Zhao G, Xu XB, Kobari Y, Pritchard, K, Sessa WC, Hintze TH: Reduced gene expression of vascular endothelial NO synthase and cyclooxygenase-1 in heart failure. Circ Res 1996; 78:58-64.
36. Bračič M, Stefanovska A: Wavelet-based analysis of human blood-flow dynamics. Bull Math Biol 1998; 60(5):919-935.
37. Geyer MJ, Jan YK, Brienza DM, Boninger ML: Using wavelet analysis to characterize the thermoregulatory mechanisms of sacral skin blood flow, J Rehabil Res Dev 2004; 41(6A):797-805
38. Humeau A, Koitka A, Abraham P, Saumet JL, L'Huillier JP: Spectral components of laser Doppler flowmetry signals recorded in healthy and type 1 diabetic subjects at rest and during a local and progressive cutaneous pressure application: scalogram analyses 2004; Phys Med Biol 49(17):3957-3970.
39. Liu XD, Zeng BF, Fan CY, Jiang PZ, Hu X: Spectral analysis of blood perfusion in the free latissimus dorsi myocutaneous flap and in normal skin. Phys Med Biol 2006; 51(1):173-183.
40. Rossi M, Carpi A, Di Maria C, Galetta F, Santoro G: Spectral analysis of laser Doppler skin blood flow oscillations in human essential arterial hypertension.

Microvasc Res 2006; 72 (1-2):34-41.

41. Li ZY, Leung JY, Tam EW, Mak AF: Wavelet analysis of skin blood oscillations in persons with spinal cord injury and able-bodied subjects. Arch Phys Med Rehabil 2006; 87(9):1207-1212.
42. Segal SS: Regulation of blood flow in the microcirculation. Microcirculation 2005; 12(1):33-45.
43. Folkow B: Description of myogenic hypothesis. Circ Res 1964; 15(2S1):279-&.
44. Davis MJ: Myogenic response gradient in an arteriolar network. Am J Physiol 1993; 264(6):H2168-H2179, Part 2.
45. Golenhofen K: Slow rhythms in smooth muscle; in Buhlbring E, Brading AF, Jones AW, Tomita T (eds): Smooth Muscle. London, Edward Arnold Ltd., London, 1970, pp. 316-342.
46. Salerud EG, Tenland T, Nillson GE, et al.: Rhythmical variations in human – skin blood flow. Int J Microc Clin Exp 1983; 2(2):91-102.
47. Colantuoni A, Bertuglia S, Intaglietta M: Quantitation of rhythmic diameter changes in arterial microcirculation. Am J Physiol 1984; 246(4):H508-H517.
48. Meyer JU, Borgstrom P, Lindbom L, et al.: Vasomotion patterns in skeletal-muscle arterioles during changes in arterial-pressure. Microvasc Res 1988; 35(2):193-203.
49. Intaglietta M: Vasomotion and flow modulation in the microcirculation; in Intaglietta M (ed.): Progress in Applied Microcirculation. Basel, Karger, 1989, vol 15, pp 1-9.
50. Hoffmann U, Yanar A, Franzeck UK, Edwards JM, Bollinger A: The frequency histogram – a new method for the evaluation of laser doppler flux motion. Microvas Res 1990; 40(3):293-301.
51. Bollinger A, Hoffmann U, Franzeck UK: Evaluation of flux motion in man by the laser doppler technique. Blood Vessels 1991; 28:21-26, Suppl. 1.
52. Traube L: Ueber periodische Thätigkeits-Aeusserungen des vasomotorischen und Hemmungs-Nerven Zentrums. Zentralbl. Med. Wiss. 1865; 56:881-885.
53. Hering E: Ueber den Einfluss der Athmung auf den Kreislauf. S.-B. Akad. Wiss. Wien, math.-nat. Kl. 1869; 60:829.
54. Mayer S: Studien zur Physiologie des Herzens und der Blutgefässe 6. Abhandlung: Über spontane Blutdruckschwankungen. S.-B. Akad. Wiss. Wien, math.-nat. Kl., 1876; 74:302.
55. Killip T: Oscillation of blood flow and vascular resistance dureng mayer waves. Circ Res 1962; 11(6):987-&.
56. Anschutz S, Schubert R: Modulation of the myogenic response by neurogenic influences in rat small arteries. Br J Pharmacol 2005; 146(2):226-233.
57. Harder DR: Pressure-dependent membrane depolarization in cat middle cerebral-artery. Circ Res 1984; 55(2):197-202.
58. Osol G, Halpern W: Myogenic properties of cerebral blood-vessels from normotensive and hypertensive rats. Am J Physiol 1985; 249(5):H914-H921.
59. Berczi V, Stekiel WJ, Contney SJ, Rusch NJ: Pressure-induced activation of membrane K+ current in rat saphenous artery hypertension. Hypertension 1992; 19(6):725-729, Part 2.

60. Kvernmo HD, Stefanovska A, Bračič M, Kirkebøen KA, Kvernebo K: Spectral analysis of the laser Doppler perfusion signal in human skin before and after exercise. Microvasc Res 1998; 56(3):173-182.
61. Hubscher M, Bernjak A, Stefanovska A, Vogt L, Banzer W: Assessment of exercise-induced changes of the microcirculation by wavelet transformation. Deutsche Zeitschrift Fur Sportmedizin 2006; 57(1):14.
62. Kitney RI, Fulton T, McDonald AH, Linkens DA: Transient interactions between blood-pressure, respiration and heart-rate in man. J Biomed Eng 1985; 7(3):217-224.
63. Stefanovska A, Strle S, Krošelj P: On the overestimation of the correlation dimension. Phys Lett A 1997; 235(1):24-30.
64. Golenhofen K, Hildebrandt G: Die beziehungen des blutdruckrhythmus zu atmung und peripherer durchblutung. Pflugers Archiv fur die Gesamte Physiologie des Menschen und Tiere 1958; 267(1):27-45.
65. Schmidt JA, Intaglietta M, Borgstrom P: Periodic hemodynamics in skeletal-muscle during local arterial-pressure reduction. J Appl Physiol 1992; 73(3):1077-1083.
66. Bajrović F, Čenčur M, Hožič M, Ribarič S, Stefanovska A: The contribution of lumbar sympathetic neurones activity to rat's skin blood flow oscillations. Pflugers Arch 2000; 439(3):R158-R160, Suppl S.
67. Landsverk SA, Kvandal P, Kjelstrup T, Benko U, Bernjak A, Stefanovska A, Kvernmo H, Kirkebøen KA: Human skin microcirculation after brachial plexus block evaluated by wavelet transform of the laser Doppler flowmetry signal. Anesthesiology 2006; 105(3):478-484.
68. Landsverk SA, Kvandal P, Bernjak A, Stefanovska A, Kirkebøen KA: The effects of general anesthesia on human skin microcirculation evaluated by wavelet transform. Anesth Analg 2007; 105(4):1012-1019.
69. Westerman RA, Widdop RE, Hannford A, Low A, Roberts RGD, Kent P, Sideris K, Yip T, Hales JRS, Stephens FRN: Laser Doppler velocimetry in the measurement of neurovascular function. Australas Phys Eng Sci Med 1988; 11(2):53-65.
70. Enerothgrimfors E, Lindblad LE, Westgren M, Ihrmansandahl C, Bevegard S: Noninvasive test of microvascular endothelial function in normal and hypertensive pregnancies. Br J Obstet Gynaecol 1993; 100(5):469-471.
71. Morris SJ, Shore AC: Skin blood flow responses to the iontophoresis of acetylcholine and sodium nitroprusside in man: Possible mechanisms. J Physiol (Lond) 1996; 496(2):531-542.
72. Moncada S, Palmer RMJ, Higgs EA: Nitric-oxide – Physiology, pathophysiology, and pharmacology. Pharmacol Rev 1991; 43(2):109-142.
73. Kvernmo HD, Stefanovska A, Kirkebøen KA: Enhanced endothelial activity reflected in cutaneous blood flow oscillations of athletes. Eur J Appl Physiol 2003; 90(1-2):16-22.
74. Veber M, Bandrivskyy A, Clarkson PBM, McClintock PVE, Stefanovska A: Wavelet analysis of blood flow dynamics: effect on the individual oscillatory components of iontophoresis with pharmacologically neutral electrolytes. Phys Med Biol 2004; 49(8):N111-N117.

75. Bandrivskyy A, Bernjak A, McClintock PVE, Stefanovska A: Role of transdermal potential difference during iontophoretic drug delivery. IEEE Trans Biomed Eng 2004; 51(9):1683-1685.

Table 1: *Clinical Characteristics of the CHF Study Population before treatment*

Age, y	69±10
Sex	11 male, 6 female
Aetiology of CHF, n	
Ischaemia	6
Hypertension	2
Valvular heart disease	3
Idiopathic dilated cardiomyopathy	6
Heart rate, bpm	79±16
Blood pressure, mm Hg	
Systolic	141±16
Diastolic	77±12
Total cholesterol, mmol/l	5.0±1.2
Length of treatment, weeks	20±10
Time to maximum tolerated medication, weeks	15±6

Group mean and standard deviation are provided where relevant.

Table 2: *Frequency Intervals*

Interval	**Frequency (Hz)**	**Physiological origin**
I	0.6–2.0	heartbeat
II	0.145–0.6	respiratory activity
III	0.052–0.145	intrinsic myogenic activity
IV	0.021–0.052	neurogenic (sympathetic) activity
V	0.0095–0.021	endothelial-related metabolic activity
VI	0.005–0.0095	(reported/discussed in the present work)

FIGURES

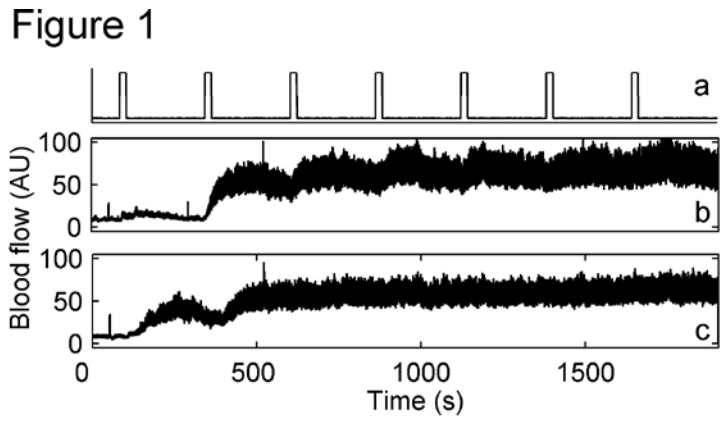

Figure 1: Simultaneously measured LDF signals showing how blood flow changes in response to iontophoresis of the two vasodilators. a, Timing of the 100μA iontophoresis current pulses; b, blood flow in response to ACh; and c, in response to SNP.

Figure 2

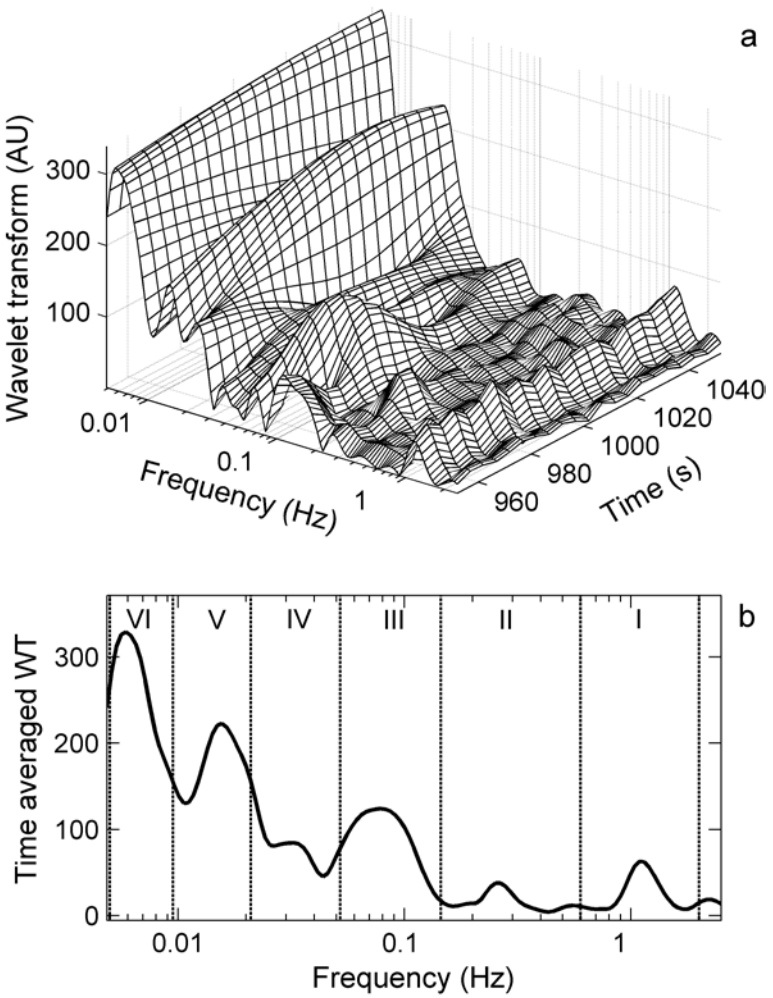

Figure 2: a, The wavelet transform of a LDF skin blood flow signal, illustrating the presence of distinct spectral peaks whose frequencies and amplitudes vary in time. The wavelet coefficients, presented in the time-frequency domain, were calculated from the basal flow of a healthy subject at rest. Only a short time section of the transform is presented. b, A time-average of the wavelet transform showing the division of the frequency scale into six intervals.

Figure 3

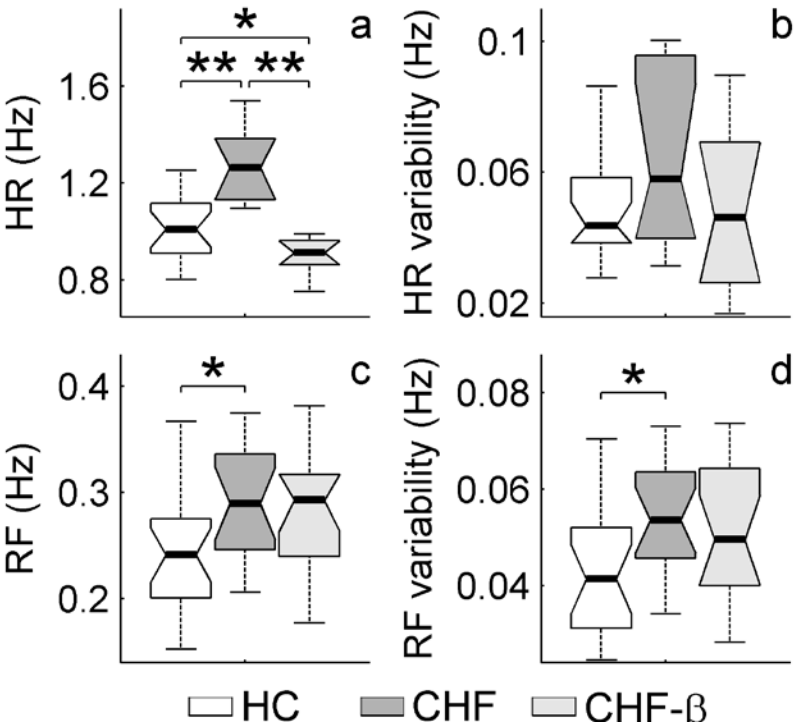

Figure 3: Summary of heart and respiration frequencies (a and c) and their variability (b and d) for the three subject groups. $0.01<p<0.05$ is indicated by * and $p<0.01$ by **. For an explanation of data presentation and error bars, see text (subsection on statistical analysis and presentation).

Figure 4

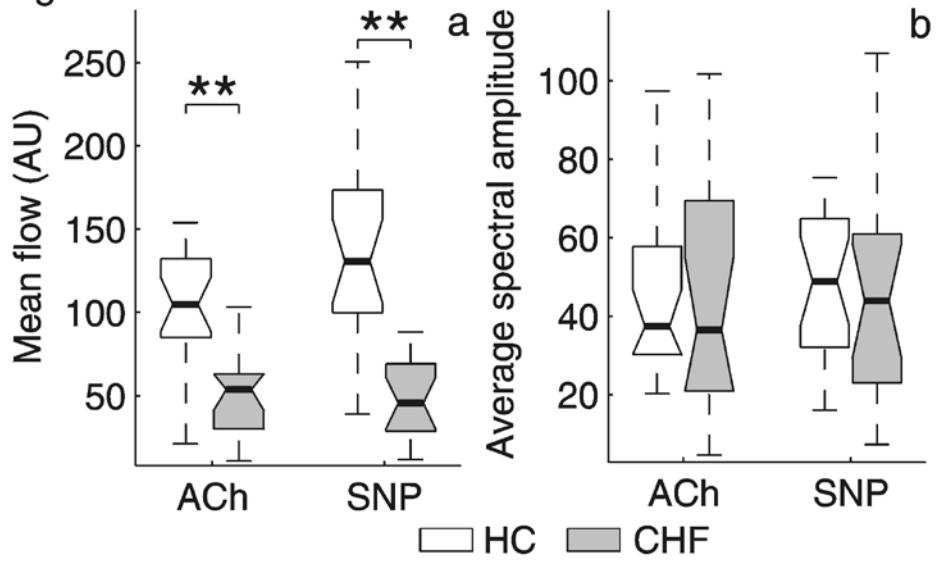

Figure 4: The effects of ACh and SNP on the mean value of the blood flow signal and the average spectral amplitude for the CHF and HC subject groups. $p<0.01$ is indicated by **. For an explanation of data presentation and error bars, see text (subsection on statistical analysis and presentation).

Figure 5

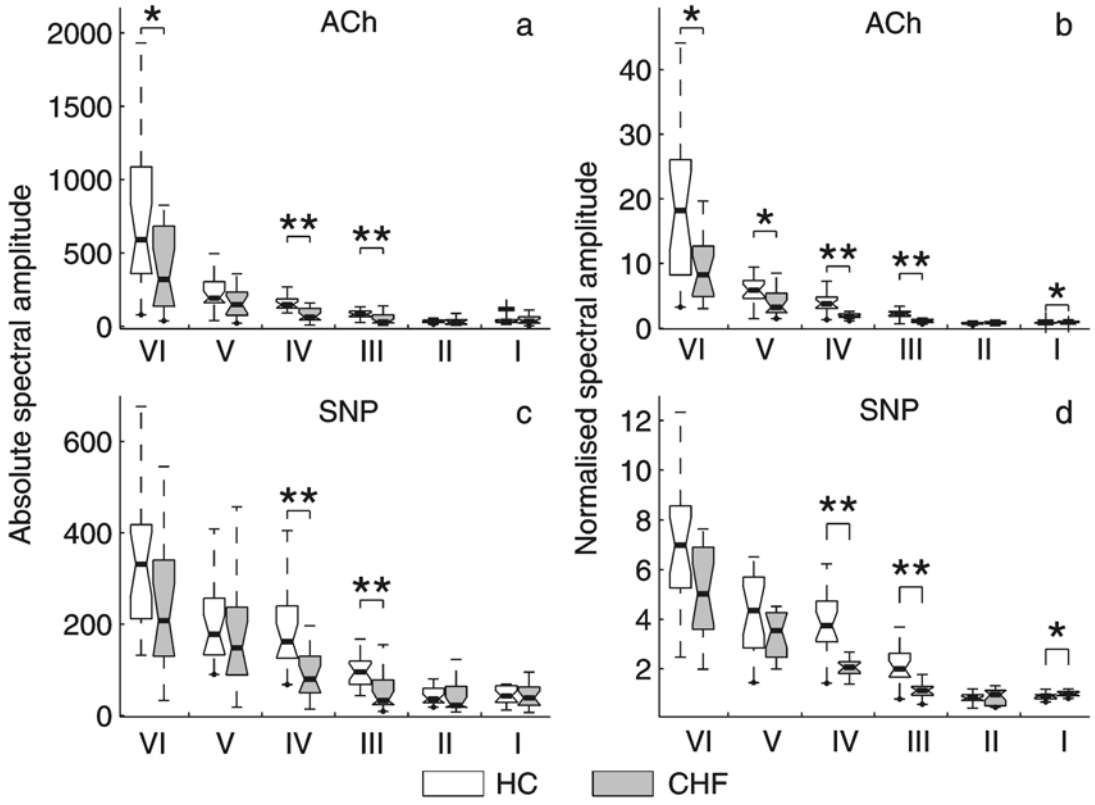

Figure 5: The effects of ACh and SNP on the individual oscillatory components in blood flow for the CHF and HC subject groups. a, effect of ACh on the absolute spectral amplitude; b, its effect on the normalised spectral amplitude; c, effect of SNP on the absolute spectral amplitude; d, its effect on normalised spectral amplitude. $0.01<p<0.05$ is indicated by * and $p<0.01$ by **. For an explanation of data presentation and error bars, see text (subsection on statistical analysis and presentation).

Figure 6

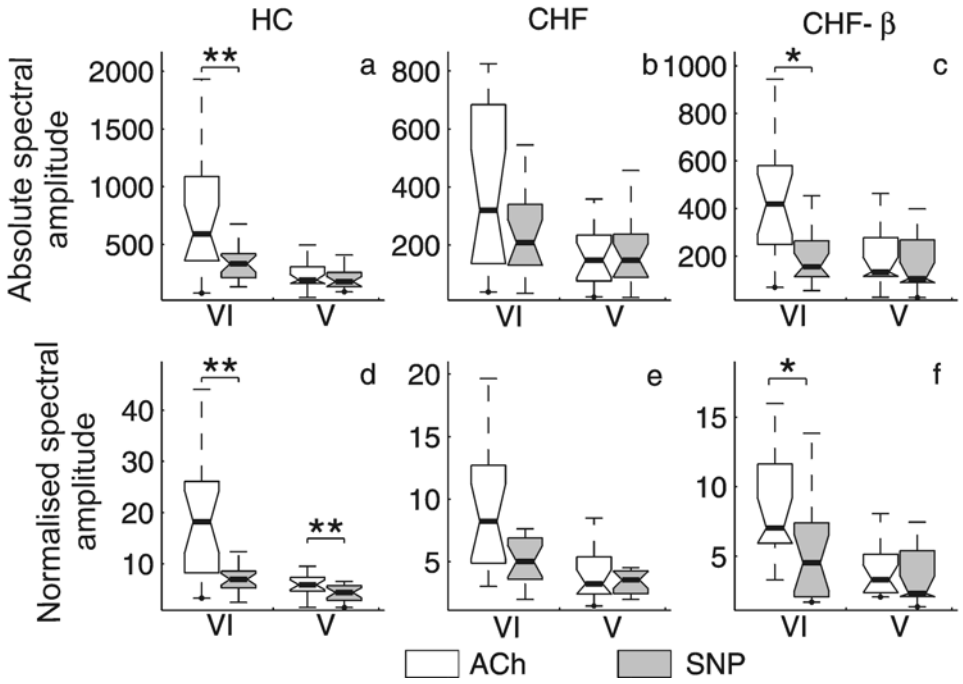

Figure 6: Effect of β1-blockers. The absolute spectral amplitude (upper row) and normalised spectral amplitude (lower row) are plotted for: a,d, the HC group; b,e, the HCF group prior to treatment; c,f, the CHF group after treatment with β_1-blockers. $0.01<p<0.05$ is indicated by * and $p<0.01$ by **. For an explanation of data presentation and error bars, see text (subsection on statistical analysis and presentation).