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SYMPOSIUM REPORT

Multiscale, multidomain analysis of microvascular flow dynamics

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Abstract

To date, time- and frequency-domain metrics of signals acquired through laser Doppler fluximetry have been unable to provide consistent and robust measures of the changes that occur in the microcirculation in healthy individuals at rest or in response to a provocation, or in patient cohorts. Recent studies have shown that in many disease states, such as metabolic and cardiovascular disease, there appears to be a reduction in the adaptive capabilities of the microvascular network and a consequent reduction in physiological information content. Here, we introduce non-linear measures for assessing the information content of fluximetry signals and demonstrate how they can yield deeper understanding of network behaviour. In addition, we show how these methods may be adapted to accommodate the multiple time scales modulating blood flow and how they can be used in combination with time- and frequency-domain metrics to discriminate more effectively between the different mechanistic influences on network properties.

KEYWORDS

blood flow, complexity, flow motion, frequency analysis, microcirculation

1 | INTRODUCTION

Investigations of blood flow in microvascular networks have shown that in many disease states, such as cardiovascular and metabolic disease, there is a reduction in the adaptive capabilities of the network and its ability to respond to an imposed stressor (Frisbee et al., 2016). The processes underlying the modulation of the blood flow are known to operate at different intensity levels and have different periodicities (Stefanovska, Bracic, & Kvernmo, 1999), which can change both temporally and spatially. Conventional time- and frequency-domain analysis techniques have proved valuable in the understanding of the network blood flow but have, so far, failed to describe mechanistically the changes in observed flow patterns between pathological conditions or haemodynamic states. Recently, non-linear methods based on ideas from information theory have been used to quantify the regularity and randomness of short lengths of physiological signals and have demonstrated the potential for diagnostic capability (Balasubramanian & Nagaraj, 2016). These studies suggest that such methods might be of benefit in the analysis of microvascular network flow dynamics to discriminate better between different influences on network functionality and flexibility.

The aim of this brief report is to describe the application of a range of analysis techniques to signals derived from skin blood flow. We review the main applications and considerations of approaches in the time, frequency and complexity domains and their use in combination to discriminate between microcirculatory blood flow signals in differing pathophysiological and/or haemodynamic states. Examples are taken from two groups of individuals at risk of cardiovascular and metabolic disease that we have reported previously in detail elsewhere (Chipperfield, Thanaj, Scorletti, Byrne, & Clough, 2019), grouped for the use (CB1, $n = 8$) or not (CB0, $n = 28$) of a calcium channel (CB) blocker for the treatment of hypertension. Calcium channel blockers are considered here because they are vasodilators that, in particular, inhibit myogenic control and can thus be expected to result in altered microvascular flow dynamics, against which the different analysis methods may be tested.

2 | ANALYSIS IN THE TIME DOMAIN

Based on the Doppler effect, laser Doppler flowmetry (LDF) was first described in 1964 for the measurement of polystyrene balls entrained in fluid (Yeh & Cummins, 1964) and first applied to retinal arteries and capillary tubes in 1972 (Riva et al., 1972). The output signal is defined in arbitrary perfusion units (PUs) as blood flux (BF) and is the product of red blood cell concentration and velocity. The volume of tissue that is sampled is dependent on the laser power, wavelength and separation between emitting and receiving fibres (Clough, Chipperfield, Byrne, de Mul, & Gush, 2009) and is subject to significant spatial variations (Wahlberg & Fagrell, 1994).

Consider the two LDF signals shown in Figure 1a,b, recorded from the forearm skin of two subjects without and with CB use, respectively. The signal in Figure 1a shows a lower mean (8.3 PU) BF but with more variation in the moving average than that in Figure 1b (mean 15.2 PU), which appears to have larger variation in the higher frequencies.

Little information can be obtained directly from examination of LDF signals (Roustit & Cracowski, 2012; Yvonne-Tee, Rasool, Halim, & Rahman, 2005) alone, because they provide a relative index of microvascular perfusion in the time domain. Thus, it is often used in conjunction with a vasoreactivity test, such as iontophoresis, local thermal warming or postocclusive reactive hyperaemia (shown in Figure 1c), to assess dilator capacity and mechanisms influencing vascular tone (Roustit & Cracowski, 2012). For the latter, resting BF (RF) is determined as the mean BF over some time period, typically 5 min, before occlusion and measurement of the fold change (MF/RF) as the ratio of mean peak (MF) of the reactive hyperaemia. In the examples shown in Figure 1, the MF/RF values are 5.28 and 4.36, respectively, indicating degraded dilatory capacity in the subject with CB. A more robust test might account for differences in mean arterial pressure (e.g. Ichinose, Nakabayashi, & Ono, 2019) to determine cutaneous vascular conductance when comparing groups, although this requires additional measurements. Although such analysis has been used in research and clinical practice (e.g. Rossi, Carpi, Galetta, Franzoni, & Santoro, 2008), its value as a diagnostic or prognostic indicator of vascular disease or pathogenesis remains disputed. As we have previously reported, reactive tests cannot discriminate reliably or sufficiently between different pathophysiological groups (Chipperfield et al., 2019) alone or mechanistically, in terms of vasocontrol properties.

3 | ANALYSIS IN THE FREQUENCY DOMAIN

Analysis of BF time series reveals local, spontaneous, rhythmic oscillatory oscillation of BF (Figure 1) occurring at different frequencies, which are taken to reflect the activity of local vasocontrol mechanisms. These repetitive oscillations are taken to represent the influence of endothelial (0.0095–0.02 Hz), sympathetic (0.02–0.06 Hz), myogenic (0.06–0.15 Hz), respiratory (0.15–0.4 Hz) and cardiac activity (0.4–1.6 Hz) activity (Stefanovska et al., 1999) and are usually made from resting BF signals. Two main methods are used to assess the spectral contribution of these component signals; one is based on the fast Fourier transform (FFT), the other on generalized wavelet analysis. The choice of method used has been discussed in detail elsewhere (Clough, Kuliga, & Chipperfield, 2017) and is thus not considered further here. With the FFT, the power spectral density of the BF signal is obtained from its discrete Fourier transform (in perfusion units squared per hertz), estimating the absolute power in the signal at a given frequency. Figure 2 shows the power spectrum, calculated using Welch's method, for the two subjects shown in Figure 1. The magnitude of BF of the person with CB was observed previously to be larger and with apparently greater variation in the higher frequencies than the person without CB. However, examination of the spectral content of the BF signals reveals that this is not the case, with all bands having higher power without CB. Differences in mean BF shown in Figure 1 in PUs are not seen in the power spectrum of Figure 2, because it does not contain the DC ($f = 0$ Hz) component and is shown in perfusion units squared per hertz.

The relative power contribution of each frequency band is often used to evaluate its influence on the overall flow-motion. In the case shown in Figure 2, large differences can be seen in all frequency bands. Much research has presented data from different pathological groups with known microvascular dysfunction (for examples, see review by Clough et al., 2017), including responses to reactive tests. However, there is a lack of consensus on the direction of change in oscillatory components of the BF signal and the balance between the absolute or relative power in the frequency bands, with much appearing to be cohort specific and varying with the measurement site (Clough et al., 2017). Direct comparison of different studies is complicated further because the choice of parameters for frequency-domain analysis (e.g. window size, overlap, number of bins) is a compromise between time and frequency resolution. Signal parameters (e.g. sample rate, length and prefiltering) may differ, and the methods can be sensitive to such variations in the parameters. Furthermore, recent work suggests that the frequency bands are not fixed and may vary, for example, with age or pathological state (Grinevich, Tankanag, Tikhonova, & Chemeris, 2019). Nevertheless, such frequency-domain analysis is a valuable tool in understanding the processes modulating BF and their relative contribution to overall flow.

New Findings

- **What is the topic of this review?**
  We describe a range of techniques in the time, frequency and information domains and their application alone and together for the analysis of blood flux signals acquired using laser Doppler fluximetry.
- **What advances does it highlight?**
  This review highlights the idea of using quantitative measures in different domains and scales to gain a better mechanistic understanding of the complex behaviours in the microcirculation.

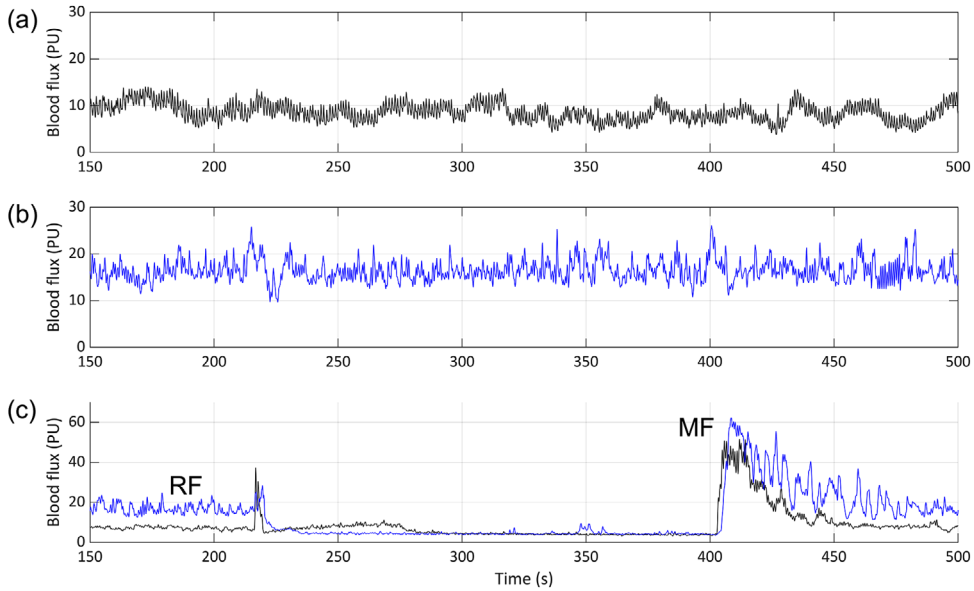

FIGURE 1 Examples of blood flux signals recorded from forearm skin at ambient room temperature in individuals at risk of cardiovascular and metabolic disease. (a) Individual without calcium channel blocker (black trace). (b) Individual with calcium channel blocker (blue trace). (c) Traces showing resting blood flux (RF) and mean maximum blood flux (MF) from both subjects rest and during the response to arterial occlusion (180 mmHg for 3 min; black trace without and blue with calcium channel blocker)

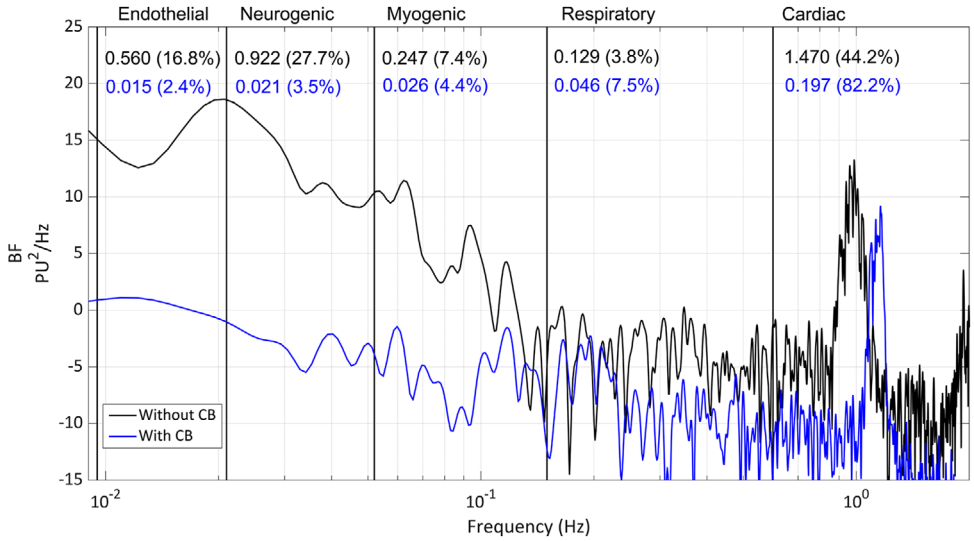

FIGURE 2 An example of blood flux (BF) absolute power spectral density plots for different individuals without (black) and with calcium channel blocker (CB) uptake (blue) showing mean and relative power per band

4 | INFORMATION AND COMPLEXITY

If the signals in Figure 1 could be described accurately by some mathematical model:

$$\mathrm{LDF}(t) = f(t, x_1, x_2, \ldots, x_n), \tag{1}$$

then all the information in LDF(t) would be described by the n parameters, x_n, and the original signal would effectively be compressed. However, if there was any error in the model then it would not describe LDF(t) accurately, and there would be a loss of information. The time- and frequency-domain analysis described previously loses, or does not use, all the information present in the LDF signals. The LDF signal is non-stationary, because the processes that produce it vary in time, and the power spectral density can yield information only on the overall signal content rather than the order or sequence in which it appears. Time-domain methods generally describe mean values or rates of change and thus do not capture

the variability in the signal. Attempts to model the data (e.g. Tigno, Hansen, Nawang, Shamekh, & Albano, 2011) have shown relatively poor descriptive power.

Complexity analysis quantifies the degree of variability or loss of spontaneity in a time series and has been applied to a range of biosignals, such as EEGs (Kalev et al., 2015) and ECGs (Valenza et al., 2017). The degree of variability in these signals reflects the physiological adaptability of the underlying system and provides established biomarkers of overall health status (Aboy, Hornero, Abásolo, & Alvarez, 2006). However, there is no single definition of complexity. Nagaraj and Balasubramanian (2017) describe three methods of quantifying complexity, which relate here to the following factors: (i) how hard it is describe the information in LDF(t), i.e. the number of unique patterns in the time series or model order; (ii) how hard it is to create or compress the information without loss; and (iii) the degree of organization or structure in LDF(t). The most widely used measures used in LDF signal analysis are Lempel–Ziv complexity (LZC), sample entropy and effort-to-compress complexity (Thanaj, Chipperfield, & Clough, 2018). However the complexity is estimated, it provides a measure of the information content of a signal (Tigno et al., 2011), and for brevity, only LZC (Lempel & Ziv, 1976) will be considered here.

Lempel–Ziv complexity provides a measure of how hard it is to describe the information contained in a signal and is the length of the shortest instruction set needed to reconstruct the signal without information loss. A simple periodic signal would have low complexity, because the same terms are repeated continually. In contrast, a random signal would have high complexity, because there are no rules, or repeating patterns, that define it. Given that LZC does not require the signal to be stationary, unlike chaos-based entropy analysis, the complexity can be normalized to the length of sample window (Hu, Gao, & Principe, 2006). Before LZC can be calculated, the original LDF signal must be transformed to a binary sequence, which can be achieved by recording a one if a sample is greater than the median and zero otherwise (Albano et al., 2008). Alternatively, a delta encoding method, whereby a zero is recoded if a value is less than the previous value in the time series or a one otherwise, which captures more of the variability in the signal, can be used (Kuliga, Gush, Clough, & Chipperfield, 2018). The signal is often divided into epochs of suitable length to examine how LZC varies over time, or a sliding window could be used to detect the time of rapid spontaneous changes in the signal.

Figure 3a shows an example of LZC calculated for 15 × 40 s epochs from forearm skin LDF captured at 40 Hz in the two different groups (CB1, n = 8 and CB0, n = 28). Given that the epochs are not synchronized in any way, direct comparison of individual or group values provides little understanding of spontaneous temporal activity. However, Figure 3a clearly shows differences in the amount of information contained in the BF signal, with the CB1 group being less variable and having fewer unique states (lower LZC) than CB0. An LZC index, calculated here as the mean of the 15 × 40 s epochs, can also be used to examine the groups (Carey et al., 2019; Chipperfield et al., 2019). In this case, the LZC index decreased from 0.362 ± 0.05 in the CB0 group to 0.302 ± 0.05 (mean ± SD) in CB1, with a significant difference between them (P = 0.0013).

5 | FREQUENCY, COMPLEXITY AND SCALE

The physiological processes that regulate flow-motion operate across multiple temporal scales, ranging from 0.001 to 2 Hz, and appear to vary with other parameters, such as skin temperature (Kuliga et al., 2018) and hypobaric hypoxia (Carey et al., 2019). For the data presented above, the LZC index is correlated positively with dilator capacity (MF/RF; r = 0.47, P = 0.001) and relative power in the respiratory band (r = 0.52, P = 0.0001) and negatively with RF (r = –0.37, P = 0.008) and relative power in the cardiac band (r = –0.56, P = 0.00004) (Chipperfield et al., 2019). The regular contribution to the information in the BF signal from the cardiac band, illustrated in Figure 2, appears to reduce the complexity in the CB1 group.

To account for these multiple, and potentially varying, process scales, LZC can be evaluated at multiple time scales (MLZC) using a coarse-graining approach (Cerutti, Hoyer, & Voss, 2009; Costa, Goldberger, & Peng, 2002). The sampling frequency is altered by a scale factor, τ, defining the scale level used to resample the original signal, reducing the scale of the time series. For the time series $\{x_1, \ldots, x_N\}$, where N is the number of samples, the coarse-grained time series, y^τ, is as follows:

$$y_i^\tau = \frac{1}{\tau} \sum_{i=(i-1)\tau+1}^{i\tau} x_j, 1 \le i \le N/\tau, \quad (2)$$

that is, the LZC is evaluated at different LDF sample rates where, for an original sampling frequency of 40 Hz, the sampling frequency is $f_\tau = 40/\tau$ where τ is the scale factor. At scale $\tau = 1$ the original signal is preserved at 40 Hz, and at scale $\tau = 24$ resampled to 1.67 Hz. At scale factor one, the time series y^1 is the original signal, and the length of each coarse-grained time series $\{y^\tau\}$ is equal to the original signal divided by the scale factor, τ. In previous work, we have investigated the length of signal required to obtain viable complexity measures and reported that a signal length > 1000 samples is required (Thanaj et al., 2018), which equates to 10 min captured at 40 Hz at scale $\tau = 24$.

Such multiscale analyses have been shown to be effective for understanding physiological signals in general (Costa et al., 2002; Humeau et al., 2010). Figure 3b shows an example of MLZC for the two groups, CB0 and CB1. As scale length increases (lower frequency corresponding to higher scale), LZC can also be seen to increase together with the separation between the groups until the Nyquist frequency of the original BF signal is reached or passed. Assuming that the maximal frequency of interest is the upper limit of the cardiac band of 1.6 Hz, the Nyquist frequency will be 3.2 Hz. (τ = 12). Above this scale, i.e. with lower sample frequencies, the influence of the relatively periodic heart rate will be diminished, and the signal will contain more information. To reach higher scales would require longer BF recordings.

One advantage of MLZC over LZC is that it can provide more features for classification of LDF data from the complexity at each scale. For example, we (Chipperfield et al., 2019) have shown that the accuracy of classification between these two groups can be improved from 77.8% using LZC to 86.1% with MLZC. Another benefit of MLZC

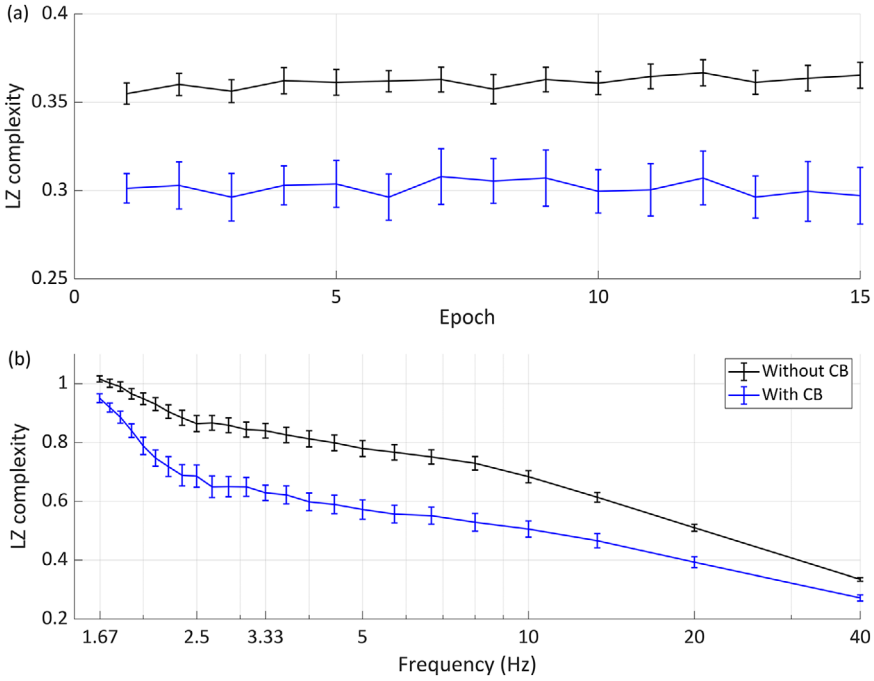

FIGURE 3 Examples of Lempel–Ziv (LZ) complexity of forearm blood flux: (a) calculated for 15 × 40 s epochs for forearm laser Doppler fluximetry signal captured at 40 Hz of two different groups of people at risk of cardiovascular and metabolic disease without (black, $n = 28$) and with (blues, $n = 8$) calcium channel blocker (CB) uptake; and (b) at multiple scales (scale = 40/frequency). Data are presented as means ± SEM

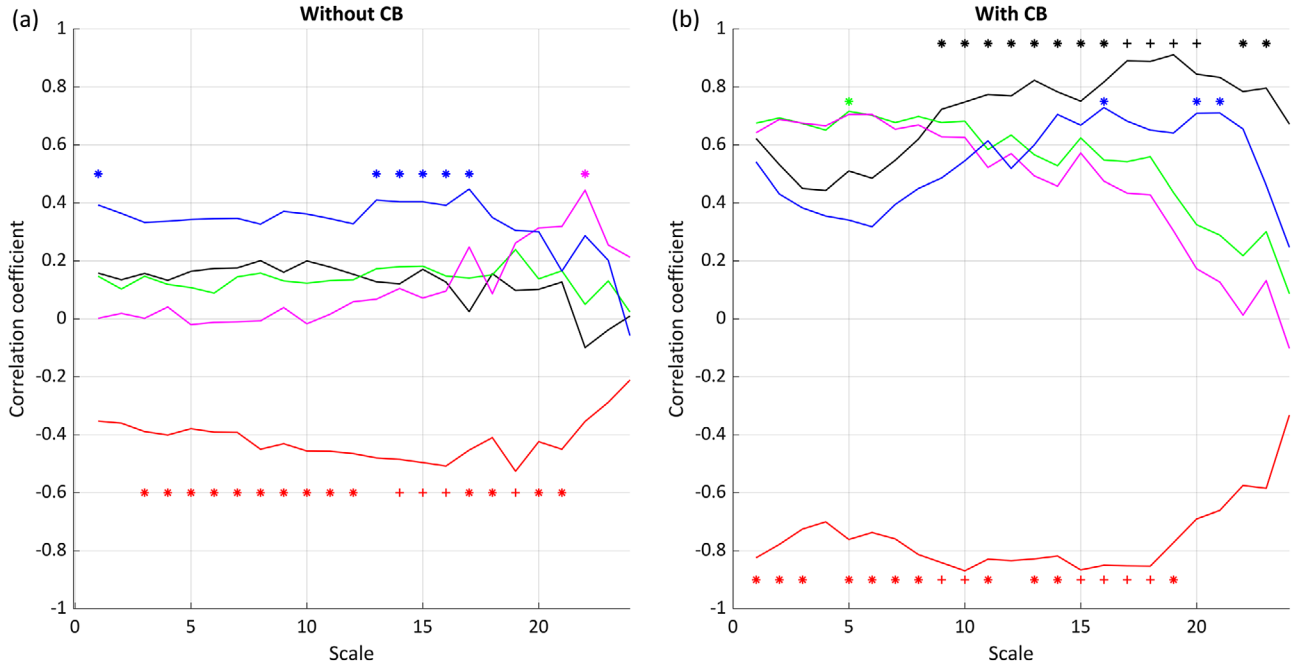

FIGURE 4 Spearman correlations between the flow-motion spectral power bands corresponding to endothelial (0.0095–0.02 Hz; black), neurogenic (0.02–0.06 Hz; green), myogenic (0.06–0.15 Hz; magenta), respiratory (0.015–0.4 Hz; blue) and cardiac (0.4–1.6 Hz; red) activity and multiscale Lempel–Ziv complexity of skin blood flow signals in $n = 28$ people without CB (a) and $n = 8$ people with CB (b). Effective sampling rate = 40 Hz/scale. $^{*}P < 0.05$, $^{+}P < 0.01$

is that it can be used to understand how the spectral components of the BF signal influence its complexity at different scales. For example, the Spearman's correlations between the power in each of the bands of the power spectra of the BF signals and the MLZC at increasing scale for the CB1 and CB0 groups described previously are shown in Figure 4. Heart beat and respiration have significant but opposite correlations with complexity until the Nyquist frequency is passed, when their influence reduces. Skin sympathetic nerve activity is known to be modulated by respiration, and cutaneous vasoconstrictor neurones are coupled temporally to cardiac and respiratory oscillations (Fatouleh & Macefield, 2013). Heart rate variability also contributes to the complexity of the BF signal (Sassi et al., 2015), and cardiac rhythm is modulated by respiration (Simms, Paton, Allen, & Pickering, 2010). This coupling of the two high-frequency components might

explain, in part, why the MLZC increase with scale, especially given that they exhibit little spontaneous variation in measurement conditions. At higher scales, the resampled BF signal covers a longer time period, and the lower frequencies associated with flow-motion, which generally contain the majority of the power of the signal, contribute proportionally more to signal variability, resulting in higher complexity. The CB changes the magnitude and relative influence of all the power bands on the signal complexity, with the cardiac pulse wave dominating.

6 | CONCLUSIONS

Traditional time- and frequency-domain analysis methods alone are unable to provide robust and consistent descriptors of the microcirculation dynamics in either the resting state or as a result of an imposed stressor. Further insight can be achieved through the combination of time-, frequency- and complexity-domain analysis, as illustrated here by the different combinations of processes modulating activity at different time scales between groups with and without CB uptake. The data presented here and elsewhere (Chipperfield et al., 2019; Frisbee et al., 2016; Tigno et al., 2011) provide further evidence that attenuation of flow-motion patterns is associated with increased cardiovascular disease risk and that prophylactic treatment results in a further decline of adaptivity through altered microvascular dynamics. The combination of techniques presented here opens new possibilities for the analysis of signals arising from the microcirculation and elsewhere. The combination of, and relationship between, metrics derived in the different domains can provide robust parameters that account, to some degree, for the temporal scales of the origin of the signal and the underlying local and systemic activity. Together, these multiple-domain analyses provide a platform for investigation of microvascular impairment in the skin and disease risk, which need to be applied to further pathophysiological states.

COMPETING INTERESTS

None declared.

AUTHOR CONTRIBUTIONS

A.J.C., M.T. and G.F.C. all contributed to the writing of this review, approved the final version of the manuscript and agree to be accountable for all aspects of this work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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