**Flow motion dynamics of microvascular blood flow and oxygenation – evidence of adaptive changes in obesity and type 2 diabetes mellitus/insulin resistance**

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**Key words:** microcirculation,blood flow, oxygenation, flow motion, frequency analysis

**Short title:** Microvascular flow motion dynamics

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**Abstract**

An altered spatial heterogeneity and temporal stability of network perfusion can give rise to a limited adaptive ability to meet metabolic demands. Derangement of local flow motion activity is associated with reduced microvascular blood flow and tissue oxygenation and it has been suggested that changes in flow motion activity may provide an early indicator of declining, endothelial, neurogenic and myogenic regulatory mechanisms and signal the onset and progression of microvascular pathophysiology. This short conference review article explores some of the evidence for altered flow motion dynamics of blood flux signals acquired using laser Doppler fluximetry in the skin in individuals at risk of developing or with cardio-metabolic disease.

**Abbreviations**

BF, blood flow

CVD, cardiovascular disease

DCS, diffuse correlation spectroscopy

DBPCT, double blind placebo controlled trial

FFT, fast Fourier transform

LDF, laser Doppler fluximetry

LF, low frequency

HF, high frequency

NIRS, near infrared spectroscopy

NO, nitric oxide

PORH, post occlusive reactive hyperaemia

PSD, power spectral density

SO2, microvascular oxygenation

WT, wavelet transform

**Introduction**

Adequate delivery of oxygen to the tissue is essential for tissue health and depends on a matching of the tissue’s requirements for O2 and the delivery of oxygenated blood to the tissue via the microvasculature. Regulation of microvascular perfusion is predominately achieved through changes in network conductance, modulated at a local level by endothelial, neurogenic and myogenic regulatory activity (88). Together these activities determine the cyclic oscillations of arteriolar diameter (vasomotion) (21, 92) that are related to changes in blood flow distribution in the microvascular networks (flow motion) (75).

Impairment of spatial and temporal regulation of network perfusion by these localised mechanisms has been shown to give rise to a mismatch between perfusion and demand, particularly at times of elevated metabolic demand (33). The consequence of such inadequate perfusion control is a compromised tissue function, such as that associated with features of the metabolic syndrome, which eventually leads to the development of retinopathy, neuropathy, skin ulcers and difficult to heal wounds (23). Thus information relevant to the multi-scale behaviour of microvascular networks and the regulatory mechanisms associated with perfusion control and tissue oxygenation may yield insights to inform diagnosis and treatment of micro-vasculopathies in a wide range of clinical settings (86).

**Measurement of tissue perfusion and its oscillatory components in health and disease**

Microcirculatory blood flow has been non-invasively investigated using techniques such as laser Doppler flowmetry (LDF), laser speckle contrast imaging (13, 83), peripheral arterial tonography (31), diffuse correlation spectroscopy (DCS) (2), side-stream dark field (36) and orthogonal polarisation spectral cameras (99), and nailfold capillaroscopy (111). The skin microcirculation offers an accessible site in which to study the physiological mechanisms involved in the regulation of tissue perfusion (43, 82) and LDF is currently the most widely used method for continuous, non-invasive monitoring of skin microcirculation in a wide range of physiological and pathological conditions (for recent review see (22). The LDF signal shows vigorous temporal and spatial variability and measures of this variability (and/or increased stability) can provide a rich source of information relating to the flexibility/responsiveness of the system.

***Analysis of the LDF signal in the time domain*** LDF is based on the Doppler Effect, first described by Christian Doppler in 1842 and applied by Buys Ballot in 1845 to sound waves (40). The output signal is defined as blood flux (BF) and is a product of red blood cell (RBC) concentration and velocity. The depth of tissue from which the LDF signal is derived depends on the laser power, wavelength and the separation of the emitting and collecting fibres (18, 40, 50, 59, 65). As LDF provides only a relative index of microvascular perfusion in the time domain, it is frequently used in conjunction with a reactivity test to allow investigation of the mechanisms underlying local control of vascular tone. Additionally, researchers may in part ameliorate the labile nature of resting skin blood flow and accommodate its intrinsic variability by expressing microvascular perfusion in terms of a relative change from baseline. The reactivity tests include post occlusive reactive hyperaemia, local thermal warming and pharmacological tools such as iontophoresis of vasoactive agents (e.g. acetylcholine, sodium nitroprusside and insulin). Skin microvascular responses in the time domain to these provocations have been recently and comprehensively reviewed (22, 80, 81) and the signalling pathways underlying the responses described (81).

LDF has been used in both research and in clinical practice to evaluate microvascular impairment in individuals at risk or with cardio-metabolic disease (19, 26, 98). These impairments include changes in endothelium-dependent and -independent function. However, to what extent impairments in microvascular reactivity can be taken as a diagnostic or prognostic indicator of vascular disease risk and pathogenesis remains disputed; as does a causal relationship between microvascular dysfunction and disease pathogenesis (41, 97).

***Analysis of the LDF signal in the frequency domain*** Recently, the use of the LDF technique has been extended to explore microvascular control mechanisms within the skin through analysis of the component frequencies of the laser Doppler signal (46). Time series analysis of LDF signals shows spontaneous, local, rhythmic oscillatory fluctuations of the blood in the microvasculature (Figure 1). These periodic oscillations are taken to reflect the activity of local vaso-control mechanisms (20, 55). The repetitive low frequency (LF) oscillations, or microvascular flow motion, represent the influence of myogenic (~ 0.05–0.15 Hz) (53), neurogenic (~ 0.02–0.05 Hz) (93) and endothelial (~ 0.0095–0.02 Hz) (56, 57, 77) activity on vascular tone. Additionally, the haemodynamic effects of the heart beat (~0.6–2.0 Hz) and respiratory activity (~0.15–0.6 Hz) (12) may be transmitted to the microcirculation (Table 1).

Two main methods of spectral analysis have been used to extract these oscillatory signals, one based on the Fast Fourier Transform (FFT) algorithm and the other on a generalized wavelet analysis (1). In FFT analysis, the power spectral density (PSD) of flow motion waves in PU2/Hz is obtained by computing the discrete Fourier transform of the LDF signal which in itself is a discrete representation of a continuous signal. In this way FFT analysis provides an estimate of the absolute power in the signal at a given frequency or the relative contribution of a frequency band to the total power of the signal. This is often used to evaluate the impact of each frequency band on the overall flow motion.

Generalized wavelet analysis, a scale-independent method with adjustable time and frequency resolution, was introduced for LDF analysis by Stefanovska et al. (95). While wavelets are not specifically designed for spectral analysis, spectral information can be recovered by analysis of the wavelets to produce the scalogram giving the energy contribution of each wavelet coefficient which can the express the flow motion activities in PU/Hz (95). A Wavelet Transform (WT) allows for analysis of time and frequency contents of an oscillatory signal (66)and has the advantage over the FFT in that it provides information about changes in frequency and power of distinct oscillatory bands over time (Figure 1B). The Morlet wavelet, a Gaussian function, has generally been used in LDF analysis as it has the best time-frequency localisation properties of generalised wavelets (34). The WT can also be averaged over time at a particular frequency to yield an average scalogram (Figure 1C) often reported in research papers.

It should be noted that data obtained using FFT and WT analysis of LDF signals cannot be compared directly as they are computed differently. Fourier transform-based power spectrum analysis is localised in frequency, being based on sine waves, and able to provide very accurate estimates of the frequencies present in the LDF signal but not when they are present in time. In contrast, the WT can discriminate the frequency content of the LDF signal and where it changes in time (figure 1B). The criteria for use of either FFT or WT and their relative advantages and disadvantages have been discussed in depth elsewhere (see, for example, (75, 96)). Direct comparison of outcomes such as those illustrated in Tables 1 and 2 should be undertaken with caution as power and scale are not the same thing. Furthermore, the parameters of the signal (length and sampling rate) and the parameters of FFT (window size and type and number of bins) have impact on PSD, therefore they should be reported alongside analysis. The choice of parameters in FFT analysis is a compromise between time and frequency resolution (10).

**Towards a mechanistic interpretation of measures of tissue perfusion and its oscillatory components in health and disease**

It has been argued that spectral analysis of the low frequency periodic oscillations in blood flux measurements obtained using LDF provides non-invasive, mechanistic information on microvascular control (53, 85, 95). To this end, spectral analysis has been performed on populations with known microvascular dysfunction such as peripheral arterial obstructive disease (69), chronic kidney disease (77), diabetes (87), essential hypertensive men and women (37, 38, 74), in chronic smokers (4, 73) and in individuals with hypercholesterolemia (72) and non-alcoholic fatty liver disease (19, 25, 89).

Figure 2 illustrates a recording of BF from the dorsum of the foot of a patient with a neuroischaemic foot ulcer showing differences in the time domain across the frequency bands suggestive of changes in the relative contribution of myogenic, neurogenic, NO-related and non-NO-related endothelium-mediated processes to the signal as compared with those seen in healthy skin (Figure 1). Variations in the amplitude and relative contribution of these oscillations have generally been associated with a decline in microvascular function (4, 19, 25, 72) with differing flow patterns according to the time course and severity of disease (Table 2). For reviews see (75, 77).

The findings in Table 2 should only be taken as illustrative of the range of studies conducted and outcomes observed. They show that amplitude and frequency of the oscillations as well as the contribution of the local, low frequency flow motion activity and higher frequency, transmitted systemic activity make to the total spectral power (and hence control of local perfusion) varies considerably when measured under basal conditions. To what extent this is attributable to the mutual interaction between the different oscillations and their synchronisation has yet to be determined. Stefanovska (94) describes the origin and nature of the coupled oscillators present in the cardiovascular system and interactions with neuronal and other oscillators. While interactions between the cardiac, oscillatory and neuronal oscillations could be described qualitatively, a long list of open questions remains unanswered and we are a long way from quantifying these known oscillatory interactions and how they might be used to differentiate with disease state such as between healthy and neuroischaemic volunteers shown Figures 1 and 2 respectively.

There is also an apparent lack of consensus of the direction of change of the relative contributions of the oscillatory signals across the spectral bands to the signal during perturbation of skin blood flow even where similar protocols have been used. Both the absolute spectral power and changes in relative spectral power appear study and cohort specific, as well as dependent on the provocation test used (see below). They may also vary with site and tissue studied and there remains an unmet need to make such measurements in tissues key to the pathogenesis of cardiovascular disease, such as muscle and adipose tissue (18, 110).

***Use of perturbation tests***

It can be noted in Table 2 that the various techniques for assessing skin blood flux in the time domain have been coupled with reactivity tests in order to explore endothelial and neurovascular function (81). Similarly, and as evidenced in Table 2, assessment of the LDF signal in the frequency domain has most usually been reported as a relative change in spectral energy in any given frequency band in response to such reactivity tests. While the use of the transient response to a reactivity test presents certain challenges for time-frequency analysis, it may be argued that the use of well-characterised reactions can better inform our understanding of PSD analysis as a surrogate of endothelial and neurovascular regulation of microvascular perfusion. The usefulness of such approaches for the evaluation of microvascular endothelial function in particular has been reviewed previously (76).

***Post occlusive reactive hyperaemia*** Mechanistically, major contributors to both the peak and time course of the post-occlusive reactive hyperaemia (PORH) response to brief (~3-5 min) arterial occlusion are local sensory nerves and, in the absence of an effect of inhibition of NO production, endothelium-derived hyperpolarising factors such as metabolites of cytochrome epoxygenase (62). Rossi et al. (71) have reported a significantly reduced PORH-induced endothelial and myogenic activity in long term smokers. The same authors have also reported variable reductions in some spectral bands in the PORH-response in individuals with untreated essential hypertension (69). The PORH response is transient and of insufficient duration to allow capture of the required 2-5 cycles (3.5 - 8.75 min) required for evaluation of the 0.01 Hz frequency band directly. However, by extending the PORH test to evoke a more sustained pressure-induced vasodilation in individuals at risk of pressure-induced ischaemic damage and ulceration, the involvement of neuro-endothelial activity has been studied (7).

Enhanced spectral analysis of the LDF signal measured in control individuals and patients with schizophrenia with an increased mortality risk due to cardiovascular events (110) suggests that while microvascular flow motion during the provoked post-ischemic stage of the PORH is significantly impaired in schizophrenic patients, alterations were more pronounced in the deeper tissue depth of about 6-8 mm (the superficial muscle tissue) compared with near-surface skin. They were also influenced by gender and age.

***Local skin warming*** Local heating of the skin evokes a biphasic response, with an initial transient (2-3 min) peak which is mostly mediated by a sensory nerve axon reflex (64) followed by a prolonged, predominantly endothelial NO-mediated plateau (15, 16) that provides a more stable and sustained differential output for time-frequency analysis. Warming to 43oC for 20 min results in a fold increase in total signal power of more than (45 ± 13) measured using a standard LDF probe in healthy forearm skin (18). When normalised to baseline, the most marked increase was in the endothelial and heart beat activity. Others have reported that warming the hand results in a decrease in normalised spectral energy in the myogenic band and increase in the endothelial band (91). In long-term smokers, attenuation of the response to warming has been associated with a reduction in relative spectral power around 0.01 Hz, reflecting a reduced endothelial/metabolic activity (4). Conversely, cooling-induced vasoconstriction and decline in microvascular perfusion is associated with increased normalised spectral energy in the myogenic band and decreased the normalised spectral energy in the cardiac band (91).

Regional differences in thermally-induced vasodilatation and flow motion activity in human skin microvascular responses have also been reported in individuals with type 2 diabetes with lower extremity neuropathy (5) that are consistent with variations in local flow motion control as well as susceptibility to disease and therapeutic intervention (6). Interestingly, significantly greater increases in respiratory, cardiac and total spectral activity of flow motion were seen on the ankle as well the dorsum of the foot in patients without a foot ulcer compared with those with a foot ulcer. To what extent this is indicative of transmission of these HF oscillations through a more dilated vascular network has yet to be explored.

***Insulin induced hyperaemia*** Evidence of adaptive flow motion changes in obesity and insulin resistance have possibly most successfully been demonstrated using acute steady-state hyperinsulinaemia (19, 25, 89). Table 2 illustrates that while the protocols and outcomes vary, during insulin administration absolute total spectral power and mean relative PSD in the LF bands are increased in both healthy and at risk individuals. In healthy individuals the insulin-induced increase in neurogenic flow motion was positively correlated with capillary recruitment (24) and both were related to insulin-induced glucose uptake. In obese individuals or those with two or more features of the metabolic syndrome the contribution of the LF intervals representative of endothelial and neurogenic activity to basal microvascular flow motion appeared lower in obese than in lean women and (26) and the insulin-induced change in relative PSD at ∼0.01 Hz correlated with insulin-induced glucose uptake (16). However, physiologically, hyperinsulinemia is usually transient and dynamic and under such conditions (e.g. following ingestion of a glucose drink and a liquid mixed-meal drink) skin microvascular LF flow motion between 0.01 and 0.02 Hz appears only mildly and variably attenuated in obese compared with lean individuals (51).

LDF parameters of microvascular reactivity in the time domain, offer characterization of endothelial dysfunction which may be used to improve CV risk assessment. However, to what extent the sensitive but difficult to measure changes in spectral energy, taken as indicative of flow motion, constitute adaptive outcomes in obesity and type 2 diabetes mellitus/insulin resistance remains uncertain and the extent to which they are reflective of changing microvascular pathology unclear. As Rossi wrote in a review in 2008 (59) ‘*Further studies are needed to evaluate whether the investigation of skin endothelial-dependent vasomotion can predict clinical and therapeutic outcomes of patients with vascular diseases’.*  This remains the case today.

***Impact of therapeutic intervention on flow motion* *responses to reactivity tests*** Table 3 summarises the studies revealed in our literature search in which the impact of therapeutic intervention on flow motion activity measured either under basal conditions or during a reactivity test in patient cohorts with or at risk of cardio metabolic disease. They are not numerous and the impact of intervention on LF flow motion limited; this is generally in spite of there being an observed effect of intervention in other key variables associated with vascular health. Additionally the numbers of individuals studied are generally small and only one was conducted as double blind placebo controlled trial. In this trial in obese individuals with two or more features of the metabolic syndrome, Clough et al. (19) reported no effect of 6 months high dose statin. Thus few conclusions can be drawn at this time as to the usefulness of including such measures in clinical trials.

**Flow motion dynamics of microvascular oxygenation**

Recent studies on frequency domain analysis of simultaneously recorded skin blood flux and oxygenation show that this approach may yield unique information on the processes determining the special heterogeneity and temporal stability of tissue perfusion. White light reflectance spectroscopy, light guide tissue spectrophotometry and visible light spectroscopy (many of which have been used in a clinical setting (84)) are all essentially based on the same principle and measure light absorption spectra of oxygenated haemoglobin (oxyHb) and deoxygenated haemoglobin (deoxyHb) between 500 and 650 nm (52). Microvascular oxygenation (SO2) parameter is a normalised measure

𝑆𝑂2=𝑜𝑥𝑦𝐻𝑏/ [𝑜𝑥𝑦𝐻𝑏+𝑑𝑒𝑜𝑥𝑦𝐻𝑏] (1)

They thus derive their signal from the same source (RBCs) as LDF and the techniques have been coupled into single systems to allow the simultaneous measurement of skin tissue blood flux and oxygenation (32, 39, 48, 54, 103). Combined blood flow and oxygenation in the deeper tissue (brain, muscle or breast tissue) has also been achieved using near infrared spectroscopy (NIRS) (3, 27), (61, 90).

Skin BF and SO2 signals measured in healthy human participants at rest, oscillate over broad, generally similar frequency ranges (~0.0095-1.6 Hz) with varying total power and relative contribution from low and high frequency PSD bands (54). These data, together with those from Bernjak et al. (11) have led to the suggestion that the LF oscillatory activities associated with microvascular perfusion and oxygenation may be modulated in a similar manner. The consequences of altered flow motion for nutrition and the oxygen supply to tissue remain largely unknown (107) but there is emerging evidence of an association between altered vasomotion and oxygen extraction in the unperturbed microvasculature in individuals at risk of CVD (103).

**Other descriptors of time- and frequency-domain characteristics in the microvascular blood flux signal**

There are a growing number of publications of the analysis of other time and frequency characteristics of LDF signals. For example, empirical mode decomposition is a method of decomposing a signal in the time domain, which may be nonlinear and non-stationary, into a set of functions allowing the varying frequencies in time to be preserved. Humeau-Heurtier & Klonizakis (45) used this approach to find instantaneous frequencies from intrinsic mode functions, in healthy subjects and patients with varicose veins. Liao & Jan (60) analysed nonlinear properties of LDF signals in subjects at risk for pressure ulcers using ensemble empirical mode decomposition to examine the self-phase synchronisation between the component frequencies of blood flow oscillations. Perhaps the two most promising areas of current investigation are coherence and complexity methods.

***Time localised phase coherence*** Studying phase coherence is another approach to studying interactions between different time series. High phase coherence synchronisation can be understood as connectivity/congruence between studied signals. This approach has been applied to BF and OXY signals by Bernjak et al. (11). These authors reported a phase coherence between skin BF and SO2 measured on the forearm of healthy volunteers. The phase coherence was estimated at two depths (~1 mm and 7 mm) as the difference in instantaneous phases at each frequency and each time point using a wavelet transform. The output result indicated how much the phase difference changes over time. The less change the higher the coherence. They reported significant phase coherence at low frequencies and also in the cardiac frequency band in the superficial dermis. They did not find significant phase coherence in deeper tissues (11). Similarly, Tankanag et al. (102) investigating wavelet phase coherence of oscillations between two different skin sites in 20 healthy subjects demonstrated both local and central mechanisms regulating low-frequency blood flow oscillations.

Thorn et al. (104) investigated the spontaneous oscillations in SO2 in relation to Hb signals (oxyHb, deoxyHb and totalHb) recorded from healthy human skin. They identified two types of swings; a type I swing when a change in SO2 resulted from a change in oxyHb and totalHb with no change in deoxyHb, and a type II swing which resulted in antiphase changes in oxyHb and deoxyHb with no change in totalHb. Thorn et al. (104) proposed that these swings reflect constant metabolic demand and oxygen usage (type I swing) and oxygen extraction due to change in oxygen demand or cutaneous blood flux (type II swing).

***Complexity*** Tigno et al. (106) have investigated the complexity of LDF signals from nondiabetic, prediabetic and diabetic monkeys. They report a Lempel-Ziv complexity in baseline data (34°C) and warmed data (44°C) and they reported that with progression of diabetes the complexity of the signal decreased (106). These findings suggest that the impact of diabetes on spontaneous oscillation may be reflected in the complexity of these oscillations rather than, or as well as in their oscillatory power content.

Hsiu et al. (44) compared the approximate entropy of beat-to-beat LDF signal in nondiabetic, prediabetic and diabetic humans. Contrary to Tigno et al (106), they showed an increased complexity in diabetic subjects compared to nondiabetic subjects. However, the complexity reported by Hsiu et al. (44) relates to complexity between consecutive heart beats rather than to low frequency oscillations. Perhaps, the adaptation of microcirculation to diabetes involves a decrease in complexity of the low frequency oscillations and an increase in the complexity of conducted signal representing the cardiac band. Further studies are required to confirm these hypotheses.

Humeau et al (47) investigated age-related changes in microvascular flows using wavelet-based representations; Hölder exponents to measure of regularity of the LDF signal and sample entropy to assess complexity. They showed that endothelium-related activity decreased with age while microvascular perfusion became more regular and less complex, although not significantly. Figueiras et al. (30) investigated whether the sample entropy of LDF signals can discriminate between healthy subjects, subjects with Raynaud’s phenomenon and subjects with systemic sclerosis. Esen et al. reported a fractal complexity measure, α, of detrended fluctuation analysis in healthy subjects (28) and subjects with essential hypertension (29). These studies demonstrate the diagnostic potential of these descriptors of time- and frequency-domain characteristics in the microvascular blood flux and oxygenation signals. Other approaches to time series analysis have been reviewed elsewhere (1).

**Conclusions**

In this brief article we set out to describe some of the quantitative measures of the time dependent behaviour of the blood flux signals acquired using laser Doppler fluximetry. Our aim was to review reports of these measures, which are generally associated with microvascular flow motion dynamics, and to explore whether measures of the variability (and/or increased stability) of these outputs offer a source of information relating to the flexibility/responsiveness of the system, particularly in individuals at risk of developing or with cardio-metabolic disease.

So far, the time and frequency descriptors of the LDF signal are unable to provide a consistent and robust reflection of changes occurring within the microcirculation either in healthy individuals during standard provocation of microvascular perfusion, or in patient cohorts. Furthermore, the diverse protocols and analysis methods used preclude direct comparison between the majority of these studies.

There appears some (if still limited) consensus on the physiological role of flow motion, and the changes in spectral energy reflective of flow motion, within the microvasculature. However, the relationship between the oscillatory dynamics of the BF signal, microvascular network perfusion heterogeneity and microvascular oxygenation has yet to be fully elucidated (17) and if we are to achieve this the quest for the time dependent behaviours of the microvasculature, standardised methods and parameters of analysis can be a direction towards the robust and more comprehensive assessment of flow motion.

**Acknowledgements**

KZK was supported by an EPSRC CASE PhD studentship.

**Figure Legends**

**Figure 1** An example of resting blood flux (BF) and oxygenation(SO2) signals recorded in healthy human skin at the forearm at ambient room temperature in the time domain (A) and frequency domains obtained using a Morlet wavelet transform (WT) (B) and as average scalogram of WT (C).

**Figure 2** An example of resting skin blood flux (BF) and oxygenation (SO2) signals recorded at ambient room temperature in the dorsum of the foot of a patient with a neuroischameic foot ulcer in the time domain (A) and frequency domains obtained using a Morlet wavelet transform (WT) (B) and as average scalogram of WT (C).

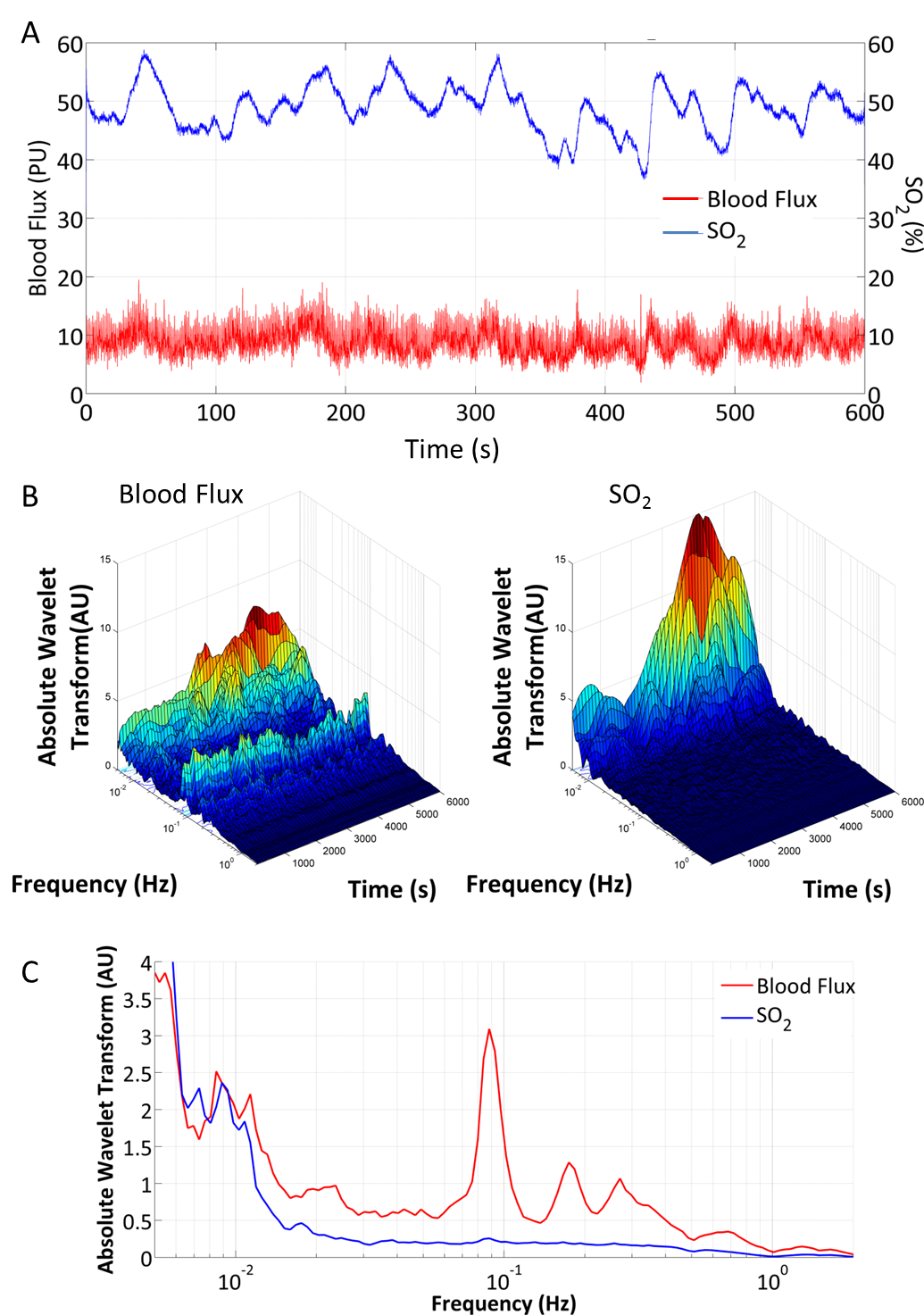
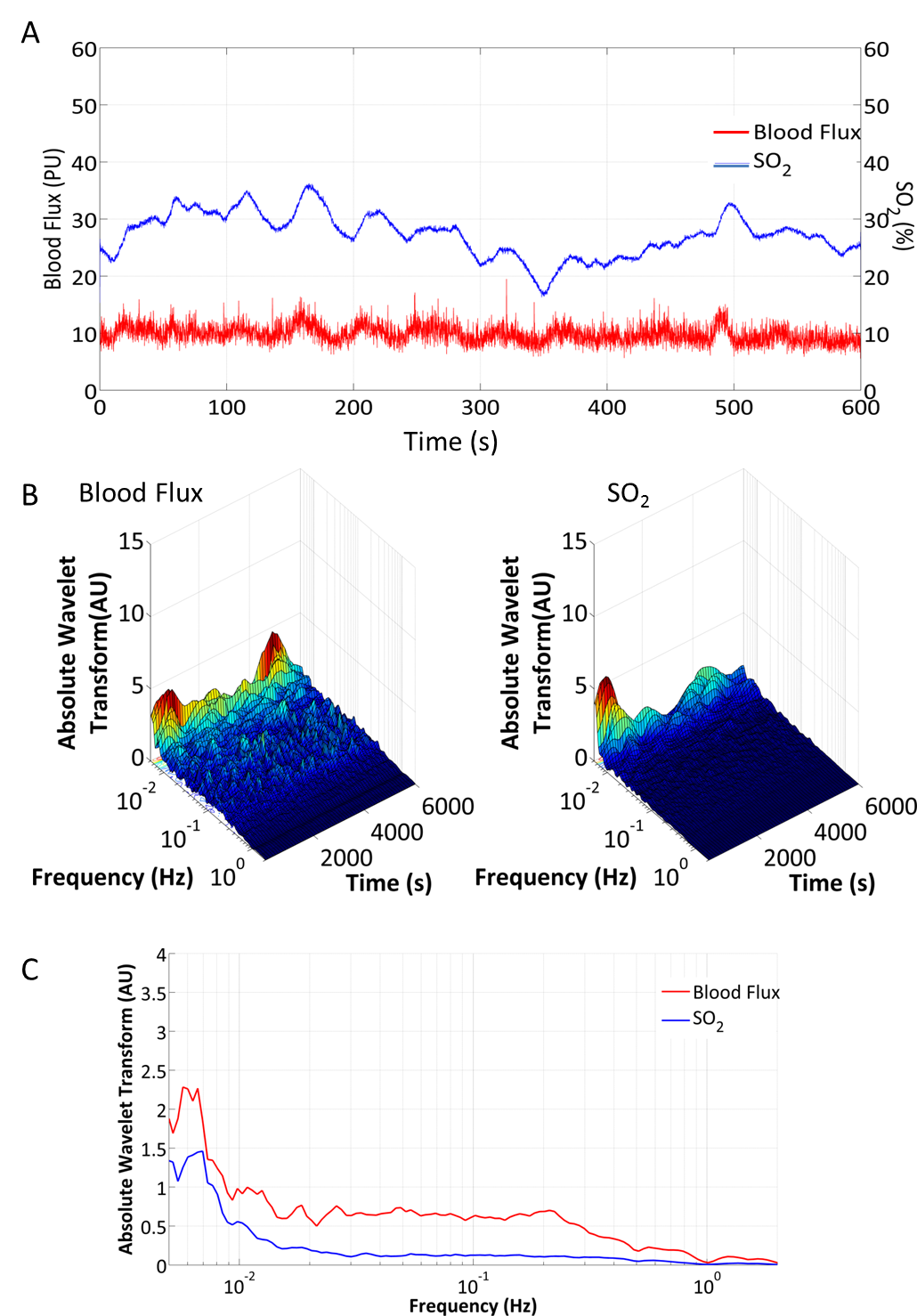
Figure 1

Figure 2



**Tables**

**Table 1** Periodic activity of the laser Doppler blood flux trace and its potential origins

**Table 2** Summary of illustrative studies reporting analysis of laser Doppler skin blood flux signals in the frequency domain.

**Table 3** Impact of intervention on blood flow motion in CVD risk cohorts.

**Table 1 Periodic activity of the laser Doppler blood flux trace and its potential origins**

|  |  |  |  |
| --- | --- | --- | --- |
| **Periodic activity** | **Time constant (s)** | **Frequency (Hz)** | **Origin** |
| Endothelial NO- independent | >105 | <0.0095 | Mechanisms other than NO-mediated originating from endothelial cells, e.g. endothelial derived hyperpolarizing factor (EDHF) |
| Endothelial NO-dependent | 48-105 | 0.0095-0.02 | NO production by endothelial cells |
| Neurogenic | 19-48 | 0.02-0.05 | Sympathetic nervous system |
| Myogenic | 7-19 | 0.05-0.15 | Vascular smooth muscle (VSM) cells |
| Respiratory | 1.6-7 | 0.15-0.620 | Breathing |
| Cardiac | 0.5-1.6 | 0.60-2.00 | Heartbeat |

**Table 2** Summary of illustrative studies reporting analysis of laser Doppler skin blood flux signals in the frequency domain

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| .**Study** | **Cohort** | **n** | **Measurements** | **Instrumentation** | **Reactivity test** | **Analysis methods** | **Outcomes** |
| Hoffmann et al 1994 (42) | Healthy/ peripheral arterial occlusive disease (PAOD) | 12 healthy, 24 PAOD | LDF dorsum of the foot |  |  | Pattern analysis: 'periodic', 'aperiodic' or 'no flux motion' | Severe claudication - increased 'periodic LF', Severe ischaemia - 'decreased or absent periodic LF' |
| Martin & Norman 1997 (63) | Infants after vaginal delivery(VD)/Caesarean section (CS) | VD 20, CS 10 | Dorsum of hand, 2, 6 and 24 hours after birth | Periflux PF 2B, Perimed AR, Sweden | Arterial occlusionLocal warming to 37°C | Perisoft, version 4.41, Perimed AR, Sweden | Higher degree of skin perfusion, vasomotion and reactive hyperaemia in CS compared to VD at 2h. Increase in vasomotion between 2 and 24h postnatal |
| Kvernmo et al 1998 (58) | Healthy adults | 9 | Forearm | MBF 3D, Moor Instruments, Axminster, UK | Aerobic exercise | WT Morlet (0.009 -1.6 Hz) | Increased contribution of ~0.1 Hz and decreased contribution ~0.04 and ~0.01 Hz  following exercise |
| Kvernmo et al 1999 (57) | Healthy adults | 9 athletes  9 controls | Forearm | MBF 3D, Moor Instruments with P10A optic probe  (Moor Instruments) | Iontophoresis of ACh, SNP | WT Morlet (0.009 -1.6 Hz) | ACh increased relative amplitude ~0.01Hz >than SNP |
| Serne et al 2002 (89) | Healthy adults | 18 | Dorsal side of the wrist | Periflux 4000 laser Doppler system in combination with a Periflux tissue heater set to 30°C | Local administration of insulin | FT in range 0.01-1.6Hz with short-time Fourier transform with different window lengths for each interval | Hyperinsulinemia increased total spectral energy density, relative energy contribution of heart rate and endothelial band |
| De Jongh et al 2004 (25) | Healthy adults | 12 | Intramuscular (anterior tibialis) | Periflux and Perimed needle probe (model 402) | Acute hyperinsulinaemia | FT 0.01 -1.6 Hz | Increase in intramuscular vasomotion by increasing the contribution of frequencies between 0.01 and 0.04 Hz |
| Urbancic-Rovan et al 2004 (108) | Diabetes | 36 healthy 43 + diabetes | Both arms and legs | floLAB, Moor Instruments, Axminster UK |  | WT | Mean amplitude of the total spectrum and at all frequency intervals highest in C, and lowest in D at left arm only |
| Bari et al 2005 (8) | Healthy aged and Alzheimer's disease (AD) | 77 healthy (4 age groups) + AD | Arm and forehead | Two-channel Periflux 4000 | PORH (1min) on the forearm | Perisoft 1.30, FFT Welch Method | Forehead age-dependent flow motion pattern. No difference in flow motion in AD |
| Rossi et al 2006 (74) | Essential hypertension (EHT) | 20 untreated EHT  20 long standing EHT  30 healthy | Forearm | Periflux PF4, probe PF 408 Perimed | PORH (3 min) | Spectral analysis on 5 min long signals before PORH and 5 min after peak PORH in Perisoft (FFT with Parsen windowing, 5 frequency bands) | Post-ischemic increase in total PSD in all groups with variable decline in some spectral bands in EHT. Long standing EHT patients showed a post- ischemic amplification only in the myogenic band |
| Rossi et al 2007 (73) | Current, long term smokers | 14 smokers 14 non smokers | Forearm | Periflux PF4 | PORH (3 min) |  | No significant difference in total basal PSD. Reduced PORH-induced endothelial and myogenic activity in smokers. |
| Jaffer et al 2008 (49) | Type 1 Diabetes Mellitus (T1DM) | 25 T1DM  13 controls | Pulp of great toe | Moor LDF and | PORH (3 min) | FT DRTSOFT (Moor Instruments) | Increased resting frequency of vasomotion and peak vasomotion in T1DM |
| Avery et al 2009 (4) | Current long term smokers | 28 smokers  28 healthy | Forearm measurements at | DRT4 with temp sensor DP12-v2 and heating unit SH02 (Moor Instruments) | Local thermal warming (43oC) | FFT for 5 frequency bands, total power and normalised power | No difference between baseline BF between healthy and smokers. Attenuated hyperaemia in smokers associated reduced relative spectral power around 0.01 Hz, reflecting a reduced endothelial/metabolic activity |
| Gryglewska et al 2010a (37) | Familial disposition and newly diagnosed hypertension (HT) | 70 (17NT-/22NT+/31HT) | Forearm | Periflux System 5000 | Local thermal warming | Perisoft Perimed Software with FFT | Differing total power and myogenic origin flowmotion with the lowest values in the NT(+) group |
| Gryglewska et al 2010b (38) | Masked hypertension | 82 (29NT/17MH/36HT) | Forearm | Periflux System 5000 |  | Perisoft Perimed Software with FFT and STFT | Increased myogenic (absolute and relative) and sympathetic in MH. Daytime systolic BP most consistent predictor of sympathetic and myogenic flow motion |
| Sun et al 2012 (100) | T2DM/sudomotor dysfunction | 68 T2DM  25 controls | Dorsum of right big | Laser Doppler flowmetry (MSP310XP Oxford Optronix, Oxford, UK) |  | Time averaged WT for low frequency only | Early impairment in LF flow motion (mainly in neurogenic and endothelial components) correlated with sudomotor dysfunction |
| Sun et al 2013  (101) | T2DM with/without peripheral neuropathy (PN) | 25 controls  24 clinical PN  24 subclinical PN  26 without neuropathy | Dorsum of right big toe | Laser Doppler flowmetry (MSP310XP Oxford Optronix, Oxford, UK) |  | Time averaged WT for low frequency only | Reduced average and endothelial and neurogenic vasomotion in clinical neuropathy group. Relative increase of myogenic and decrease of neurogenic activity in subclinical neuropathy group. |
| Rossi et al 2014 (79) | Current long term smokers | 100 smokers  66 controls | Medial surface of right forearm | Periflux PF4001 (probe PF408) Perimed | PORH | FT in Perisoft | Reduced basal myogenic vasomotion and a reduced PORH-induced increase in endothelial and sympathetic vasomotion |
| Bruning et al 2015 (14) | Essential hypertension (EHT) | 18 EHT18 NT controls | Forearm | Moor LDF and SH02 heater | Local warming with pharmacological inhibition (nitric oxide synthase, NOS blockade) | FT analysis on 10 min long signals | EHT had a lower total PSD, with reduced neurogenic and augmented myogenic contributions. HT + NOS blockade lower absolute endothelial, neurogenic (p<0.05), and total PSD (p<0.001) vs NT |

**Table 3** Impact of intervention on blood flow motion in CVD risk cohorts

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Study** | **Cohort** | **Intervention** | **n** | **Measurements** | **Instrumentation** | **Reactivity test** | **Analysis methods** | **Outcomes** |
| Bernjak et al 2008 (9) | Congestive heart failure (CHF) | Bisoprolol 10 mg max  20 weeks | 17 newly diagnosed CHF  21 controls | Forearm | DRT4 LDF monitor (MPI-V2 probe) (Moor Instruments, UK) | Iontophoretically-administered ACh and SNP | WT analysis | Improved 0.005-0.0095 Hz and 0.0095-0.021Hz |
| Rossi 2009 (72) | Hypercholesterolemia (HP) | Rosuvastatin 10mg/day  10 weeks | 15 HP15 controls | Forearm | Periflux PF4001 (probe PF408) Perimed, Sweden | Iontophoretically-administered ACh and SNP | Perisoft (FFT with Parsen windowing, 5 frequency bands) | No difference in baseline PSD. Lower ACh-induced increase in 0.01-0.02 Hz band in HP. No effect of intervention in either group |
| Rossi et al 2011 (70) | Newly diagnosed essential hypertension (EHT) | Antihypertensive therapy  8 weeks | 26 EHT 20 NT | Forearm | Periflux PF4001 (probe PF408) | PORH | WT analysis in Matlab, absolute and relative spectral amplitude | Lower relative amplitude in myogenic band in HT No effect of intervention on low frequency bands |
| Clough et al 2011 (19) | Central obesity | DBPCT Atorvastatin  40mg/day  6 months | 40 | Over tibialis anterior muscle | LDF with 785 nm, 20 mW laser and 4 mm separation (DP1-V2-HP) probe (Moor Instruments) | Acute hyperinsulinaemia + PORH (3 min) | FFT in Matlab | Increase in relative PSD around 0.01 Hz. Change in relative PSD at ∼0.01 HZ during insulin infusion was correlated with insulin sensitivity. No effect of intervention |
| Rossi et al 2012 (68) | Hypercholesterolaemic with systemic sclerosis | Simvastatin (20mg/day) 9-11 weeks | 13 patients 15 controls | Dorsal aspect of the third right finger | Periflux PF4001 (probe PF408) | PORH (3min) | FT analysis in Perisoft on 10min long signals | Simvastatin enhanced the PORH-induced increase in PSD in myogenic band in patient group |
| Rossi et al 2012 (78) | Morbidly obese | Gastric bypass (and weight loss)  1 year follow-up | 16 obese10 lean controls | Subcutaneous adipose tissue | Periflux PF4001 |  | FT analysis within 3 frequency intervals | Higher normalised PSD in obese before intervention.  Reduced after intervention |
| Ticcinelli et al 2014 (105) | Critical limb ischemia | Revascularisation  Before and no later than 30 days after | 15 | Dorsum of the foot | PF5000 LDF system with Probe 457 (Perimed, Stockholm, Sweden) | revascularisation | WT analysis | Shift from prevailing endothelial towards sympathetic activity |
| Gijsbers et al 2015 (35) | Untreated (pre)hypertensives | Supplemental Na (3·0 g/d), supplemental K (2·8 g/d) or placebo, 4 weeks each, in random order | 36 | 2 cm distal to the wrist on back of left hand | PF5000 LDF system with Probe 457 (Perimed, Stockholm, Sweden) |  | FT analysis in Perisoft | No effect on skin vasomotion vs placebo |
| Popa et al 2015 (67) | Stage II PAOD with HT and high cholesterol | Frequency Rhythmic Electrical Modulation System (FREMS, 10 sessions in 10 days | 5 | Dorsum of the affected foot | PF5000 LDF system with Probe 457 (Perimed, Stockholm, Sweden) | PORH (max 5 min) | WT | FREMS enhancedPORH-induced ineurogenic activity |
| Vinet et al  2015 (109) | Metabolic syndrome  RESOLVE study | Lifestyle (diet & physical activity programme)  6 months | 38 MetS  18 healthy controls | Arm | PF5000 LDF system with Probe 457 (Perimed, Stockholm, Sweden) | Ionophoresis of insulin | FT analysis in Perisoft | MetS patients had lower insulin-induced total PSD and depressed low frequency (myogenic) band activity which became similar to control values with Intervention |

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