People With Long COVID and Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) Exhibit Similarly Impaired Vascular Function as Determined by Flow Mediated Dilation

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This article is formatted in British English

Abstract

This study aimed to compare flow-mediated dilation (FMD) values between individuals with Long COVID, Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS), and healthy age-matched controls to assess the potential implications for clinical management and long-term health outcomes.

A case-case-control approach was employed, and FMD measurements were obtained from 54 participants (17 Long COVID patients, 17 ME/CFS patients, and 17 healthy age-matched controls). FMD values were analysed using one-way ANOVA for between-group comparisons.

Results revealed significantly impaired endothelial function in both Long COVID and ME/CFS groups compared to healthy age-matched controls as determined by maximum % brachial artery diameter post-occlusion compared to pre-occlusion resting diameter (6.99 ± 4.33% and 6.60 ± 3.48% vs. 11.30 ± 4.44%, respectively, both $p < 0.05$). Notably, there was no difference in FMD between Long COVID and ME/CFS groups ($p = 0.949$), despite significantly longer illness duration in the ME/CFS group (ME/CFS: 16 ± 11.15 years vs. Long COVID: 1.36 ± 0.51 years, $p < 0.0001$).

The study demonstrates that both Long COVID and ME/CFS patients exhibit similarly impaired endothelial function, indicating potential vascular involvement in the pathogenesis of these post-viral illnesses. The significant reduction in FMD values suggests an increased cardiovascular risk in these populations, warranting careful monitoring and the development of targeted interventions to improve endothelial function and mitigate long-term health implications.

Key words

Myalgic Encephalomyelitis, Chronic Fatigue Syndrome, Long-COVID, Flow Mediated Dilation

Introduction

Following a viral infection, individuals may experience post-viral illness, which is characterised by prolonged feelings of unwellness and fatigue [1–3]. Post-viral illnesses exhibit various symptoms[4–7,] involving cardiovascular, respiratory, neurological, and musculoskeletal systems, significantly impacting the quality of life and functional capacity of affected individuals[1]. The term "Long COVID" has gained prominence in the last three years, as it refers to persistent symptoms lasting over 12 weeks after the acute phase of COVID-19 infection, drawing attention to post-viral fatigue. Long COVID shares similarities with Myalgic Encephalomyelitis (ME), Chronic Fatigue Syndrome (CFS), and/or ME/CFS[8], conditions that have been known in the medical literature for decades[9], with several overlapping symptoms [8,10,11]. ME/CFS and long COVID are debilitating conditions characterised by post-exertional malaise (PEM), fatigue, cognitive impairments, and pain, for which there is currently no known cure or definitive treatment [12–14]. Interestingly, both long COVID and ME/CFS are associated with vascular effects which may drive some of the persistent symptoms experienced[15–20]. A dominantly proposed mechanism concerning post-viral syndromes involves vascular damage and endothelial dysfunction-driven inflammatory response, leading to microclots which block capillaries, leading to hypoxic tissues[21–23]. This may well be a mechanistic pathway responsible for symptoms including fatigue, cognitive impairment, and pain[24].

Endothelial dysfunction, characterised by impaired nitric oxide bioavailability and altered vascular reactivity, is a key hallmark of various cardiovascular and metabolic disorders[25,26], and can be quantified using flow-mediated dilation (FMD). This technique involves measuring the diameter of an artery before and after a period of occlusion with an inflation cuff[27]. Upon deflation, blood flow is elevated downstream through the artery causing arterial dilation. This elevation in blood flow causes shear stress across the endothelium which stimulates eNOS activity, resulting in NO production and release, subsequently causing vasodilation as the smooth muscle relaxes[28]. Therefore, FMD is an indirect measure of NO bioavailability[28]. Reduced FMD has been associated with increased cardiovascular risk, including hypertension, atherosclerosis, and endothelial dysfunction[25]. Investigating FMD in individuals living with post-viral illnesses may provide valuable insights into potential vascular abnormalities and their relevance to the persistent symptoms experienced by these individuals. In this way, theories suggest that vascular damage and subsequent dysfunction may play a leading role in long COVID[15] and reduced FMD has been

observed in this population (8.2% vs 10.3%)[19]. Similarly, ME/CFS patients had markedly reduced FMD compared to healthy controls (5.1% vs. 8.2%)[18].

Whilst vascular function impairments have been reported in people with long COVID[19] and people with ME/CFS[18], these two patient groups have never been compared directly in the same study. This could be of interest as long COVID is a relatively new condition, and therefore it could be speculated that people with ME/CFS would have poorer vascular function as they have been suffering from the post-viral illness longer, therefore experiencing the multi-systems disease and deconditioning for a longer time[29].

Given the considerable overlap with long COVID and ME/CFS, we sought to examine FMD, compared to healthy controls. The objective of this case-control study was to investigate the effects of Long COVID and ME/CFS on vascular function. The study compared FMD between individuals with Long COVID or ME/CFS and age-matched healthy controls. By unravelling the link between vascular health and post-viral illnesses, our study aimed to provide a deeper understanding of the underlying pathophysiology of these debilitating conditions.

Methods

Participants

51 participants (long-COVID, n = 17 ME/CFS, n = 17; and healthy controls, n = 17, Table 1) were recruited for this study via social media advertisement. Participants attended a one-off visit to the Cardiovascular Imaging laboratory at the University of the West of Scotland, Lanarkshire, between March 2022 and January 2023. This study was carried out in accordance with the Declaration of Helsinki and approved by the Institutional Ethics Committee. Written informed consent was obtained from all participants prior to study commencement. Descriptive statistics for participants are shown in **Table 1**, and further described in the results section.

Sample size was calculated based on clinically meaningful changes in FMD from previous literature[30]. The effect size of persistent symptoms following COVID 19 infection, in terms of FMD, is large (Cohen's d = 1.08). To achieve 90% statistical power, with an alpha level of 0.05, 17 participants were required per group. To allow for 20% attrition, 20 participants were recruited to each group.

Participant Characteristics

Stature was measured using a wall mounted stadiometer (SECA, CE0123, Germany). Participants were required to remove their shoes and stand in the anatomical position keeping a straight back and ensuring their heels were in contact with the floor, stature was recorded in centimetres. Body mass of each participant was recorded using electronic scales (SECA 876, CE0123, Germany). Participants wore minimal clothing (shorts and t shirt where possible), and body mass was recorded in kilograms. Body composition determination was conducted in accordance with the International Society for the Assessment of Kinanthropometry (ISAK). Each participant's BMI was then calculated (BMI = Kg/m^2) from the body composition values. Resting blood pressure (BP) of participants was measured using an automated sphygmomanometer (Omron, the Netherlands), in accordance with the international society of hypertension (ISH) protocol. The participant was seated, and the BP cuff was secured on their left arm, a few centimetres above the elbow crease. The machine was then initialised, and the cuff inflated to 200 mmHg and subsequently deflated to 0 mmHg, this process was repeated three times and recorded. A one-minute rest period between repetitions was used, deemed optimal for BP accuracy.

Flow Mediated Dilation Measurement

Ultrasound examinations were performed in a semi-darkened room, with participants supine. Vascular reactive function was measured using flow mediated dilatation of the brachial artery, using high resolution 12 MHz linear ultrasound transducer (Siemens, Erlangen, Germany) and rapid cuff inflators. This protocol required imaging of a short non-branching segment of the brachial artery proximal to the antecubital fossa, using duplex sonography. The dominant limb was comfortably extended in a horizontal position to allow consistent imaging of the brachial artery. A segment of the brachial artery above the antecubital crease was imaged in the longitudinal plane, ensuring the lumen diameter was maximised and the light-dark contrast optimised to provide clear visualization of the double lines of Pignoli. After 1 min of baseline data incorporating brachial artery diameter and doppler flow was collected, a pressure cuff around the upper forearm was inflated to suprasystolic pressure (180 mmHg), using a rapid cuff inflator (Hockanson, Washington, USA) for 5 min. Subsequently, the cuff was deflated, and 5 min of post-deflation diameters and Doppler flow data was recorded in accordance with expert-consensus guidelines[27].

Flow Mediated Dilation Calculation

All scans were recorded in the internal memory of the ultrasound equipment, exported to USB devices, and transferred to the core laboratory computer. Video files were condensed to 10 frames per second (fps) using MediaCoder 0.8.65 video analysis software (Microsoft, USA). An automatic edge detection system (BrachialAnalyser, Vascular Tools 5, MIA-LLC, Iowa, USA), was used to calculate FMD, taken as the maximal percentage increase in diameter above baseline (mean of measures obtained during the first minute). Scans were rejected in case of poor quality and/or instability of the images due to inconsistency of clear artery borders and anatomical markers.

Statistical Analysis

All data were assessed for normal distribution and homogeneity of variance. Data were analysed using Jamovi (Version 2.3.21) and figures were created using GraphPad Prism (Version 9.4.1). To

assess the differences dependent variables, Welch's one-way analyses of variance (ANOVA) were performed. The relation between percent flow-mediated dilatation and vessel diameter was assessed by linear regression, for vessel size alone and then with adjustment for age, BMI, diastolic and systolic BP, and resting HR. Pearson's correlations were used to assess the relationship between FMD values and baseline diameter, age, BMI, systolic BP, diastolic BP, and resting HR. Effect size for paired comparisons was conducted using Cohen's d whereby the difference in means between two samples was divided by the pooled standard deviation (SD). Thresholds of 0.2, 0.5, and 0.8 for small, moderate, and large effects were used for Cohen's d [52]. Thresholds of 0-0.29, 0.3-0.49, and ≥0.5 for small, moderate, and large effects were used to interpret Pearson's correlation coefficients. Data are presented without subjective terminology and alpha levels are reported as exact P values, without dichotomous interpretation of 'significant' or 'non-significant' as advised by the American Statistical Association[51]. Data are presented as mean ± SD. For bar graphs, individual data points are presented as recommended by Drummond and Vowler [31,32].

Table 1: Descriptive data of participants

Variable	Group	Mean	SD	ANOVA P value for effect of group
Age (years)	LC (n=21)	47.52	9.60	0.8084 (LC vs ME)
	ME/CFS (n=20)	49.7	9.78	0.9005 (LC vs controls)
	Control (n=20)	49.05	13.77	0.9816 (ME vs controls)
Height (cm)	LC (n=21)	168	9.925	0.9735 (LC vs ME)
	ME/CFS (n=20)	168.7	9.464	0.6643 (LC vs controls)
	Control (n=20)	170.6	8.874	0.8007 (ME vs controls)
Body Mass (kg)	LC (n=21)	97.04	23.04	0.2720 (LC vs ME)
	ME/CFS (n=20)	86.79	23.9	0.0006 (LC vs controls)***
	Control (n=20)	70.93	15.04	0.0531 (ME vs controls)
BMI ($kg \cdot m^2$)	LC (n=21)	34.19	6.14	0.4058 (LC vs ME)
	ME/CFS (n=20)	31.49	9.21	<0.0001 (LC vs controls)****
	Control (n=20)	24.23	3.62	0.0033 (ME vs controls)**
Systolic |Blood Pressure (mmHg)	LC (n=21)	140	19	<0.0001 (LC vs ME)***
	ME/CFS (n=20)	102	33	<0.0001 (LC vs controls)***
	Control (n=20)	94	40	0.2958 (ME vs controls)
Diastolic Blood Pressure (mmHg)	LC (n=21)	95	15	0.9503 (LC vs ME)
	ME/CFS (n=20)	87	12	0.0928 (LC vs controls)
	Control (n=20)	77	8	0.0501 (ME vs controls)
Resting Heart Rate (bpm)	LC (n=21)	80	14	0.3882 (LC vs ME)
	ME/CFS (n=20)	82	19	0.3191 (LC vs controls)
	Control (n=20)	65	10	0.0215 (ME vs controls)*

Results

Descriptive participant parameters are displayed in **Table 1**. Illness duration of Long COVID group was 1.36±0.51 years and ME/CFS was 16±11.15 years ($p < 0.0001$). Pairwise differences between long COVID, ME/CFS, and controls for age and height were trivial to small ($p>0.67$, $d<0.31$). Long COVID participants were heavier than ME/CFS ($p=0.272$, $d=0.55$; medium effect) and controls ($p<0.001$, $d=2.10$; large effect). ME/CFS were heavier than controls ($p=0.053$, $d=0.80$; large effect). As a result of the differences in body mass, long COVID participants had a higher BMI than ME/CFS ($p=0.406$, $d=0.39$; small effect) and controls ($p<0.001$, $d=2.56$; large effect). ME/CFS were heavier than controls ($p=0.003$, $d=1.44$; large effect). Long COVID participants had higher diastolic BP than ME/CFS ($p<0.001$, $d=1.41$; large effect) and controls ($p<0.001$, $d=1.47$; large effect). The ME/CFS group had higher diastolic BP than controls ($p=0.296$, $d=0.22$; small effect). Systolic BP was not different between long COVID and ME/CFS ($p=0.950$, $d=0.09$; trivial effect), whilst controls had lower systolic BP than long COVID ($p=0.093$, $d=1.50$; large effect) and ME/CFS groups ($p=0.050$, $d=0.65$; medium effect). Resting heart rate was not different between long COVID and ME/CFS groups ($p=0.970$, $d=0.12$; trivial effect), whilst controls had lower resting heart rate than long COVID ($p<0.001$, $d=1.23$; large effect) and ME/CFS cohorts ($p=0.005$, $d=1.12$; medium effect).

After rejecting poor quality, 51 FMD videos were analysed (Long COVID n =17, 13 females, 4 males, ME/CFS n=17, 10 females, 7 males, Controls n=17, 10 females, 7 males). Baseline diameter of brachial artery (Long COVID: 0.43 ± 0.11 cm, ME/CFS: 0.40 ± 0.05 cm, and Controls: 0.40 ± 0.08 cm) was similar across groups (Long COVID vs ME/CFS $p = 0.683$ Cohen's $d = 0.137$, Long COVID vs Control $p = 0.828$, Cohen's $d = -0.076$, and ME/CFS vs Control $p = 0.863$, Cohen's $d = 0.137$) (**Figure 1A**). The maximum diameter of vessels was 0.464 ± 0.12 cm, 0.428 ± 0.05 cm, and 0.440 ± 0.08 cm in Long COVID, ME/CFS, and controls, respectively.

ANOVA and Post-Hoc tests revealed between group difference for FMD of Long COVID vs Controls (6.99 ± 4.33% vs 11.30 ± 4.44%, $p = 0.022$, Cohen's $d = 0.98$); and for ME/CFS vs Controls (6.60 ± 3.48% vs 11.30 ± 4.44%, $p = 0.019$, Cohen's $d = 1.18$). There was no between group difference for Long COVID vs ME/CFS (6.99 ± 4.33% vs 11.30 ± 4.44%, $p = 0.949$, Cohen's $d = 0.983$). The R^2 value revealed a small effect of FMD between groups ($R^2 = 0.22$). FMD data are displayed in **Figure 1B**.

Pearson's correlations revealed no relationship between FMD values and age (r = -0.179, p = 0.21), BMI (r = -0.097, p = 0.50), systolic BP (r = -0.045, p = 0.75), diastolic BP (r = -0.191, p = 0.18) or heart rate (r = -0.072, p = 0.62). However, there was a moderate Pearson's correlation coefficient between FMD and baseline diameter (r = -0.324, p = 0.02).

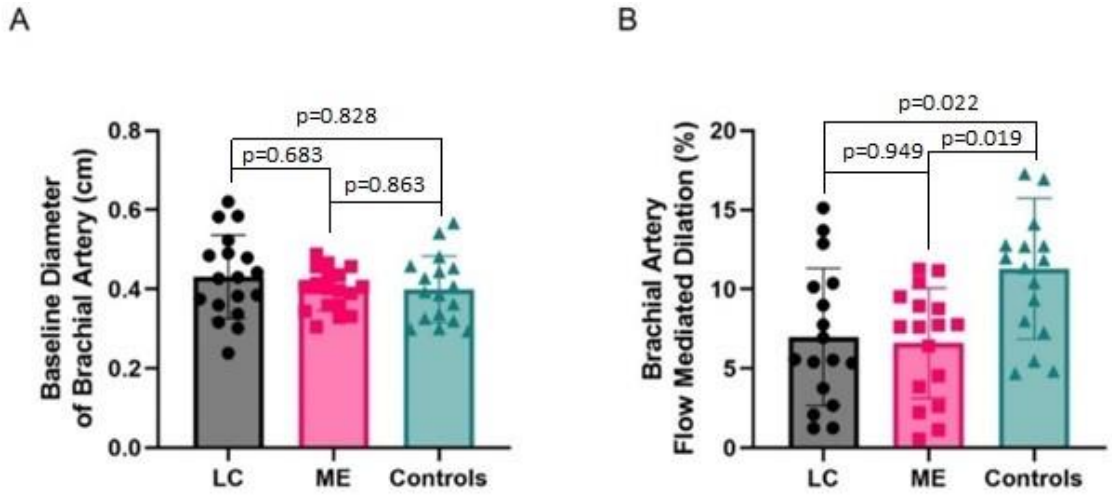

Figure 1. Baseline diameter of brachial artery (cm; A) and percentage change of brachial artery diameter upon cuff deflation in relation to baseline diameter (B). *Data presented are mean ± SD, with individual data points displayed. Long COVID (n =17, 13 females, 4 males), ME/CFS, (n=17, 10 females, 7 males), Controls (n=17, 10 females, 7 males).*

Discussion

The objective of this study was to compare FMD between individuals with Long COVID, ME/CFS, and healthy age-matched controls. The main findings from this case-case-control study show that Long COVID and ME/CFS both have similarly impaired endothelial function, as determined by lower FMD values as compared to healthy age-matched controls (**Figure 1**). This has significant implications for both the clinical management and long-term health outcomes of individuals living with long COVID and ME/CFS, including clinical assessment, management, and treatment of endothelial dysfunction to prevent subsequent cardiovascular disease progression [33,34]. Understanding the potential vascular contributions to persistent symptoms can guide the development of targeted interventions aimed at improving endothelial function and mitigating the burden of post-viral illnesses.

FMD is a clinically meaningful measurement and has prognostic value for cardiovascular events[33]. A meta-analysis of 35 studies found every 1% increase in FMD associated with a 12% reduced risk for cardiovascular events, with even higher risk reductions in disease populations[33]. Remarkably, cardiovascular disease risk was halved for those in high vs. low FMD categories[34]. Previous studies have observed lower FMD in Long COVID vs healthy controls (8.2% vs 10.3%)[19]. Similarly, another previous study found ME/CFS patients had markedly reduced FMD compared to healthy controls (5.1% vs. 8.2%)[18]. The current findings show that, in comparison to controls, Long COVID and ME/CFS groups had similar FMD values which were significantly lower than controls (6.99 ± 4.33% and 6.60 ± 3.48% vs 11.30 ± 4.44%, respectively), which may be indicative of ~50% increased cardiovascular risk[33,34]. This highlights the need for individuals with post-viral illnesses to be carefully monitored for cardiovascular risk and potentially prescribed treatments to lower risk.

It is worth noting that the large spread of the data shows that some individuals in Long COVID and ME/CFS groups had very severely impaired endothelial function, whilst others had comparable FMD to that of the controls. This suggests possibly different disease trajectories and symptomology amongst individuals with post-viral illnesses which is yet to be explored. This is emphasised by the divergent symptoms experienced by people with ME/CFS and long COVID [1,35,36]. The lack of uniform symptomology must be considered when considering personalised

medicine treatment options, as data presented herein suggest endothelial dysfunction may not be ubiquitous in post-viral conditions[37].

Whilst FMD impairments have been reported in people with long COVID[19] and people with ME/CFS[18], these two patient groups have never had FMD compared directly in the same study. It would have seemed reasonable *a priori* to hypothesise people with ME/CFS would have poorer vascular function than people with long COVID, due to post-viral illness duration, and multi-system deconditioning for longer (which is known to reduce FMD)[29]. However, the current study opposes this contention as Long COVID and ME/CFS groups exhibited similar FMD values despite significantly different illness duration (ME/CFS: 16±11.15 years vs Long COVID: 1.36±0.51 years, $p < 0.0001$). Therefore, the impairment in FMD in the current study is unlikely due to deconditioning. It is more likely that endothelial damage is experienced in the early post-viral phase, without further reduction in FMD after this initial diminution.

Although endothelial dysfunction may be a consequence of post-viral illnesses, vascular damage and subsequent dysfunction may play a leading role in development of the conditions and their persistent symptoms[15]. It has been well established that the SARS-CoV-2 virus enters eukaryotic cells via the angiotensin-converting enzyme (ACE)-2 receptor[38]. Since the vascular endothelium possesses ACE-2 receptors, vascular damage and endothelial dysfunction is likely caused by the virus[39,40]. This damage subsequently drives an inflammatory response, stimulating microclot formation within the blood due to hyperactivation of platelets, subsequently blocking capillaries from delivering oxygen and nutrients to local tissues[21–23]. This is a highly plausible mechanistic pathway which could explain several symptoms of long COVID including fatigue, cognitive impairment, and pain[24]. As ME/CFS is likely to have a similar disease aetiology to Long COVID, endothelial damage and formation of microclots[23] may also explain, at least partly, ME/CFS development and symptomology. Notably, a study utilising hyperaemia index to determine endothelial function found that a subset of both Long COVID and ME/CFS groups had endothelial dysfunction as compared with healthy controls[21]. This observation occurred alongside significantly reduced serum angiotensin-2 and elevated circulating endothelin-1[21]. Reduced levels of angiotensin-2 may reflect high shear stress due to chronic inflammation or endothelial damage[41]. Endothelin-1 plays a key role in vasoconstriction and elevated levels is an aggravating factor for hypertension and cardiovascular disease states[42]. Therefore, there is clear evidence for

cardiovascular risk within both long COVID and ME/CFS [18, 19, 21]. Promisingly, treatment to resolve mircoclots has successfully reduced symptoms, including fatigue, within Long COVID sufferers[43]. However, controlled clinical trials are still required to confirm initial findings and further research is required within ME/CFS populations.

The current study found no relationship between FMD and age ($r = -0.179$, $p = 0.21$), BMI ($r = -0.097$, $p = 0.50$), systolic BP ($r = -0.045$, $p = 0.75$), diastolic BP ($r = -0.191$, $p = 0.18$) or heart rate ($r = -0.072$, $p = 0.62$). In contrast, a large study investigating FMD reference intervals ($n = 5362$) found that age was strongly associated with FMD[44]. Perhaps larger participant numbers were required to confirm this effect in the current study. Furthermore, previous studies have found that obesity has an association with FMD [45]. In the current study, BMI was high in both ME/CFS and long COVID groups (34.19 ± 6.14 kg·m^2 and 31.49 ± 9.21 kg·m^2, respectively) compared to control (24.23 ± 3.62 kg·m^2). This may explain the lack of interactions between BMI and FMD in this study and highlights the requirement for further investigation in BMI-matched individuals. Similarly, previous studies have found FMD to relate to systolic and diastolic BP in healthy individuals[44] which is in contrast to the findings in this study. There is no clear explanation for this divergence, warranting further investigation and potential explanations for these differences.

Limitations

This study has certain limitations that necessitate acknowledgment. Firstly, the findings may not be readily applicable to the broader population of individuals with Long COVID, especially those who are unable to visit a laboratory, such as those severely affected by the condition. It is important to note that our experimental methods constrained participation to individuals who could travel to our laboratory, navigate stairs or lifts, and undergo the testing process. This approach was not entirely inclusive for people with Long COVID and ME/CFS, considering that according to NICE, approximately 25% of individuals with ME/CFS are bedbound or housebound[46], making it impossible for them to visit a laboratory. As a result, the extent of vascular function deficits observed in this study likely underestimates the true effect due to the inherent recruitment bias.

Expert-consensus guidelines for FMD acquisition were followed where possible[27]. As such, baseline diameter was presented. Baseline diameter is the biggest determinant of FMD and has a strong negative correlation with FMD[27,44]. The current study also found a correlation between FMD and baseline diameter ($r = -0.324$, $p = 0.02$). To reduce participant burden, participants were

not advised to fast before the test as recommended[27]. Hence, there is potential for confounding effect of dietary intake immediately prior to the FMD acquisition.

Conclusion

Both Long COVID and ME/CFS display notable impairment in endothelial function, evidenced by lower FMD values compared to the age-matched control group. FMD has prognostic value for cardiovascular events, highlighting the importance of monitoring individuals with Long COVID and ME/CFS for cardiovascular risk[33,34]. The large variability in FMD values within the Long COVID and ME/CFS groups suggests the presence of different disease trajectories and etiological mechanisms among individuals with post-viral illnesses, emphasizing the need for individualised care[37]. This study provides valuable insights into the vascular implications of Long COVID and ME/CFS, contributing to a growing body of knowledge that can aid healthcare professionals in monitoring and managing these conditions effectively. Findings strongly agree with a potential link between endothelial dysfunction[39,47], microclot formation[21–23], and persistent symptoms[24] opens avenues for targeted interventions, providing hope for improved outcomes for those affected post-viral illnesses. Further research in this area is essential to refine our understanding and develop evidence-based therapeutic approaches to address the long-term consequences of these conditions.

Authorship contributions according to the CRediT taxonomy

Conceptualization, M.M., N.E.M.S-H., L.D.H., E.C.B., and N.F.S.; methodology, M.M, N.E.M.S-H., L.D.H, and N.F.S.; software, M.M and N.F.S.; validation, M.M, and N.F.S.; formal analysis, M.M; investigation, M.M, N.E.M.S-H., L.D.H, E.C.B., and N.F.S.; resources, M.M.; data curation, M.M.; writing—original draft preparation, M.M, ; writing—review and editing, M.M, N.E.M.S-H., L.D.H, E.C.B., and N.F.S.; visualisation, M.M.; supervision, N.F.S; project administration, M.M, N.E.M.S-H., L.D.H, E.C.B., and N.F.S.; funding acquisition, L.D.H, and N.F.S.. All authors have read and agreed to the published version of the manuscript.

Acknowledgements

This work was supported by grants from The Chief Scientist Office for Scotland (COV/LTE/20/08) and the National Institute for Health and Care Research (COV-LT2-0010).

Conflict of interest statement

The submitted work was not carried out in the presence of any personal, professional, or financial relationships that could potentially be construed as a conflict of interest.

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