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**DEVELOPMENT OF A SYSTEM TO ANALYZE
PHYSIOLOGICAL PARAMETERS THAT ARE
INFLUENCED BY ENDOTHELIAL
DYSFUNCTION TO ASSESS RISKS OF DIABETES**

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Master of Philosophy

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University of Moratuwa
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Thesis submitted in partial fulfillment of the requirements for the degree
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DECLARATION

I declare that this is my own work and this Thesis does not incorporate without acknowledgement any material previously submitted for a Degree or Diploma in any other University or Institute of higher learning and to the best of my knowledge and belief it does not contain any material previously published or written by another person except where the acknowledgement is made in the text. I retain the right to use this content in whole or part in future works (such as articles or books).

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The above candidate has carried out research for the Master of Philosophy Thesis under our supervision. We confirm that the declaration made above by the student is true and correct.

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ABSTRACT

Vasodilatory Endothelial Dysfunction (VED) is identified as a precursor to cardiovascular diseases (CVDs) and atherosclerosis. Therefore, early detection of VED is important to prevent the development of severe CVDs and prescribe lifestyle changes or make clinical interventions. Though coronary angiography is the gold standard to detect VED, specialized devices have provided alternative means of detecting VED non-invasively. With these, they have detected VED in patients who are already diagnosed with CVD or Diabetes Mellitus (DM). Here, we investigated early detection of VED under four subject categories: individuals with pre-diabetes (PDM); healthy individuals with CVD risk factors (HRF); individuals with DM; and individuals with CVD. Non-invasively measured physiological parameters were used to detect VED. We developed a custom-made device to record non-invasive physiological parameters from the distal end of upper limbs namely, Digital Body Temperature, Peripheral Arterial Tone, Photo Plethysmography and Peripheral Bio-impedance. Multiple indices which represent unique features of these parameters were calculated considering both long-term and short-term variations. Using these, the performance of this device was validated against the flow-mediated dilation (FMD) procedure in a preliminary study with 10 volunteers (age range: 35 - 60 years): 5 healthy subjects (control) and 5 CVD patients with/without DM. Then the clinical study was conducted with 69 participants (age range: 35-60 years) with all the subject categories: healthy (n=9), HRF (n=15), PDM (n=6), type 2 DM (n=15), CVD with/without DM (n=24). CVD risk factors identified for the HRF category were physical inactivity, unhealthy diet, smoking, alcohol consumption, body mass index, and family history of CVD. Participants with type-1 DM, liver cirrhosis, renal failure, thyroid disease, spinal cord injuries, and finger deformities were excluded from the study. In the preliminary study, an unpaired t-test performed between FMD readings and indices derived from parameters calculated from the custom-made device validated device performance. In the clinical study, the ANOVA analysis showed promising results ($p < 0.05$) in distinguishing long-term trend variations and short-term variations of VED-associated physiological parameters in test subjects diagnosed with CVD or DM along with preclinical stages compared to the healthy conditions. Thereby, we have demonstrated that it may be possible to detect the early development of CVD in preclinical stages through monitoring these VED-associated physiological parameters.

Keywords: Vasodilatory Endothelial Dysfunction, Cardiovascular diseases, Risk factors, Pre-Diabetes, Peripheral Bio-impedance

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CHAPTER 1

INTRODUCTION

To live a healthy life is the goal of every person. However, unknowingly, they may be living with risk factors which lead to the development of cardiovascular diseases (CVDs) and eventually end up with life-threatening conditions. CVDs are disorders that are caused by coronary (heart) and peripheral (blood vessels) circulatory systems which lead to apprehending vital functionalities of human organs [1]. The death rate is approximately 32% from CVDs among global deaths [1] and is identified as the main cause of hospital deaths in Sri Lanka [2]. Current treatment modalities can only suppress the CVD condition momentarily thus, the patients must bear the consequences for the rest of their life. Therefore, early identification of the risk of CVD is a promising way to move forward to prevent developing severe CVDs [3, 4].

It has been identified that the early stage of development of CVDs is initiated through atherosclerosis [5]. This condition is caused by the oxidation of low-density lipoproteins due to impaired production of Nitric Oxide (NO) from the endothelium layer [6]. This shortage of NO leads to endothelial dysfunction (ED) which promotes resistance to vasodilation in vascular flow [7]. Studies have shown that ED is a precursor of the initial pathogenesis of atherosclerosis [5, 8, 9].

It has been a consistent finding that the presence of ED in many forms of CVDs, including both coronary and peripheral artery diseases [5]. Therefore, ED is a promising diagnostic tool for early assessment of the risk of developing CVD. In addition, Diabetes Mellitus (DM) and Prediabetes Mellitus (PDM) conditions and CVD risk factors can induce the progression of ED [5, 10]. The overall linkage of ED towards the development of severe CVDs and the link through hyperglycemic conditions are shown in Fig.1.1. The presence of ED has been experimentally demonstrated in those having DM and CVDs compared with healthy conditions. However, it is yet to be investigated the presence of ED in preclinical (marginal) stages such as healthy with CVD risk factors and PDM. If changes in ED are shown, it would be a crucial diagnostic parameter to identify the development of CVDs in advance and initiate prevention remedies to prevent morbidity and mortality.

At present, detection methods of ED are primarily categorized into two main approaches which include the analysis of standard biomarkers and the assessment of resistance to vasodilation as depicted in Fig.1.2. Most of the pathological conditions of ED are detectable through the standard biomarkers and commonly used to diagnose CVD risks and meta-bolic abnormalities [11, 12]. However, these tests are relatively

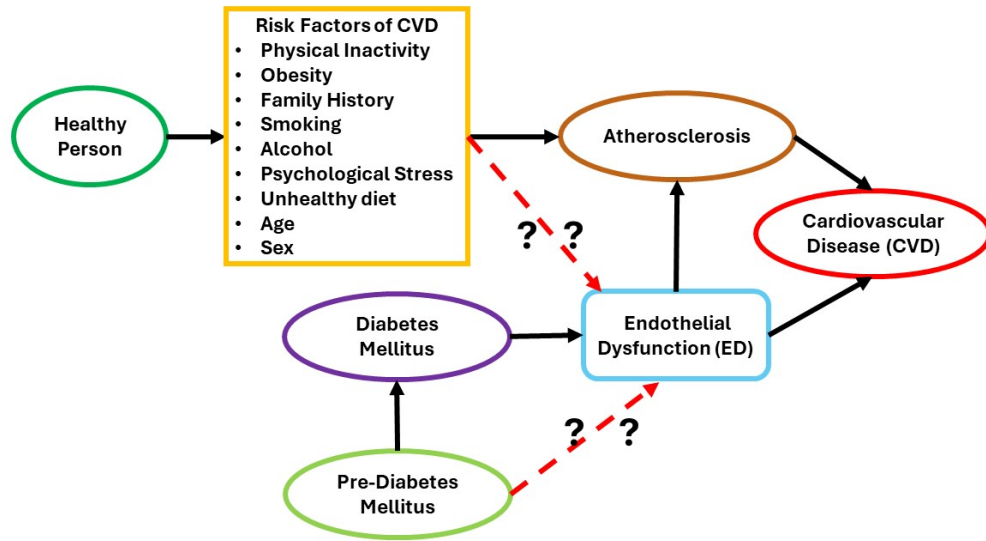


Fig. 1.1: Overview diagram of Linkage of ED towards the development of CVD

expensive and limited by scarcity of laboratory facilities. On the other hand, it has been widely reported that the effect of ED on the resistance to vasodilation can be assessed by either invasive or non-invasive procedures associated with physiological parameters [5, 13]. The vasodilation refers to the widening or dilation of blood vessels, particularly the arteries and arterioles which are triggered and controlled by the NO concentration released from the endothelial layer. Therefore, it can be explicitly stated that these methodologies are assessing vasodilatory endothelial dysfunction (VED).

The invasive VED assessment method is involved with screening of coronary arteries through coronary angiography which is known to be the gold standard for invasive VED assessment [5, 14]. Even though the measurement accuracy is high, this involves with high risk of cardiac catheterization surgery, dedicated medical expertise, specialized equipment and infrastructure [15, 16]. Therefore, coronary angiography is prescribed only to patients with substantial CVD risks. However, at this level it is useless to implement preventive measurements and require disease management medication schemes. Therefore, non-invasive, less risky and less expensive indirect measurements of VED have emerged to overcome the drawbacks of coronary angiography. Also these techniques were developed focusing on analyzing affected physiological parameter variations to estimate VED with improved patient comfort.

Non-invasive assessment of VED is carried out by examining the peripheral arterial vasculature (including capillary network) instead of coronary arteries. However, the

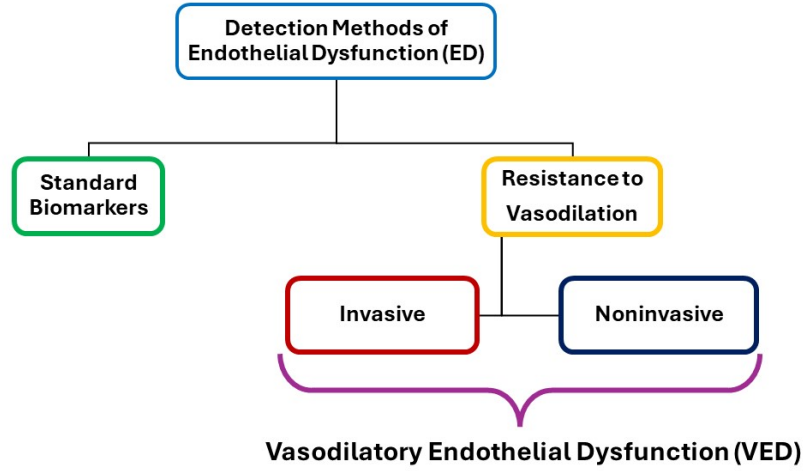


Fig. 1.2: Detection methods of Endothelial Dysfunction

endothelium requires an external stimulation by inducing reactive hyperemia (RH) in the peripheral vasculature to enhance the vasodilation process. The VED is assessed through the variations occurred during RH and post reactive hyperemia (PRH) stages. Though, there are several methods to induce RH, the brachial artery occlusion is the commonly used method [17].

Brachial artery flow mediated dilation (FMD) assessment by ultrasonography during post reactive hyperemia (PRH) is considered as the non-invasive gold standard for assessing VED [18, 19]. Thus the VED parameter results have shown a high correlation with the Coronary endothelial functionality [7, 20] which facilitates an alternative method to assess VED. However, the accessibility to perform this procedure has been restricted by the lack of availability of high skilled operators and equipment. Therefore, researchers have investigated other physiological parameters which reflects VED.

The alternative physiological parameters for noninvasive assessment of VED are identified as peripheral arterial tonometry, body surface thermal monitoring and finger photo plethysmography. Also, these physiological parameters have been used individually and in combined formation in commercial devices [21–23]. Although, these devices are reluctant to record multiple physiological parameters simultaneously during a single stimulated RH cycle. Furthermore, peripheral bio-impedance plethysmography which has shown promising results at research level [21] has not been incorporated in commercial products thus far.

The current research study has focused on investigating the presence of VED in pre-clinical stages such as Healthy with CVD risk factors (HRF) and PDM with respect to healthy conditions. Therefore, a custom-made device was developed containing VED associated physiological parameters of peripheral arterial tonometry (PAT), digital body temperature (DBT), pulse plethysmography (PPG) along with novel peripheral bio-impedance (PBI) which can be recorded simultaneously. A preliminary study was conducted to validate the custom-made device and data acquisition procedure through the FMD protocol. Then the clinical study data collection was performed and the VED indices were calculated with respect to long term and short-term variations of the physiological parameters. Finally, the feasibility of detecting the presence of VED in preclinical stages was shown through the statistical analysis.

The general objective of the current research study was to investigate feasibility of detecting the presence of VED in preclinical stages which leads into severe cardiovascular diseases through the associated physiological parameters. To achieve this, the following specific objectives will be imperative.

1. Development of a customized system to record and analyze the physiological parameters associated with vasodilatory endothelial dysfunction.
2. Perform a preliminary study on vasodilatory endothelial dysfunction indices between healthy subjects and patients diagnosed with cardiovascular diseases with/without diabetes to verify the developed system and methodology.
3. Investigate the degree of VED in healthy subjects with CVD risk factors, persons with prediabetes and diabetes.

1.1 Outline of the Thesis

The organization of the chapters is as follows:

- Chapter 1: Introduction
This chapter presents the overall knowledge, research gap and implementation procedure to fulfill the objectives on the assessment of VED in preclinical stages for the early detection of Cardiovascular diseases.
- Chapter 2: Literature Review and Research Objectives
This chapter summarizes the relationship of VED towards the physiological aspects of VED, related research conducted and existing methodologies used in the assessment of VED while introducing the current study research objectives.

- Chapter 3: Device Development
This chapter describes the selection of the VED associated physiological parameters and the procedure used to develop the custom-made devices.
- Chapter 4: Peripheral Bio-impedance Measurement
This chapter introduces a novel parameter of peripheral bio-impedance and its linkage towards the detection of VED. Also, this chapter introduces the experimental outcomes of time domain variations of peripheral bio-impedance.
- Chapter 5: Device Integration and Data Preprocessing
This chapter introduces the custom-made device integration with multiple physiological parameters associated with VED, data acquisition process and data preprocessing relevant to each of the physiological parameter raw data.
- Chapter 6: Device Validation and Preliminary Study
This chapter explains the validation procedure of custom-made device through a preliminary study performed using healthy (control) and participants diagnosed with CVD. Also, it explains the VED index calculation and statistical analysis.
- Chapter 7: Clinical Study
This chapter explains the extension of preliminary study data acquisition process while expanding test subject categories with preclinical stages: healthy but having CVD risk factors, prediabetes and clinical stages: diagnosed with diabetes and diagnosed with CVD. Furthermore, this chapter explains the VED index calculation and statistical analysis.
- Chapter 8: Discussion
This chapter presents the results obtained from both preliminary study and clinical study where the VED indices were calculated and analyzed with respect to test subject categories. The presence of VED in preclinical categories compared to healthy condition was shown through the statistical significance.
- Chapter 9: Conclusion
This chapter presents the summary of the overall research study and proposed improvements for the study limitations which can be implemented in future work.

CHAPTER 2

LITERATURE REVIEW AND RESEARCH OBJECTIVES

The following chapter explains the information gathered from relevant literature to provide the basis for the assessment of VED to early detect the risks of CVD along with the intended research objectives.

- Interpretation of CVD and its associated risk factors
- Correlation between CVD and VED
- Methods of assessing VED
- Medical graded devices to assess VED

The main focus of this chapter is to provide evidence that there is a consistent presence of VED in CVD patients. This correlation opens a new path to identify the development of CVD by assessing VED. There are several invasive and non-invasive techniques to assess VED using standard biomarkers and associated physiological parameters. Most of the commercially available medical graded devices have implemented for the noninvasive approaches with endothelial stimulations is required to assess VED. Even though there are few drawbacks and limitations need to be addressed before using VED as a diagnostic tool for early identification of CVD.

2.1 Cardiovascular Diseases

Cardiovascular diseases (CVDs) are a range of conditions associated with coronary and peripheral vasculature including hypertension, myocardial infarction (Coronary heart disease), cerebrovascular disease (stroke), peripheral vascular disease and many other disease forms which interrupt the functional organs such as brain, kidneys and liver [1]. It has been reported that the mortality rate is approximately 32% from CVDs among the global deaths [1]. In Sri Lanka, the ischemic heart disease has been recorded as the leading cause of hospital deaths throughout the last decade which was reported as 15.1% in 2019 [2]. Additionally, pulmonary heart diseases and cerebrovascular disease deaths were also among the leading causes with individual percentage of 7.6% respectively [2].

Majority of the individuals possess with the risk of CVDs may have shown elevated blood pressure, hyperglycemia and high lipid profiles continuously. It has been investigated that the development of CVDs can be triggered by several risk factors such as physical inactivity, obesity and emaciation, family history (parents and siblings),

smoking, consumption of alcohol, unhealthy diet, psychological stress, age and sex [5]. Collectively, these are known as the CVD risk factors.

Physical activity is essential component of a healthy lifestyle. However, the advancement of technological aspects to the daily routing, there is a lack of engaging in physical activities such as exercising, walking and running (less than 30 minutes per day) which can lead to develop CVD [24]. Obesity is mostly a consequence of physical inactivity and uncontrolled diet, which is defined by a body mass index (BMI) of greater than 29 kg/m^2 , thus elevate the risk of CVD with the increase of BMI [25]. On the other hand, emaciation which leads to decrease the BMI less than 18.5 kg/m^2 has been also considered as a CVD risk factor [26]. The family history of parents who has diagnosed for CVD can widen the probability (30% to 70% of chance) of developing CVD in their children [27]. The research studies have reported that 53% of any CVD deaths caused by smoking or usage of tobacco products, thus it has shown a strong correlation in developing CVDs [28]. Alcohol is another CVD risk factor that affects on lipids and insulin sensitivity which amplifies the risk of developing CVDs [29]. Unhealthy diet with a low intake of nuts, seeds, vegetables, and grains as well as excess usage of salt can increase the risk of developing CVDs [30]. In addition, psychological stress can adversely affect heart functionality and it can be quantified through standard questionnaire based methods such as patient health questionnaire (PHQ 9) and perceived stress scale (PSS) [8].

Echocardiograms and laboratory blood sample tests have been used as diagnostics tools to detect CVDs onset and monitor the progress. Also the clinicians are more focused towards treating on post-diseases stage. Lifelong Medication, bypass surgeries, pacemakers and stents implant surgeries and lifestyle modification are commonly prescribed remedies for CVD patients. These modalities momentarily suppress the symptoms of CVDs with multiple side-effects thus not a permanent solution. There is a promising way to identify the development of CVD even in a healthy person with a proper assessment of CVD risk factors. Thereby, treatment modalities can be implemented for preventing CVD before it gets affected.

2.2 Endothelium and Vasodilatory Endothelial Dysfunction

Many of the CVDs are affected through the circulatory system. Therefore, research studies have been focused on investigating the cellular level composition and functionalities of the blood vessels. The endothelium is the inner cell layer of blood vessels composed of endothelial cells which is located in between circulating blood and the media and adventitia of the blood vessel as shown in Fig.2.1 [31, 32]. This cell layer responds to blood flow changes, vessel stretching, circulating substances and inflam-

matory mediators. The endothelial layer is discontinuous with gaps between endothelial cells which permits permeability to large molecules such as plasma proteins [33]. The health of blood vessels is largely determined by the endothelium. It serves as a barrier and as an active influencer that modifies the structure of the vessel wall [34].

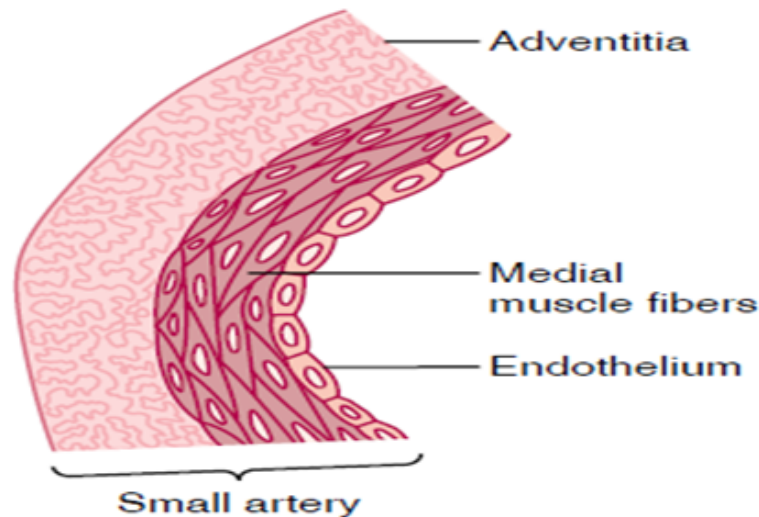


Fig. 2.1: Anatomical structure of Endothelium adapted from Medical Physiology by Guyton and Hall

A healthy endothelium contributes to regulation of vascular tone, cellular adhesion, smooth muscle proliferation and vessel wall inflammation. Of these, the vascular tone (vasodilation) mediated by the endothelium (through the production and release of vasoactive molecules) is recognized as a key marker of endothelial function [35]. On the contrary, its dysfunction is characterized by reduced bioavailability of vasodilators especially Nitric Oxide (NO) and increased endothelium-derived contracting factors. This imbalance leads to vasodilatory endothelial dysfunction (VED) [5]. Thereby VED can be defined as a condition that produces an imbalance the production of vasodilator factors and predisposes the vasculature towards prothrombic and proatherogenic effect [36, 37]. In other words, VED is characterized by a disturbed endothelium-dependent vasodilation and a decrease of the bioavailability of Nitric Oxide [38, 39].

2.3 Relationship between CVD and VED

It has been identified that VED is a consistent finding in many types of the CVD patients [5]. VED correlates with many CVDs such as atherosclerosis, pulmonary hypertension, peripheral vascular disease, coronary artery disease and many more [13, 40–42]. It has been identified that VED appears as an early marker at the initial pathogenesis of atherosclerosis [5, 43–45]. This relationship can be further elaborated from the

associated risk factors of CVD [5].

Endothelial dysfunction (ED) is induced by multiple factors as shown in Fig 2.2. Many of the CVD risk factors considered in previous section such as physical inactivity, obesity, smoking, alcohol consumption, unhealthy diet and dyslipidemia are categorized as traditional factors which induce ED [34]. Local factors such as shear stress, hyperglycemia and the rate of cholesterol deposition promote ED while genetic factors can mutate poor skeletal muscle capillarization and free radical generation which leads to ED [35]. However, there are non-traditional factors such as insulin resistance, oxidative stress, advanced glycation end products and homocysteine which can cause ED [5]. It is reported that unknown factors of ED have a substantial role in atherogenesis [46]. Most of the above-mentioned factors have been considered as CVD risk factors by the health organizations [1]. Therefore, these factors strongly justify the correlation between CVD and ED.

According to the Fig.2.2, vascular lesion and remodeling, inflammation, thrombosis, plaque rupture and resistance to vasodilation are resulted as consequences of ED. However, many of these consequences can be assessed by invasively using standard chemical and biological markers, thus literature provides strong evidence of non-invasive methods to quantify the resistance to vasodilation. Therefore, the intention of the proposed research study is to estimate ED by measuring the resistance to vasodilation which can be rephrased as vasodilatory endothelial dysfunction (VED).

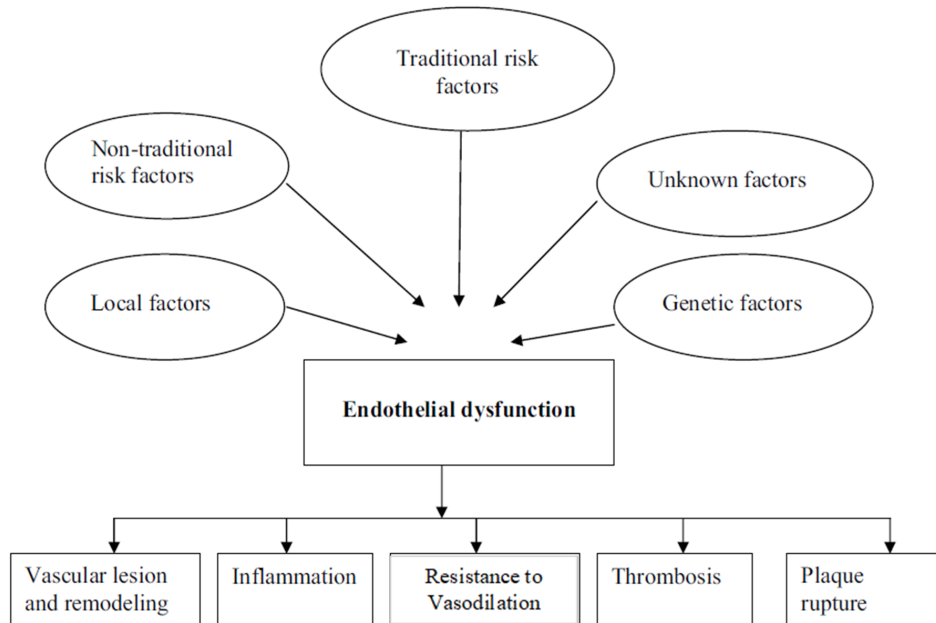


Fig. 2.2: Factors influencing endothelial dysfunction and its consequences

2.4 VED and initiation of Atherosclerosis

Atherosclerosis is the buildup of hyperlipidemic substances in and on the arterial vessel walls. It is known to be an early stage of developing CVDs which is caused by oxidation of low-density lipoproteins due to impaired production of Nitric Oxide (NO) from the endothelium layer [25]. The shortage of NO is caused due to endothelial dysfunction (ED) which promotes resistance to vasodilation in vascular flow [27]. Therefore, atherosclerosis has been considered as a preclinical stage in developing severe CVDs. The progression stages of atherosclerosis within the arterial wall is shown in Fig.2.3 [31]. It has been reported that VED is present at the initiation of atherosclerosis and also contributes to every progression phase towards plaque formation [5, 45]. Therefore, VED appears as a precursor of the initial pathogenesis of atherosclerosis.

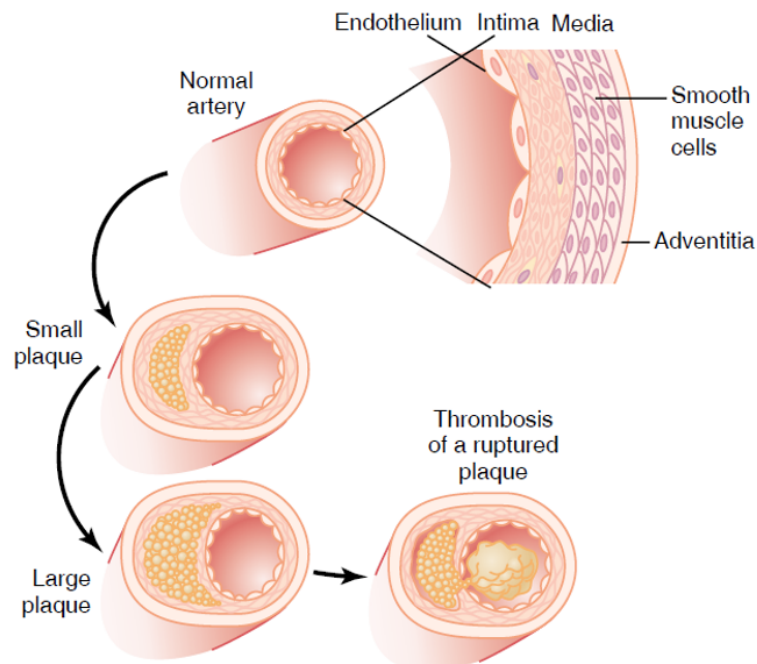


Fig. 2.3: Progression of the Atherosclerosis in the Arterial Wall adapted from Medical Physiology by Guyton and Hall

2.5 Assessment methods of ED and VED

2.5.1 Standard Biomarkers

Standard biomarkers tests are used as direct measures of chemical changes due to ED. These standard biomarkers can be used to assess the consequences of ED except for resistance to vasodilation. Asymmetric Dimethylarginine (ADMA), Vascular cellular adhesion molecule-1 (VCAM-1), intracellular adhesion molecule-1 (ICAM-1), Homocysteine, Dimethylarginine dimethylaminohydrolase (DDAH) and E-Selectin [12, 13, 38, 47–49] are reported as the widely used biomarkers while Superoxide dismutase, L-Arginine, endothelin, von Willebrand factor (VwF) and Symmetric Dimethylarginine (SDMA) are also used to assess the ED [7, 48, 50]. However, these tests are relatively expensive compared to common blood tests and ED biomarker tests are not performed routinely in Sri Lanka. Therefore, there is a place to develop alternative methods of assessing ED and VED.

2.5.2 Invasive and Non-invasive assessment of VED

The assessment of VED is done with coronary and peripheral circulations [5]. Since endothelial-dependent vasodilation is the most widely used method to assess VED, it has been used by either invasive or non-invasive methods in clinical settings [35]. The invasive method of assessing VED is with coronary arterial catheterization and coronary angiogram [5, 15, 16]. This procedure requires invasive surgery, risk, expensive medical devices and expert knowledge. Therefore, alternatively, non-invasive techniques were developed to estimate the peripheral VED focusing on analyzing affected physiological parameters with improved patient comfort. Brachial artery flow-mediated dilation (FMD) measured by ultrasonography machine has been considered as the gold standard of assessing VED non-invasively [5, 15, 50]. Other physiological parameters have been used for assessing VED such as arterial flow rate (measured by Doppler flow meter), body temperature, peripheral arterial tonometry (arterial pressure) and photoplethysmography [51–58].

2.6 Stimulation of Endothelium for Noninvasive Assessment

The noninvasive assessment of VED requires an external mechanism to stimulate the endothelium to create reactive hyperemia in peripheral blood flow. Hence, with the development of ischemic condition stimulates the endothelial cells, producing Nitric Oxide which regulates the vasodilation and vasoconstriction enabling fast recovery of the ischemic artery. The approaches recorded in literature includes from brachial artery occlusion, local heating and drug administration which induce NO release [17, 50, 59, 60]. Out of these methods, brachial artery occlusion is the widely used technique for

endothelium stimulation.

2.7 Impedance plethysmography and VED

Human body parts are permeable to conduct electrical current. However, the different body segments show different electrical conduction due to variation of bio-impedance. Bio-impedance analysis (BIA) is used to estimate the body compositions of the body [61]. BIA has been used as a physiological parameter to assess VED noninvasively by measuring skin impedance (800 ohms in wet skin to 10000 ohms in dry skin.) placed over peripheral vessels while a stimulation [5]. This process is known as impedance plethysmography where blood flow changes will be examined via BIA. However, impedance plethysmography assessment is still at research level and hasnt been implemented to any VED assessing commercial device thus far[62].

2.8 Commercially available devices that used to measure VED with physiological parameters

There are commercially available non-invasive VED assessment devices which have incorporated some of the physiological parameters mentioned in section 2.5. To stimulate the endothelium, the following commercially available devices have used reactive hyperemia by occlusion of an upper limb using a standard blood pressure cuff or local heating by wrapping a heating pad around the forearm. Note that these devices have been approved by the Food and Drugs Administration (FDA) of the USA.

2.8.1 PeriFlux System 5000

This is a multi-parameter analysis system designed to evaluate peripheral endothelial function non-invasively with three types of endothelial stimuli; reactive hyperemia, iontophoresis and local heating [63]. Physiological changes are recorded through a modular connected laser Doppler unit, pressure unit, oxygen saturation unit and a temperature unit. Further analysis is carried out with the support of PeriCam PSI system as shown in Fig. 2.4 which is capable of capturing infrared images of real-time micro-circulation noninvasively. Thereby, blood perfusion can be visualized to evaluate the endothelial function. The variation of blood perfusion before, during and after the occlusion is monitored continuously to assess the endothelial function. The endothelial function can be viewed with the help of software analysis results.

One of the limitations of this device is that, at a given time, only one stimulation method can be used and only one parameter can be measured. Also, this is an expensive system requiring the expert analyst to operate the system.

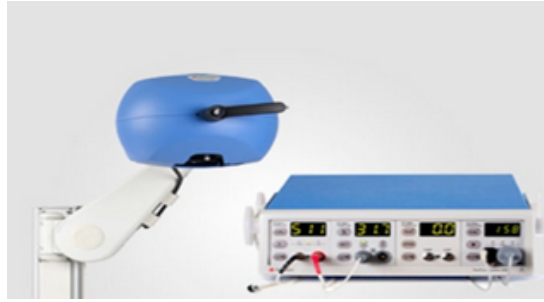


Fig. 2.4: Perifluc 5000 system with PeriCam PSI unit

2.8.2 EndoPAT 2000

This system is developed to assess the endothelial function using the Peripheral Arterial Tone (PAT) signal. The endothelium is only stimulated by reactive hyperemia on an arm and the PAT signal is acquired from the index fingers of both the arms. The system module contains a blood pressure unit and two PAT biosensors as shown in Fig. 2.5. Both the arms are used to obtain the PAT signals where one arm is used for baseline measurements and the contralateral arm is used for the occlusive reactive hyperemia measurement. The analysis algorithm is performed on a PC with a proprietary software and PAT signal will be analyzed in a post reactive hyperemia process to assess the endothelial function [54].



Fig. 2.5: Endo-PAT 2000 System

2.8.3 Heart Sentry

This is a portable device which is wrapped around upper arm that mainly focused on assessing endothelial function in larger arteries (refer Fig.2.6). This device is currently used for early detection of cardiovascular diseases. The system is incorporated with a blood pressure unit where the endothelium is stimulated by reactive hyperemia. This can analyze the endothelium function within a minimum of three minutes [64]. Furthermore, this device can be integrated with nearby smart mobile via a Bluetooth connection which is useful to transfer recorded data to smart mobile.



Fig. 2.6: Heart Sentry Portable Device

2.8.4 VENDYS II Endothelix Inc

This system is designed to assess endothelial function by monitoring the temperature variation at the fingertips during reactive hyperemia (refer Fig. 2.7). This device has a fully automated cuff inflation and a deflation process while measuring blood pressure. Both arms of the test subject are used to obtain the measurements where the arm with blood pressure cuff will be used to monitor the reactive hyperemia condition and contralateral arm will be used as the baseline reading. The level of endothelial functionality is calculated by vascular reactivity index [65]. Assessment results have been used to identify patients with a high risk of developing cardiovascular diseases.



Fig. 2.7: VENDYS II Endothelix, Inc

2.9 Drawbacks of the existing devices compared to proposed study

Even though there are commercially available devices to assess the VED, there are some limitations and drawbacks. The existing devices are unable to record multiple physiological parameters during a single stimulation (RH or thermal) cycle. Most of the devices have considered only capillary blood pressure and temperature parameters while only a few record photo-plethysmography [66]. However, blood vessel impedance which has shown promising results in research has not been incorporated in any commercial device thus far concerning the section 2.8 [62]. The proposed system in the study can record four simultaneous physiological parameters namely; impedance, oxygen saturation, arterial pressure and temperature in the process of detecting VED as described in chapter 3.

TABLE 2.1: COMPARISON OF COMMERCIAL DEVICES WITH PROPOSED SYSTEM

Commercial Device	Physiological Parameters					Stimulation Method		
	Flow rate	Temperature	Arterial Pressure	Oxygen Saturation	Bio-impedance	Occlusion	Local Heating	Iontophoresis
Periflux 5000	✓	✓	✓	✓		✓	✓	✓
EndoPAT 2000			✓			✓		
Heart Sentry			✓			✓		
Vendys II		✓				✓		
Proposed Device		✓	✓	✓	✓	✓		

2.10 Evidence of the presence of ED in healthy people with CVD risk factors

There are several research studies which have investigated the presence of ED on healthy subjects with CVD risk factors. There, ED was estimated from biomarkers and using non-invasive methods.

Research studies conducted to enquire the presence of ED with healthy obese children and healthy obese adolescents including physical inactivity criterion has shown positive signs [33]. Landgraf et al. has performed a study with ED biomarkers and Endo-PAT test on 69 lean and 105 obese children. Results showed there is a significant difference (ICAM-1 ($p<0.001$), E-selectin ($p<0.001$) and Reactive Hyperemia Index

($p < 0.001$) among the two study groups [67]. Müller-Ehmsen J et al. used cell-based ED evaluation biomarkers such as endothelial progenitor cells (EPCs) with CD34 on 149 obese people in a weight loss program and noted that there is a reduction in number of EPCs ($p < 0.005$) in obese people compared to healthy people [68]. Arnold et al. has used same assessment method on 24 healthy obese adolescents and verified the EPCs reduction [69]. Alternatively, using non-invasive assessments such as FMD test on brachial artery and Endo-PAT test with similar test subject groups have reported that obese population shows impaired endothelial vasodilation [67, 70–74].

Furthermore, FMD studies have been conducted with healthy subjects based on their dietary intake. Bae J H et al. has conducted a study on 10 healthy young people showed that there is a variation between high-fat meal and low-fat meal on endothelial vasodilation [75]. This study concludes that there is an occurrence of transient ED after a high fat meal in which it shows significant difference of FMD values compared to baseline ($p < 0.05$).

Several correlation studies have performed to investigate the impact on ED with different healthy test subject combinations such as overweight healthy people with alcohol consumption and healthy smokers [76, 77]. Padro et al. has conducted a study on 36 healthy overweight and obese adults to assess the relationship between alcohol intake and CVD. Results showed a significance on total cholesterol ($p < 0.04$) and high density lipoprotein (HDL) ($p < 0.01$) after 4 weeks on continues alcohol consumption compared to the starting point [76]. Nagaya et al. has conducted a study on 20 healthy male smokers and showed that there is a significant decrease on forearm blood flow compared to healthy non-smokers ($p < 0.001$) [77]. With these evidence, there is a possibility on establishing a correlation between ED and healthy people with CVD risk factors which is highlighted on the overview of the research study (refer Fig. 1.1).

The overall objective of the research study is to investigate the correlation between impaired vasodilation caused by vasodilatory endothelial dysfunction (VED) and cardiovascular disease risk. To achieve this, the following specific objectives will be imperative as shown in section 2.11.

2.11 Specific objectives

1. Development of a customized system to assess vasodilatory endothelial dysfunction.

In this research, four (04) physiological parameters were chosen to identify and analyze VED. A multi-parameter acquisition system was developed to acquire data on digital body temperature, peripheral arterial tonometry, photoplethys-

mography and peripheral bio-impedance from the participants non-invasively. A Specially designed printed circuit board assembled with development hardware embedded customized device will be used to acquire relevant data. MATLAB (Release 2018a) and open-source software integrated algorithms were used to analyze the physiological parameters and graphical representation in real-time.

2. Verify the developed system and methodology with healthy subjects and patients diagnosed with cardiovascular diseases with/without diabetes.

The preliminary study was carried out between healthy (Control) and CVD patients with/without diabetes (extreme case). Flow-mediated dilation (FMD) values obtained from the brachial artery using a Doppler ultrasound machine were used as the gold standard to verify the developed device. In this study, both brachial artery diameter and flow rate readings were recorded simultaneously from each test subject along with the developed device's physiological parameters. FMD and flow rate variations were used to compare and verify the developed device. Furthermore, this validation was able to prove the presence of VED in CVD with/without diabetes patients.

3. Investigate the degree of VED in preclinical stages.

This objective will address the research gap in investigating the presence of impaired vasodilation in preclinical stages such as healthy with CVD risk factors, PDM and DM. If these test subjects show signs of VED implying a risk of developing CVD in future. Our methodology would enable early identification of VED allowing early interventions to prevent CVD.

Note that this study does not attempt to provide the underlying physiological mechanism which leads to this correlation because it is out of the purview and expertise of the researcher with an engineering background. (However, these findings will be helpful for medical experts to further investigate and derive conclusions about the underlying physiological mechanism).

CHAPTER 3

DEVICE DEVELOPMENT

Vasodilatory Endothelial Dysfunction (VED) can be detected non-invasively by analyzing its associated physiological parameters as explained in sections 3.2, 3.3, 3.4 and 3.5. Therefore, a custom-made device was implemented embedding sensors for acquiring four (04) physiological parameters namely:

1. Digital Body Temperature (DBT)
2. Peripheral Arterial Tonometry (PAT)
3. Photo Plethysmography (PPG)
4. Peripheral Bio-impedance (PBI)

To assess the endothelial functionality under the reactive hyperemia (RH) condition, the physiological measurements were obtained from the custom-made devices' sensors connected to the dominant arm of the test subject as shown in Fig. 3.1. An adult pressure cuff is wrapped around the arm to stimulate the endothelium by momentary occlusion of vascular flow. The contralateral arm of the test subject was connected to another identical custom-made device as reference measurement depicted in Fig. 3.1 which was helpful in accurately isolating the relationship between VED and the physiological parameters being measured while eliminating the systemic and confounding factors,

Both the devices were powered and operated by a PC where every sensor is controlled through a dedicated microcontroller for data communication and acquisition. Collected data were stored in the PC and were analyzed offline to estimate VED. The following schematic diagram shown in Fig. 3.1 elaborates on the overview of the device development. The experimental setup was designed for 13 minutes to simultaneously acquire data from all the physiological parameters in both arms. This period was divided into three distinct phases: 5 minutes for baseline, 3 minutes for occlusion and 5 minutes for the post-reactive hyperemia (PRH). The specifications of inducement of RH condition and physiological parameters are explained in sections 3.1, 3.2, 3.3, 3.4 and 3.5.

3.1 Occlusion duration of Reactive Hyperemia

Many research studies have suggested inducing Reactive Hyperemia (RH) by occluding the brachial artery with an adult pressure cuff inflated to supra-systolic pressure

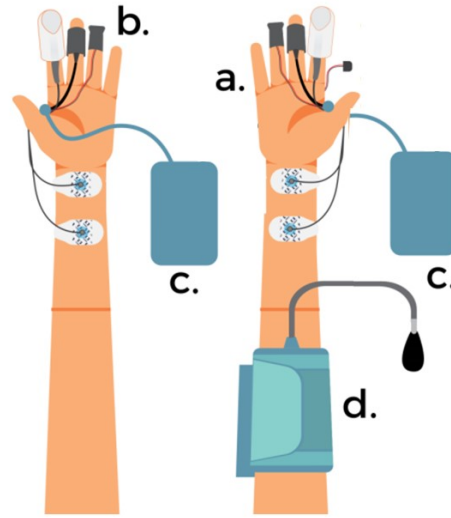


Fig. 3.1: Block Diagram of Custom-made Device: (a) Dominant arm
(b) Contralateral arm (c) Custom-made device (d) Adult pressure cuff

value (200 mmHg) for 5 minutes [52, 54, 60]. Then observe post-reactive hyperemia (PRH) following 5 minutes after deflating the applied pressure.

However, the test subjects experienced discomfort and numbness due to the 5-minute occlusion duration, highlighting a significant drawback of this approach. Some subjects reported symptoms of faintness, and many exhibited skin-related issues such as bruising, chafing, ecchymosis, and blistering as a result of the cuff's application [78].

In response to these issues, reducing the occlusion duration to 3-4 minutes, which has been shown to be sufficient for inducing ischemia in the brachial artery while mitigating adverse effects [79, 80]. Consequently, for the present study, an RH induction time of 3 minutes was selected to enhance test subject safety and minimize the risk of discomfort and skin lesions. Additionally, proper techniques for cuff application were practiced to further reduce the likelihood of skin damage.

3.2 Digital Body Temperature

In the context of a non-invasive VED detection approach, the skin surface digital body temperature (DBT) will be monitored to assess the variations in the peripheral arterial circulation during reactive hyperemia (RH). DBT measurements were obtained from each test subject's dominant arm and control arm from the sensors connected to the fingertips surrounding the capillary network and the ambient temperature was also

measured proximal to the fingertip by a separate sensor to isolate effects on fingertip measurements. Additionally, all these measurements were taken in a temperature-controlled enclosed room to eliminate the environmental impact on RH variation on surface body temperature.

TSIC506F TO92 (Innovative Sensor Technology, Switzerland) digital temperature sensor shown in Fig. 3.2 was selected due to its high accuracy of $\pm 0.1^{\circ}\text{C}$ and prompt response time of 100 milliseconds [54, 81]. Also, the sensor operating range lies between -10°C to $+60^{\circ}\text{C}$ which is ideal for measuring both body temperature and ambient temperature.

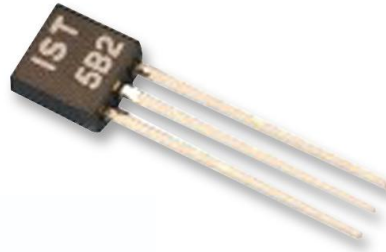


Fig. 3.2: TSIC506F TO92 Digital Temperature Sensor

This digital temperature sensor is precalibrated with absolute measurements, hence calibration is not required. It operates on a supply voltage range of 2.97 V to 5.5 V and provides a ZACwire (single-wire) protocol 11-bit digital serial signal output that is compatible with various types of microcontrollers. Given that physiological body temperature variations are relatively slow, a sampling rate of 10Hz was adequate for capturing the variations resulting from RH. Furthermore, the sensor has low power consumption and is resistant to electromagnetic interference, making it suitable for use in various applications, including the current study.

Each custom-made device was embedded with two (02) temperature sensors to monitor the DBT and ambient temperature for a dedicated arm. Both the sensors were operated and communicated through an Arduino Nano ATmega328 microcontroller. Arduino Integrated Development Environment (IDE) software and TSIC506F libraries were used to obtain continuous DBT and ambient temperature readings.

3.3 Peripheral Arterial Tonometry

Peripheral Arterial Tonometry (PAT) is a noninvasive measure of pulsatile arterial volume changes in terms of pressure variations at the peripheral arterial capillary network

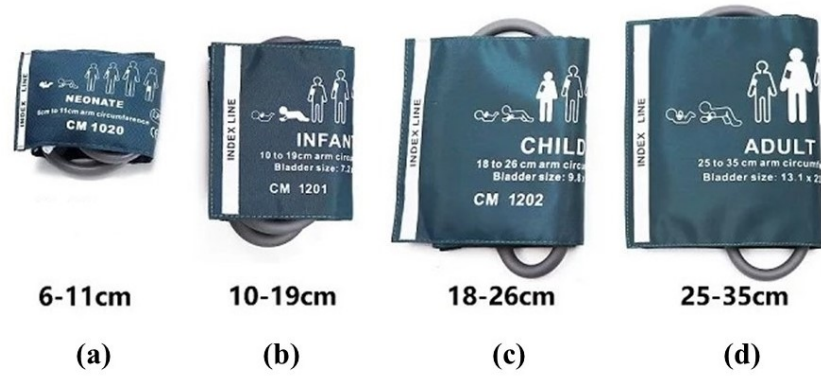


Fig. 3.3: Size Comparison of Blood Pressure Cuffs: (a) Neonatal (b) Infant (c) Child (d) Adult



Fig. 3.4: ABPDANT005PGAA5 Analog Pressure Sensor

(fingertips). Each custom-made device was equipped with a neonatal inflatable cuff used as the fingertip cuff as shown in Fig. 3.3 (a). This was compatible with the finger size of adults (6 to 11 cm circumference). Each cuff was connected to a pneumatic pressure sensor and a pneumatic motor through the pneumatic tubing system. The rate of airflow was controlled by a potentiometer connected to the pneumatic motor for maintaining the pressure of the finger cuff at a sub-diastolic pressure (20-60 mmHg) in each test subject [54]. A manual air release valve was connected to control the deflation and for safety.

The pneumatic pressure sensor used here was ABPDANT005PGAA5 (Honeywell Sensing and Productivity Solutions, USA) analog pressure sensor shown in Fig. 3.4. It has an operating range of 0-225 mmHg, a response time of 0.5 milliseconds and an accuracy of $\pm 0.25\%$ [54, 82]. This sensor has demonstrated the capacity to detect pulsatile blood flow with a sampling rate of 90 Hz. The pneumatic motor used in the device was a miniature pneumatic motor with an operating voltage of 5V and a current of 0.18A.



Fig. 3.5: PCB designed for PAT signal Data acquisition

The raw pressure signal was filtered with a 2nd order analog Butterworth low pass filter with a cut-off of 15 Hz to enhance the pulse variation. Therefore, PCB was designed to accommodate the pressure sensor, analog filter and pneumatic motor with the control unit as shown in Fig. 3.5. The PCB was powered by an Arduino Uno with a power supply of 5V. The pre-processed signals were digitized on the Arduino Uno and sent to the PC through UART to USB communication protocol.

The finger cuff pressure was pre-adjusted before the data acquisition by connecting finger-size cuffs to the test subject and adjusting the inflated level until stabilized in sub-diastolic pressure to visualize a pulsatile pattern. Like DBT, Arduino IDE (Release 1.8) was used for the algorithm development for continuous measurement of PAT signal. The Arduino Uno features a 10-bit resolution ADC (Analog-to-Digital Converter), resulting in digitized sensor values ranging from 0 to 1023 (2^{10}). The ADC value is initially converted to voltage using the following equation 3.1. According to the datasheet of the ABPDANT005PGAA5 sensor, the voltage is converted to pressure in pounds per square inch (psi). To convert this pressure from psi to millimeters of mercury (mmHg), it is multiplied by 51.7149326, (1 psi = 51.7149326 mmHg) as detailed in equation 3.2.

$$V_0 = \text{Sensor ADC Value} * \left(\frac{5}{1024}\right) \quad (3.1)$$

$$\text{Pressure} = \left(\frac{V_0 - 0.5}{0.8}\right) * 51.7149326 \quad (3.2)$$

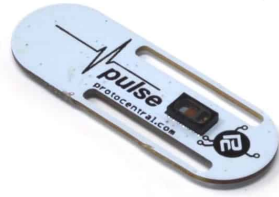


Fig. 3.6: MAX30102 Photoplethysmography Sensor

3.4 Photo Plethysmography

Photoplethysmography (PPG) is an optical measurement technique used to measure peripheral arterial blood flow, heart rate and oxygen saturation in clinical diagnostics. PPG sensors can be divided into two primary categories: transmission type and reflection type. In transmission-type PPG sensors, the receiving sensor is located on the opposite side of the skin from the infrared (IR) and red LED light sources, whereas in reflection-type PPG sensors, both the receiving sensor and light sources are located on the same side of the skin.

The current research study used a reflective PPG sensor MAX30102 (ProtoCentral Electronics, India) shown in Fig. 3.6 which can be worn on an adult's fingertips. It consists of IR and Red LED light sources which emit wavelengths of 880nm and 660nm respectively. This PPG sensor has an operating range of 1% to 100% saturation which is measured as a percentage with an accuracy of $\pm 1\%$ and a response time of 20 milliseconds. It requires an input voltage of 3.3V and uses I2C data communication protocol with ADC resolution of 18 bits for data acquisition.

The PPG sensor was configured with Arduino Nano board using 4 pins of VIN, GND, SDA and SCL. The sensor is powered by the VIN with 3.3V and GND is connected to the ground. The I2C communication was stabilized by Serial data line (SDA) and serial data clock (SCL) pins for the data acquisition.

The PPG sensor data acquisition algorithm was derived from Arduino IDE (Release 1.8) libraries and customized to acquire the raw data of both IR and RED light reflective ADC values continuously. A sampling rate of 25 Hz was sufficient to acquire the pulsatile blood flow in raw data. Additionally, an algorithm was designed including a pre-calibration step where the PPG sensors were attached to the fingertips to establish reference parameters for the test subject before data collection.

3.5 Peripheral Bio-Impedance

Peripheral bio-impedance (PBI) represents an emerging physiological parameter that offers a novel approach to assessing the electrical activity of the vasculature. While limited research has been conducted to explore the dynamic behaviour of the peripheral vasculature, the investigation of PBI behaviour under reactive hyperemia (HR) condition remains unexplored in the literature.

The current study has focused on investigating the behaviour of PBI under RH condition and the feasibility of detecting the presence of VED. However, there are undefined essential preliminaries such as the selection of compatible acquisition devices, preliminary experimental setup accessories, PBI behaviour in frequency and time domains, selection of stimulation frequencies and the mathematical modelling of PBI under RH condition for an accurate PBI data acquisition which are elaborately discussed under chapter 4.

CHAPTER 4

PERIPHERAL BIO-IMPEDANCE

Peripheral Bio-impedance (PBI) is a novel physiological parameter which is introduced through the current study to investigate the feasibility of identifying the presence of VED. This parameter reflects the change in blood flow with respect to electrical conductivity. Measurements are obtained by applying a small electrical current (in μA range) to the body (stimulation) and measuring the resulting voltage across two electrodes. Due to the novelty of this physiological parameter, many prerequisites have to be fulfilled prior to the data acquisition. The choice of electrode type, electrode configuration and anatomical positioning needed to be determined before selecting a suitable PBI acquisition device.

4.1 Electrodes selection for PBI measurement

Unlike the other physiological parameters used for the current study, electrodes are mandatory to acquire the PBI measurements. During the initial testing, the PBI measurements were acquired using conventional disk electrodes and long strip electrodes [4.1](#) (BIOPAC systems Inc, USA). However, the literature has stated that there are specially designed PBI electrodes with a contact area greater than 4 cm^2 that should be used for accurate measurements [\[62, 83\]](#). Therefore, Bio Impedance Analysis (BIA) adhesive electrodes (Sanibel TM, Denmark) shown in [Fig. 4.2](#) were selected for the current study which comprised of a contact area of 5.5 cm^2 .

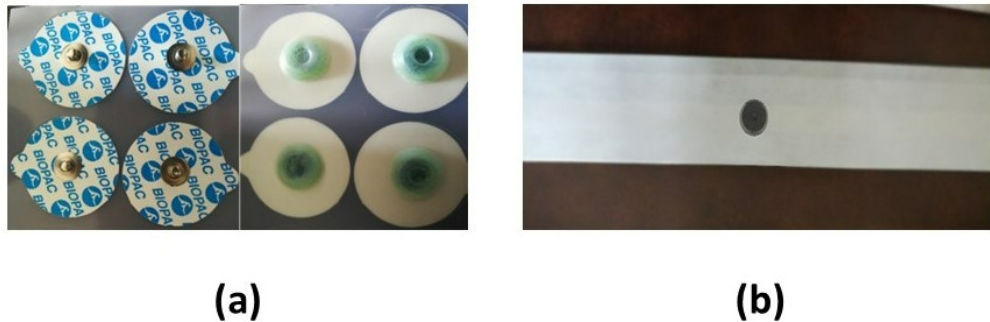


Fig. 4.1: Conventional Electrodes: (a) Disk Electrodes (b) Long strip Electrode



Fig. 4.2: Sanibel BIA Electrodes

4.2 Electrode Configuration for PBI measurement

Previous research studies have highlighted that bipolar and tetra-polar electrode configuration methods shown in Fig. 4.3 are commonly employed for PBI measurements. The tetra-polar electrode configuration shown in Fig. 4.3(a) involves the use of four (04) electrodes, with two dedicated electrodes for current injection ($800\ \mu\text{A}$) and the other two electrodes dedicated to voltage measurement [84]. Though this configuration eliminates the electrode-skin contact impedance, it is hard to maintain constant inter-electrode distance by separating the current injection and voltage measurement points while minimizing the error. PBI measurements from the tetra-polar electrode configuration are greatly influenced by “Negative Sensitivity” where a change in a physiological parameter results in a decrease in the measured impedance value. This results in, PBI measurements underestimating the actual impedance value [85].

On the other hand, the bipolar electrode configuration shown in Fig. 4.3(b) is simple and requires only two (02) electrodes to be placed on the body where one electrode acts as the current source ($400\ \mu\text{A}$) and the other as the voltage measurement point. This configuration eliminates the common mode noise and negative sensitivity from PBI measurements [85]. Additionally, it is easy to maintain constant inter-electrode distances in bipolar electrode configuration. Therefore, based on the accuracy and ease of placing electrodes, the bipolar electrode configuration was chosen over the tetra-polar electrode configuration for the current study.

Proper anatomical positioning of electrodes is indispensable for achieving precise PBI

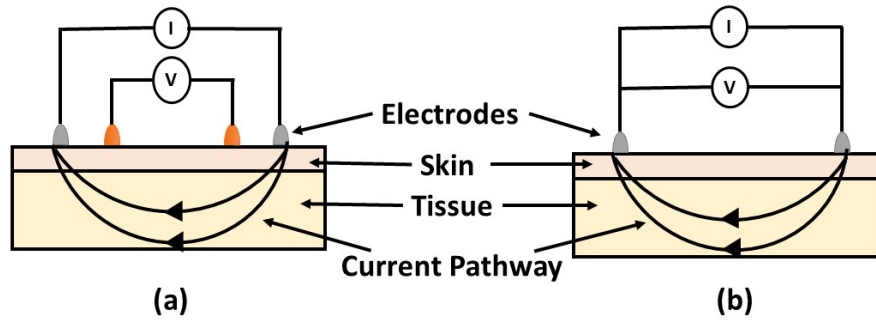


Fig. 4.3: Electrode Configurations: (a) Tetrapolar Configuration
(b) Bipolar Configuration

measurements, complementing the chosen electrode configuration. The current study is intended to acquire PBI measurements from the upper limb peripheral vascular flow and corresponding to the bipolar electrode configuration, the two (02) electrodes were placed on the skin along the radial artery between the wrist and the elbow [86–88].

4.3 Inter Electrode Distance

The inter-electrode distance for PBI measurements was determined based on the following 02 criteria. The first criterion specifies eliminating diffusion resistance generated from the electrode placement. The diffusion resistance can influence the PBI by creating a large potential difference between electrode placement areas, resulting in overestimated measurements. Yamamoto’s study has provided criteria to calculate inter-electrode distance based on the electrode type to eliminate the involvement of diffusion resistance to PBI readings [89]. The radius (R) of the limb at the position of the sensing electrode is derived from the circumference measurement (assuming that the cross-sectional area of the limb is circular) at the same limb location. Once the R is calculated, it should be multiplied by a factor: $1.5R$ for tape electrodes and $2.3R$ for disk electrodes to obtain the inter-electrode distance.

The second criterion is recommended by research studies done on PBI measurements which imply keeping a minimum of 5 cm inter-electrode distance for optimum output [90, 91]. However, all these research studies have been focused on static PBI mea-

surements. Since we are using impedance in the Dynamic platform the recommended values may not be the optimum values. Considering the first criterion, the average inter-electrode distance for the current study sample was calculated as 5.85 ± 0.5 cm. To satisfy the second criterion, it was decided to use 7 cm as the inter-electrode distance for the PBI measurement acquisition in the current study.

4.4 Acquisition device for PBI measurement

Upon determining the electrode type, electrode configuration and anatomical positioning, the study proceeded to identify a suitable PBI acquisition device. From a scant number of commercially available devices and development boards, the following specific devices were evaluated for their feasibility for PBI measurements in the current study.

4.4.1 Keysight E4990A LCR meter

The Keysight E4990A LCR meter shown in Fig. 4.4 is a commercially available device which is capable of measuring Impedance from 20Ω to $2 \text{ M}\Omega$ range with a stimulating frequency range of 20 Hz to 300 kHz. This system was only designed to output the impedance and phase angle relative to the corresponding frequency cycle. Each frequency cycle is pre-programmed to specifically select discrete stimulating frequencies in a non-linear manner covering the entire range. It took approximately 3 minutes to complete a frequency cycle while storing 201 impedance and phase angle readings for discrete stimulating frequencies in the device.

The pilot study was conducted to observe the behaviour of PBI against the stimulating frequency. Three test subjects were used for the data collection and acquired 3 frequency cycles based on the study protocol explained in the section 6.1. A plot of averaged data with standard deviation presented in Fig. 4.5 was obtained to examine the relationship between PBI and stimulation frequency. It shows an exponential decay behaviour with lower stimulation frequencies showing a larger standard deviation while higher stimulation frequencies show much more stable PBI measurements. Consequently, it was highlighted that the stimulating frequencies beyond 3 kHz (range indicated in Fig. 4.5 by dotted lines) have shown more linear PBI variation in the entire curve.

However, this device was not suitable to be used in our study due to bulkiness, poor response time and factory-programmed stimulation frequencies. Therefore, an alternative PBI device was explored to be compatible with the custom-made device. The choice of electrode type and configuration had to be determined before selecting an



Fig. 4.4: Keysight E4990A LCR meter

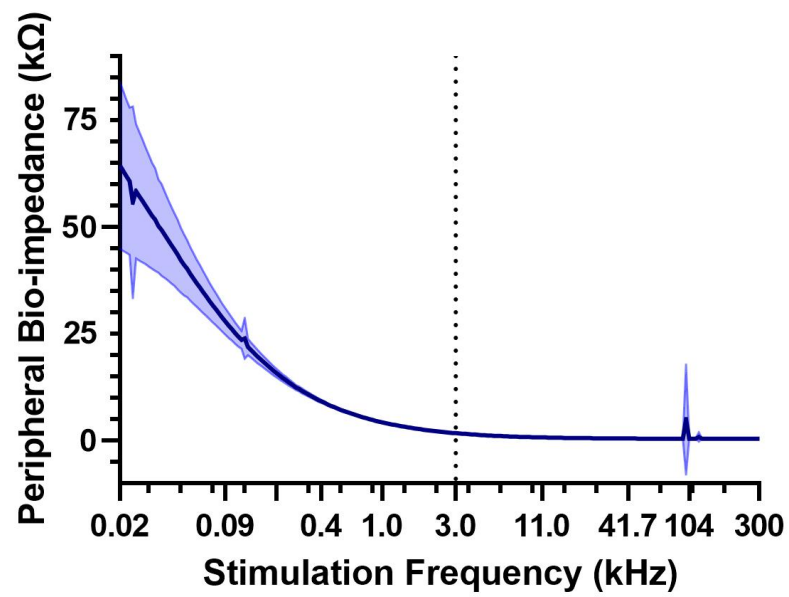


Fig. 4.5: Relationship between Peripheral Bio-impedance Vs Stimulation Frequency



Fig. 4.6: EVAL-AD5933EBZ Evaluation board

alternative PBI device.

4.4.2 EVAL-AD5933EBZ Evaluation Board

Considering the factors outlined in sub-section 4.4.1, the EVAL-AD5933EBZ evaluation board (Analog Devices, USA) shown in Fig. 4.6 was assessed as a potential alternative PBI measurement device for the current study. This evaluation board composed of a high-precision AD5933 impedance converter IC with a bipolar electrode configuration. It can measure impedance from $1\text{ k}\Omega$ to $10\text{ M}\Omega$ with an accuracy up to 0.5 %. The stimulation frequencies are adjustable from 3 kHz to 100 kHz with a resolution of 0.1Hz. The on-chip crystal oscillator provides an accurate and stable internal clock of 16.667 MHz for stimulation frequencies to acquire a better measurement. The sampling rate is varied based on the number of stimulation frequencies required to measure Bio-impedance. The system is operated by a DC power supply voltage ranging from 2.7V to 5.5V and a communication protocol of I²C [92]. Typically, it was highly recommended to operate the evaluation board at 3.3V for optimum output.

The design of the EVAL-AD5933EBZ evaluation board is a compact and portable device which compatible with the current research study requirements. The board also supports USB connectivity, allowing it to be easily connected to a PC or other devices

for data acquisition, control and visualization along with the dedicated evaluation software. Unlike in the Keysight E4990A LCR meter, the frequency sweeping cycle can be adjusted as per the user's preferences. However, the built-in software operation is only capable of demonstrating the frequency-dependent variations of impedance for a specified frequency cycle from 20 Hz to 300 kHz.

In addition, the EVAL-AD5933EBZ evaluation board was configured to perform a pre-calibration test before the data acquisition involving multiple steps. Firstly, the external calibration resistor is required to be connected to the board. The standard calibration resistor values given in the datasheet are listed in Table 4.1 with the optimum impedance measuring range [93]. For the current study, a 1 k Ω calibration resistor was chosen since the PBI measurements fall within the range of 1 k Ω to 10 k Ω at higher stimulation frequencies beyond 3 kHz. This selection was verified in the pilot study conducted with the Keysight E4990A LCR meter (refer Fig. 4.5). Secondly, a known impedance value (standard resistor) should be connected to the bipolar electrode configuration and then proceed with the calibration from the software. If the calibration was done successfully, all the stimulating frequencies should show similar impedance measurements to the known impedance value.

TABLE 4.1: CALIBRATION RESISTOR VALUES FOR IMPEDANCE RANGES

Impedance Range	Calibration Resistor
0.1 k Ω - 1 k Ω	100 Ω
1 k Ω - 10 k Ω	1 k Ω
10 k Ω - 100 k Ω	10 k Ω
100 k Ω - 1 M Ω	100 k Ω
1 M Ω - 2 M Ω	1 M Ω
9 M Ω - 10 M Ω	9 M Ω

The evaluation board software consists of a graphical user interface (GUI) as shown in Fig. 4.7 where the initial parameters such as calibration impedance, start stimulation frequency, frequency resolution and the number of increments can be set up before the calibration step. After calibration, clicking the start sweep tab allows for the acquisition, real-time visualization, and storage of impedance data for further analysis.

However, the EVAL-AD5933EBZ Evaluation board software is programmed to store the data for only one complete frequency stimulation cycle. Therefore, the user had to manually operate the software to store multiple rounds of frequency stimulation cycles. Using the same 3 test subjects, the pilot study proceeded with the study protocol and the PBI measurements obtained for the stimulating frequency range of 3 KHz to 100 kHz with increments of 500 Hz along with the calibration impedance measurement are

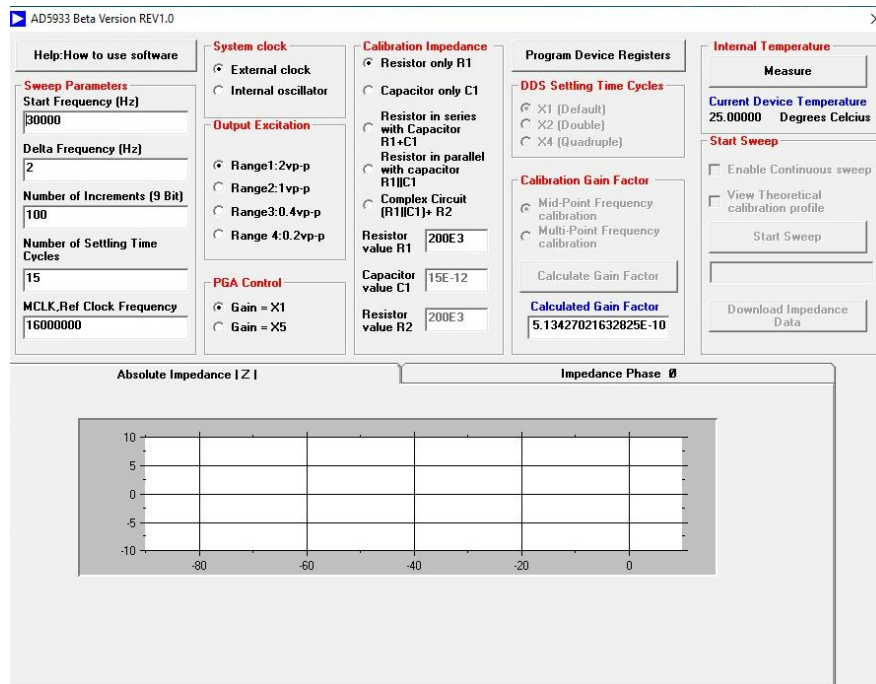


Fig. 4.7: EVAL-AD5933EBZ Evaluation board software Graphical User Interface

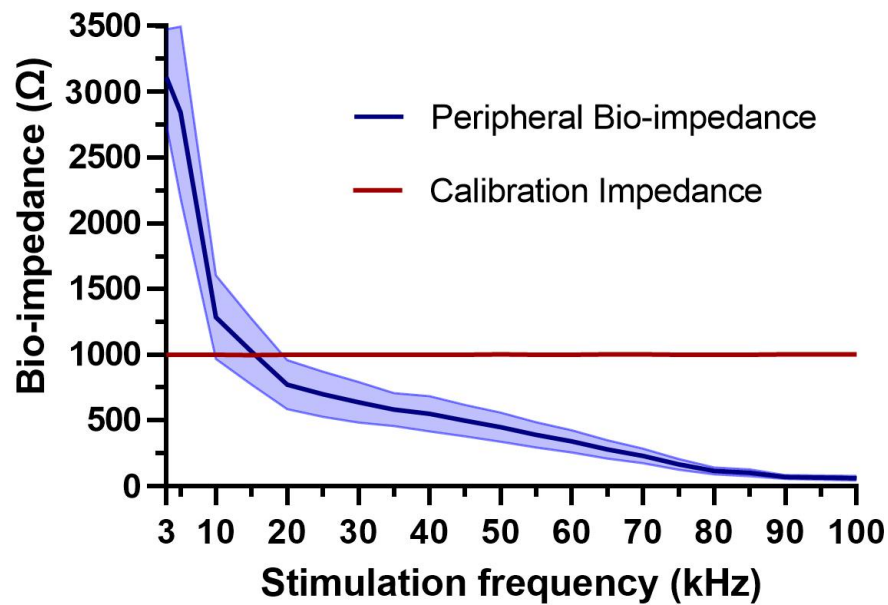


Fig. 4.8: EVAL-AD5933EBZ Evaluation board frequency stimulation cycle

plotted with mean and standard deviation in Fig, 4.8.

It was highlighted that the EVAL-AD5933EBZ evaluation board also demonstrated the characteristic exponentially decaying of PBI with the increase of the stimulation frequency, consistent with the results obtained from the Keysight E4990A LCR meter in subsection 4.4.1. The compact and portable design of the EVAL-AD5933EBZ evaluation board makes it convenient for embedding into customized devices. Consequently, the EVAL-AD5933EBZ was selected as the PBI measurement acquisition device for the current study.

4.5 Time domain variation of Peripheral Bio-impedance

Even though the initial PBI measurements were obtained in the frequency domain, it was essential to monitor the time domain variations of PBI to study the dynamic behaviour for the current study investigations. However, it was hardly evident the dynamic behaviour of PBI and variations during reactive hyperemia (RH) conditions in previous research studies. Therefore, to simplify the understanding of the dynamic behaviour of PBI, a mathematical model for PBI in the forearm was developed based on the evidence from the available literature.

4.5.1 Peripheral Bio-impedance Model for the Forearm

Peripheral Bio-impedance (PBI) is a novel parameter introduced through the current research study to assess the presence of VED through the induced RH condition. It has revealed a new dimension of assessing VED based on the electrical activity of the peripheral vascular flow. However, previous literature has only reported the static nature of the PBI. Therefore, a comprehensive study of the dynamic nature of the PBI parameter was analyzed based on Magnitude variation and Frequency dependency and developed a mathematical model for the forearm PBI.

The magnitude variation of PBI is highly dependent on the anatomical position and the tissue structure of the forearm. For the current study, PBI electrodes in Bipolar configuration were placed on the skin over the radial artery of the test subject's forearm. The conduction pathway of stimulation current I_S lies across 3 layers of tissue structure: fat, blood and muscle, as shown in Fig.4.9(a). Each individual tissue layer is composed of different impedance values of Z_F , Z_B and Z_M respectively connected in parallel as shown in Fig.4.9(b). The order of the magnitudes of these impedances at normal blood flow is $Z_F > Z_B > Z_M$ [61].

With reference to Fig.4.9 (b), the equivalent impedance of the forearm can formulated

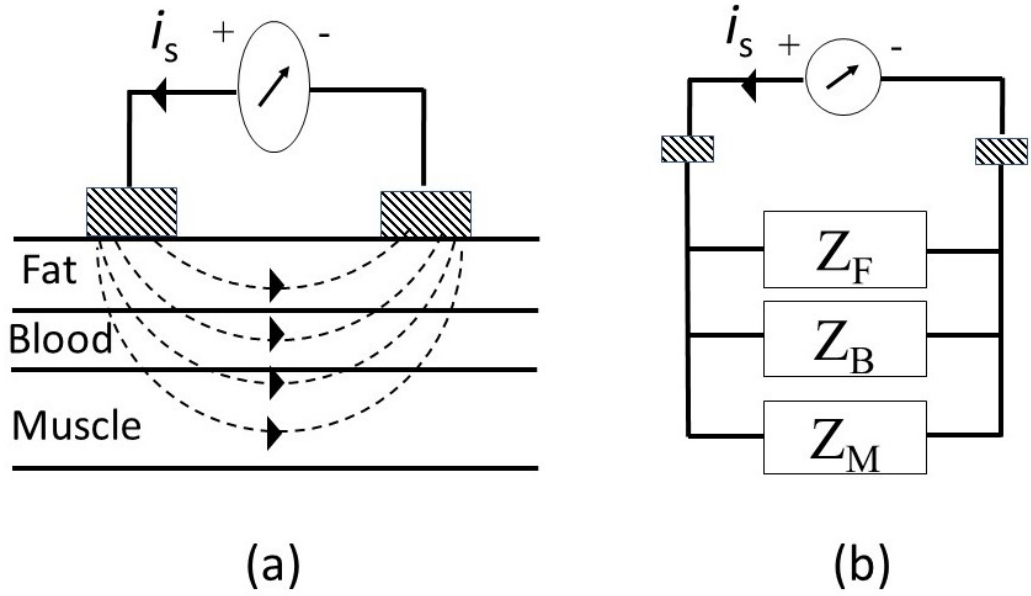


Fig. 4.9: (a) Conduction Pathway of Stimulation Current in the Forearm (b) Peripheral Bio-impedance Equivalent Circuit Model of forearm

using the basic fundamentals of parallel impedances as in the equation 4.1.

$$Z_{eq} = \frac{1}{\frac{1}{Z_B} + \frac{1}{Z_F} + \frac{1}{Z_M}} \quad (4.1)$$

An assumption was made that the impedance values won't be changed in fat (Z_F) and muscle (Z_M) tissues significantly in a smaller time period and only the impedance in the blood (Z_B) will be varied with the change in the blood flow, where $Z_{FM} = \frac{Z_F Z_M}{Z_F + Z_M}$ becomes a constant for a given frequency. Thereby, the further simplified equation can be derived as shown in equation 4.2.

$$Z_{eq} = \frac{1}{\frac{1}{Z_B} + \frac{1}{Z_{FM}}} \quad (4.2)$$

In an ideal scenario, the equivalent impedance (Z_{eq}) remains constant during the base-line period. When the occlusion of the brachial artery is initiated, the blood flow rate is gradually reduced to zero level. Thereby the impedance of blood (Z_B) also gradually increases and settles at a maximum value. Therefore, the equivalent impedance (Z_{eq}) increases proportional to the change caused by Z_B . Soon after the cuff release (post reactive Hypermia), the brachial artery blood flow rate increases rapidly and the impedance of Z_B decreases at a higher rate of slope. Consequently, the equivalent impedance (Z_{eq}) also decreases at a higher rate.

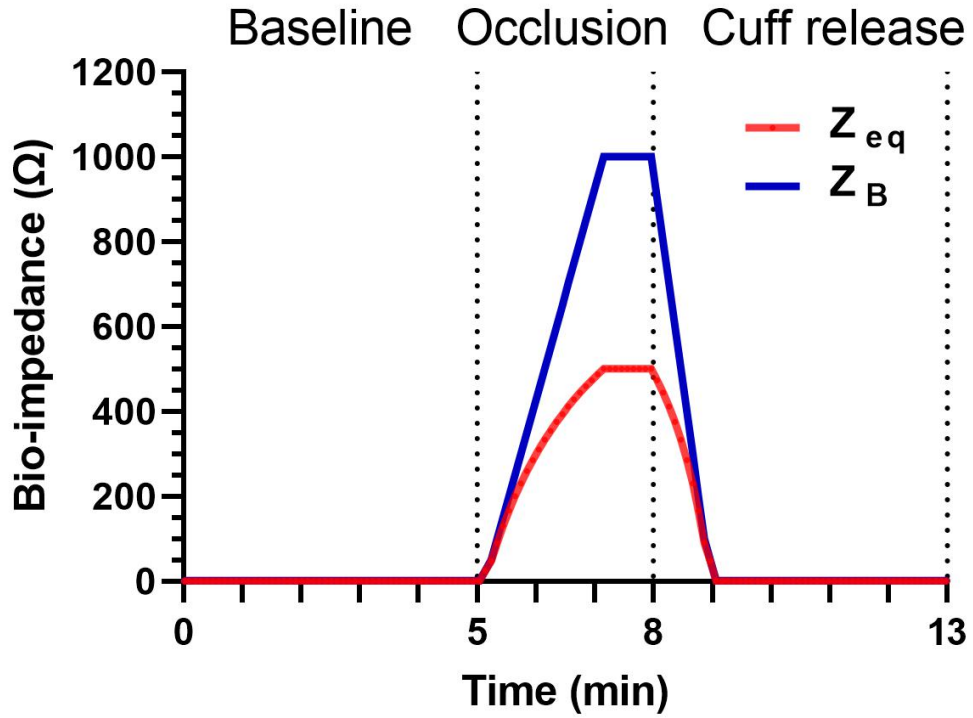


Fig. 4.10: Magnitude Variation of blood impedance (Z_B) and equivalent impedance (Z_{eq}) of the Peripheral Bio-impedance Model for the Forearm

The magnitude variations of Z_B and Z_{eq} during baseline, occlusion and post-reactive hyperemia are graphically visualized as shown in Fig.4.10. Thereby, the peripheral bio-impedance (PBI) model for the forearm has demonstrated the magnitude variation of a gradual increase during occlusion and a rapid decrease during post-reactive hyperemia which is inversely proportional behaviour compared to the observed pattern of the DBT, PAT and PPG parameters. Therefore, a preliminary study was conducted to validate the PBI variation during reactive hyperemia condition.

4.5.2 Device Integration of PBI

The default setup of EVAL-AD5933EBZ Evaluation board is pre-programmed to acquire stimulation frequency cycle based on the PBI measurements which didn't facilitate the requirement of studying the dynamic behaviour of PBI in the time domain. Since the pre-programmed method was reluctant to capture continuous time domain variations of PBI with respect to selected stimulation frequencies, both hardware and software adjustments were made to fulfil the expected outcome. Therefore, a customized algorithm was developed to operate the EVAL-AD5933EBZ Evaluation board through an Arduino Nano ATmega328 microcontroller.

To operate the evaluation board through the microcontroller, a few onboard adjustments were made to the circuitry of the EVAL-AD5933EBZ Evaluation board. The USB power supply path was changed to a microcontroller power supply path by changing the onboard jumper switch and additional circuit wires were connected to VCC and GND. For I²C communication, the SDA and SCL pins were intercepted and connected to the microcontroller by additional circuit wires.

After these onboard adjustments, the algorithm was developed in the Arduino integrated development environment (IDE) by accessing the chip registries given in the data sheet to obtain the continuous variations of PBI measurements concerning the stimulation frequencies. It was highlighted that the sampling rate to complete a stimulation frequency cycle was reduced as the number of stimulation frequencies increased. Therefore, to maintain a reasonable sampling rate, ideal stimulation frequencies were investigated for PBI data acquisition from the previous research studies.

4.5.3 Selection of Stimulation Frequencies

The frequency domain PBI data acquisition approach was proven incompatible with the other physiological parameters used in the current research study, as they predominantly exhibit variations in the time domain. Therefore, the analysis of PBI shifted towards examining time-domain variations, focusing on specific stimulation frequencies. The choice of these frequencies was determined by prior research conducted on bio-impedance.

Following an exhaustive review of the literature, it was evident that the 50 kHz stimulation frequency has been predominantly employed in the analysis of PBI [61, 94]. However, recognizing the necessity for a more comprehensive understanding of PBI dynamics, there arose a need to explore alternative stimulation frequencies. Consequently, four (04) arbitrary stimulation frequencies, namely 5 kHz, 10 kHz, 20 kHz, and 50 kHz were selected to proceed further with the remainder of the research.

4.5.4 PBI Measurement Validation

Numerous prior research studies on PBI focused solely on its static nature. However, the current study has paved the way to explore the dynamic behaviour of PBI with the inducement of reactive hyperemia. According to the PBI model outlined in section 4.5.1, the validation of magnitude variation was deemed essential, considering the innovative application of PBI in the current research study. Thus, the PBI measurements obtained from the customized device were compared with the clinically employed Bio-impedance measuring device, serving to validate both the measurement and the PBI model.



Fig. 4.11: Bodystat 500 Bio-impedance Measuring Device

The Health and Wellness Unit attached to the Faculty of Medicine, University of Colombo has facilitated the opportunity to experiment with Bodystat 500 as shown in Fig. 4.11 which is a professional body composition analyzer. It is orchestrated with four (04) BIA electrode configuration and is capable of measuring the PBI of any body part at 50 kHz Stimulation frequency. As the device is specifically tailored for static measurements, 100 samples were manually gathered from voluntary participants over 13 minutes, following the study protocol detailed in Section 6.1. After a 15-minute rest period for participants, data collection from the custom-made device was repeated for the same duration, following the established protocol.

The collected PBI data from both the Bodystat 500 and custom-made devices at 50 kHz stimulation frequency were graphically represented as shown in Fig. 4.12 to visualize the magnitude variations during the reactive hyperemia condition. Both plots have demonstrated congruent variations to the PBI model as shown in Fig. 4.10. It was observed that the delayed and prolonged response in PBI variations can be ascribed to the physiological status of the participants, in contrast to the ideal scenario depicted in the PBI model. Hence, the PBI measurements obtained by the custom-made device were validated by comparing them with the PBI model and the Bodystat 500 device plots. Moreover, the Bodystat 500 readings exhibited a higher standard deviation compared to the custom-made device, indicating the impact of discrepancies in the PBI electrode configurations.

With reference to Fig. 4.12, the bodystat 500 device results have shown higher stan-

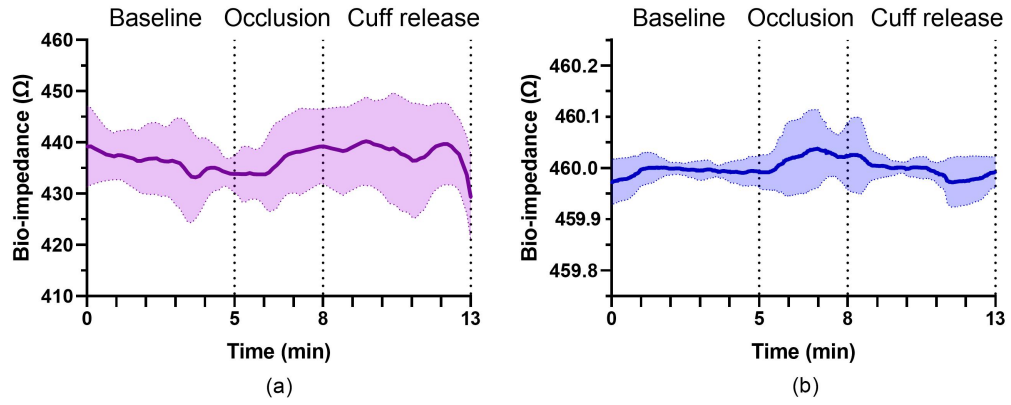


Fig. 4.12: PBI Comparison of Bodystat 500 Vs Custom made Device;
(a) PBI variation of BodyStat 500 (b) PBI variation of Custom made device

dard error due to the electrode placement, where 4 electrodes required to be stabilized for a single measurement and the discrete data acquisition

CHAPTER 5

DEVICE INTEGRATION AND DATA PREPROCESSING

5.1 Device Integration

Two Plastic enclosures with dimensions of 157x89x47mm (length, width and height respectively) were used to house the components into two compact devices as shown in Fig. 5.1. Each of the enclosures were filled with three (03) Arduino Nano boards connected to DBT, PPG and PBI sensors and one (01) Arduino Uno board mounted with PAT signal data acquisition PCB. The enclosures were drilled to create outlets for sensors, air pump and USB powering for each Arduino board. Additionally, a two-way toggle switch outlet was created which was connected to the EVAL-AD5933EBZ Evaluation board to toggle between the calibration pathway (where the known 1 k Ω resistor) to the data acquisition pathway of bipolar electrode configuration. The adult pressure cuff was manually operated and monitored separately by the investigator during the experiment.

Sensors were connected on the participant as shown in Fig. 5.2. The PBI electrodes are attached to the skin surface over the radial artery while the PAT, DBT and PPG sensors are connected to the fingertips from index to ring respectively in both the upper limbs. Additionally, the ambient temperature sensors are attached proximally to the DBT sensor in the middle finger without touching the skin surface.

5.2 Data Acquisition

The eight (08) USB outlets were connected to the PC through a USB hub to power up the sensors and data acquisition procedure. The initial data acquisition algorithm for each sensor component was developed based on the Arduino IDE (Release 1.8) and a graphical user interface (GUI) based real-time acquisition setup was integrated by the Processing (Release 3.5) to be compatible with synchronizing, parallel processing and storing the raw data of all eight (08) sensor inputs simultaneously.

Separate graphical plots were created in GUI for each physiological parameter as shown in Fig. 5.3 (DBT, PAT, PPG and PBI) for real-time visualization in both arms. On the same GUI, push buttons were included to start the data acquisition process, and terminate and store the raw data of each physiological parameter in separate files. Additional three (03) push buttons were created to timestamp the beginning of the baseline, cuff inflation (Occlusion) and cuff deflation (Release) points during the experimental protocol. All the physiological parameters were acquired for 13 minutes

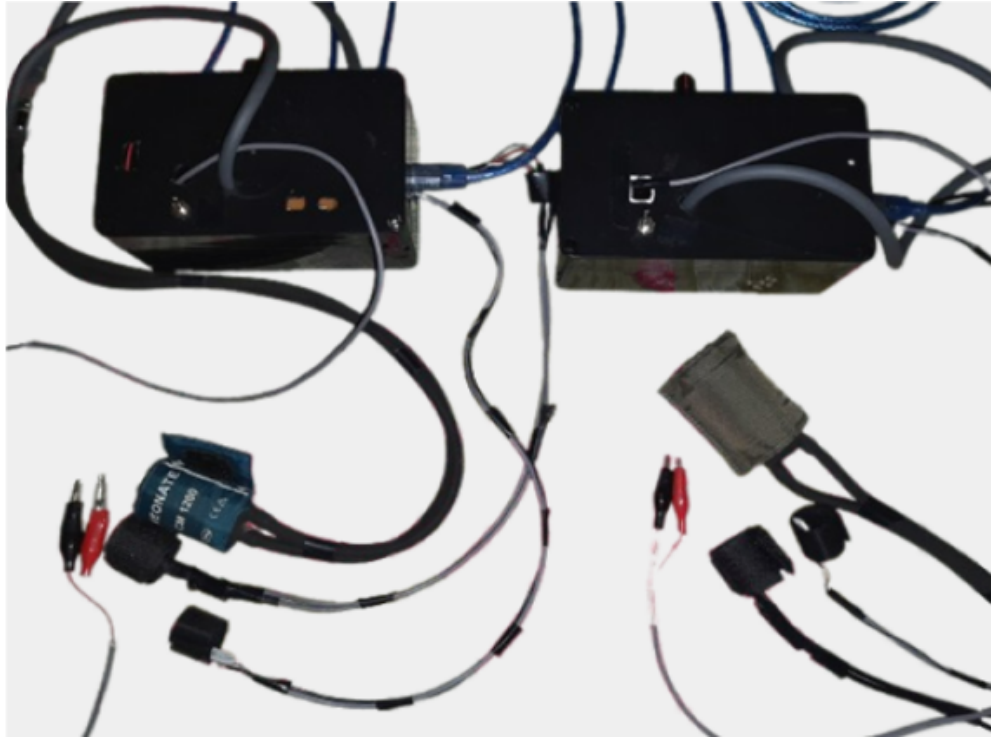


Fig. 5.1: The Complete Integrated Devices



Fig. 5.2: Sensor connections to Test subject

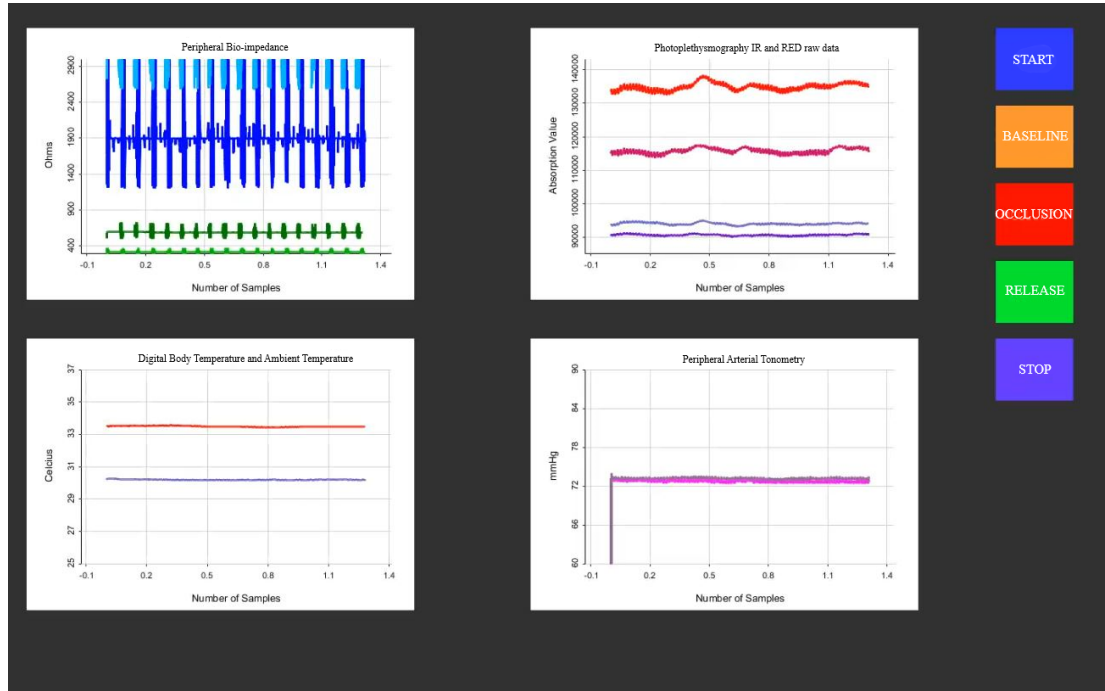


Fig. 5.3: GUI developed from Processing Software

and saved on the PC.

5.3 Data Preprocessing

The recorded data were saved in CSV file format and organized in individual serial-coded folders corresponding to each test subject. MATLAB (Release 2018a) software was employed to visualize the raw data and perform data preprocessing. At the initial screening of the raw data, outliers were excluded before the data preprocessing stage. Then the raw data pertaining to the experimental procedure were segregated by utilizing timestamps at the 0th and 13th minutes and further categorized into the test subject's occluded arm and control arm data sets. The data were analyzed in terms of long-term variations as well as short-term variations with the inducement of RH. However, the short-term variations were observed in PAT and PPG signals only.

The raw data of DBT consisted of 04 data sets per test subject: occluded arm temperature, control arm temperature and 02 ambient temperatures measured at the proximal of both forearm fingertips. Unlike other physiological parameters, DBT did not undergo any preprocessing for the raw data and the long-term trend of DBT is illustrated in Fig. 5.4. Notably, the recorded DBT measurements consistently indicated values below 37°C, attributing this observation to the fact that readings were obtained from the skin surface.

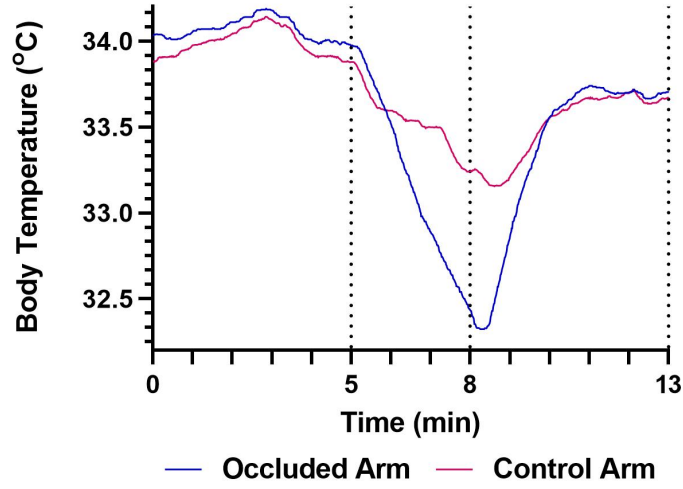


Fig. 5.4: Preprocessed DBT Measurements for Occluded Arm and Control Arm

The PAT signal was acquired from the neonatal cuffs wrapped in the forearm fingertips inflating in between 60 to 90 mmHg range. The PAT signal was possessed with both long-term and short-term variations. However, the raw data was inherited with random noise regardless of the analog filtering at the data acquisition. Therefore, a moving average filter was used to suppress the noise and enhance the pulsatile pattern of the signal. In addition, the detrending was used to eliminate the baseline drifting of the signal. The PAT signal's long-term and short-term trends are shown in Fig. 5.5.

The PPG raw data consisted of 02 parts: the Infrared light (IR) data set and the Red light (RED) data set. To obtain the PPG wave pattern, the R index has to be calculated using the equation 5.1 and then converted into SpO_2 using the manufacturer's conversion equation shown in the equation 5.2. The Manufacturer's calibration coefficients (a,b and c) are obtained from the application guide manual [95]. The long-term and the short-term variations of the PPG parameter extracted from SpO_2 are depicted in Fig. 5.6.

$$R = \frac{AC_{rmsRED}/AC_{rmsIR}}{DC_{RED}/DC_{IR}} \quad (5.1)$$

$$SpO_2 = aR^2 + bR + c \quad (5.2)$$

The PBI dataset is composed of bio-impedance measurements of 04 stimulation frequencies (5 kHz, 10 kHz, 20 kHz and 50 kHz) per test subject. However, only 50 kHz was used for further analysis as per the literature findings explained in section 4.5.3. The raw data plots of PBI have possessed mutual noise interference in periodic

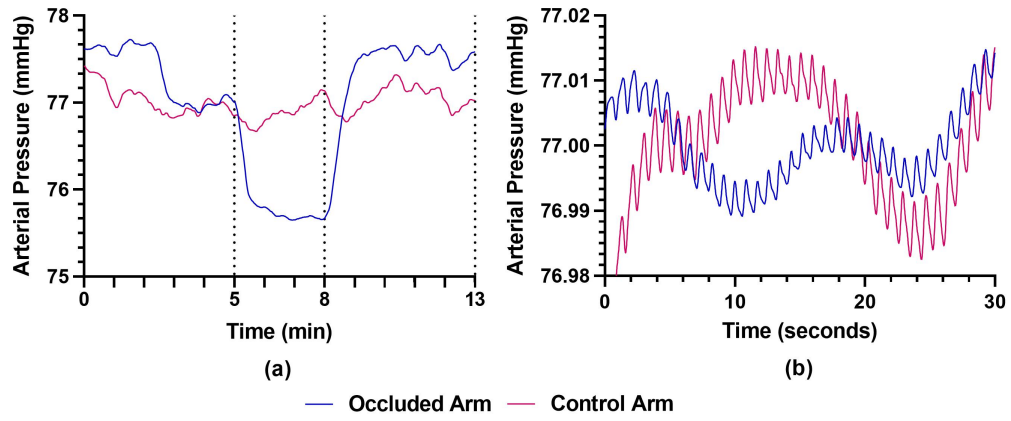


Fig. 5.5: Preprocessed PAT Measurements for Occluded Arm and Control Arm:
(a) Long-term variation (b) Short-term variation

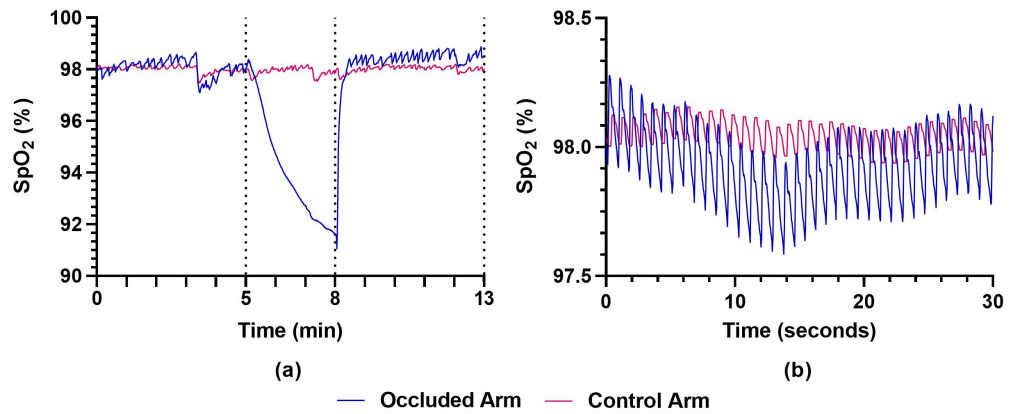


Fig. 5.6: Preprocessed PPG Measurements for Occluded Arm and Control Arm:
(a) Long-term variation (b) Short-term variation

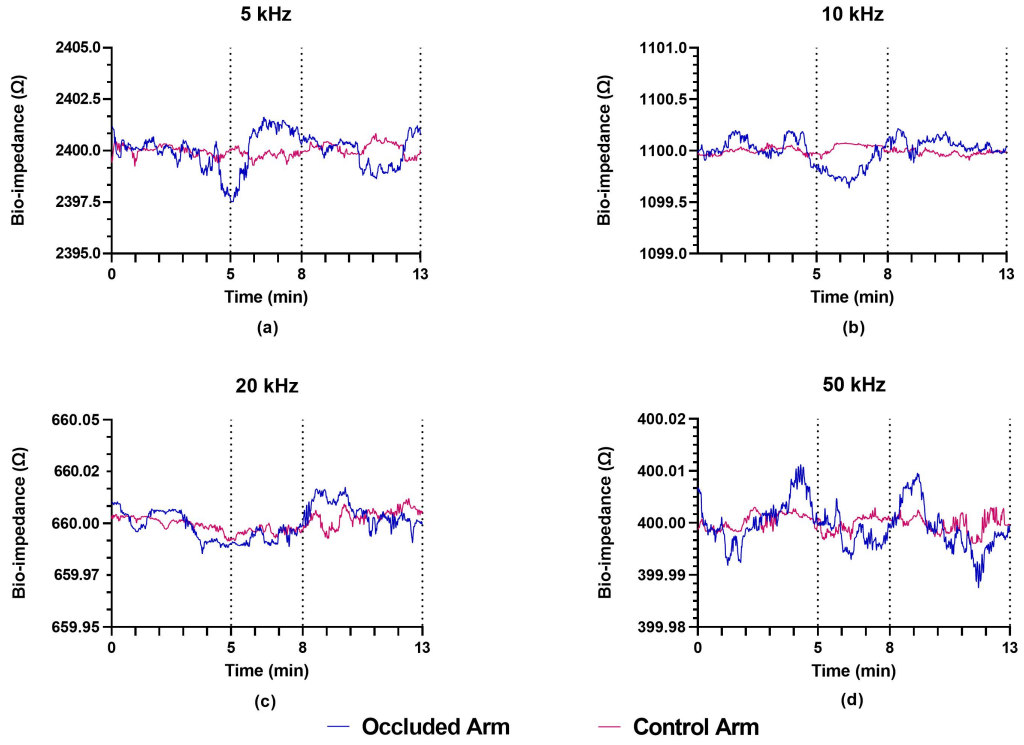


Fig. 5.7: Preprocessed PBI measurements for Occluded arm and Control on Stimulation Frequencies: (a) 5 kHz (b) 10 kHz (c) 20 kHz (d) 50 kHz

intervals due to the simultaneous data acquisition of PBI measurements from both the test subject's forearms using 02 custom-made devices. Therefore, PBI data were processed through a median filter to suppress the mutual noise interference. Furthermore, the baseline drifting was removed by using the detrending command. The preprocessed long-term variation of PBI for 4 stimulation frequencies is shown in Fig. 5.7 for a randomly selected Healthy participant.

CHAPTER 6

DEVICE VALIDATION AND PRELIMINARY STUDY

Device validation is essential for ensuring that a device is safe, effective, and ready for its intended use, thereby protecting both the operator and participants while facilitating an accurate data acquisition process. Therefore, a preliminary study was conducted to validate the newly developed custom-made devices comprised of digital body temperature (DBT), peripheral arterial tonometry (PAT), photoplethysmography (PPG) and peripheral bio-impedance (PBI) physiological parameters along with flow-mediated dilation (FMD) procedure which is the non-invasive gold standard of assessing the presence of vasodilatory endothelial dysfunction (VED). The validation study was conducted between healthy and CVD participants.

This study mainly focused on validating the long-term trend variations of physiological parameters (DBT, PAT, PPG and PBI) in response to reactive hyperemia (RH) compared to gold standard parameters (brachial artery diameter and flow rate). Previous research studies have individually employed DBT, PAT, and PPG parameters to investigate VED [22, 52, 96]. The identified pattern, characterized by a progressive decrease in amplitude during occlusion followed by a swift increase post-reactive hyperemia, elucidates the response of these parameters to reactive hyperemia condition. Nevertheless, there is a lack of documented evidence regarding the utilization of PBI in studies focused on VED.

This chapter discusses the study protocol of data collection, inclusion and exclusion criteria for test subject categories(Healthy and CVD), FMD procedure, data normalization, long-term variation index calculation and statistical significance of indices between test subject categories.

6.1 Study Protocol

The preliminary study was involved with human test subjects, the ethical approval was obtained from the Ethical Review Committee in the Faculty of Medicine attached to the University of Colombo under protocol No: EC/18/038 before the data collection. The preliminary study was conducted at the vascular laboratory of National Hospital of Sri Lanka (NHSL) premises. The potential participants who arrived at the laboratory premises were provided with the information sheet of the study protocol and consent form (refer to Appendix A). Only those giving written consent were recruited. After a period of physical rest lasting 30 minutes the data collection process began.

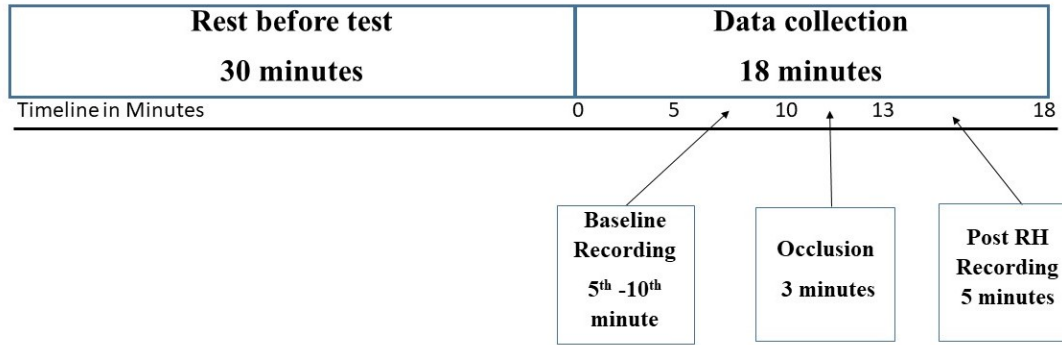


Fig. 6.1: Experimental Procedure of the Current Research Study

The experimental protocol was verbally explained to each participant by the investigator and demographic data was gathered by interview using a questionnaire. Based on the questionnaire, the participants with any evidence of type 1 DM, liver cirrhosis, renal failure, thyroid disease, spinal cord injuries and finger deformities were excluded from the study, as per protocol. Further data collection was initiated by recording the test subjects anthropometric data of age, gender, height, weight and noninvasive blood pressure. The body mass index (BMI) was derived later from the recorded data. However, the blood samples were not obtained from the participants in the preliminary study.

Each participant was then escorted individually to an enclosed room to conduct the experimental procedure. The room was temperature controlled at 26°C to ensure minimal environmental impact for consistent temperature recordings throughout the experimental session. The participant was asked to be seated in a comfortable posture and place both arms in parallel on a cushioned armrest. The skin area over the radial artery and brachial artery of both arms was cleaned using alcohol swabs to remove any remaining dust and sweat which may influence the sensor measurements. Then the sensors were attached to the test subject as shown in Fig. 5.2. The adult pressure cuff was wrapped around the dominant arm covering the brachial artery of the test subject to induce reactive hyperemia by occlusion.

The data collection was initiated using the customized GUI shown in Fig. 5.3 which operated on a PC. A total time of 18 minutes was taken to complete the data collection from a single test subject. The entire experimental procedure is sketched in Fig. 6.1 along with the data collection phases. The initial 5 minutes were used for sensor calibrations and settling of all the physiological parameter values into a steady state. The initial phase of data collection was used to obtain the baseline readings from the 5th minute to the 10th minute (5 minutes duration). During the second phase, the pressure

cuff was inflated to supra systolic pressure (200 mmHg) to induce reactive hyperemia by occluding the brachial artery and holding it for 3 minutes. During this period, the test subject may feel numbness in the dominant arm. Soon after 3 minutes, the pressure cuff was rapidly deflated and the data were recorded for a further 5 minutes during the last phase. If a participant is required to repeat the experimental setup due to interruption of data collection, a sufficient resting period of 15 minutes was given to recover the peripheral vascular system from reactive hyperemia condition [97]. All the raw data were stored in the PC with a serial code based on the test subject's anthropometric parameters for further processing.

6.2 Preliminary Study

The preliminary study was conducted to validate the custom-made devices against the FMD as the benchmark. The FMD protocol is explained in section 6.3. Data collection for the study was conducted at the Vascular laboratory attached to the National Hospital of Sri Lanka (NHSL), using 10 volunteers between ages 35 to 60 years. Two groups of participants were recruited: 05 healthy individuals (control) and 05 patients affected by CVD with/without DM. The selection of participant categories was based on the hypothesis that patients affected by CVD with/without DM would show variations inductive to the presence of VED through the physiological parameters in contrast to healthy participants. This experiment was implemented to investigate the validity of this hypothesis.

The experimental procedure in the section 6.1 was explained to each participant and written consent was obtained before the commencement of data collection. A questionnaire was filled along with anthropometric data which was used to classify the participant to one of the groups. The summary of anthropometric data (Mean $\pm$ Standard Deviation) of the preliminary study test subjects are tabulated in Table 6.1. As part of the validation process, each participant underwent two (02) rounds of data collection procedures: one with the Flow-Mediated Dilation (FMD) protocol and another with the custom-made device.

TABLE 6.1: ANTHROPOMETRIC DATA OF THE PARTICIPANTS RECRUITED FOR THE PRELIMINARY STUDY

Parameter	Healthy (n=5)	CVD (n=5)
Age (years)	48 $\pm$ 5	50 $\pm$ 7
Gender (Male/Female)	1 4	3 2
Height (cm)	160 $\pm$ 11	161 $\pm$ 16
Weight (kg)	64 $\pm$ 14	72 $\pm$ 9
Body Mass Index (BMI)	25 $\pm$ 6	28 $\pm$ 3
Systolic Pressure (mmHg)	120 $\pm$ 2	131 $\pm$ 10
Diastolic Pressure (mmHg)	80 $\pm$ 2	89 $\pm$ 12

6.3 Experimental protocol with Flow Mediated Dilation

Flow-mediated Dilation (FMD) Protocol is known as the noninvasive gold standard of VED assessment validated as a reliable and reproducible measurement where the Brachial artery diameter and flow velocity parameters are measured using Doppler-Ultrasonography [19, 21, 57, 98, 99]. In this protocol, the occlusion method is commonly used to induce reactive hyperemia condition in the Brachial artery using an adult pressure cuff.

It is recommended to have a Doppler ultrasound machine and well-trained sonographer who is fluent in measuring the Brachial arterial parameters within a few seconds with correct probe placement to perform FMD protocol [100, 101]. Therefore, the Vascular laboratory attached to the National Hospital of Sri Lanka (NHSL), Colombo 07 was accessed with prior approval to perform the FMD protocol. With the help of the sonographer, the Toshiba Nemio 35 SSA-550A diagnostic ultrasound machine shown in Fig. 6.2 (National Ultrasound, USA) was utilized to acquire the relevant data from the participants.

Since the Brachial artery is anatomically placed superficial to the skin, the linear ultrasound probe specialized for vascular detection with a higher frequency (8 MHz) with less penetration was used to detect the Brachial artery diameter and flow velocity. The ultrasound gel was applied to the skin area over the Brachial artery to match the acoustic impedances between the skin and the ultrasound probe.

The experiment setup was conducted similarly to the procedure explained in the section 6.1. The measurements were recorded from both the occluded arm and control arm of the test subject over 13 minutes of data collection period. A photograph of taking measurements is given in Fig 6.3. Two physiological parameters were obtained from the FMD protocol namely Brachial Artery Diameter and Brachial Artery Flow Rate.



Fig. 6.2: Toshiba Nemio 35 SSA-550A Diagnostic Ultrasound Machine

The vessel diameters were read by observing the real-time Brachial arterial images and velocity graphs through the ultrasound monitor as shown in Fig. 6.4 to record the data. Measurements were manually recorded every 30 seconds (due to the unavailability of continuous measurement storage within the ultrasound machine). The manually recorded data were then fed into a PC and time series graphs were plotted with respect to the individual FMD parameter.

6.4 Experimental protocol with Custom-made device

All the participants who underwent the FMD protocol were rested for 15 minutes before connecting them to the custom-made device. The study protocol mentioned in the section 6.1 were repeated for the data acquisition from the custom-made devices. Sensors corresponding to DBT, PAT, PPG and PBI were connected to the test subject's both forearms while the adult pressure cuff was wrapped around the brachial artery region of the dominant arm as shown in Fig. 6.5. Time stamps of the starting points of the baseline, occlusion and cuff release were manually entered through the software GUI. Soon after the termination of real-time data acquisition, the data were stored in a PC as explained in section 5.3 for data preprocessing.



Fig. 6.3: FMD measurements acquired from the Brachial Arteries of both occluded arm (with the pressure cuff) and control arm of the Test Subject

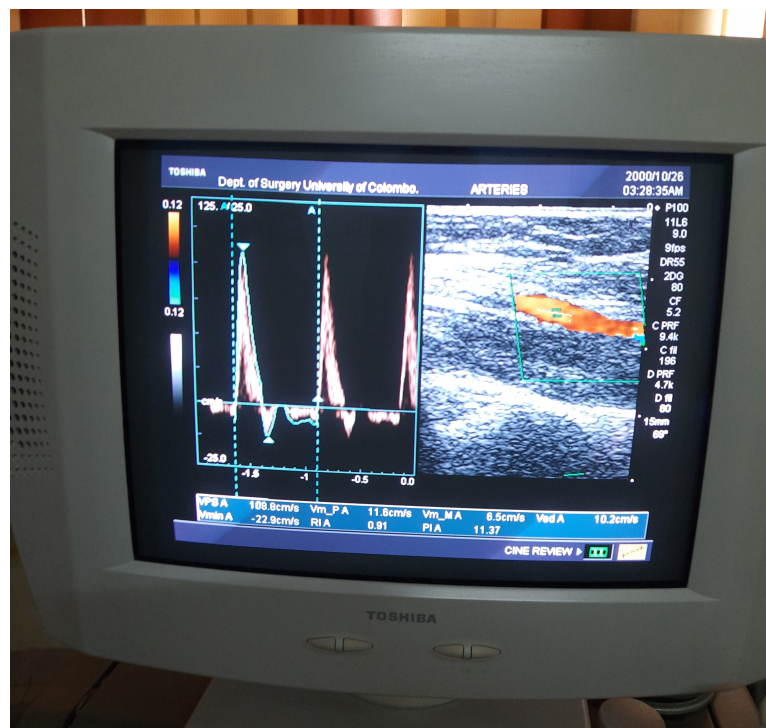


Fig. 6.4: Real-time FMD measurements observing and freezing the optimum segment to record the data



Fig. 6.5: Data Acquisition from Custom-made devices

6.5 Data Normalization

To assess the presence of VED and validate the custom-made devices, data normalization is essential for eliminating test subject-biased variations and enabling comparison across different test subject categories. All the data should be preprocessed before initiating the Data Normalization procedure. The mean values were not removed from the signals to avoid the zero crossing points which impact on resulting in undefined values when performing division.

FMD data were recorded in discrete time stamps of 30 seconds, data were resampled through interpolation to observe the long-term trend during the RH condition. Then data were normalized utilizing both occluded arm and control arm data adhering to the equation 6.1. The averaged plots are shown in Fig. 6.6 for each normalized FMD parameter distinguishing the variability between Healthy (Blue) and CVD with/without DM (Red) test subject categories. It was observed that both FMD parameters have shown comparable variations where the reduction of ratios in the occlusion stage due to lack of blood circulation through the brachial artery. This led to shrink the brachial artery diameter in the occluded arm and through diameter ratio is observed at the latter part of the occlusion stage. Furthermore, a rapid increase can be observed here soon after the cuff release in the PRH stage as blood rushes through the brachial artery until

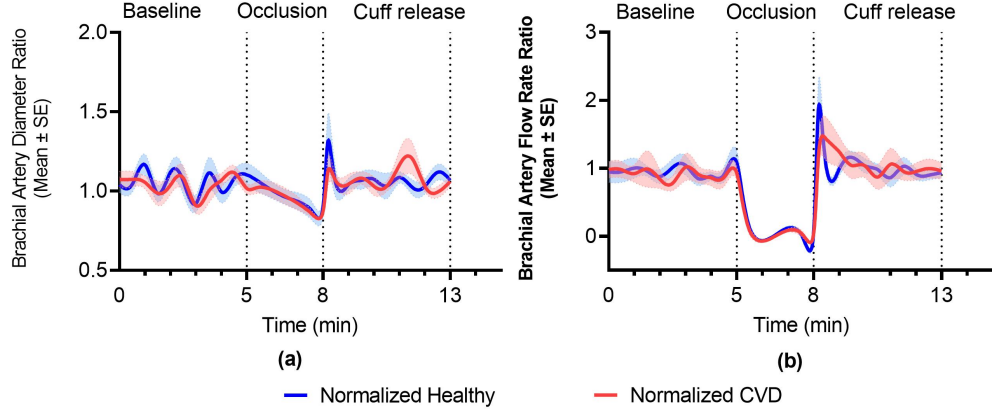


Fig. 6.6: Normalized Long-term Variations of FMD Parameters:
(a) Brachial Artery Diameter Ratio (b) Brachial Artery Flow Rate Ratio

gradually stabilizing the circulation.

$$Data\ Normalization = \frac{Resampled\ Occluded\ Arm\ Data}{Resampled\ Control\ Arm\ Data} \quad (6.1)$$

In the custom-made devices sampling rates for respective sensors have been noted to be slightly different due to individual hardware setup variability. Therefore, the data were resampled to equalize the sampling rate in occluded arm data with control arm data before performing normalization. The resampled data of PAT, PPG and PBI measurements were subtracted from the respective minimum value of the data to remove the DC baseline shift. However, the ambient temperature readings were deducted from both occluded arm and control arm measurements of DBT to eliminate the environmental temperature impact on the measurement and the DC baseline shift. The Normalized data were obtained by dividing the resampled occlude arm data from the resampled control arm data as shown in equation 6.1. Once the normalization is performed, all the parameters are represented as ratios. The averaged plots of normalized data of both subject categories are shown for each physiological parameter in Fig. 6.7. All the physiological parameters of the custom-made devices except PBI have shown a compatible variation to that of FMD parameters. However, the PBI has shown inversely proportional behaviour due to an increase in blood impedance (Z_B) during the occlusion stage where the blood flow is temporarily restricted on the radial artery resulting in an increment of the equivalent impedance (Z_{eq}). Once the cuff is deflated, blood flow is restored and equivalent impedance (Z_{eq}) decreases corresponding reduction of blood impedance (Z_B).

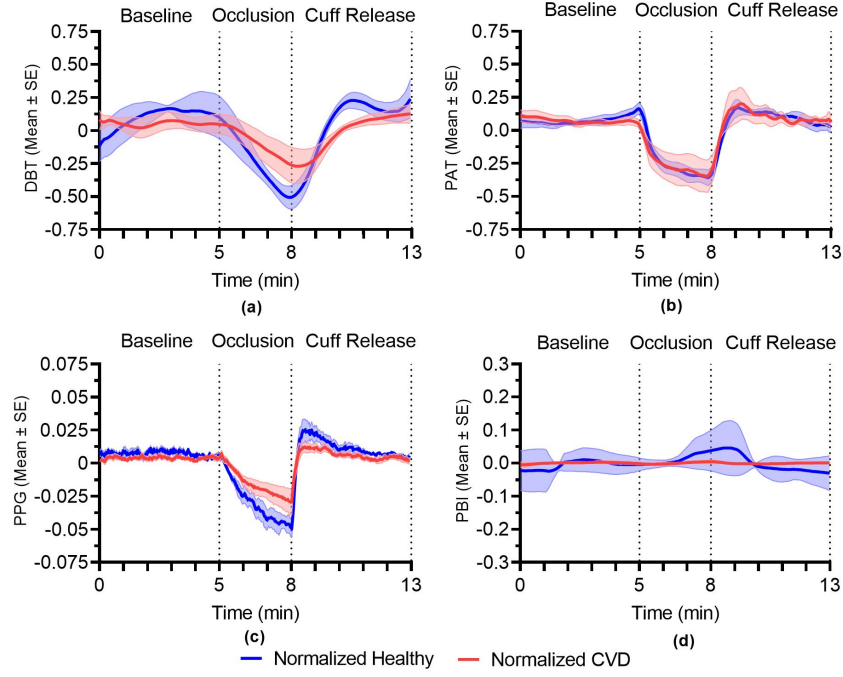


Fig. 6.7: Normalized Long-term Variations of Custom-made device Parameters:
(a) DBT Ratio (b) PAT Ratio (c) PPG Ratio (d) PBI Ratio

6.6 Index Calculation

The aim of the preliminary study is to validate the custom-made device with the FMD (Non-invasive Gold Standard) protocol to investigate the presence of VED within the CVD with/without DM category compared to the healthy category. To achieve this objective, VED indices are derived based on the long-term variation trend, which involves analyzing time-domain fluctuations in physiological parameters observed over an extended period (13 minutes for the current study). All the physiological parameters (DBT, PAT, PPG, and PBI) used for the current study exhibited long-term variations in both occlusion and PRH stages compared to the baseline.

Standardized VED indices are available for both the Brachial Artery Diameter and Brachial Artery Flowrate parameters within the FMD protocol [99–101]. The equations of calculating these VED indices for the Brachial Artery Diameter and Doppler Flowrate are detailed in equations 6.2, 6.3 and 6.4. These indices are mainly derived from the characteristics that occurred in the PRH stage compared with the baseline.

$$FMD\ Peak\ \% = \left(\frac{Peak\ Diameter - Baseline\ Diameter}{Baseline\ Diameter} \right) \% \quad (6.2)$$

$$FMD\ Trough\ \% = \left(\frac{Trough\ Diameter - Baseline\ Diameter}{Baseline\ Diameter} \right) \% \quad (6.3)$$

$$DFI\ \% = \left(\frac{Peak\ Flowrate - Baseline\ Flowrate}{Baseline\ Flowrate} \right) \% \quad (6.4)$$

According to previous research studies, the DBT is the main physiological parameter that has derived VED indices for the long-term variation assessment [55, 102–105]. Therefore, 07 long-term variation VED indices:

1. Falling gradient
2. Rising gradient
3. Ratio of the slopes
4. Area Under the Curve (AUC)
5. Rebound
6. Nadir to Peak
7. Rebound time

were calculated on each physiological parameter recorded. The following Fig. 6.8 has illustrated the regions of interest used for index calculations. The falling gradient is computed by considering the time-stamped occluded region (Red) from 5th minute to the 8th minute. Conversely, the rising gradient is calculated using the time-stamped PRH region (Blue) from 8th minute to the reach of the peak. The ratio of slopes is determined by the ratio between Rise to Fall gradients. The AUC index was calculated through the confined triangular area (Green) in PRH as shown in Fig. 6.8. The rebound index is calculated through the following equation 6.5 where the Temp_i is denoted as the baseline temperature recorded at 5th minute and Temp_{peak} represents the peak at PRH region. The rebound time is determined by the time difference of time stamps corresponding to peak and nadir (8th minute). Furthermore, nadir to peak is calculated by the equation 6.6.

$$Rebound\ Index = \left(\frac{Temp_{peak} - Temp_i}{Temp_i} \right) * 100 \quad (6.5)$$

$$Nadir\ to\ Peak = Temp_{peak} - Temp_{nadir} \quad (6.6)$$

6.7 Preliminary Study Results

VED indices calculated from preliminary study data were utilized to validate the custom-made device including the novel PBI parameter against the FMD protocol and assess

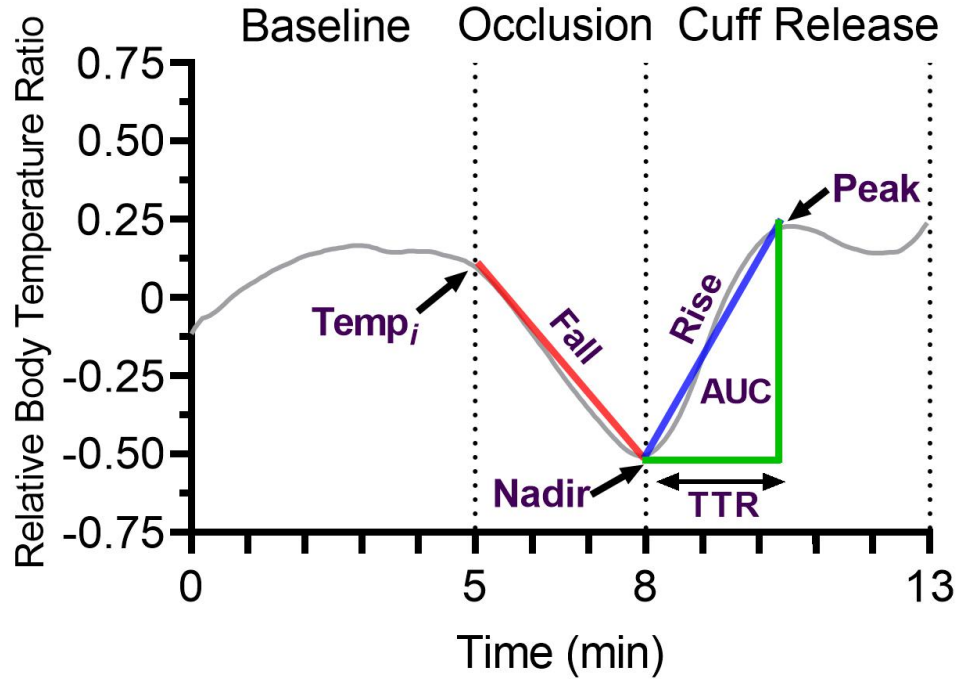


Fig. 6.8: Long-term variations Index Calculation

the ability to differentiate the healthy participants from CVD with/without DM participants based on VED. The VED indices were computed for each participant in healthy and CVD groups and compared through unpaired t-tests to assess the statistical significance. The averaged indices (mean $\pm$ standard error) are tabulated in Table 6.2 with 95% of significance ($p < 0.05$).

The FMD-Peak and DFI indices (except FMD-Trough) exhibited a statistically significant difference between healthy and CVD subjects as shown in Fig. 6.9, indicating the presence of VED in CVD test subjects. This disparity is evidenced by the lower peak diameter (18.02 % < 25.68 %) and Doppler flow rates (62.49 % < 105.37 %) in CVD test subjects during PRH compared to healthy individuals. This observation implies that individuals with CVD have a prolonged recovery period from the reactive hyperemia condition in contrast to healthy due to a lack of NO production from Endothelium and the presence of CVD risk factors.

Many VED indices calculated for each physiological parameter including PBI (novel parameter) obtained from custom-made devices have shown a statistically significant difference between healthy and CVD categories as depicted in Figures 6.10, 6.11, 6.12 and 6.13. Concerning Table 6.2, it was observed that the rebound index computed for the physiological parameters highly correlated with the FMD-Peak % index where the

**TABLE 6.2: PRELIMINARY STUDY STATISTICAL ANALYSIS
FOR VED INDICES (UNPAIRED T-TEST)**

VED Index		Healthy (n=5)	CVD (n=5)	p-value
Parameters from the FMD protocol				
FMD-Peak %		25.68 ± 2.04	18.02 ± 1.76	0.0131
FMD-Trough %		-4.93 ± 2.11	-4.81 ± 1.94	0.9674
DFI %		105.37 ± 5.50	62.49 ± 5.03	0.0002
Parameters from custom made device				
DBT	Falling Gradient	-0.25 ± 0.04	-0.19 ± 0.07	0.4479
	Rising Gradient	0.32 ± 0.08	0.08 ± 0.04	0.0314
	R/F Ratio	-1.23 ± 0.34	-0.34 ± 0.04	0.0409
	AUC	9.41 ± 1.22	4.40 ± 1.55	0.0346
	Rebound	185.7 ± 54.63	26.05 ± 20.29	0.0255
	Nadir to Peak	0.93 ± 0.16	0.50 ± 0.18	0.1015
	Rebound Time	2.06 ± 0.22	1.92 ± 0.38	0.7606
PAT	Falling Gradient	-0.16 ± 0.03	-0.08 ± 0.04	0.0396
	Rising Gradient	0.23 ± 0.04	0.13 ± 0.07	0.0207
	R/F Ratio	-1.49 ± 0.14	-1.70 ± 0.21	0.1712
	AUC	4.69 ± 0.76	3.08 ± 1.50	0.1080
	Rebound	4.89 ± 6.71	16.95 ± 12.42	0.3687
	Nadir to Peak	1.74 ± 0.17	1.68 ± 0.48	0.9145
	Rebound Time	1.14 ± 0.18	1.97 ± 0.22	0.0280
PPG	Falling Gradient	-0.02 ± 0.001	-0.01 ± 0.002	0.0098
	Rising Gradient	0.03 ± 0.004	0.01 ± 0.005	0.0461
	R/F Ratio	-1.65 ± 0.08	-1.68 ± 0.14	0.8702
	AUC	0.68 ± 0.10	0.31 ± 0.11	0.0417
	Rebound	204.6 ± 12.01	157.6 ± 16.47	0.0850
	Nadir to Peak	7.86 ± 0.99	3.95 ± 1.07	0.0453
	Rebound Time	1.05 ± 0.15	0.61 ± 0.03	0.0189
PBI	Falling Gradient	-0.06 ± 0.030	-0.01 ± 0.002	0.1114
	Rising Gradient	0.05 ± 0.020	0.01 ± 0.002	0.0326
	R/F Ratio	-1.19 ± 0.28	-1.23 ± 0.25	0.9147
	AUC	1.75 ± 0.43	0.21 ± 0.09	0.0076
	Rebound	181.2 ± 63.64	22.58 ± 22.26	0.0443
	Nadir to Peak	10.26 ± 4.36	0.66 ± 0.38	0.0417
	Rebound Time	2.67 ± 0.21	2.50 ± 0.27	0.6404

peak value during the PRH stage compared against the baseline of each physiological parameter for the rebound index calculation and many of the rebound indices (except for PAT rebound) have shown a statistically significant difference between the two subjects categories. CVD test subjects demonstrated lower values in rebound indices in each physiological parameter compared to the same indices of the healthy group. Additionally, the rising gradient as shown in Table 6.2 for all physiological parameters

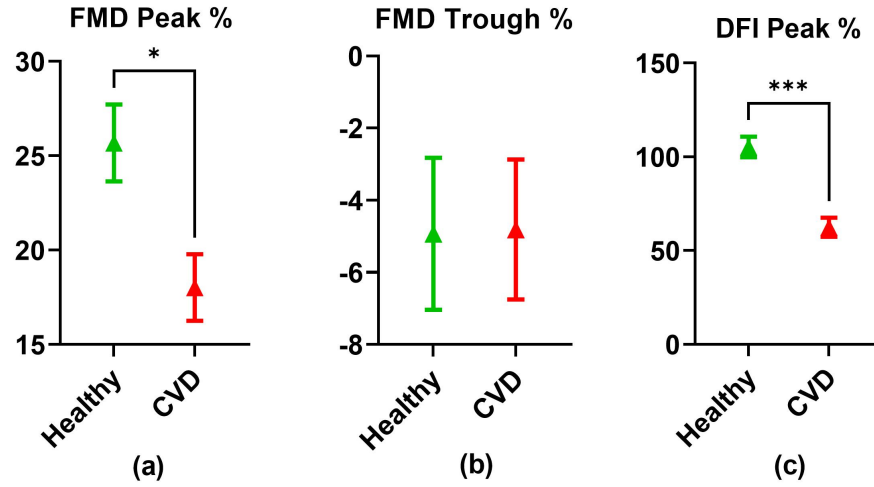


Fig. 6.9: Unpaired t-test plots of FMD based VED Indices: (a) FMD-Peak % (b) FMD-Trough % (c) DFI Peak % (Significance Level: * $p < 0.05$, *** $p < 0.001$)

displayed significant differences achieving higher gradient values for the healthy group in contrast to CVD. For the rest of the VED indices, at least one physiological parameter has shown a statistically significant difference. Since PBI has shown inversely proportional variations, rising and falling gradients were calculated in the occlusion stage and PRH stage respectively.

These results have indicated the validity of the data of each physiological parameter compared to FMD data and the capability of the custom-made device to detect the presence of VED in the CVD category. Additionally, it was determined that the PBI is suitable as a novel physiological parameter to assess VED. Therefore, the current study was further expanded to investigate the presence of VED in the intermediate test subject categories such as healthy but having CVD risk factors (HRF), Prediabetes Mellitus (PDM) and Diabetes Mellitus (DM) using custom-made devices.

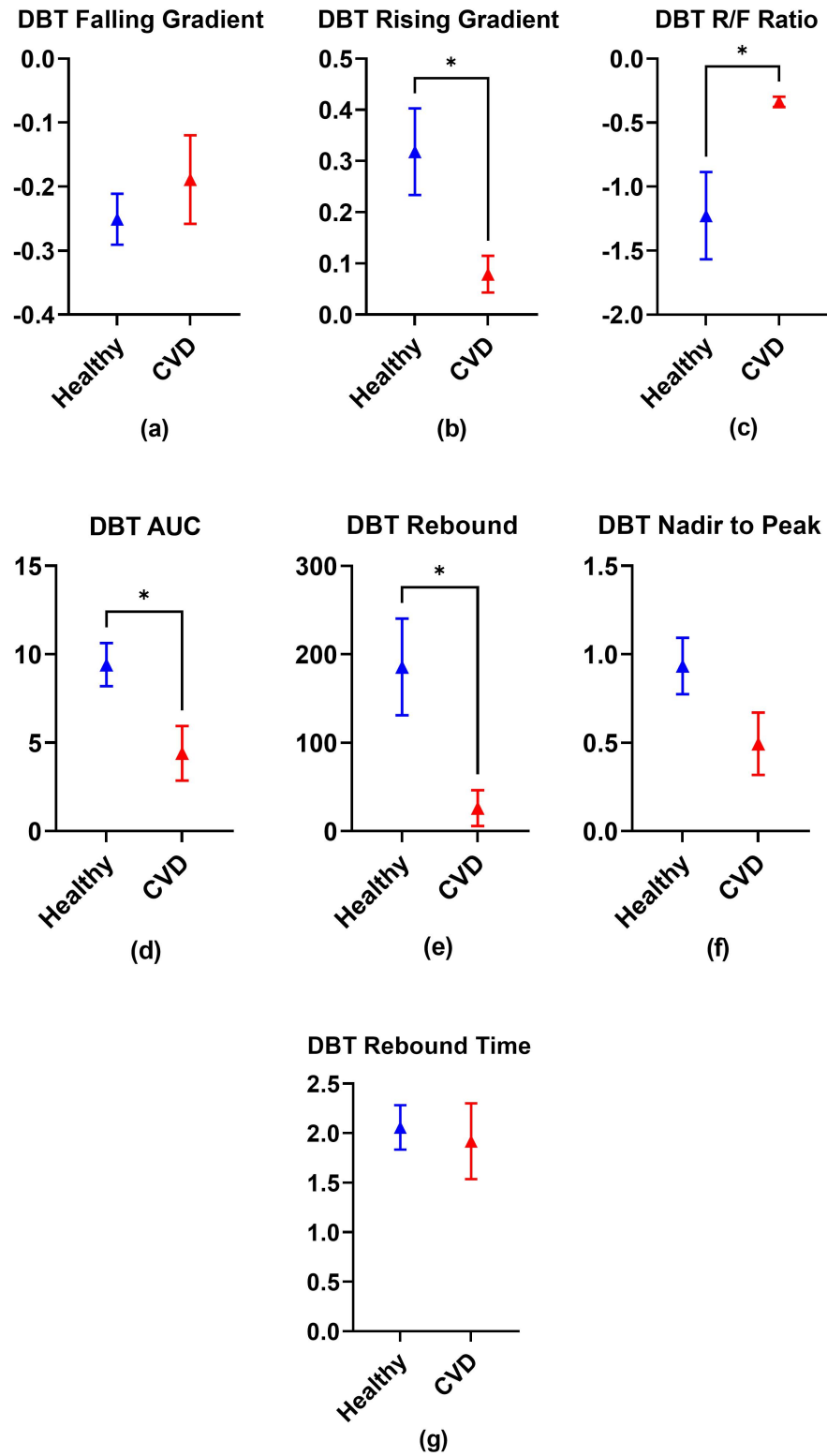


Fig. 6.10: Unpaired t-test plots of DBT Long-term Variation VED Indices: (a) Falling Gradient (b) Rising Gradient (c) R/F Ratio (d) AUC (e) Rebound (f) Nadir to Peak (g) Rebound Time (Significance Level: * $p < 0.05$)

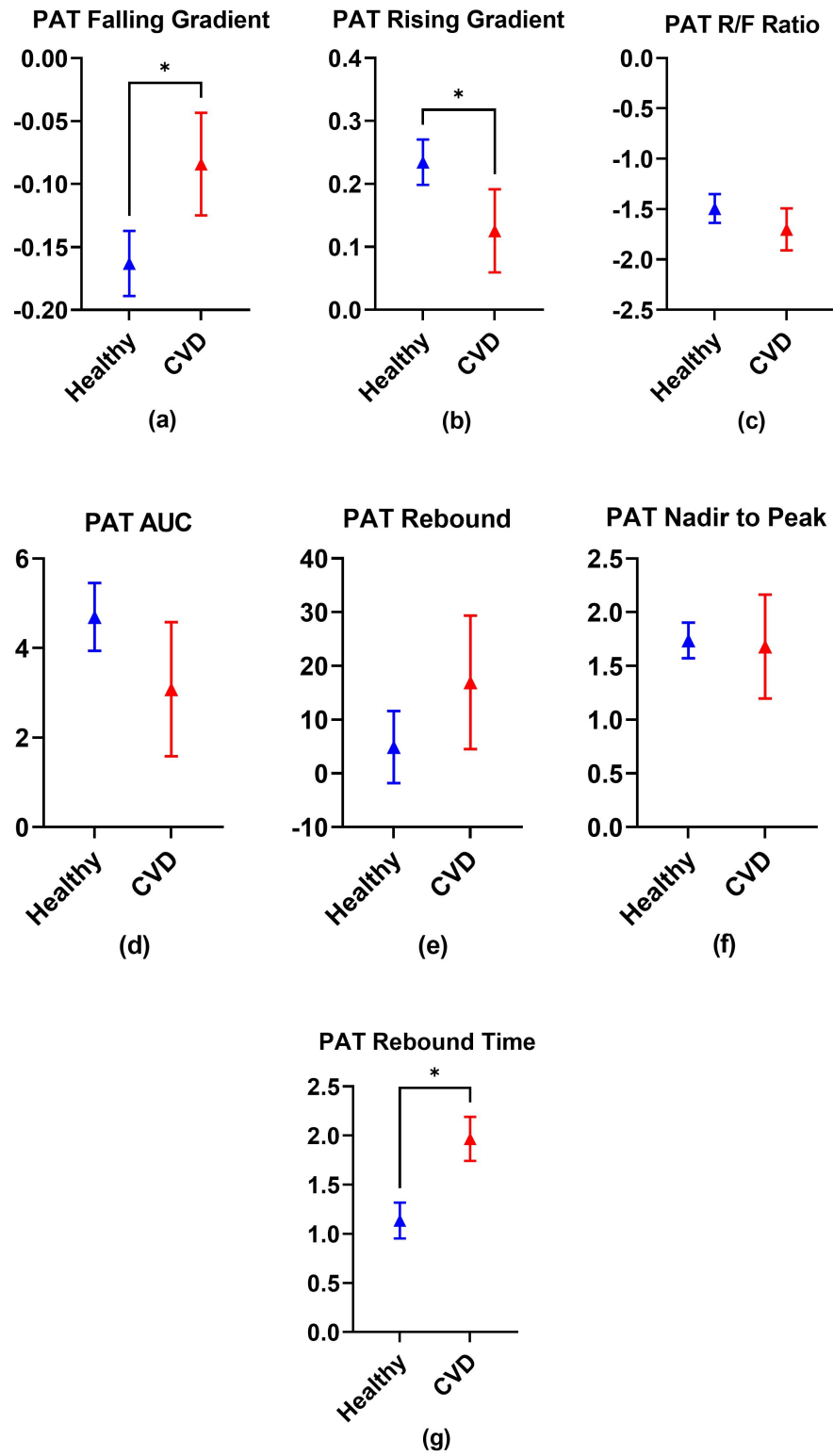


Fig. 6.11: Unpaired t-test plots of PAT Long-term Variation VED Indices: (a) Falling Gradient (b) Rising Gradient (c) R/F Ratio (d) AUC (e) Rebound (f) Nadir to Peak (g) Rebound Time (Significance Level: * $p < 0.05$)

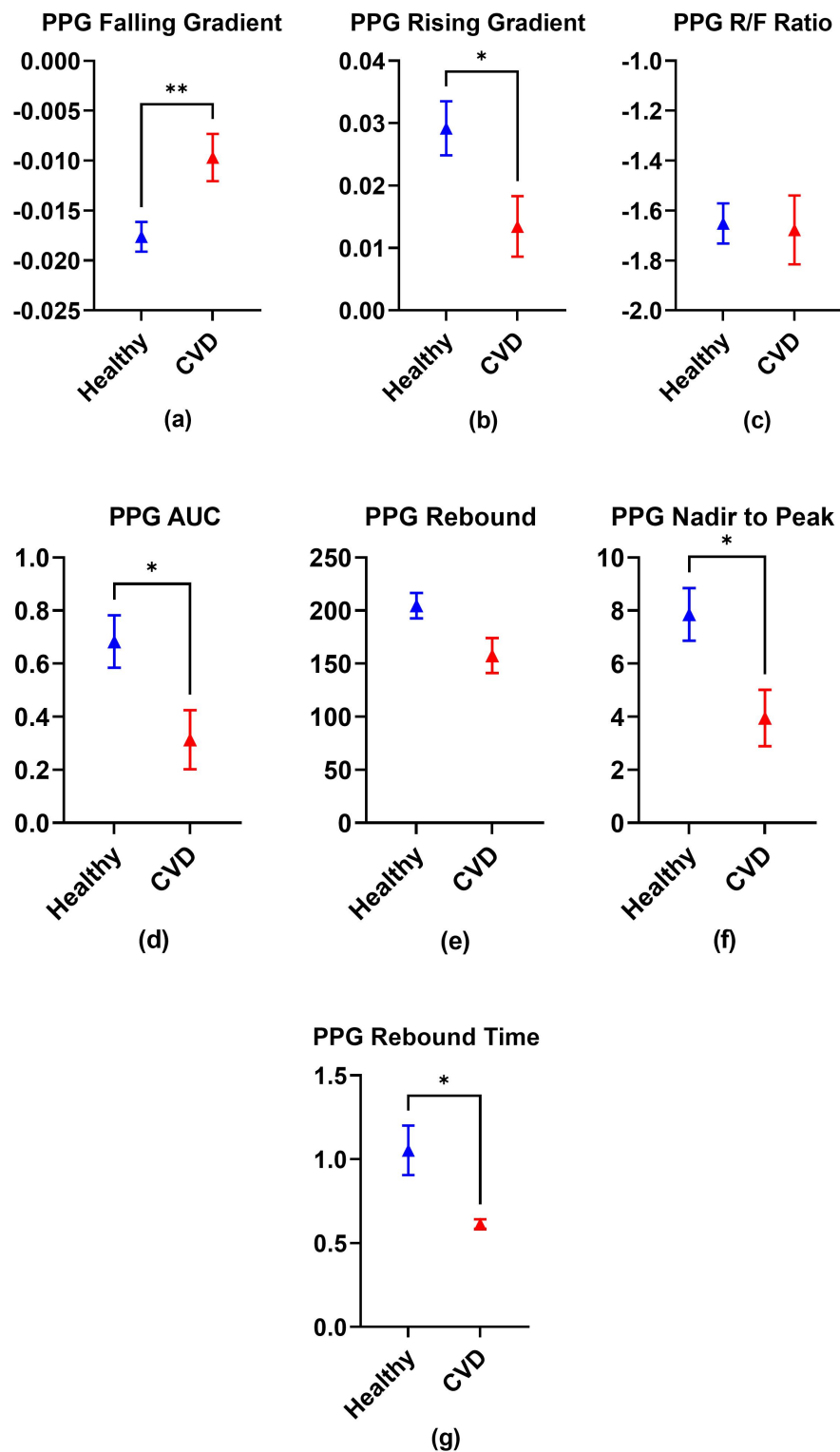


Fig. 6.12: Unpaired t-test plots of PPG Long-term Variation VED Indices: (a) Falling Gradient (b) Rising Gradient (c) R/F Ratio (d) AUC (e) Rebound (f) Nadir to Peak (g) Rebound Time (Significance Level: * p<0.05, ** p<0.01)

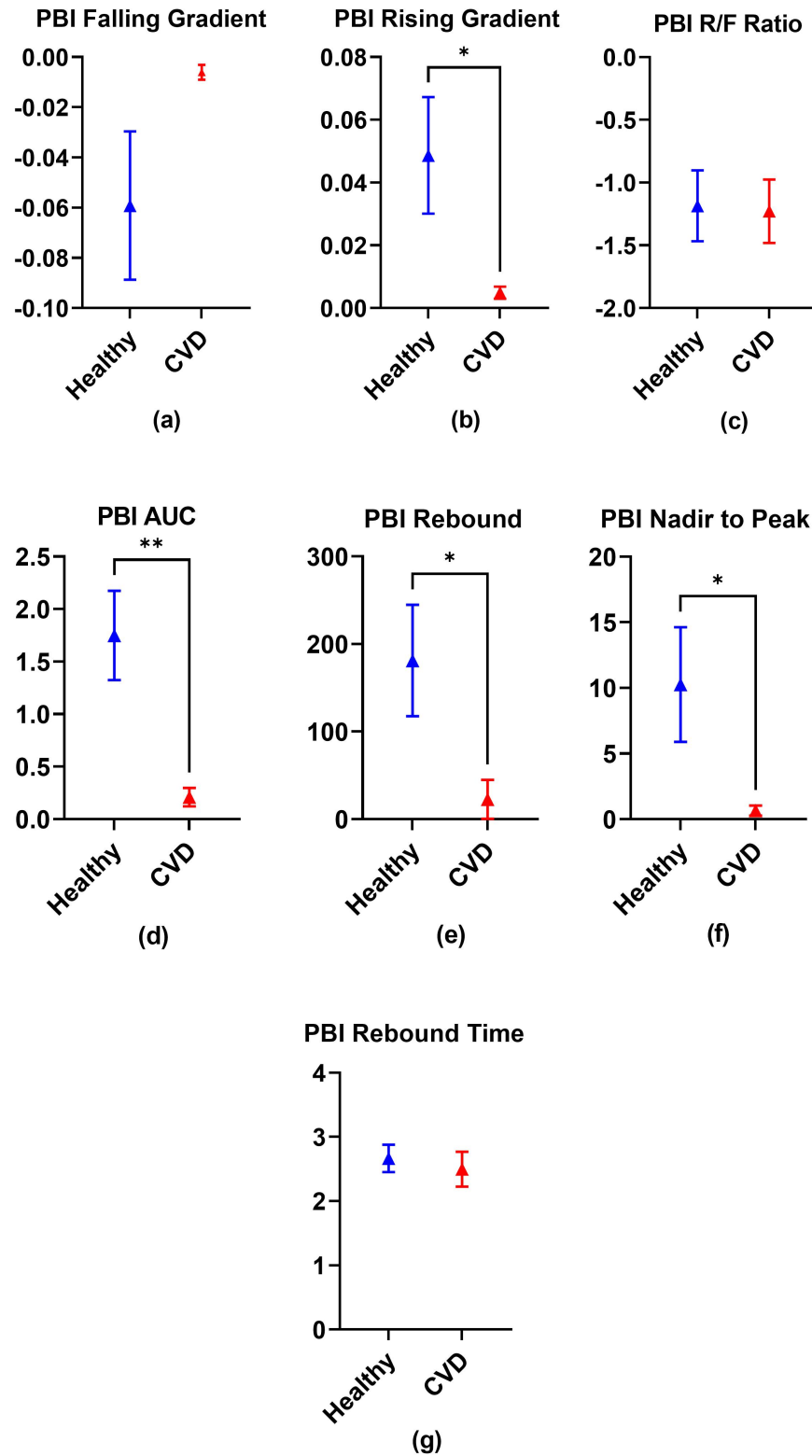


Fig. 6.13: Unpaired t-test plots of PBI Long-term Variation VED Indices: (a) Falling Gradient (b) Rising Gradient (c) R/F Ratio (d) AUC (e) Rebound (f) Nadir to Peak (g) Rebound Time (Significance Level: * p<0.05, ** p<0.01)

CHAPTER 7

CLINICAL STUDY

The clinical study was conducted including five comprehensive participant categories of healthy, healthy but having CVD risk factors (HRF), Prediabetes Mellitus (PDM), Diabetes Mellitus (DM) and CVD with/without DM. This was an extension of the preliminary study explained in chapter 6 which specifically focuses on identifying the presence of Vascular Endothelial Dysfunction (VED) in intermediate stages of HRF, PDM and DM. In contrast to the preliminary study, the inclusion and exclusion criteria for each participant category were explicitly determined by combining both anthropometric data (from the questionnaire) and biomarkers obtained from a blood sample of each participant.

7.1 Blood Sampling

Participants were instructed to undergo twelve hours of fasting prior to the collection of blood samples. In addition, they were advised to refrain from extensive physical activity and consume caffeine, vitamin supplements, nicotine, alcohol and medical drugs during this period. The following tests were done with the blood sample obtained from each participant.

1. Fasting Plasma Glucose (FPG) - Measure of glucose level
2. Glycated Haemoglobin (HbA1c) - Measures the average glucose level over the past 3 months
3. Lipid Profile - Measure of the total amount of cholesterol and its categories
4. C-reactive protein (CRP) - Measures the level of inflammation or infections
5. Serum Creatinine (SCr) - Measures the renal functionality
6. Full blood count (FBC) - Measure of the composition of blood cells

The blood samples were drawn from the participants by a well-qualified phlebotomist at the National Diabetes Centre, Sri Lanka. The biomarker standards of the Diabetes Association of Sri Lanka were followed for the classification of subject categories.

7.2 Determination of the Test Subject Category

7.2.1 Healthy (Control)

The healthy condition is defined by the presence of the following inclusion criteria: physical activity in daily routine; healthy diet; abstinence from alcohol and tobacco; body mass index ($19 \leq \text{BMI} \leq 24$); and not being under medication for or diagnosed for any CVD or DM related conditions. In addition, the participants parents and siblings should also be clear of the diagnosis of any CVD. Laboratory tests were performed to verify the FPG in between the normoglycemic (75 mg/dl to 110 mg/dl), HbA1c in between 4.3% to 5.6% and normal range of lipid profile, CRP, SCr and FBC.

7.2.2 Healthy with CVD risk Factors

The HRF condition is defined by the presence of the following: physical inactivity; unhealthy diet; smoking; alcohol consumption; body mass index ($\text{BMI} \geq 25$: Overweight and $\text{BMI} \leq 18.5$: Underweight); and family history of CVD and DM (in parents and siblings). These are considered the main CVD risk factors that could initiate the development of CVD [5]. In addition, the laboratory tests were verified to be compatible with healthy criteria.

7.2.3 Pre-Diabetes Mellitus

The participant should have only been diagnosed for PDM without any CVD complications, and also be under medication for PDM only. In addition, laboratory tests were performed to verify the fasting plasma glucose level between 110 mg/dl to 125 mg/dl, HbA1c in between 5.7% to 6.4% and normal range of lipid profile, CRP, SCr and FBC.

7.2.4 Diabetes Mellitus

The participant should have been diagnosed only for DM without any CVD complications and be under medication only for DM. In addition, laboratory tests were performed to verify the FPG greater than 125 mg/dl, HbA1c beyond 6.5% with normal lipid profile, CRP, SCr and FBC. Type 1 DM people were excluded from the study.

7.2.5 CVD

It was observed that most of the test subjects diagnosed with CVD also have DM. Therefore, such participants were also included under the CVD category. Taking medication for CVD and diabetes (if present), and the usage of alcohol and tobacco products were accepted as inclusion criteria. Laboratory tests were performed to confirm the lipid profile and SCr ranges within the intermediate/high and normal range of CRP and FBC.

7.3 Anthropometric Data

Mirroring the approach in the preliminary study, anthropometric data were gathered through a questionnaire in the clinical study. In addition to this, waist circumference and Body Fat Index parameters were also collected for the clinical investigation. A total of 69 test subjects Healthy (n=9), HRF (n=15), PDM (n=6), DM (n=15) and CVD (n=24) participated in the experiment and their anthropometric data statistics (Mean $\pm$ Standard Deviation) are tabulated in Table 7.1, revealing a noticeable pattern of parameter values increasing with greater risk association across the subject categories.

TABLE 7.1: ANTHROPOMETRIC DATA OF THE CLINICAL STUDY

Parameter	Healthy (n=9)	HRF (n=15)	PDM (n=6)	DM (n=15)	CVD (n=24)
Age (years)	46 $\pm$ 9	49 $\pm$ 5	53 $\pm$ 8	52 $\pm$ 6	54 $\pm$ 5
Gender (M/F)	2 7	4 11	2 4	9 6	11 13
Height (cm)	159 $\pm$ 7	162 $\pm$ 9	155 $\pm$ 7	162 $\pm$ 9	160 $\pm$ 9
Weight (kg)	63 $\pm$ 9	64 $\pm$ 9	59 $\pm$ 6	60 $\pm$ 15	71 $\pm$ 13
Waist (cm)	92 $\pm$ 6	89 $\pm$ 9	87 $\pm$ 8	93 $\pm$ 11	95 $\pm$ 12
Body Mass Index	24.9 $\pm$ 3.7	24.8 $\pm$ 3.8	25.0 $\pm$ 4.4	26.4 $\pm$ 4.0	27.7 $\pm$ 5.0
Body Fat Index	31.8 $\pm$ 4.3	31.3 $\pm$ 7.7	31.5 $\pm$ 8.1	31.3 $\pm$ 6.3	33.4 $\pm$ 7.3
Systolic (mmHg)	120 $\pm$ 9	119 $\pm$ 11	114 $\pm$ 10	127 $\pm$ 16	131 $\pm$ 15
Diastolic (mmHg)	78 $\pm$ 4	80 $\pm$ 8	81 $\pm$ 7	83 $\pm$ 12	85 $\pm$ 9

7.4 Laboratory Biomarkers

Each participant recruited for the clinical study was given a blood sample as mentioned in section 7.1 to examine the biomarkers to verify the test subject categorization. The Biomarker statistics (Mean $\pm$ Standard Deviation) are tabulated in Table 7.2. The data show that individuals with pre-diabetes mellitus and diabetes mellitus have higher FPG and HbA1c levels compared to healthy individuals. They also have higher triglycerides, Very low-density lipoprotein (VLDL), and cholesterol/HDL ratios, while their direct high density lipoprotein (dHDL) levels are lower. Individuals with CVD risk factors, pre-diabetes mellitus, diabetes mellitus, and CVD with or without diabetes mellitus also have higher low-density lipoprotein (LDL) levels compared to healthy individuals. However, there are no significant differences in creatinine (Scr), White Blood cell count (WBC), Red Blood cell count (RBC), Hemoglobin (HGB), platelet count (PLT), and CRP levels among the different groups.

7.5 Data Collection Procedure

The data collection procedure was performed at the National Diabetic Association, Sri Lanka premises using entirely custom-made devices. Compared to the preliminary

TABLE 7.2: BIOMARKER DATA OF THE CLINICAL STUDY

Parameter	Healthy (n=9)	HRF (n=15)	PDM (n=6)	DM (n=15)	CVD (n=24)
FPG (mg/dl)	98 $\pm$ 8	99 $\pm$ 8	122 $\pm$ 11	186 $\pm$ 77	149 $\pm$ 79
HbA1c (%)	5.4 $\pm$ 0.3	5.6 $\pm$ 0.3	6.5 $\pm$ 0.7	9.1 $\pm$ 2.6	7.2 $\pm$ 1.7
Cholesterol (mg/dl)	206 $\pm$ 22	238 $\pm$ 40	234 $\pm$ 38	214 $\pm$ 49	179 $\pm$ 49
Triglyceride (mg/dl)	101 $\pm$ 23	114 $\pm$ 47	151 $\pm$ 67	139 $\pm$ 54	103 $\pm$ 38
dHDL (mg/dl)	48 $\pm$ 10	52 $\pm$ 9	42 $\pm$ 5	47 $\pm$ 7	43 $\pm$ 10
LDL (mg/dl)	138 $\pm$ 29	164 $\pm$ 36	162 $\pm$ 29	140 $\pm$ 46	115 $\pm$ 39
VLDL (mg/dl)	20 $\pm$ 5	23 $\pm$ 9	30 $\pm$ 13	28 $\pm$ 11	21 $\pm$ 8
Chol/dHDL Ratio	4.5 $\pm$ 1.3	4.7 $\pm$ 1.1	5.6 $\pm$ 0.8	4.7 $\pm$ 1.2	4.2 $\pm$ 0.8
SCr (mg/dl)	0.9 $\pm$ 0.1	0.9 $\pm$ 0.1	1.0 $\pm$ 0.1	0.9 $\pm$ 0.1	0.9 $\pm$ 0.1
WBC ($10^3 \mu\text{l}$)	8.5 $\pm$ 2.3	7.1 $\pm$ 1.3	8.8 $\pm$ 3.2	8.8 $\pm$ 2.6	7.4 $\pm$ 1.8
RBC ($10^3 \mu\text{l}$)	4.6 $\pm$ 0.3	4.6 $\pm$ 0.5	4.5 $\pm$ 0.5	4.8 $\pm$ 0.6	4.4 $\pm$ 0.5
PLT ($10^3 \mu\text{l}$)	262 $\pm$ 52	277 $\pm$ 58	285 $\pm$ 96	260 $\pm$ 60	269 $\pm$ 85
HGB (g/dl)	13.1 $\pm$ 2.2	13.0 $\pm$ 1.4	12.6 $\pm$ 2.7	14.3 $\pm$ 1.9	13.0 $\pm$ 1.4
CRP (mg/L)	3.5 $\pm$ 2.9	3.7 $\pm$ 3.4	4.8 $\pm$ 5.2	3.4 $\pm$ 3.2	4.5 $\pm$ 5.1

study, the inclusion and exclusion criteria of test subjects were explicitly determined as explained in section 7.2. Similar to the preliminary study the experimental protocol in section 6.1 was explained to each individual and obtained the written consent before the data collection procedure. All the test subjects initially underwent the questionnaire filled out for anthropometric data and the blood sampling for laboratory biomarker data to verify the test subject categorization. Thereby, a total of 69 volunteered test subjects aged between 35 to 60 years categorized in Healthy (n=9), HRF (n=15), PDM (n=6), DM (n=15) and CVD (n=24) participated in the clinical study. Both validated custom-made devices were used to for the data acquisition of DBT, PAT, PPG and PBI physiological parameters with the inducement of RH for the VED assessment.

7.6 Data Preprocessing and Data Normalization

The collected data stored and preprocessed exactly following the preliminary study procedure as explained in section 5.3 and followed by the data normalization explained in section 6.5 before the data analysis. The long-term variations of all the physiological parameters concerning each test subject category were graphically plotted compared to the Healthy.

7.6.1 Long term variations of Physiological Parameters

The long term variations of DBT, PAT, PPG and PBI physiological parameters were plotted contrasting test subject categories (HRF, PDM, DM and CVD) against healthy in Figures 7.1, 7.2, 7.3 and 7.4 respectively. The solid line represents the mean long-

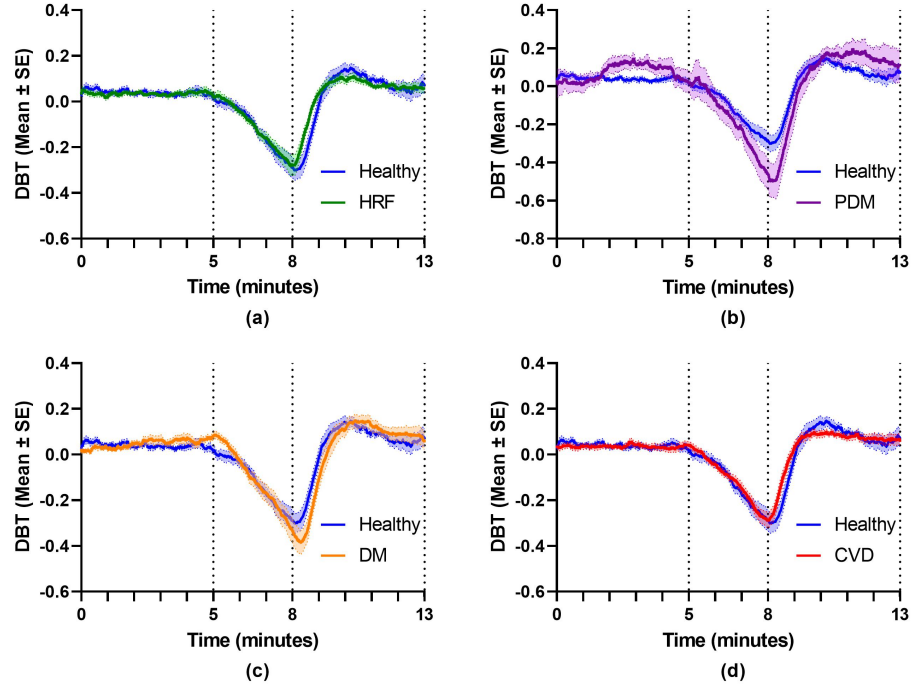


Fig. 7.1: Normalized Long-term Variations of DBT :
(a) HRF Vs Healthy (b) PDM Vs Healthy (c) DM Vs Healthy (d) CVD Vs Healthy

term variation, while the shaded region indicates the standard error. The initial five minutes (0 to 5) represent the baseline variation, the following three minutes (5 to 8) correspond to the occlusion phase, and the final five minutes (8 to 13) illustrate the variation of PRH.

7.6.2 Short-term variations of Physiological Parameters

In addition to the long-term variations of physiological parameters, the short-term variations were assessed from PAT and PPG pulsatile signals. The pulsatile patterns were observable only in baseline and PRH stages while no response occurred during the occluded stage. However, the analysis was conducted using the pulsatile patterns in PRH stage. The averaged pulsatile signals of PAT and PPG are plotted in Fig. 7.5 for the occluded arm and control arm respectively.

Following the methodology adopted in the long-term variation analysis, the short-term data were normalized as in equation 6.1. The normalized short-term pulsatile variations at the initial minute of the PRH stage for both PAT and PPG are shown in Fig. 7.6. It was noted that immediately following cuff release, faint pulses were recorded, and during the post-reactive hyperemia (PRH) stage, the pulse amplitude gradually increased until stabilizing alongside restoring physiological conditions caused by re-

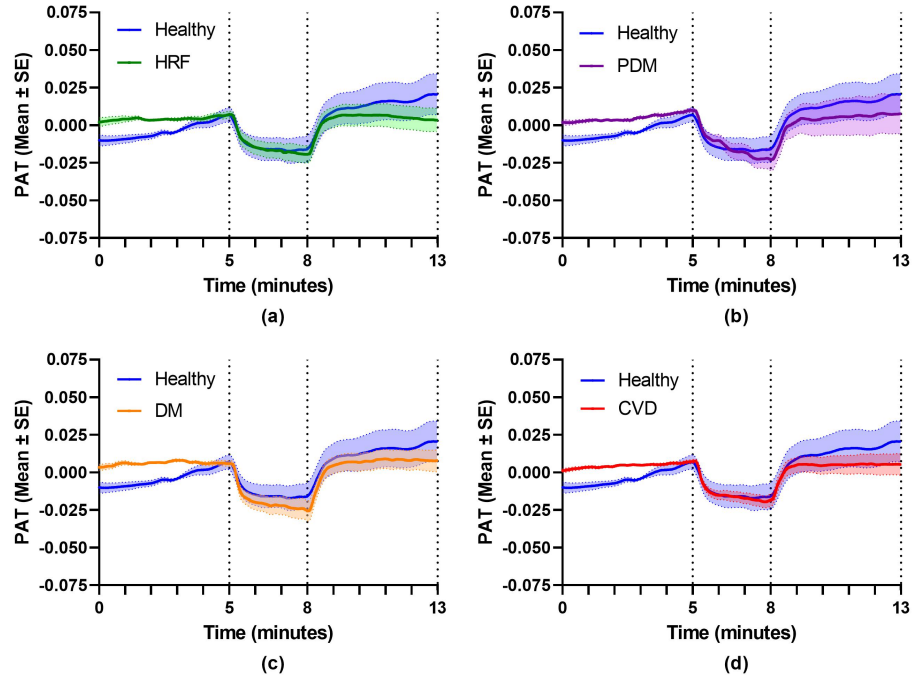


Fig. 7.2: Normalized Long-term Variations of PAT :
(a) HRF Vs Healthy (b) PDM Vs Healthy (c) DM Vs Healthy (d) CVD Vs Healthy

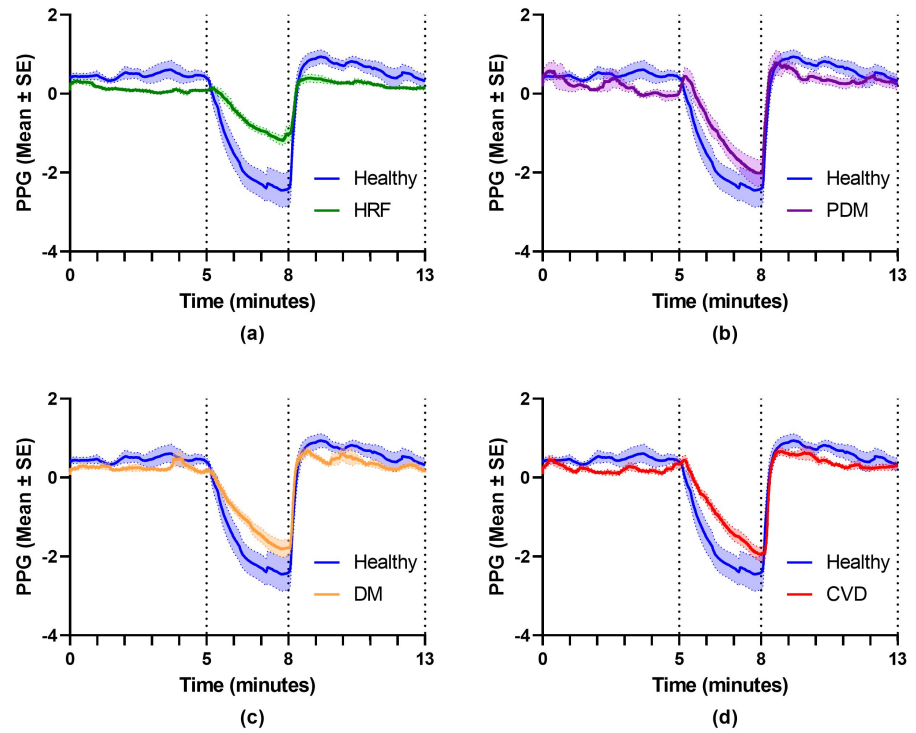


Fig. 7.3: Normalized Long-term Variations of PPG :
(a) HRF Vs Healthy (b) PDM Vs Healthy (c) DM Vs Healthy (d) CVD Vs Healthy

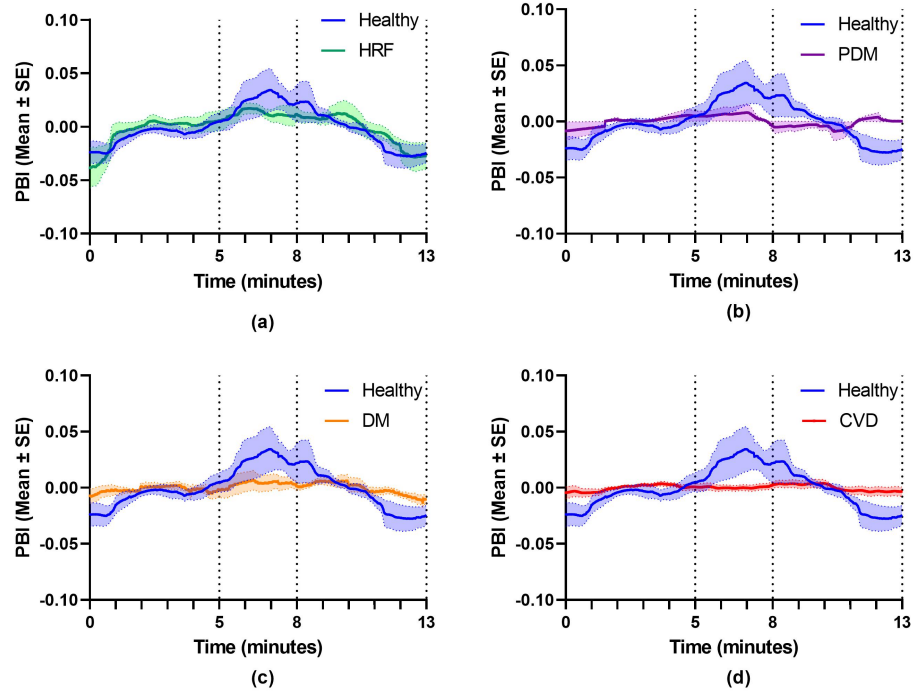


Fig. 7.4: Normalized Long-term Variations of PBI :
(a) HRF Vs Healthy (b) PDM Vs Healthy (c) DM Vs Healthy (d) CVD Vs Healthy

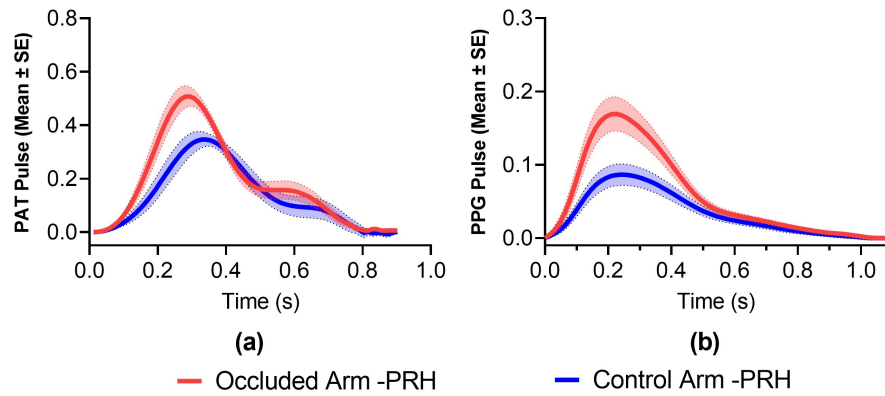


Fig. 7.5: Averaged Pulsatile Short-term Variations at PRH stage
(Occluded Arm Vs Control Arm):
(a) Short-term Variations of PAT (b) Short-term Variations of PPG

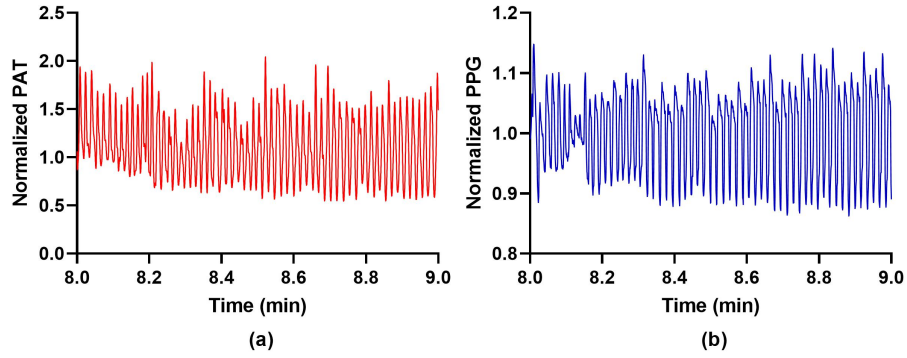


Fig. 7.6: Short-term Pulsatile variations at the initial minute of the PRH stage:

- (a) Normalized PAT Short-term Pulsatile variations
- (b) Normalized PPG Short-term Pulsatile variations

active hyperemia in the peripheral vasculature. Therefore, short-term variation analysis was conducted throughout the PRH stage, with indices calculated for each time-stamped pulsatile from 8th minute to 11th minute rather than analyzed from an averaged pulsatile.

7.7 Index Calculation

7.7.1 Long-term variation Indices

Similar to the preliminary study, 07 VED indices were calculated individually for each physiological parameter to assess long-term variations. Apart from long-term trends, PAT and PPG signals showed short-term pulsatile patterns, hence the analysis was expanded to investigate the short-term variations using specific indices to assess the VED. However, these short-term trends were not visualized in both DBT and BI parameters. Temperature would not change to subtle pulsatile variations of the blood flow. Conversely, it was impossible to capture the short-term variations in PBI due to the limitation of using the bipolar electrode configuration instead of the tetrapolar electrode configuration when developing the device.

7.7.2 Short-term variation Indices

Additionally, twelve VED indices: 1) Reflective index (RI), 2) Logarithmic Reflective index (LnRI), 3) Pulse Amplitude (Di-Amp), 4) Pulse Area (Di-Area), 5) Stiffness index (SI), 6) Crest Time (CT), 7) Crest Time Ratio (CTR), 8) Delta Time (DT), 9) Slope Transit Time (STT), 10) Augmented index (AI), 11) Pulse width to Amplitude Ratio (PAR) and 12) Buckberg Sub-Endocardial Viability Ratio (BSEVR) [96, 106–108] were chosen for the short-term variation analysis of both PAT and PPG physiologi-

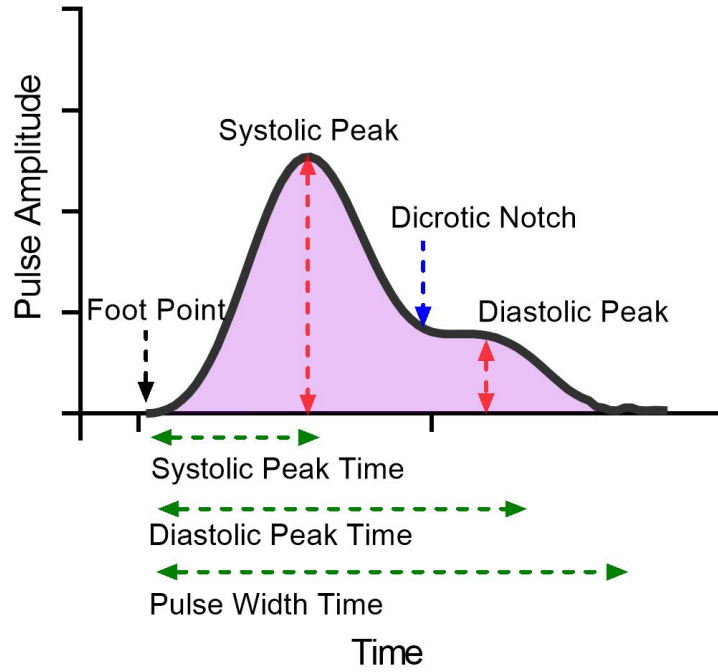


Fig. 7.7: Parameters and Regions of Pulse for Short term variation Indices

cal parameters. The following Fig. 7.7 has illustrated the parameters and regions of interest used for short-term variation index calculations. It was highlighted that the short-term variation indices are mainly derived from either pulse amplitude or pulse time parameters.

The RI is computed using equation 7.1 and the natural logarithmic value of RI (LnRI) is obtained from the equation 7.2.

$$\text{Reflective Index (RI)} = \left(\frac{\text{Diastolic Peak}}{\text{Systolic Peak}} \right) * 100 \quad (7.1)$$

$$\text{Logarithmic Reflective Index (LnRI)} = \ln(\text{Reflective Index}) \quad (7.2)$$

Both of these indices are used to assess the shift in vascular tone from vasoconstriction to vasodilation at PRH. Elevated RI/LnRI values indicate vasoconstriction, while a subsequent decrease in these values signifies a transition toward vasodilation.

The Di-Amp is determined by assessing the amplitude variation during the PRH stages in both the occluded arm and control arm as described in equation 7.3. Higher Di-Amp values leads to increased arterial stiffness and lower Di-Amp values leads to reduced

stroke volume. Both conditions lead into risk of developing CVD.

$$\text{Pulse Amplitude Index (Di - Amp)} = \frac{\text{Pulse Amplitude in Occluded Arm}}{\text{Pulse Amplitude in Control Arm}} \quad (7.3)$$

The Pulse area (Di-Area) is calculated by the ratios of the area under the pulse of the occluded arm and control arm as outlined in equation 7.4. The higher Di-Area signifies the robust and healthy endothelial function and endothelium is strongly responsive to brief ischemic conditions. The lower Di-Area reflects the signs of VED and high risk of development of CVDs.

$$\text{Pulse Area Index (Di - Area)} = \left(\frac{\text{Pulse Area in Occluded Arm}}{\text{Pulse Area in Control Arm}} \right) \quad (7.4)$$

Concurrently, the stiffness index (SI) is calculated by dividing the height of each test subject by the time interval between the diastolic and systolic peaks, as illustrated in equation 7.5. Higher SI values are indicative of increased arterial stiffness and promoting vascular aging, whereas lower SI values suggest reduced arterial stiffness and better vascular health.

$$\text{Stiffness Index (SI)} = \left(\frac{\text{Height}}{\text{Diastolic Peak Time} - \text{Systolic Peak Time}} \right) \quad (7.5)$$

The crest time (CT) is computed by the time difference between the foot point and the maximum peak point of the pulse (Systolic Peak Time), as specified in equation 7.6. CT is then divided by the pulse width time to derive the CT ratio index, as demonstrated in Equation 7.7. The Delta time is determined by the ratio between the Systolic Peak Time and the Diastolic Peak Time, as shown in equation 7.8. All these three indices determine the compliance of the arteries where the increased values reflects healthy elastic arteries and the decreased values indicates higher arterial stiffness which lead into arteriosclerosis.

$$\text{Crest Time Index (CT)} = (\text{Systolic Peak Time} - \text{Foot Point Time}) \quad (7.6)$$

$$\text{Crest Time Ratio (CTR)} = \left(\frac{CT}{\text{Pulse Width Time}} \right) \quad (7.7)$$

$$\text{Delta Time Index (DT)} = \left(\frac{\text{Systolic Peak Time}}{\text{Diastolic Peak Time}} \right) \quad (7.8)$$

Additionally, the slope transit time (STT) is derived from the ratio between the Systolic Peak Time and the Systolic Peak Amplitude, as indicated in Equation 7.9. Higher

STT values are indicative of healthy endothelial function and effective vasodilation, whereas lower STT values suggest increased arterial stiffness and the presence of VED.

$$\text{Slope Transit Time Index (STT)} = \left(\frac{\text{Systolic Peak Time}}{\text{Systolic Peak}} \right) \quad (7.9)$$

The augmented index is determined by normalizing the amplitude difference between the Systolic Peak and Diastolic Peak by the Systolic Peak, as depicted in equation 7.10. Pulse width to Amplitude Ratio (PA) is computed following equation 7.11. Both indices are used to assess endothelial function, where higher index values indicate a healthy vascular tone and optimal endothelial function. Conversely, lower index values are associated with VED and increased arterial stiffness.

$$\text{Augmented Index (AI)} = \left(\frac{\text{Systolic Peak} - \text{Diastolic Peak}}{\text{Systolic Peak}} \right) \quad (7.10)$$

$$\text{Pulse Width to Amplitude Index (PA)} = \left(\frac{\text{Pulse Width Time}}{\text{Systolic Peak}} \right) \quad (7.11)$$

Lastly, BSEVR is calculated by taking the ratio of the area under the curve of the diastolic portion to the area under the curve of the systolic portion, as described in Equation 7.12. The areas are split from the dicrotic notch as shown in Fig. 7.7. Higher BSEVR values indicate that the heart is receiving an comfortable oxygen supply with presence of healthy cardiovascular function. When the presence of lower BSEVR values reflect poor oxygen supply leading into myocardial ischemia and arterial stiffness.

$$\text{Buckberg Sub Endocardial Viability Ratio (BSEVR)} = \left(\frac{\text{Diastolic Area}}{\text{Systolic Area}} \right) \quad (7.12)$$

7.7.3 Short-term variations of PAT and PPG Parameters

As outlined in Section 7.6.2, the indices were computed for the entire normalized PRH stage in discrete intervals of the pulse sequence. Subsequently, separate graphs were generated for PAT and PPG for each index, with comparisons made to Healthy individuals within each test subject category.

7.7.3.1 short-term variation of PAT Indices

The short-term variation indices graphs plotted for PAT are as shown in Figures 7.8, 7.9, 7.10, 7.11, 7.12, 7.13, 7.14, 7.15, 7.16, 7.17, 7.18 and 7.19 respectively.

The RI and LnRI indices plots of PAT depicted in Fig. 7.8 and 7.9 have shown gradually decrease of values towards the latter part of the PRH stage. It has been observed that the healthy group demonstrates a greater rate of decay compared to the other test

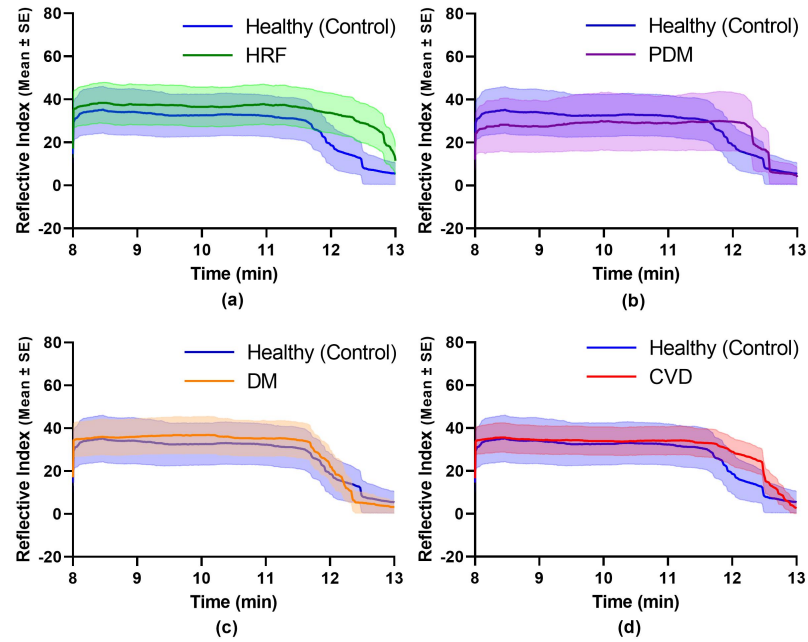


Fig. 7.8: Normalized Short-term Variations of PAT Reflective Index (RI):
(a) HRF Vs Healthy (b) PDM Vs Healthy (c) DM Vs Healthy (d) CVD Vs Healthy

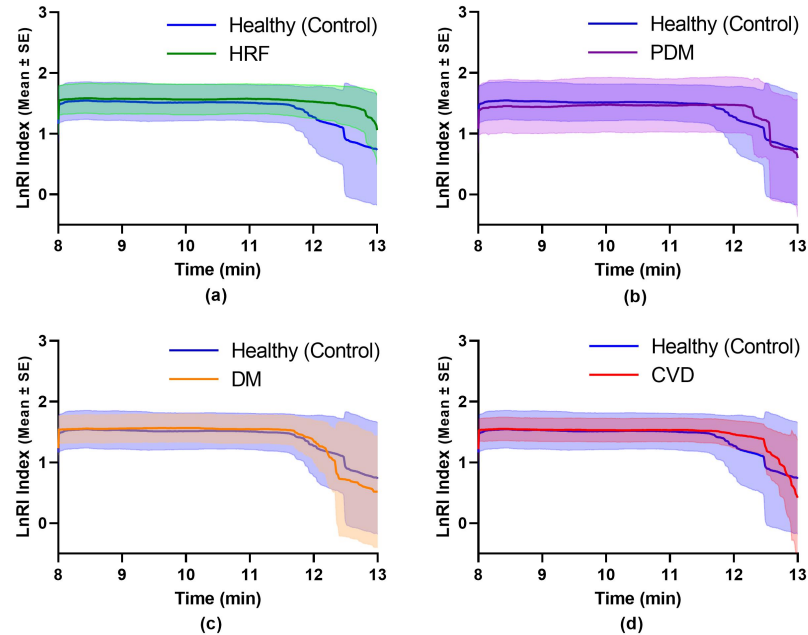


Fig. 7.9: Normalized Short-term Variations of PAT Natural logarithmic Reflective Index (LnRI): (a) HRF Vs Healthy (b) PDM Vs Healthy (c) DM Vs Healthy (d) CVD Vs Healthy

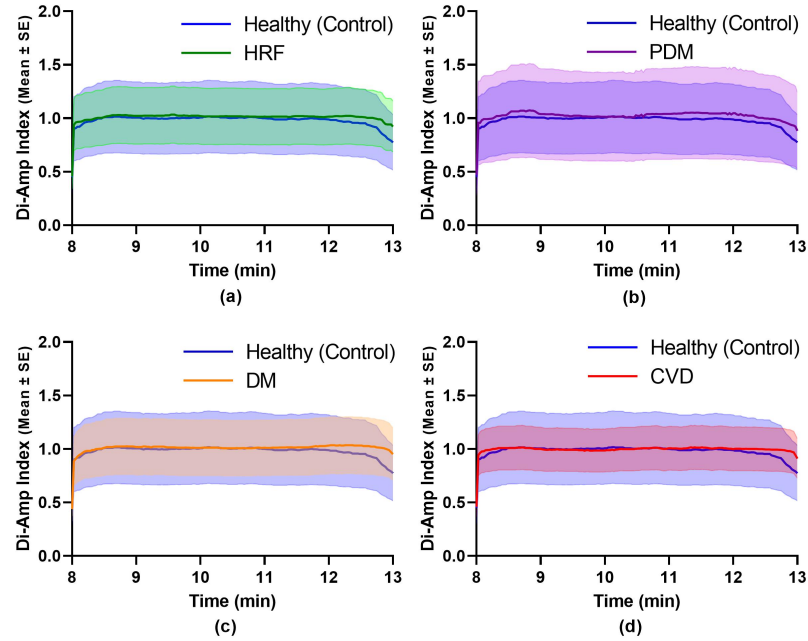


Fig. 7.10: Normalized Short-term Variations of PAT Pulse Amplitude Index (Di-Amp): (a) HRF Vs Healthy (b) PDM Vs Healthy (c) DM Vs Healthy (d) CVD Vs Healthy

subject categories, indicating more robust endothelial function. In contrast, a delayed decline in the HRF, PDM and CVD categories is clearly evident in the graphical representations.

The Di-Amp graphical plots of PAT depicted in Fig. 7.10 demonstrate consistent variations between the healthy group and the other subject categories. However, a slight decline in the healthy category becomes evident during the later stage of PRH.

The Di-Area graphical plots of PAT shown in Fig. 7.11 illustrate a sharp increase in pulse area at the onset of PRH, followed by a gradual decrease toward the later stages of PRH. The healthy plot has significantly demonstrated high values through the PRH reflecting the healthy endothelial function compared to remaining categories.

The SI graphical plots of PAT depicted in Fig. 7.12 demonstrate the slight incline and gradual decrement towards the latter part of PRH. Due to the normalization applied to the SI values, the resulting trends appear inversely in the graphs, with the healthy group exhibiting higher values compared to the other categories. This pattern indicates better vascular health and lower arterial stiffness within the healthy group.

The CT, CTR and DT indices of PAT depicted in Figures 7.13, 7.14 and 7.15 have

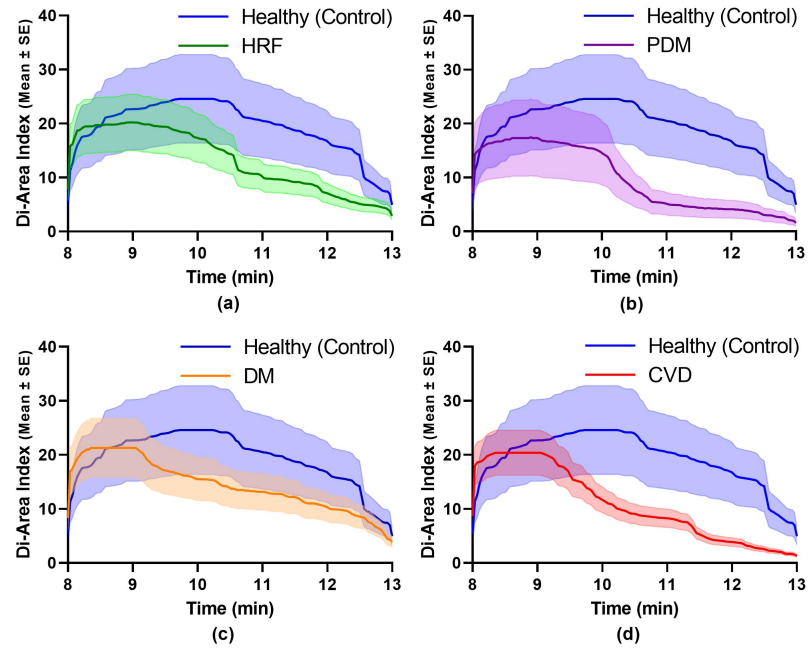


Fig. 7.11: Normalized Short-term Variations of PAT Pulse Area Index (Di-Area):
(a) HRF Vs Healthy (b) PDM Vs Healthy (c) DM Vs Healthy (d) CVD Vs Healthy

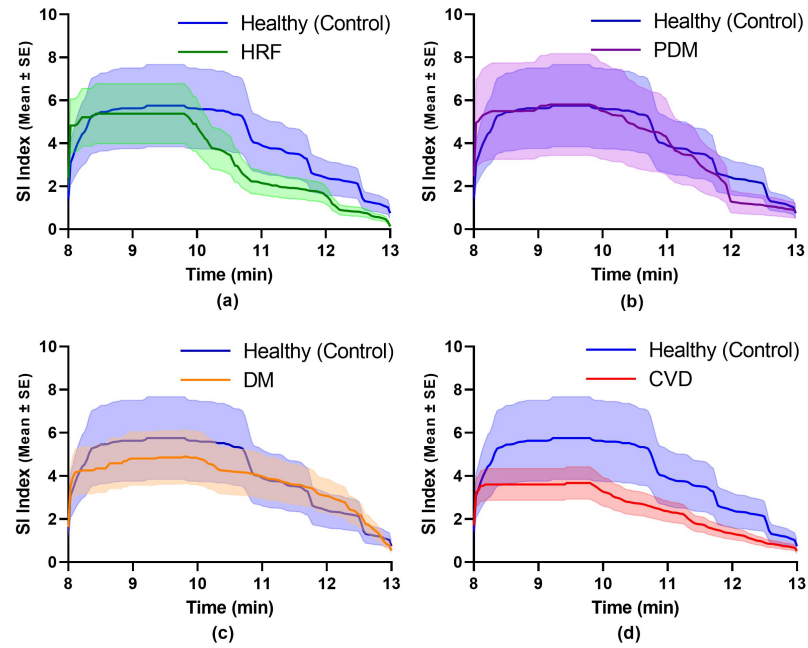


Fig. 7.12: Normalized Short-term Variations of PAT Stiffness Index (SI):
(a) HRF Vs Healthy (b) PDM Vs Healthy (c) DM Vs Healthy (d) CVD Vs Healthy

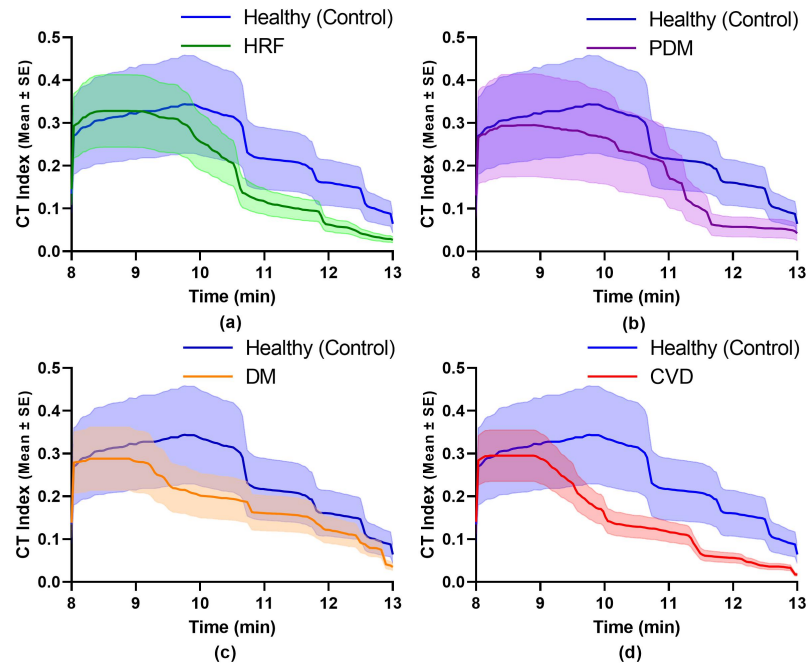


Fig. 7.13: Normalized Short-term Variations of PAT Crest Time Index (CT):
(a) HRF Vs Healthy (b) PDM Vs Healthy (c) DM Vs Healthy (d) CVD Vs Healthy

demonstrated similar declining trend patterns during the PRH. In these three figures, the healthy category demonstrates higher index values compared to the other test subject groups. This observation suggests that healthy individuals possess greater arterial compliance, enabling their vasculature to better adapt to brief ischemic conditions. In contrast, the remaining categories exhibit a more rapid decline in index values, reflecting a reduced capacity to adapt to transient ischemia, which may be attributed to increased arterial stiffness.

The STT, AI and PA indices plots of PAT presented in Figures 7.16, 7.17 and 7.18 demonstrate significant trend variations in the healthy category when compared only with the DM and CVD groups. The healthy, HRF, and PDM groups exhibit higher values for all three indices with similar trend patterns, indicating prompt vasodilation and relatively well-preserved endothelial function during the PRH phase.

The BSEVR plots of PAT depicted in Fig.7.19 demonstrate significant differences in trend patterns between the healthy group and the remaining test subject categories. The higher index values observed in the healthy category reflect better cardiovascular health and an adequate myocardial oxygen supply.

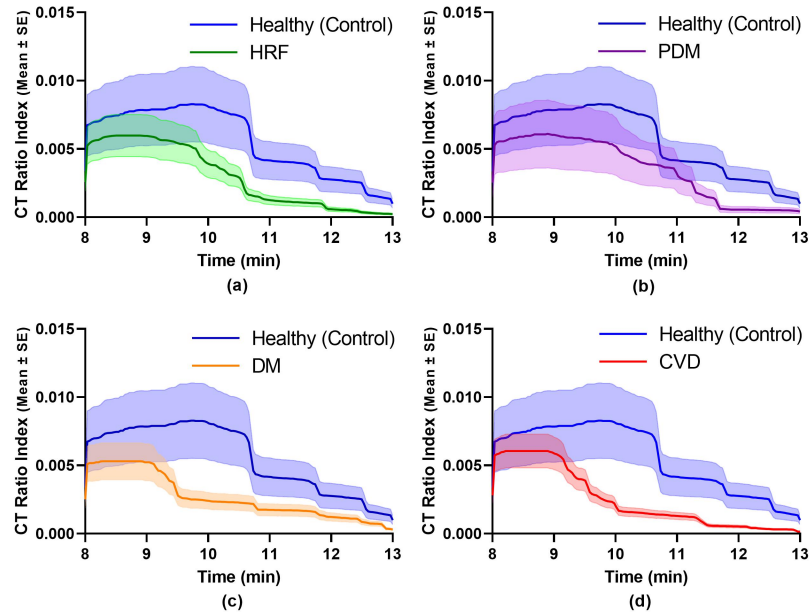


Fig. 7.14: Normalized Short-term Variations of PAT Crest Time Ratio (CTR):
(a) HRF Vs Healthy (b) PDM Vs Healthy (c) DM Vs Healthy (d) CVD Vs Healthy

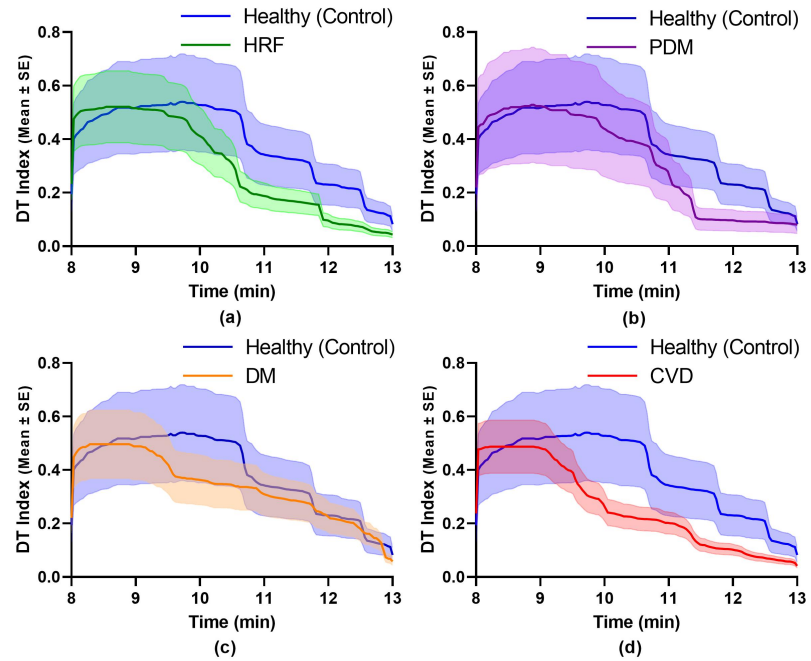


Fig. 7.15: Normalized Short-term Variations of PAT Delta Time Index (DT):
(a) HRF Vs Healthy (b) PDM Vs Healthy (c) DM Vs Healthy (d) CVD Vs Healthy

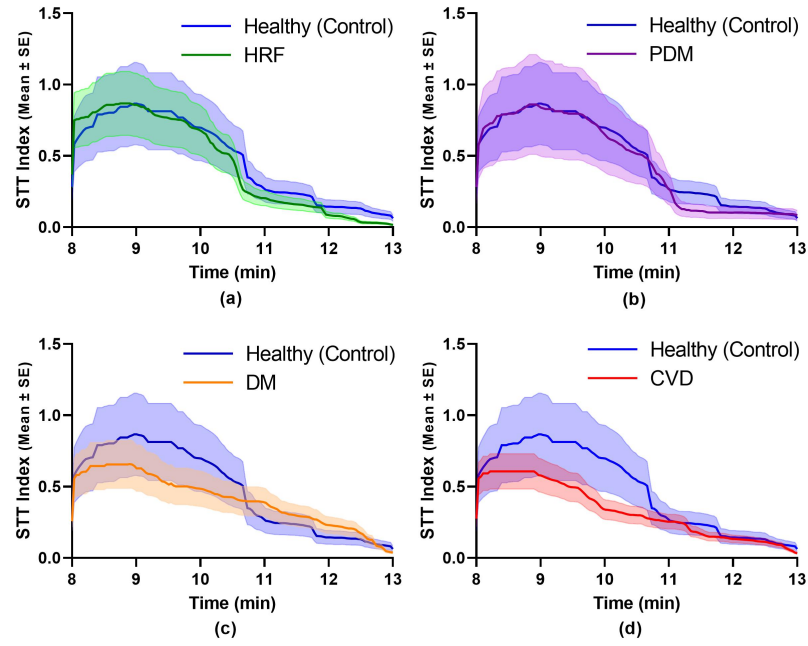


Fig. 7.16: Normalized Short-term Variations of PAT Slope Transit Time Index (STT):
(a) HRF Vs Healthy (b) PDM Vs Healthy (c) DM Vs Healthy (d) CVD Vs Healthy

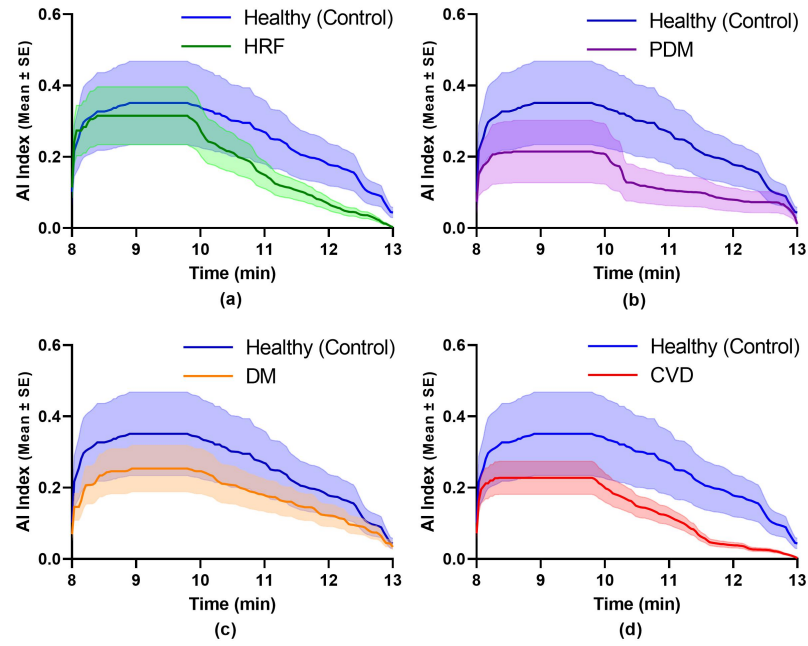


Fig. 7.17: Normalized Short-term Variations of PAT Augmented Index (AI):
(a) HRF Vs Healthy (b) PDM Vs Healthy (c) DM Vs Healthy (d) CVD Vs Healthy

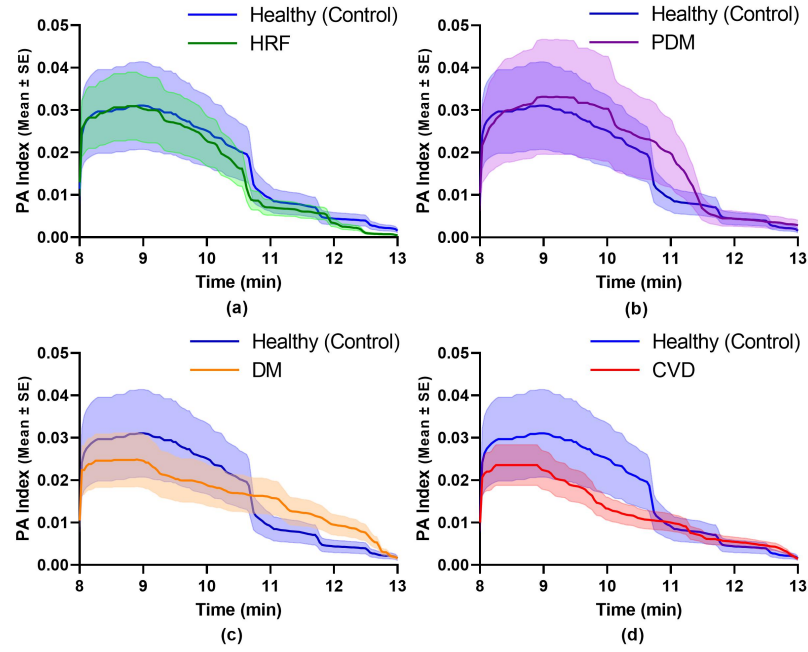


Fig. 7.18: Normalized Short-term Variations of PAT Pulse Width to Amplitude Index (PA): (a) HRF Vs Healthy (b) PDM Vs Healthy (c) DM Vs Healthy (d) CVD Vs Healthy

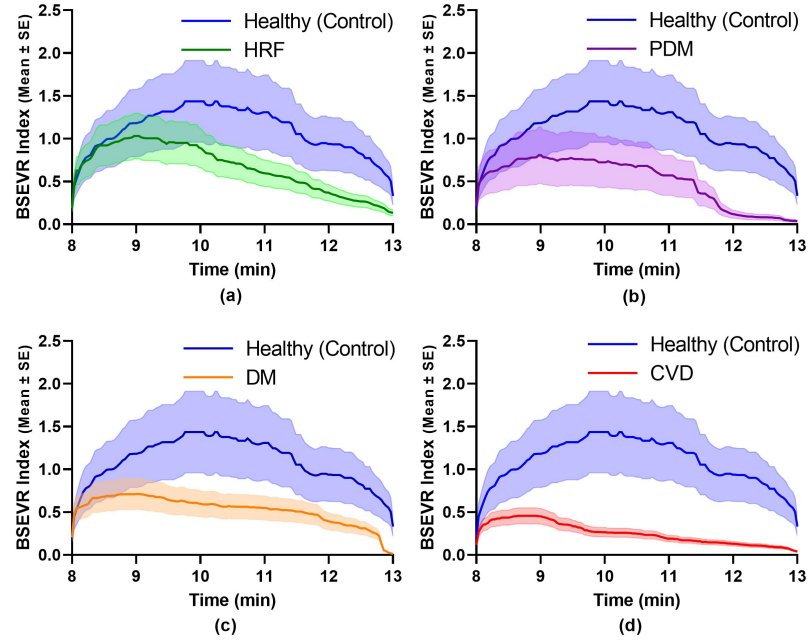


Fig. 7.19: Normalized Short-term Variations of PAT Buckberg Sub Endocardial Viability Ratio (BSEVR): (a) HRF Vs Healthy (b) PDM Vs Healthy (c) DM Vs Healthy (d) CVD Vs Healthy

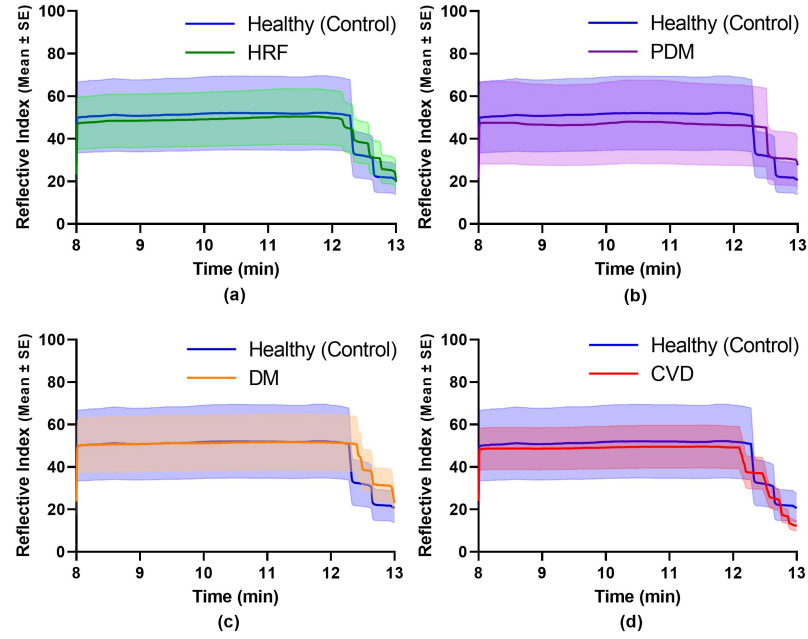


Fig. 7.20: Normalized Short-term Variations of PPG Reflective Index (RI):
(a) HRF Vs Healthy (b) PDM Vs Healthy (c) DM Vs Healthy (d) CVD Vs Healthy

7.7.3.2 short-term variation of PPG Indices

In addition, the short-term variation indices graphs plotted for PPG are as shown in Figures 7.20, 7.21, 7.22, 7.23, 7.24, 7.25, 7.26, 7.27, 7.28, 7.29, 7.30 and 7.31 respectively.

The RI and LnRI indices of PPG, depicted in Figures 7.20 and 7.21, demonstrate a slight responsiveness toward the latter part of the PRH phase, as the index values of the healthy category decrease more steeply compared to the other subject groups, implying a more prompt vasodilatory response.

The Di-Amp PPG plots illustrated in Fig. 7.22 exhibit a relatively steady variation, indicating no significant observable impact across the subject groups.

The Di-Area index plots of PPG depicted in Fig. 7.23 has demonstrated that higher index values in healthy category compared to the other test subject categories indicating that capability of healthy individuals to adapt to brief ischemic conditions due to healthy endothelial function whereas the remaining subject categories reflect poorer responsiveness, likely associated with VED.

The SI index plots of PPG shown in Fig. 7.24 demonstrate a parabolic variation in

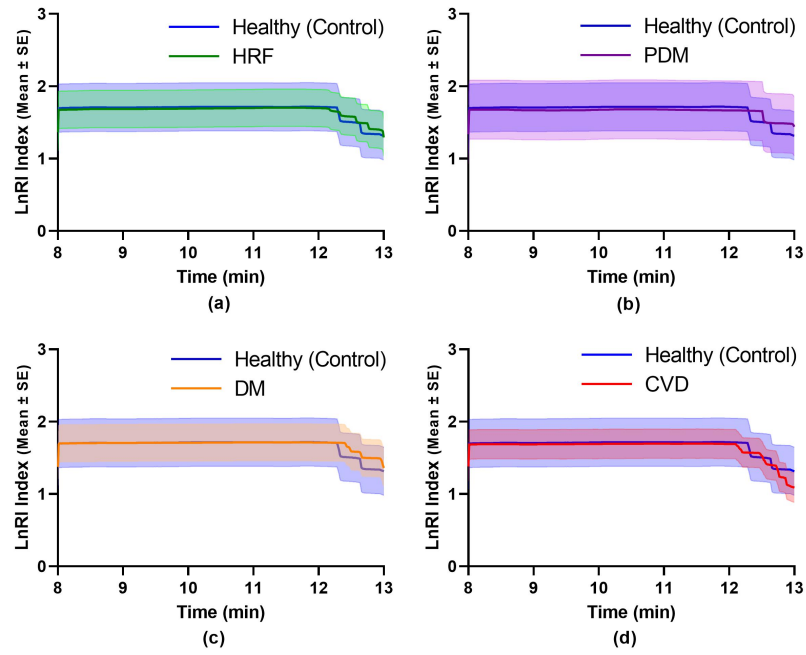


Fig. 7.21: Normalized Short-term Variations of PPG Natural logarithmic Reflective Index (LnRI): (a) HRF Vs Healthy (b) PDM Vs Healthy (c) DM Vs Healthy (d) CVD Vs Healthy

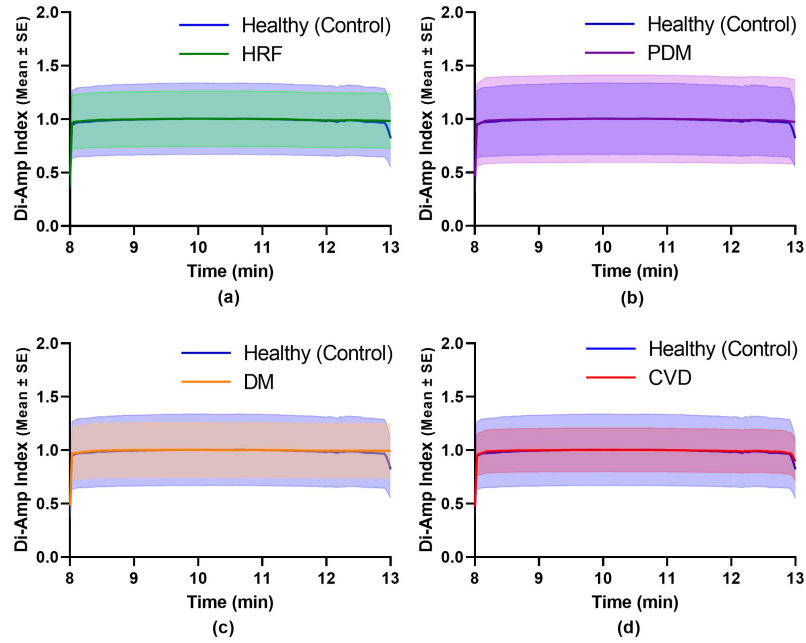


Fig. 7.22: Normalized Short-term Variations of PPG Pulse Amplitude Index (Di-Amp): (a) HRF Vs Healthy (b) PDM Vs Healthy (c) DM Vs Healthy (d) CVD Vs Healthy

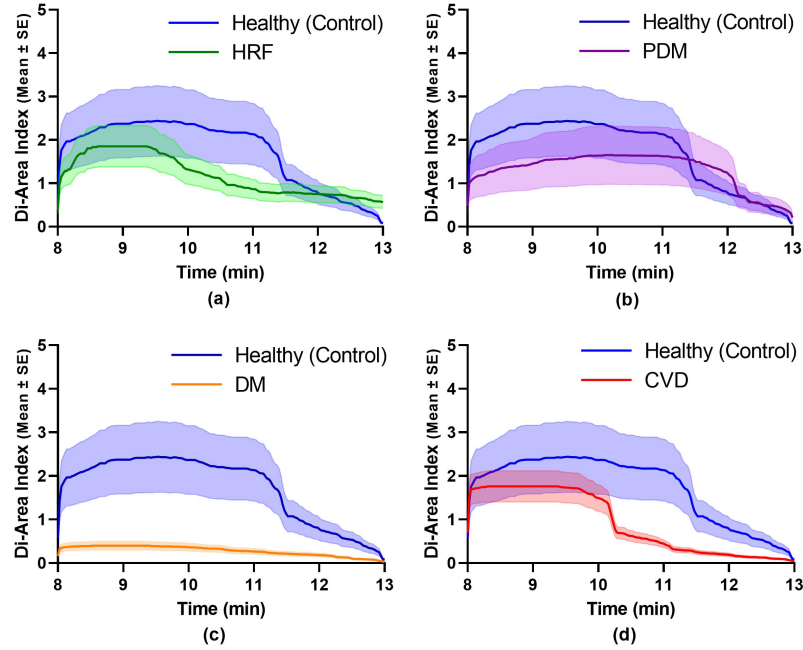


Fig. 7.23: Normalized Short-term Variations of PPG Pulse Area Index (Di-Area):
(a) HRF Vs Healthy (b) PDM Vs Healthy (c) DM Vs Healthy (d) CVD Vs Healthy

the healthy and PDM groups, with greater index values compared to the remaining categories, throughout the PRH phase. Due to the normalization applied to the SI values, the resulting trends appear inversely in the graphs. This pattern indicates better vascular health and lower arterial stiffness in the healthy and PDM categories.

The CT and CTR index plots of PPG, depicted in Figures 7.25 and 7.26, exhibit similar trend patterns. The healthy group demonstrates a gradual increase followed by a sudden decline toward the latter stage of the PRH phase, whereas the remaining categories show a decline during the early stage of PRH. This reflects that healthy individual possesses with more compliance arteries with low arterial stiffness.

The DT index plots of PPG illustrated in Fig. 7.27 reveal that the healthy and PDM categories exhibit similar variation patterns, with a gradual decline observed during the latter stage of the PRH phase. This suggests that individuals in these groups possess more compliant arteries with lower arterial stiffness. In contrast, the remaining categories demonstrate a rapid decline during the early stage of PRH which reflects the high arterial stiffness of arteries.

The STT, AI, and PA index plots of PPG, depicted in Figures 7.28, 7.29, and 7.30, exhibit smooth variations with higher index values observed in the healthy category,

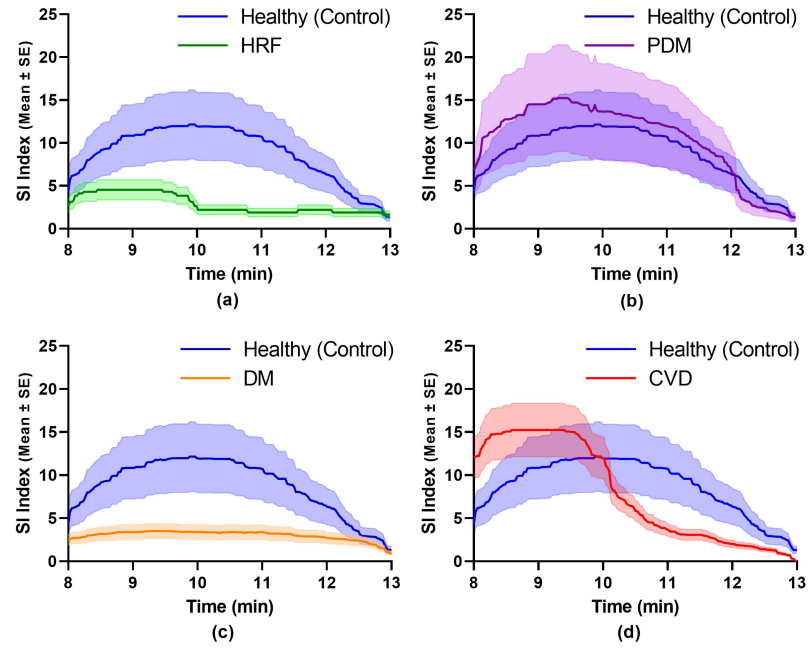


Fig. 7.24: Normalized Short-term Variations of PPG Stiffness Index (SI):
(a) HRF Vs Healthy (b) PDM Vs Healthy (c) DM Vs Healthy (d) CVD Vs Healthy

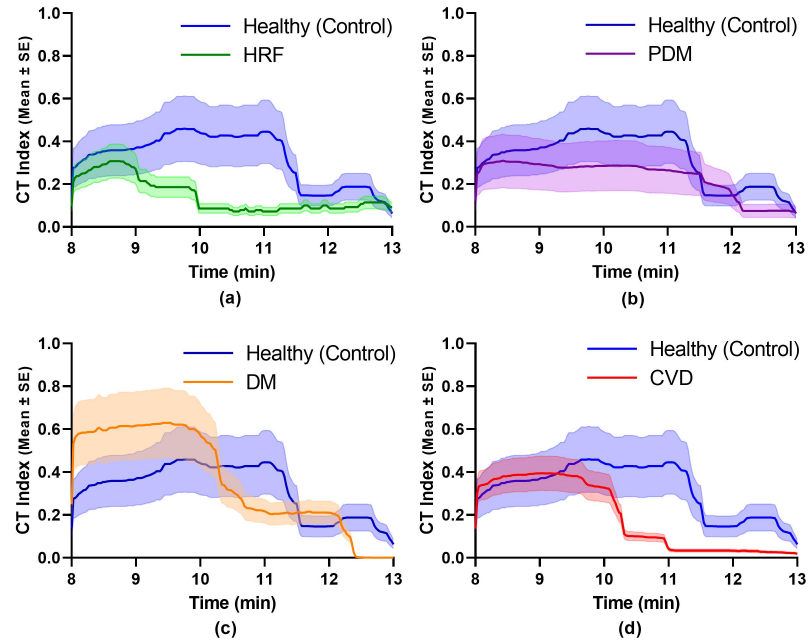


Fig. 7.25: Normalized Short-term Variations of PPG Crest Time Index (CT):
(a) HRF Vs Healthy (b) PDM Vs Healthy (c) DM Vs Healthy (d) CVD Vs Healthy

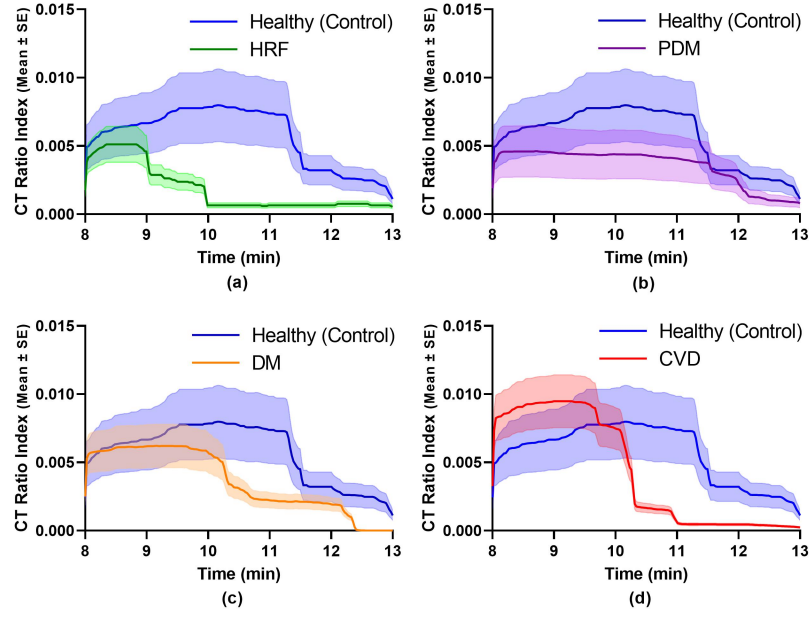


Fig. 7.26: Normalized Short-term Variations of PPG Crest Time Ratio (CTR):
(a) HRF Vs Healthy (b) PDM Vs Healthy (c) DM Vs Healthy (d) CVD Vs Healthy

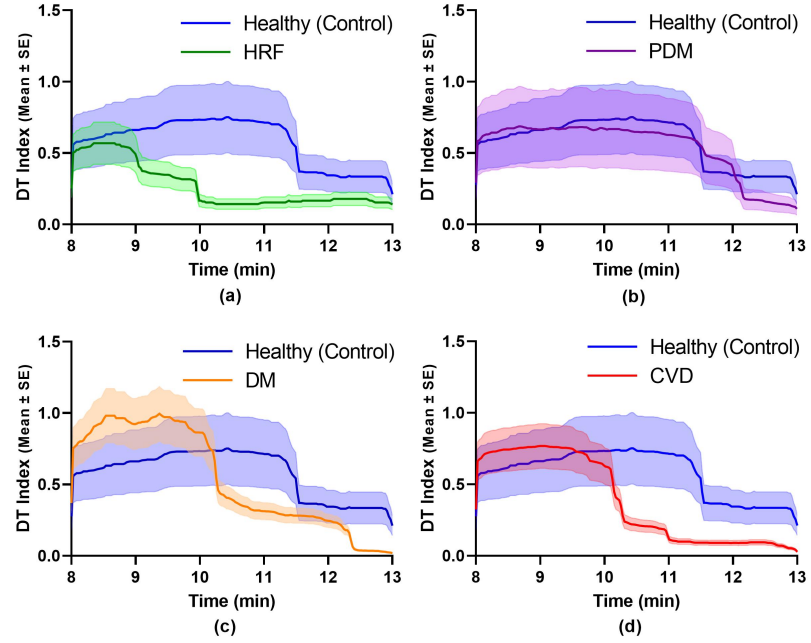


Fig. 7.27: Normalized Short-term Variations of PPG Delta Time Index (DT):
(a) HRF Vs Healthy (b) PDM Vs Healthy (c) DM Vs Healthy (d) CVD Vs Healthy

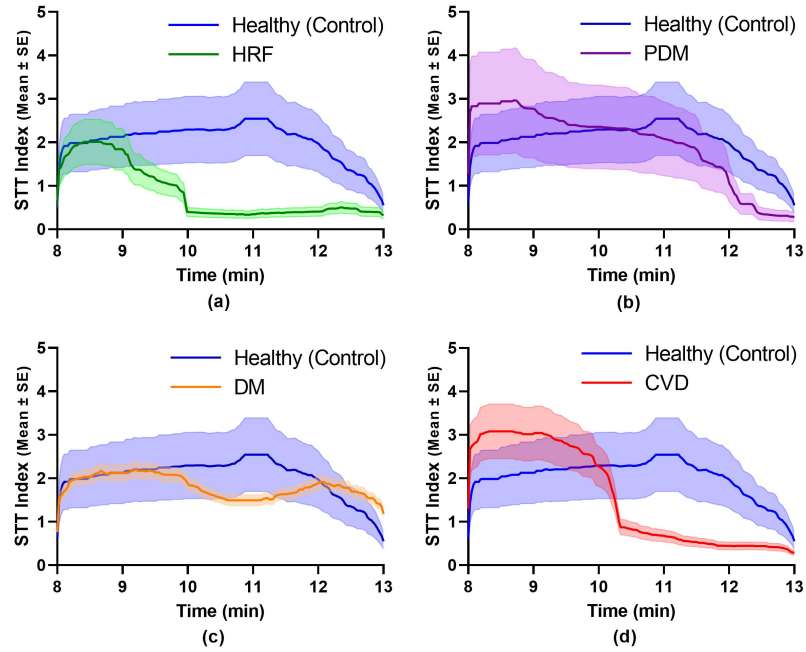


Fig. 7.28: Normalized Short-term Variations of PPG Slope Transit Time Index (STT):
(a) HRF Vs Healthy (b) PDM Vs Healthy (c) DM Vs Healthy (d) CVD Vs Healthy

followed by a gradual decline toward the latter part of the PRH phase compared to the remaining categories. This pattern suggests better vascular tone and preserved endothelial function in response to brief ischemic conditions. In contrast, the DM and CVD categories demonstrate a rapid decline in index values during the mid-phase of PRH, indicating increased arterial stiffness and the presence of VED in these individuals.

The BSEVR index plots of PPG shown in Fig. 7.31 illustrate a parabolic variation in the healthy category, with higher index values and noticeable variation compared to the other subject groups. This suggests that healthy individuals possess an adequate myocardial oxygen supply and better endothelial function compared to the remaining test subject categories.

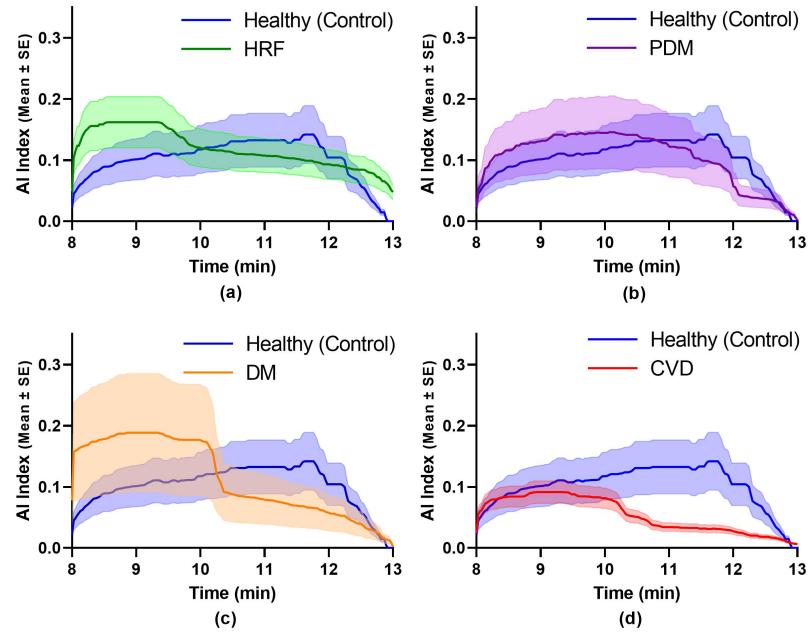


Fig. 7.29: Normalized Short-term Variations of PPG Augmented Index (AI):
(a) HRF Vs Healthy (b) PDM Vs Healthy (c) DM Vs Healthy (d) CVD Vs Healthy

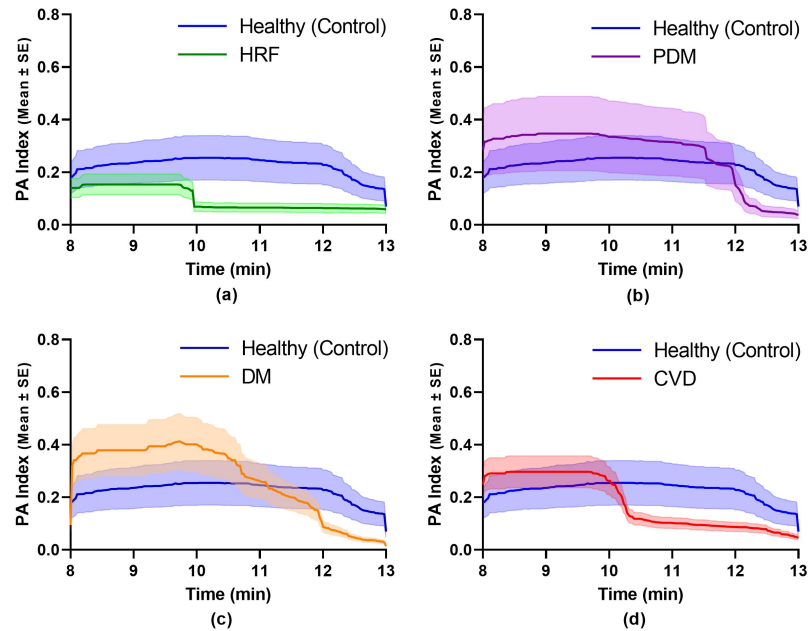


Fig. 7.30: Normalized Short-term Variations of PPG Pulse Width to Amplitude Index (PA): (a) HRF Vs Healthy (b) PDM Vs Healthy (c) DM Vs Healthy (d) CVD Vs Healthy

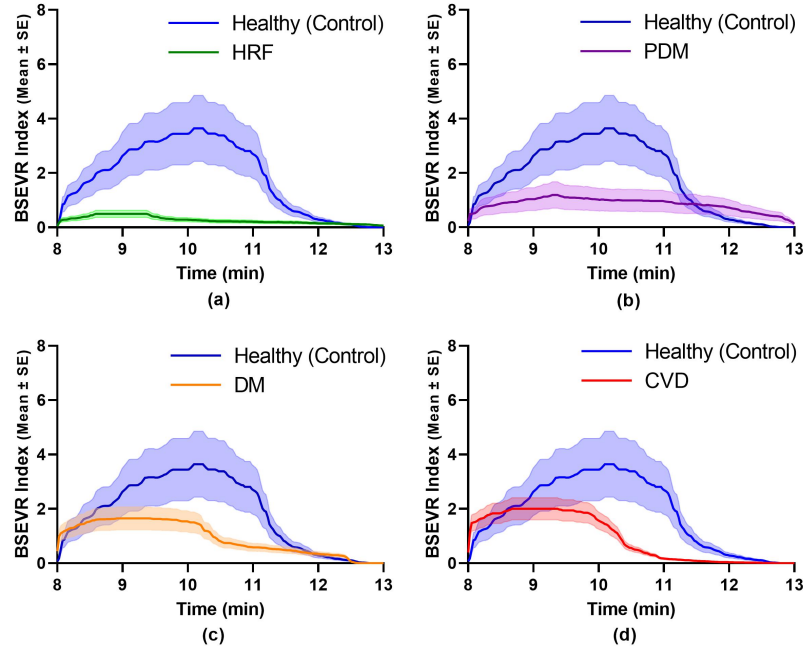


Fig. 7.31: Normalized Short-term Variations of PPG Buckberg Sub Endocardial Viability Ratio (BSEVR): (a) HRF Vs Healthy (b) PDM Vs Healthy (c) DM Vs Healthy (d) CVD Vs Healthy

7.7.4 Statistical Analysis of Long-term VED Indices

The statistical analysis was carried out to examine the significance of the indices ($p < 0.05$) among the test subject categories compared to the healthy condition. The clinical study data were analyzed by One-way ANOVA (Analysis of Variance) using GraphPad Prism 9 software.

The long-term variation VED indices calculated for each physiological parameter (DBT, PAT, PPG and PBI) were statistically analyzed using ANOVA. The corresponding results generated for each physiological parameter are presented in sections 7.7.4.1, 7.7.4.2, 7.7.4.3 and 7.7.4.4 respectively.

7.7.4.1 DBT Long-term VED Indices

The seven long-term variation VED indices of DBT, along with their mean and standard error values, are presented separately in Tables 7.3, 7.4, 7.5 and 7.6 where they are compared across the Healthy group and other subject categories (HRF, PDM, DM, and CVD), with corresponding significance levels determined using ANOVA (p-values). The one-way ANOVA statistical plots of DBT long-term variation VED indices are depicted in Fig. 7.32 with level of significance indicated in asterisk (*) symbols.

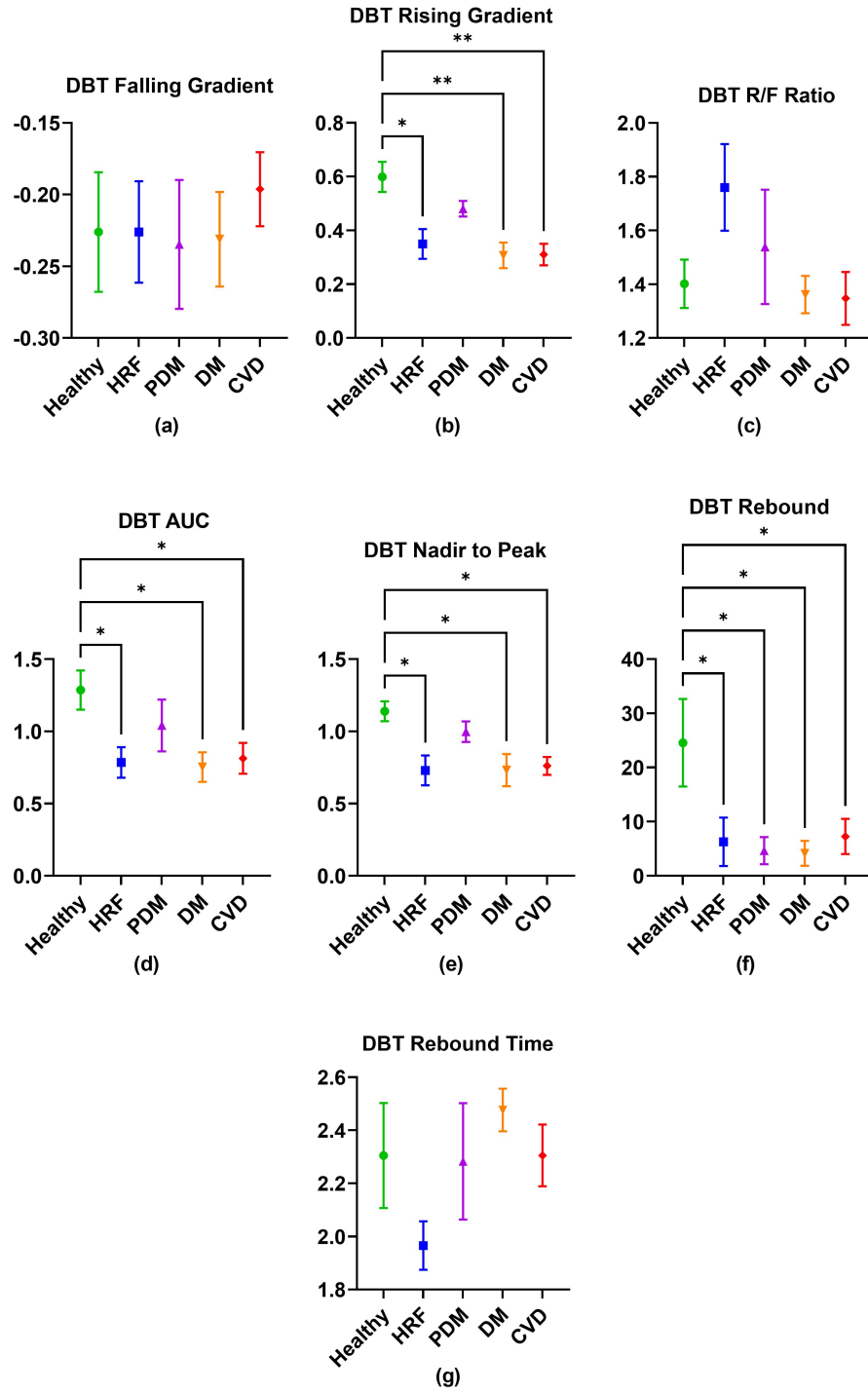


Fig. 7.32: ANOVA plots of DBT long-term Variation VED Indices: (a) Falling Gradient (b) Rising Gradient (c) R/F Ratio (d) AUC (e) Nadir to Peak (f) Rebound (g) Rebound Time (Significance Level: * $p < 0.05$, ** $p < 0.01$)

TABLE 7.3: CLINICAL STUDY: DBT LONGTERM VARIATION VED INDICES ANOVA STATISTICAL ANALYSIS (HEALTHY VS HRF)

	VED Index	Healthy (n=9)	HRF (n=15)	p-value
DBT	Falling Gradient	-0.23 ± 0.12	-0.23 ± 0.13	0.9999
	Rising Gradient	0.60 ± 0.13	0.35 ± 0.17	0.0147
	R/F Ratio	-1.40 ± 0.22	-1.80 ± 0.51	0.1876
	AUC	1.30 ± 0.36	0.79 ± 0.38	0.0276
	Rebound	24.60 ± 22.80	6.29 ± 16.10	0.0264
	Nadir to Peak	1.10 ± 0.17	0.73 ± 0.34	0.0205
	Rebound Time	2.30 ± 0.59	1.97 ± 0.35	0.2703

TABLE 7.4: CLINICAL STUDY: DBT LONGTERM VARIATION VED INDICES ANOVA STATISTICAL ANALYSIS (HEALTHY VS PDM)

	VED Index	Healthy (n=9)	PDM (n=6)	p-value
DBT	Falling Gradient	-0.23 ± 0.12	-0.23 ± 0.10	0.9998
	Rising Gradient	0.60 ± 0.13	0.48 ± 0.07	0.4985
	R/F Ratio	-1.40 ± 0.22	-1.50 ± 0.42	0.9297
	AUC	1.30 ± 0.36	1.01 ± 0.36	0.6836
	Rebound	24.60 ± 22.80	4.65 ± 6.14	0.0494
	Nadir to Peak	1.10 ± 0.17	1.01 ± 0.14	0.8237
	Rebound Time	2.30 ± 0.59	2.28 ± 0.54	0.9999

TABLE 7.5: CLINICAL STUDY: DBT LONGTERM VARIATION VED INDICES ANOVA STATISTICAL ANALYSIS (HEALTHY VS DM)

	VED Index	Healthy (n=9)	DM (n=15)	p-value
DBT	Falling Gradient	-0.23 ± 0.12	-0.20 ± 0.11	0.9200
	Rising Gradient	0.60 ± 0.13	0.31 ± 0.16	0.0020
	R/F Ratio	-1.40 ± 0.22	-1.30 ± 0.37	0.9929
	AUC	1.30 ± 0.36	0.81 ± 0.44	0.0306
	Rebound	24.60 ± 22.80	7.26 ± 14.20	0.0247
	Nadir to Peak	1.10 ± 0.17	0.76 ± 0.26	0.0228
	Rebound Time	2.30 ± 0.59	2.31 ± 0.57	0.9999

7.7.4.2 PAT Long-term VED Indices

The seven long-term variation VED indices of PAT, along with their mean and standard error values, are presented separately in Tables 7.7, 7.8, 7.9 and 7.10 where they are compared across the Healthy group and other subject categories (HRF, PDM, DM, and CVD), with corresponding significance levels determined using ANOVA (p-values). The one-way ANOVA statistical plots of PAT long-term variation VED indices are depicted in Fig. 7.33 with level of significance indicated in asterisk (*) symbols.

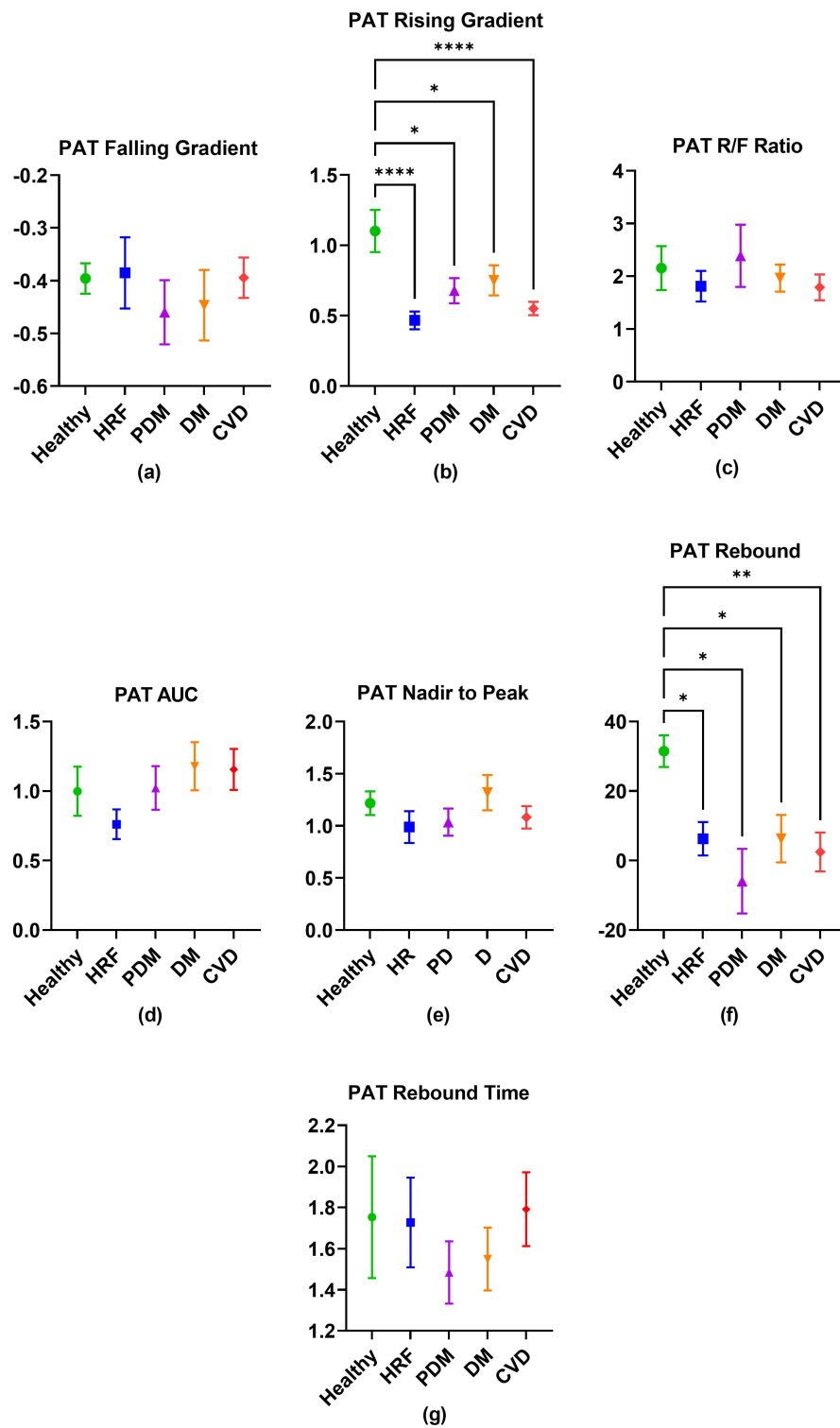


Fig. 7.33: ANOVA plots of PAT long-term Variation VED Indices:
 (a) Falling Gradient (b) Rising Gradient (c) R/F Ratio (d) AUC
 (e) Nadir to Peak (f) Rebound (g) Rebound Time
 (Significance Level: * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$)

TABLE 7.6: CLINICAL STUDY: DBT LONGTERM VARIATION VED INDICES ANOVA STATISTICAL ANALYSIS (HEALTHY VS CVD)

	VED Index	Healthy (n=9)	CVD (n=24)	p-value
DBT	Falling Gradient	-0.23 ± 0.12	-0.20 ± 0.11	0.9200
	Rising Gradient	0.60 ± 0.13	0.31 ± 0.16	0.0020
	R/F Ratio	-1.40 ± 0.22	-1.30 ± 0.37	0.9929
	AUC	1.30 ± 0.36	0.81 ± 0.44	0.0306
	Rebound	24.60 ± 22.80	7.26 ± 14.20	0.0247
	Nadir to Peak	1.10 ± 0.17	0.76 ± 0.26	0.0228
	Rebound Time	2.30 ± 0.59	2.31 ± 0.57	0.9999

TABLE 7.7: CLINICAL STUDY: PAT LONGTERM VARIATION VED INDICES ANOVA STATISTICAL ANALYSIS (HEALTHY VS HRF)

	VED Index	Healthy (n=9)	HRF (n=15)	p-value
PAT	Falling Gradient	-0.40 ± 0.09	-0.39 ± 0.25	0.9998
	Rising Gradient	1.1 ± 0.48	0.47 ± 0.22	0.0001
	R/F Ratio	-2.16 ± 1.25	-1.81 ± 0.97	0.8828
	AUC	0.99 ± 0.53	0.76 ± 0.37	0.7572
	Rebound	31.47 ± 12.86	6.27 ± 16.65	0.0306
	Nadir to Peak	1.22 ± 0.32	0.98 ± 0.51	0.6504
	Rebound Time	1.75 ± 0.89	1.73 ± 0.82	0.9999

TABLE 7.8: CLINICAL STUDY: PAT LONGTERM VARIATION VED INDICES ANOVA STATISTICAL ANALYSIS (HEALTHY VS PDM)

	VED Index	Healthy (n=9)	PDM (n=6)	p-value
PAT	Falling Gradient	-0.40 ± 0.09	-0.46 ± 0.15	0.9229
	Rising Gradient	1.1 ± 0.48	0.68 ± 0.20	0.0410
	R/F Ratio	-2.16 ± 1.25	-2.39 ± 1.44	0.9818
	AUC	0.99 ± 0.53	3.08 ± 2.59	0.1080
	Rebound	31.47 ± 12.86	-5.93 ± 20.77	0.0132
	Nadir to Peak	1.22 ± 0.32	1.04 ± 0.26	0.9099
	Rebound Time	1.75 ± 0.89	1.48 ± 0.37	0.8980

7.7.4.3 PPG Long-term VED Indices

The seven long-term variation VED indices of PPG, along with their mean and standard error values, are presented separately in Tables 7.11, 7.12, 7.13 and 7.14 where they are compared across the Healthy group and other subject categories (HRF, PDM, DM, and CVD), with corresponding significance levels determined using ANOVA (p-values). The one-way ANOVA statistical plots of PPG long-term variation VED indices are depicted in Fig. 7.34 with level of significance indicated in asterisk (*) symbols.

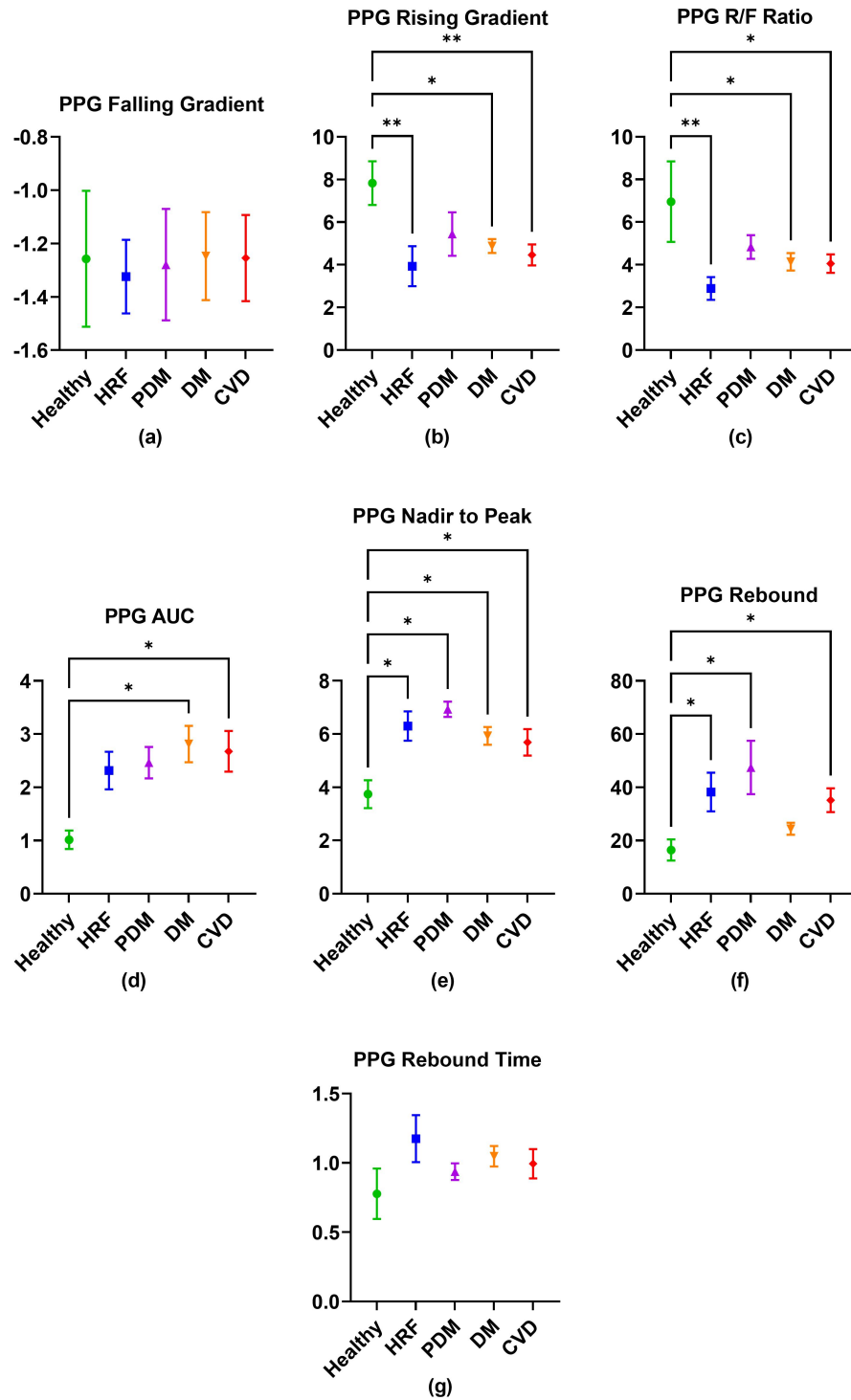


Fig. 7.34: ANOVA plots of PPG long-term Variation VED Indices:
(a) Falling Gradient (b) Rising Gradient (c) R/F Ratio (d) AUC (e) Nadir to Peak
(f) Rebound (g) Rebound Time (Significance Level: * $p < 0.05$, ** $p < 0.01$)

TABLE 7.9: CLINICAL STUDY: PAT LONGTERM VARIATION VED INDICES ANOVA STATISTICAL ANALYSIS (HEALTHY VS DM)

	VED Index	Healthy (n=9)	DM (n=15)	p-value
PAT	Falling Gradient	-0.40 ± 0.09	-0.45 ± 0.24	0.9309
	Rising Gradient	1.1 ± 0.48	0.75 ± 0.34	0.0377
	R/F Ratio	-2.16 ± 1.25	-1.96 ± 0.81	0.9849
	AUC	0.99 ± 0.53	3.08 ± 2.59	0.1080
	Rebound	31.47 ± 12.86	8.09 ± 24.79	0.0306
	Nadir to Peak	1.22 ± 0.32	1.32 ± 0.54	0.9699
	Rebound Time	1.75 ± 0.89	1.55 ± 0.55	0.9210

TABLE 7.10: CLINICAL STUDY: PAT LONGTERM VARIATION VED INDICES ANOVA STATISTICAL ANALYSIS (HEALTHY VS CVD)

	VED Index	Healthy (n=9)	CVD (n=24)	p-value
PAT	Falling Gradient	-0.40 ± 0.09	-0.39 ± 0.19	0.9999
	Rising Gradient	1.1 ± 0.48	0.55 ± 0.20	0.0001
	R/F Ratio	-2.16 ± 1.25	-1.79 ± 1.10	0.8102
	AUC	0.99 ± 0.53	3.08 ± 2.59	0.1080
	Rebound	31.47 ± 12.86	2.47 ± 25.68	0.0103
	Nadir to Peak	1.22 ± 0.32	1.08 ± 0.47	0.9145
	Rebound Time	1.75 ± 0.89	1.79 ± 0.88	0.9998

TABLE 7.11: CLINICAL STUDY: PPG LONGTERM VARIATION VED INDICES ANOVA STATISTICAL ANALYSIS (HEALTHY VS HRF)

	VED Index	Healthy (n=9)	HRF (n=15)	p-value
PPG	Falling Gradient	-1.26 ± 0.77	-1.32 ± 0.52	0.9974
	Rising Gradient	7.83 ± 2.90	3.93 ± 3.38	0.0028
	R/F Ratio	-6.96 ± 4.99	-2.89 ± 1.85	0.0020
	AUC	1.02 ± 0.46	2.24 ± 1.14	0.2056
	Rebound	16.46 ± 11.87	38.22 ± 22.97	0.0281
	Nadir to Peak	3.74 ± 1.39	5.77 ± 1.50	0.0338
	Rebound Time	0.78 ± 0.55	1.17 ± 0.66	0.1745

7.7.4.4 PBI Long-term VED Indices

The seven long-term variation VED indices of PBI, along with their mean and standard error values, are presented separately in Tables 7.15, 7.16, 7.17 and 7.18 where they are compared across the Healthy group and other subject categories (HRF, PDM, DM, and CVD), with corresponding significance levels determined using ANOVA (p-values). The one-way ANOVA statistical plots of PBI long-term variation VED indices are depicted in Fig. 7.35 with level of significance indicated in asterisk (*) symbols.

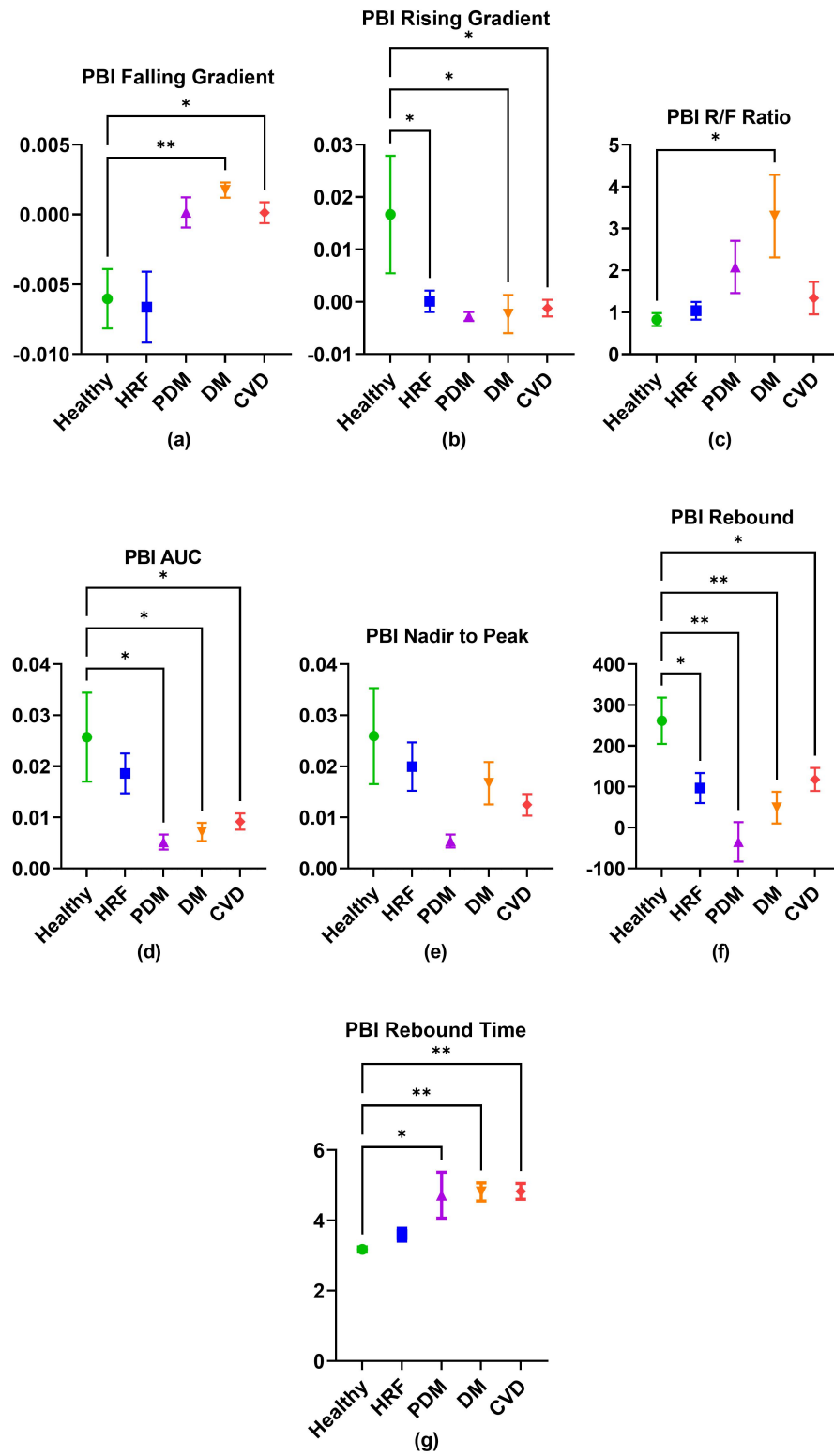


Fig. 7.35: ANOVA plots of PBI Long-term Variation VED Indices:
(a) Falling Gradient (b) Rising Gradient (c) R/F Ratio (d) AUC (e) Nadir to Peak
(f) Rebound (g) Rebound Time (Significance Level: * $p < 0.05$, ** $p < 0.01$)

TABLE 7.12: CLINICAL STUDY: PPG LONGTERM VARIATION VED INDICES ANOVA STATISTICAL ANALYSIS (HEALTHY VS PDM)

	VED Index	Healthy (n=9)	PDM (n=6)	p-value
PPG	Falling Gradient	-1.26 ± 0.77	-1.28 ± 0.51	0.9999
	Rising Gradient	7.83 ± 2.90	5.44 ± 2.29	0.2481
	R/F Ratio	-6.96 ± 4.99	-4.83 ± 1.36	0.2760
	AUC	1.02 ± 0.46	2.46 ± 0.72	0.1956
	Rebound	16.46 ± 11.87	40.74 ± 19.58	0.0302
	Nadir to Peak	3.74 ± 1.39	6.39 ± 1.15	0.0327
	Rebound Time	0.78 ± 0.55	0.94 ± 0.15	0.9154

TABLE 7.13: CLINICAL STUDY: PPG LONGTERM VARIATION VED INDICES ANOVA STATISTICAL ANALYSIS (HEALTHY VS DM)

	VED Index	Healthy (n=9)	DM (n=15)	p-value
PPG	Falling Gradient	-1.26 ± 0.77	-1.25 ± 0.64	0.9999
	Rising Gradient	7.83 ± 2.90	4.88 ± 1.18	0.0307
	R/F Ratio	-6.96 ± 4.99	-4.14 ± 1.58	0.0343
	AUC	1.02 ± 0.46	2.81 ± 1.23	0.0292
	Rebound	16.46 ± 11.87	24.44 ± 6.85	0.7196
	Nadir to Peak	3.74 ± 1.39	5.93 ± 1.00	0.0479
	Rebound Time	0.78 ± 0.55	1.05 ± 0.29	0.4760

TABLE 7.14: CLINICAL STUDY: PPG LONGTERM VARIATION VED INDICES ANOVA STATISTICAL ANALYSIS (HEALTHY VS CVD)

	VED Index	Healthy (n=9)	CVD (n=24)	p-value
PPG	Falling Gradient	-1.26 ± 0.77	-1.25 ± 0.79	0.9999
	Rising Gradient	7.83 ± 2.90	4.46 ± 2.09	0.0070
	R/F Ratio	-6.96 ± 4.99	-4.05 ± 1.85	0.0234
	AUC	1.02 ± 0.46	2.68 ± 1.87	0.0277
	Rebound	16.46 ± 11.87	35.18 ± 18.43	0.0372
	Nadir to Peak	3.74 ± 1.39	5.69 ± 2.15	0.0444
	Rebound Time	0.78 ± 0.55	0.99 ± 0.52	0.5967

7.7.5 Statistical Summary of Long-term VED Indices

A heat map of summarized ANOVA results for the long-term VED indices of the physiological parameters (DBT, PAT, PPG, and PBI), comparing the test subject categories (HRF, PDM, DM, and CVD) against the healthy group, is presented in Fig. 7.36. In this heat map, statistically significant ($p < 0.05$) combinations of long-term VED indices for each physiological parameter across test subject compared against healthy are shaded in gray.

TABLE 7.15: CLINICAL STUDY: PBI LONGTERM VARIATION VED INDICES ANOVA STATISTICAL ANALYSIS (HEALTHY VS HRF)

	VED Index	Healthy (n=9)	HRF (n=15)	p-value
PBI	Falling Gradient	-0.006 ± 0.006	-0.007 ± 0.009	0.9971
	Rising Gradient	0.017 ± 0.027	-0.003 ± 0.005	0.0332
	R/F Ratio	0.83 ± 0.44	1.04 ± 0.59	0.9978
	AUC	0.026 ± 0.026	0.019 ± 0.012	0.6084
	Rebound	261.5 ± 150.3	96.99 ± 109.9	0.0320
	Nadir to Peak	0.026 ± 0.027	0.020 ± 0.015	0.8028
	Rebound Time	3.18 ± 0.17	3.60 ± 0.63	0.7231

TABLE 7.16: CLINICAL STUDY: PBI LONGTERM VARIATION VED INDICES ANOVA STATISTICAL ANALYSIS (HEALTHY VS PDM)

	VED Index	Healthy (n=9)	PDM (n=6)	p-value
PBI	Falling Gradient	-0.006 ± 0.006	0.001 ± 0.005	0.2471
	Rising Gradient	0.017 ± 0.027	-0.003 ± 0.002	0.0739
	R/F Ratio	0.83 ± 0.44	2.08 ± 1.08	0.6965
	AUC	0.026 ± 0.026	0.005 ± 0.003	0.0418
	Rebound	261.5 ± 150.3	-34.80 ± 96.64	0.0012
	Nadir to Peak	0.026 ± 0.027	0.005 ± 0.003	0.0927
	Rebound Time	3.18 ± 0.17	4.72 ± 1.31	0.0386

TABLE 7.17: CLINICAL STUDY: PBI LONGTERM VARIATION VED INDICES ANOVA STATISTICAL ANALYSIS (HEALTHY VS DM)

	VED Index	Healthy (n=9)	DM (n=15)	p-value
PBI	Falling Gradient	-0.006 ± 0.006	0.002 ± 0.002	0.0098
	Rising Gradient	0.017 ± 0.027	-0.002 ± 0.014	0.0132
	R/F Ratio	0.83 ± 0.44	3.30 ± 3.11	0.0223
	AUC	0.026 ± 0.026	0.007 ± 0.006	0.0127
	Rebound	261.5 ± 150.3	49.00 ± 128.9	0.0026
	Nadir to Peak	0.026 ± 0.027	0.017 ± 0.014	0.4597
	Rebound Time	3.18 ± 0.17	4.81 ± 1.00	0.0020

7.7.6 Statistical Analysis of Short-term VED Indices

The short-term variation VED indices computed for PAT and PPG data from the clinical study underwent statistical analysis, comparing their significance ($p < 0.05$) among the test subject categories (HRF, PDM, DM and CVD) with the healthy group. One-way ANOVA was utilized as the statistical method, and the resulting p-values of the short-term variation VED indices (mean ± standard error along with p-values) contrasted with the healthy group are presented in the following subsections [7.7.6.1](#) and [7.7.6.2](#) for PAT and PPG respectively.

TABLE 7.18: CLINICAL STUDY: PBI LONGTERM VARIATION VED INDICES ANOVA STATISTICAL ANALYSIS (HEALTHY VS CVD)

VED Index		Healthy (n=9)	CVD (n=24)	p-value
PBI	Falling Gradient	-0.006 ± 0.006	0.0001 ± 0.003	0.0316
	Rising Gradient	0.017 ± 0.027	-0.003 ± 0.005	0.0113
	R/F Ratio	0.83 ± 0.44	1.34 ± 1.60	0.9096
	AUC	0.026 ± 0.026	0.009 ± 0.007	0.0133
	Rebound	261.5 ± 150.3	117.9 ± 109.3	0.0412
	Nadir to Peak	0.026 ± 0.027	0.017 ± 0.014	0.1168
	Rebound Time	3.18 ± 0.17	4.83 ± 1.04	0.0011

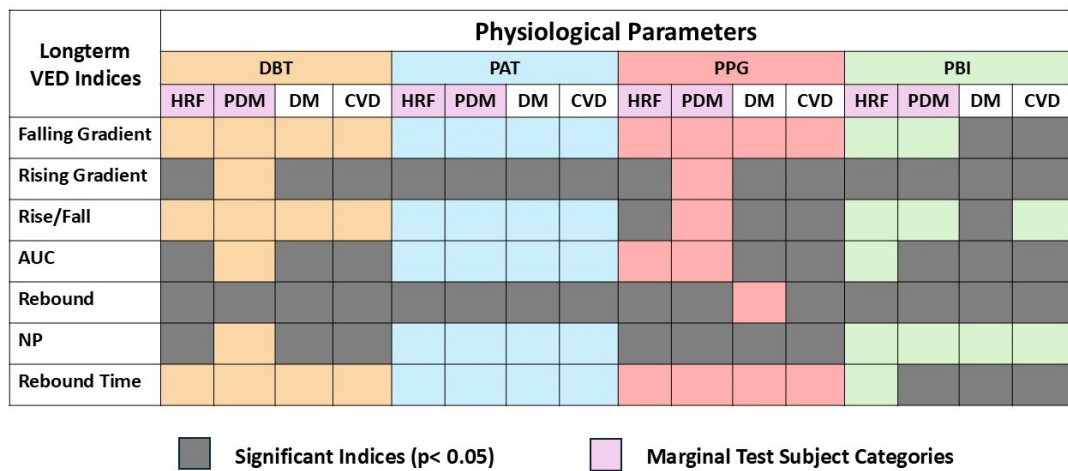


Fig. 7.36: Heat map of summarized ANOVA results for the long-term VED indices

7.7.6.1 PAT Short-term VED Indices

The twelve short-term variation VED indices of PAT, along with their mean and standard error values, are presented separately in Tables 7.19, 7.20, 7.21 and 7.22 where they are compared across the healthy group and other subject categories (HRF, PDM, DM, and CVD), with corresponding significance levels determined using ANOVA (p-values). The one-way ANOVA statistical plots of PAT short-term variation VED indices are depicted in Figures 7.37 and 7.38 with level of significance indicated in asterisk (*) symbols.

**TABLE 7.19: CLINICAL STUDY STATISTICAL ANALYSIS (ANOVA)
HEALTHY VS HRF FOR THE PAT VED INDICES OF
SHORT-TERM VARIATIONS**

VED Index		Healthy (n=9)	HRF (n=15)	p-value
PAT	RI	28 ± 0.40	35 ± 0.20	0.00001
	LnRI	1.4 ± 0.23	1.5 ± 0.003	0.00001
	Di-Amp	0.98 ± 0.004	1.0 ± 0.004	0.00001
	Di-Area	19 ± 0.42	13 ± 0.48	0.00001
	SI	4.2 ± 0.13	3.3 ± 0.16	0.00001
	CT	0.25 ± 0.007	0.19 ± 0.010	0.00001
	CTR	0.005 ± 0.0002	0.003 ± 0.0002	0.00001
	DT	0.39 ± 0.01	0.30 ± 0.02	0.00001
	STT	0.48 ± 0.02	0.44 ± 0.03	0.5936
	AI	0.26 ± 0.008	0.19 ± 0.009	0.0001
	PA	0.017 ± 0.001	0.016 ± 0.001	0.5134
	BSEVR	1.1 ± 0.02	0.66 ± 0.02	0.00001

**TABLE 7.20: CLINICAL STUDY STATISTICAL ANALYSIS (ANOVA)
HEALTHY VS PDM FOR THE PAT VED INDICES OF
SHORT-TERM VARIATIONS**

VED Index		Healthy (n=9)	PDM (n=6)	p-value
PAT	RI	28 ± 0.40	26 ± 0.30	0.0030
	LnRI	1.4 ± 0.23	1.4 ± 0.01	0.5090
	Di-Amp	0.98 ± 0.004	1.0 ± 0.004	0.00001
	Di-Area	19 ± 0.42	9.7 ± 0.49	0.00001
	SI	4.2 ± 0.13	4.0 ± 0.15	0.8010
	CT	0.25 ± 0.007	0.19 ± 0.008	0.00001
	CTR	0.005 ± 0.0002	0.004 ± 0.0002	0.00001
	DT	0.39 ± 0.01	0.32 ± 0.02	0.0007
	STT	0.48 ± 0.02	0.45 ± 0.03	0.8351
	AI	0.26 ± 0.008	0.14 ± 0.005	0.0001
	PA	0.017 ± 0.001	0.020 ± 0.001	0.1424
	BSEVR	1.1 ± 0.02	0.51 ± 0.02	0.00001

7.7.6.2 PPG Short-term VED Indices

The twelve short-term variation VED indices of PAT, along with their mean and standard error values, are presented separately in Tables 7.23, 7.24, 7.25 and 7.26 where they are compared across the healthy group and other subject categories (HRF, PDM, DM, and CVD), with corresponding significance levels determined using ANOVA (p-values). The one-way ANOVA statistical plots of PAT short-term variation VED indices are depicted in Figures 7.39 and 7.40 with level of significance indicated in asterisk (*) symbols.

**TABLE 7.21: CLINICAL STUDY STATISTICAL ANALYSIS (ANOVA)
HEALTHY VS DM FOR THE PAT VED INDICES OF
SHORT-TERM VARIATIONS**

VED Index		Healthy (n=9)	DM (n=15)	p-value
PAT	RI	28 ± 0.40	30 ± 0.48	0.0003
	LnRI	1.4 ± 0.23	1.4 ± 0.01	0.9999
	Di-Amp	0.98 ± 0.004	1.0 ± 0.004	0.00001
	Di-Area	19 ± 0.42	14 ± 0.39	0.00001
	SI	4.2 ± 0.13	3.8 ± 0.09	0.1413
	CT	0.25 ± 0.007	0.19 ± 0.006	0.00001
	CTR	0.005 ± 0.0002	0.003 ± 0.0001	0.00001
	DT	0.39 ± 0.01	0.34 ± 0.01	0.0215
	STT	0.48 ± 0.02	0.41 ± 0.02	0.0925
	AI	0.26 ± 0.008	0.18 ± 0.005	0.0001
	PA	0.017 ± 0.001	0.016 ± 0.001	0.7777
	BSEVR	1.1 ± 0.02	0.53 ± 0.01	0.00001

**TABLE 7.22: CLINICAL STUDY STATISTICAL ANALYSIS (ANOVA)
HEALTHY VS CVD FOR THE PAT VED INDICES OF
SHORT-TERM VARIATIONS**

VED Index		Healthy (n=9)	CVD (n=24)	p-value
PAT	RI	28 ± 0.40	31 ± 0.34	0.00001
	LnRI	1.4 ± 0.23	1.5 ± 0.01	0.0001
	Di-Amp	0.98 ± 0.004	1.0 ± 0.003	0.0140
	Di-Area	19 ± 0.42	11 ± 0.56	0.00001
	SI	4.2 ± 0.13	2.5 ± 0.09	0.00001
	CT	0.25 ± 0.007	0.15 ± 0.008	0.00001
	CTR	0.005 ± 0.0002	0.003 ± 0.0002	0.00001
	DT	0.39 ± 0.01	0.26 ± 0.01	0.00001
	STT	0.48 ± 0.02	0.33 ± 0.02	0.00001
	AI	0.26 ± 0.008	0.14 ± 0.007	0.00001
	PA	0.017 ± 0.001	0.013 ± 0.001	0.0006
	BSEVR	1.1 ± 0.02	0.25 ± 0.01	0.00001

7.7.7 Statistical Summary of Short-term VED Indices

A heat map of summarized ANOVA results for the short-term VED indices of the PAT and PPG physiological parameters, comparing the test subject categories (HRF, PDM, DM, and CVD) against the healthy group, is presented in Fig. 7.41. In this heat map, statistically significant ($p < 0.05$) combinations of short-term VED indices for each physiological parameter across test subject compared against healthy are shaded in gray.

**TABLE 7.23: CLINICAL STUDY STATISTICAL ANALYSIS (ANOVA)
HEALTHY VS HRF FOR THE PPG VED INDICES OF
SHORT-TERM VARIATIONS**

VED Index		Healthy (n=9)	HRF (n=15)	p-value
PPG	RI	48 ± 0.42	47 ± 0.31	0.0241
	LnRI	1.7 ± 0.005	1.7 ± 0.004	0.5920
	Di-Amp	0.90 ± 0.02	0.91 ± 0.02	0.9991
	Di-Area	1.7 ± 0.056	1.2 ± 0.034	0.00001
	SI	8.8 ± 0.23	2.9 ± 0.08	0.00001
	CT	0.32 ± 0.009	0.15 ± 0.006	0.00001
	CTR	0.0057 ± 0.0002	0.0019 ± 0.0001	0.00001
	DT	0.58 ± 0.012	0.27 ± 0.011	0.00001
	STT	2.0 ± 0.031	1.6 ± 0.083	0.00001
	AI	0.10 ± 0.003	0.12 ± 0.002	0.0002
	PA	0.23 ± 0.003	0.10 ± 0.003	0.00001
	BSEVR	1.80 ± 0.096	0.27 ± 0.009	0.00001

**TABLE 7.24: CLINICAL STUDY STATISTICAL ANALYSIS (ANOVA)
HEALTHY VS PDM FOR THE PPG VED INDICES OF
SHORT-TERM VARIATIONS**

VED Index		Healthy (n=9)	PDM (n=6)	p-value
PPG	RI	48 ± 0.42	45 ± 0.24	0.00001
	LnRI	1.7 ± 0.005	1.7 ± 0.003	0.0219
	Di-Amp	0.90 ± 0.02	0.94 ± 0.01	0.3909
	Di-Area	1.7 ± 0.056	1.3 ± 0.030	0.00001
	SI	8.8 ± 0.23	10 ± 0.32	0.00001
	CT	0.32 ± 0.009	0.23 ± 0.006	0.00001
	CTR	0.0057 ± 0.0002	0.0035 ± 0.0001	0.00001
	DT	0.58 ± 0.012	0.54 ± 0.014	0.2854
	STT	2.0 ± 0.031	2.0 ± 0.062	0.6562
	AI	0.10 ± 0.003	0.10 ± 0.003	0.8539
	PA	0.23 ± 0.003	0.27 ± 0.008	0.00001
	BSEVR	1.80 ± 0.096	0.84 ± 0.018	0.00001

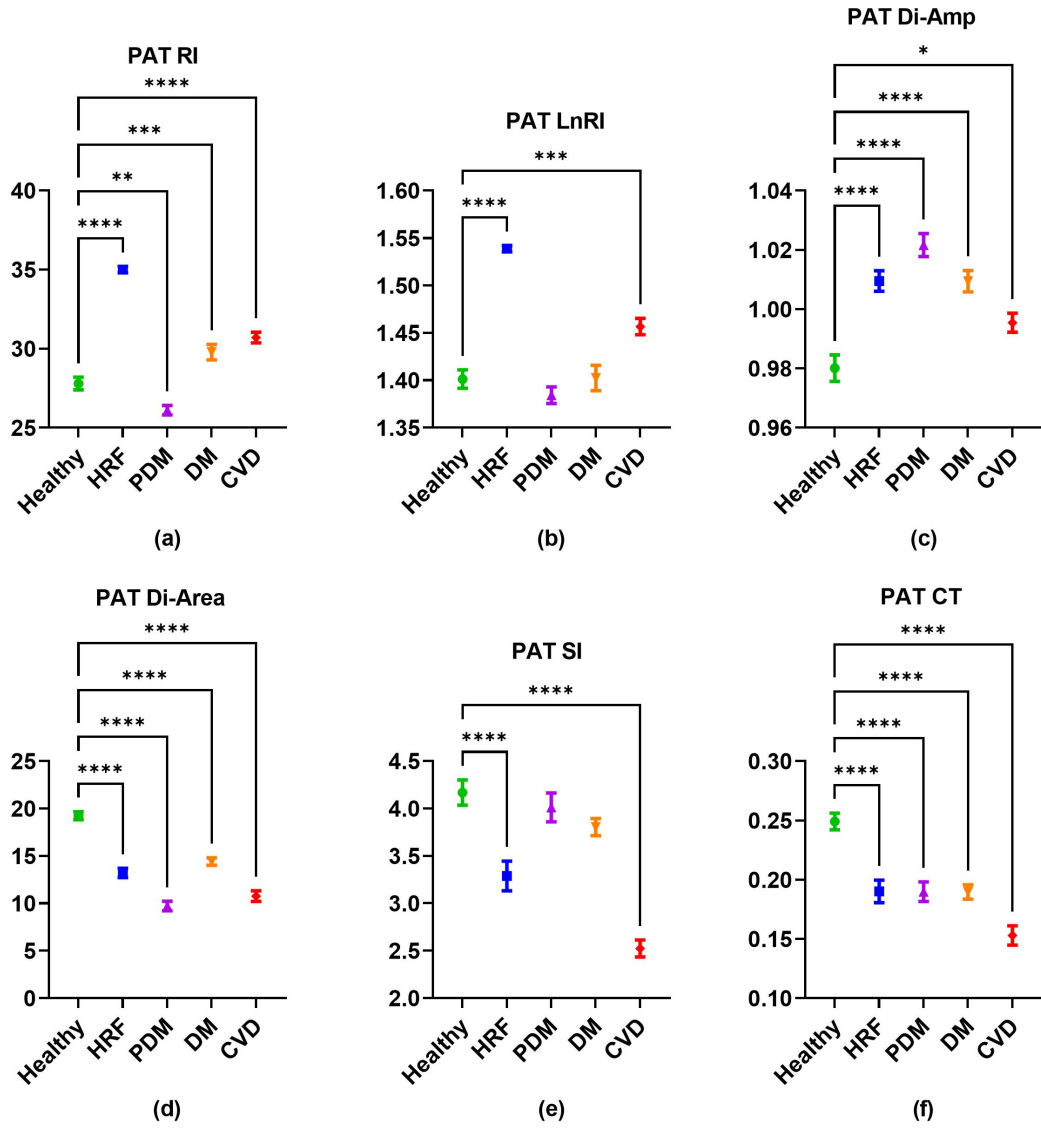


Fig. 7.37: ANOVA plots of PAT Short-term Variation VED Indices:
 (a) Reflective Index (RI) (b) Natural Logarithmic Reflective Index (LnRI)
 (c) Pulse Amplitude (Di-Amp) (d) Pulse Area (Di-Area)
 (e) Stiffness Index (SI) (f) Crest Time Index (CT)
 (Significance Level: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$)

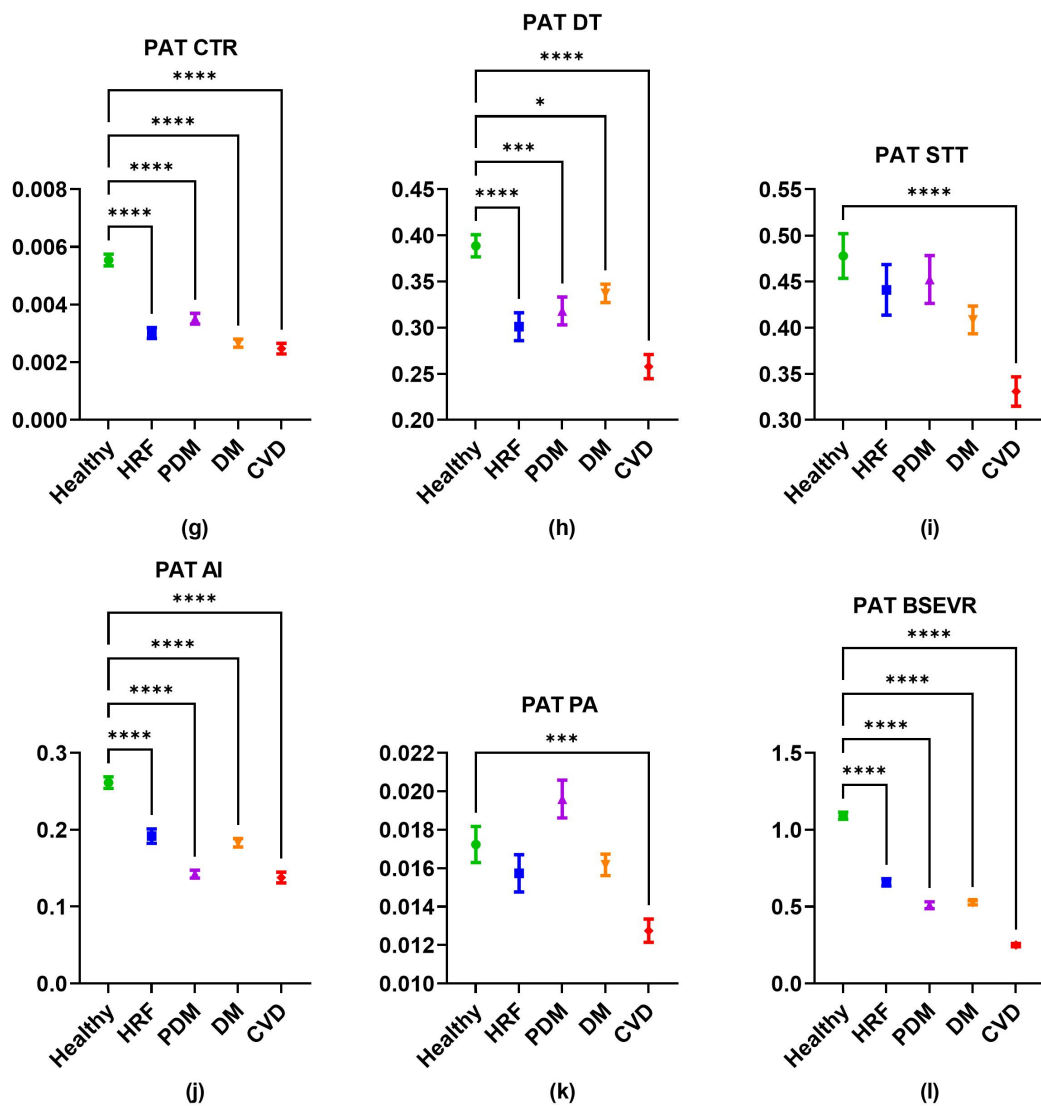


Fig. 7.38: ANOVA plots of PAT Short-term Variation VED Indices:
 (g) Crest Time Ratio (CTR) (h) Delta Time Index (DT)
 (i) Slope Transit Time Index (STT) (j) Augmented Index (AI)
 (k) Pulse Width to Amplitude Index (PA)
 (l) Buckberg Sub Endocardial Viability Ratio (BSEVR)
 (Significance Level: * $p < 0.05$, *** $p < 0.001$, **** $p < 0.0001$)

**TABLE 7.25: CLINICAL STUDY STATISTICAL ANALYSIS (ANOVA)
HEALTHY VS DM FOR THE PPG VED INDICES OF
SHORT-TERM VARIATIONS**

VED Index		Healthy (n=9)	DM (n=15)	p-value
PPG	RI	48 ± 0.42	49 ± 0.28	0.0587
	LnRI	1.7 ± 0.005	1.7 ± 0.003	0.0181
	Di-Amp	0.90 ± 0.02	0.96 ± 0.01	0.0319
	Di-Area	1.7 ± 0.056	0.28 ± 0.008	0.00001
	SI	8.8 ± 0.23	3.0 ± 0.04	0.00001
	CT	0.32 ± 0.009	0.36 ± 0.017	0.0179
	CTR	0.0057 ± 0.0002	0.0036 ± 0.0002	0.00001
	DT	0.58 ± 0.012	0.54 ± 0.026	0.3002
	STT	2.0 ± 0.031	1.8 ± 0.019	0.0099
	AI	0.10 ± 0.003	0.11 ± 0.004	0.0081
	PA	0.23 ± 0.003	0.27 ± 0.010	0.00001
	BSEVR	1.80 ± 0.096	0.93 ± 0.044	0.00001

**TABLE 7.26: CLINICAL STUDY STATISTICAL ANALYSIS (ANOVA)
HEALTHY VS CVD FOR THE PPG VED INDICES OF
SHORT-TERM VARIATIONS**

VED Index		Healthy (n=9)	CVD (n=24)	p-value
PPG	RI	48 ± 0.42	45 ± 0.44	0.00001
	LnRI	1.7 ± 0.005	1.6 ± 0.006	0.00001
	Di-Amp	0.90 ± 0.02	0.91 ± 0.02	0.9961
	Di-Area	1.7 ± 0.056	0.92 ± 0.052	0.00001
	SI	8.8 ± 0.23	7.9 ± 0.42	0.0468
	CT	0.32 ± 0.009	0.19 ± 0.012	0.00001
	CTR	0.0057 ± 0.0002	0.0043 ± 0.0002	0.00001
	DT	0.58 ± 0.012	0.38 ± 0.022	0.00001
	STT	2.0 ± 0.031	1.6 ± 0.083	0.00001
	AI	0.10 ± 0.003	0.05 ± 0.002	0.00001
	PA	0.23 ± 0.003	0.18 ± 0.007	0.00001
	BSEVR	1.80 ± 0.096	0.91 ± 0.062	0.00001

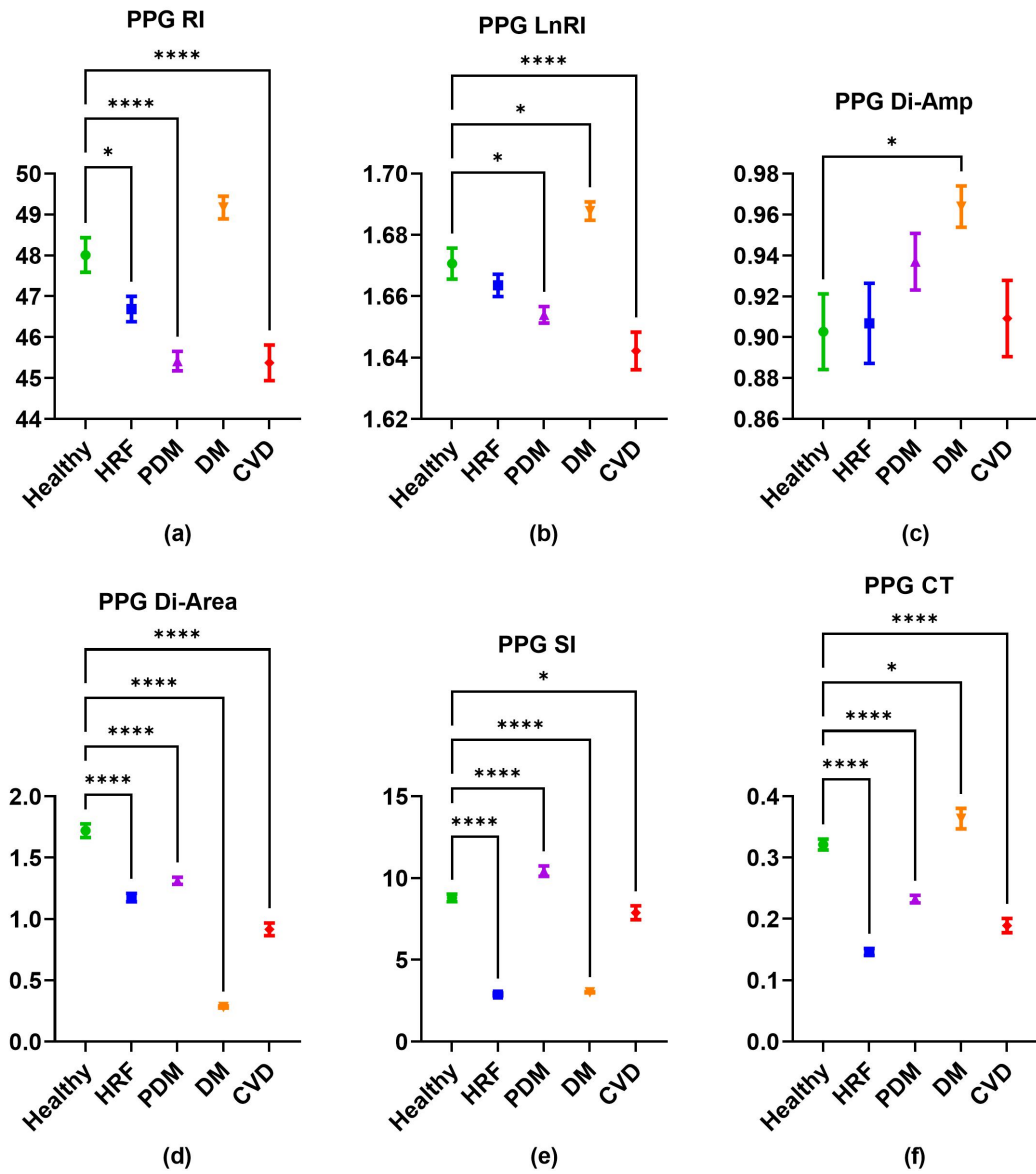


Fig. 7.39: ANOVA plots of PPG Short-term Variation VED Indices:
(a) Reflective Index (RI) (b) Natural Logarithmic Reflective Index (LnRI)
(c) Pulse Amplitude (Di-Amp) (d) Pulse Area (Di-Area)
(e) Stiffness Index (SI) (f) Crest Time Index (CT)
(Significance Level: * $p < 0.05$, **** $p < 0.0001$)

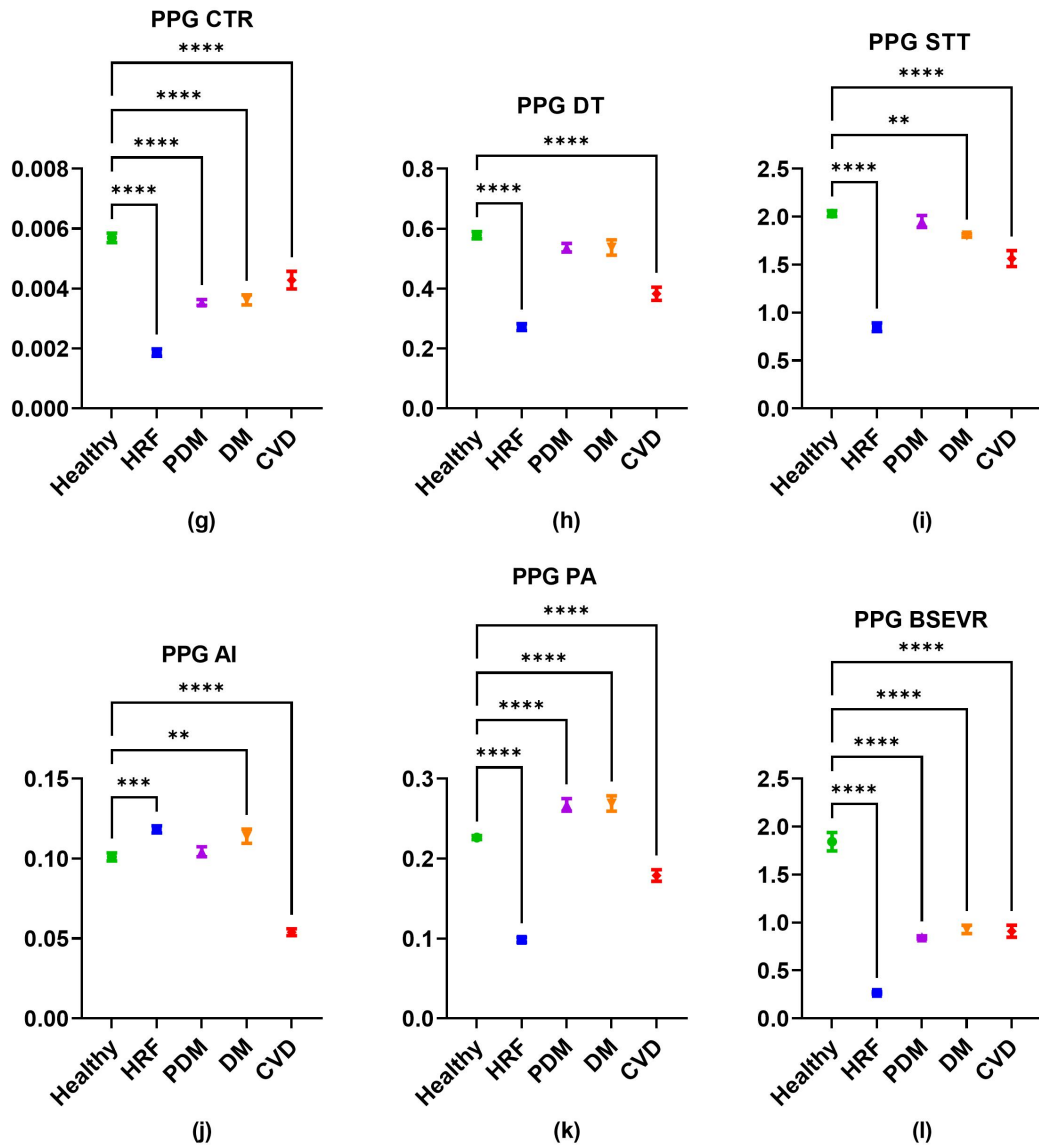


Fig. 7.40: ANOVA plots of PPG Short-term Variation VED Indices:
 (g) Crest Time Ratio (CTR) (h) Delta Time Index (DT)
 (i) Slope Transit Time Index (STT) (j) Augmented Index (AI)
 (k) Pulse Width to Amplitude Index (PA)
 (l) Buckberg Sub Endocardial Viability Ratio (BSEVR)
 (Significance Level: ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$)

Short term VED Indices	Physiological Parameters							
	PAT				PPG			
	HRF	PDM	DM	CVD	HRF	PDM	DM	CVD
RI								
LnRI								
Di-Amp								
Di-Area								
SI								
CT								
CTR								
DT								
STT								
AI								
PA								
BSEVR								

Significant Indices ($p < 0.05$)
 Marginal Test Subject Categories

Fig. 7.41: Heat map of summarized ANOVA results for the short-term VED indices

CHAPTER 8

DISCUSSION

Cardiovascular disease (CVD) being the leading hospitalization non-communicable disease in Sri Lanka, many clinical research studies have been focused on early detection interventions to prevent CVD in advance. Vasodilatory endothelial dysfunction (VED) which is caused in the endothelial tissue layer of the vascular network has been recognized as a precursor for the development of CVD, hence used to estimate the risk of CVD with the advantage of non-invasive monitoring [5, 7]. The feasibility of detecting VED through the individual association of physiological parameters has been conducted in a few previous research studies with patients diagnosed with either Diabetes Mellitus (DM) or CVD [5, 8–10]. However, preventing severe developments at these clinical stages is uncertain as the clinical approaches are orchestrated to suppress CVD conditions rest of the lifetime.

The significance of the current research is that it investigated the feasibility of detecting VED at preclinical stages by including a subject group who is healthy but with CVD risk factors (HRF) and another subject group with prediabetes mellitus (PDM). For the comparison, clinical stage subject groups included those who were diagnosed with CVD and DM in addition to healthy (control). The assessment of VED has been performed for all of these subject groups by using non-invasive physiological parameters of Digital Body Temperature (DBT), Peripheral Arterial Tonometry (PAT) and Pulse Plethysmography (PPG). Additionally, the compatibility of Peripheral Bioimpedance (PBI) as a novel physiological parameter for VED assessment was investigated in this research study. Key outcomes of the research study are explained in the following sections.

8.1 Custom made device

A device equipped with sensors to measure physiological parameters and a stimulation method to induce Reactive Hyperemia (RH) condition in endothelial functionality are essential prerequisites for conducting the VED assessment.

The assessment of VED using a Doppler-ultrasound device is widely utilized in the flow-mediated dilation (FMD) procedure and is considered the non-invasive gold standard. However, due to limitations in accessibility and adaptability for custom applications, alternative devices were explored for research purposes. Commercially available VED assessment devices are costly, reliant on proprietary software and scarce in medical facilities. Despite their ability to assess VED across multiple physiological param-

eters in separate stimulation cycles, none of these devices can simultaneously evaluate VED with multiple physiological parameters within a single stimulation cycle.

To address the aforementioned limitations, a custom device was developed, integrating four non-invasive physiological parameters: DBT, PAT, PPG, and PBI. This device allows for the simultaneous assessment of VED within a single stimulation cycle. This approach provides a comprehensive assessment of how each physiological parameter corresponds to VED, offering a better understanding of the vascular functionality. Additionally, it enables a time-efficient evaluation with reduced variability, resulting in more reliable and reproducible measurements.

To obtain a subject-independent measurement, two identical custom-made devices were built to acquire measurements from both the occluded arm and the control arm. The preliminary study was used for device validation and investigation of the suitability of PBI as a novel physiological parameter for the VED assessment is explained in section 8.3.

8.2 Data Normalization

Data normalization was performed as part of the data preprocessing, as explained in the section 6.5. The occluded arm measurement of each physiological parameter was normalized from the corresponding control arm measurement to eliminate subject bias toward outcomes. This was essential in the current study since the results were generated by comparing five distinguished categories and ensuring only reflected the effect of the VED rather than the subjective differences.

8.3 Preliminary Study for the Device Validation

The preliminary study was conducted at the National Hospital of Sri Lanka (NHSL), Colombo 07 u to validate the performance of the custom-made devices by comparing it with the FMD procedure. Healthy (control) and participants diagnosed with CVD were selected for this study to clearly distinguish the physiological effect of VED through the custom-made device measurements. Due to limited accessibility to the premises, the study was limited to 5 healthy subjects and 5 participants diagnosed with CVD.

In the morphological comparison of waveforms, the graphical plots produced for each physiological parameter using the custom-made device exhibited consistent long-term trends comparable to FMD parameters as shown in Figures 6.6 and 6.7. Furthermore, distinct variations were observed among test subject categories during the Post-Reactive Hyperemia (PRH) stage, except for PBI. Regarding the PBI model for the

forearm as elucidated in Section 4.5.1, the graphical outcomes suggested an inversely proportional long-term trend in comparison to other physiological parameters and previous research study has shown that PBI increases during RH condition [109]. Additionally, deviations in test subject categories were noted from the occluded stage onwards. Therefore, the custom-made device data validity, repeatability and reliability were verified by the preliminary study.

The preliminary study was a quantitative analysis with FMD and the VED indices calculation and statistical analysis were restricted only to long-term variations since FMD data didn't possess short-term variations. Therefore, long-term term VED indices were selected by an extensive literature review. The long-term variation FMD indices (refer Table 6.2 and Fig. 6.9) have indicated that there is a significant difference between healthy and CVD test subject categories as the presence of VED in CVD patients. For the same sample, seven long-term variation indices of physiological parameters (PAT, PPG, DBT and PBI) measured by the custom-made device (refer Table 6.2) have also shown the statistical significance between the test subject categories (refer Fig. 6.10 to 6.13). Thereby, it has been shown that the possibility of detecting VED from the associated physiological parameters of the custom-made device.

8.4 Long-term Variation Analysis of the Clinical Study

Once the performance of the custom-made device was validated, the research work was expanded for the clinical study including pre-clinical stages: Healthy with CVD risk factors (HRF), Prediabetes Mellitus (PDM), clinical stages: Diabetes Mellitus (DM) and CVD and a control group of healthy participants. Similarly to the preliminary study, the long-term variation VED indices were calculated for all five test subject categories.

As shown in subsections 7.7.4.1, 7.7.4.2, 7.7.4.3 and 7.7.4.4, the long-term variation VED indices of each physiological parameter were compared between healthy vs. test subject categories (HRF, PDM, DM and CVD) using ANOVA. The results have shown that there are significant differences between healthy (control) and the rest of the test subject categories. Most importantly, the two preclinical stages of HRF and PDM show a significant difference compared to the healthy category. However, these categories did not show significant differences between clinical stages. The homogeneity between preclinical stages and clinical stages has indicated the mean differences between them are comparatively small, making it difficult to identify a significant difference.

The summarized ANOVA results heat map of long-term VED indices as shown in Fig. 7.36, rising gradient and rebound VED indices have dominantly shown statistical

significance comparing preclinical (HRF and PDM) and clinical (DM and CVD) stages with healthy category in all the physiological parameters including novel PBI. Furthermore, the nadir to peak VED index has shown statistical significance exclusively in DBT and PPG, while the AUC index exhibited significant differences in DBT, PPG, and PBI.

Also, these long-term VED indices have shown the extent of the correlation VED has on vascular reactivity in preclinical stages where the response to RH is reduced and delayed compared to healthy subjects [5]. Therefore, this implies the possibility of early detection of VED of subjects having risk factors of CVD (HRF) and PDM.

8.5 Short-term Variation Analysis of the Clinical study

During the clinical study, short-term pulsatile patterns were visible exclusively in PAT and PPG signals, during the Baseline and PRH (cuff release) stages. However, no pulsatile pattern was evident during the Occluded stage, attributable to the temporary cessation of blood flow through the peripheral vasculature. The PRH stage presented the most significant challenge, characterized by rapid changes in short-term variations within a limited timeframe. Initially, faint pulses were detected at the onset of the PRH stage, followed by a gradual increase in pulse amplitude until reaching a threshold during the PRH stage, culminating in stabilization concurrent with the restoration of physiological conditions induced by reactive hyperemia in peripheral vasculature. This restoration process is heavily reliant on vasodilatory endothelial functionality, with any dysfunctional state identifiable through statistical analysis of short-term Vascular Endothelial Dysfunction (VED) indices. Given the impracticality of assessing indices from an average pulse, short-term VED indices were calculated for discrete-time windows throughout the PRH stage.

Twelve short-term variation VED indices were chosen for the analysis and calculated short-term VED indices sequences are plotted in subsections 7.7.3.1 and 7.7.3.2 for PAT and PPG physiological parameters respectively. All the plots show a decreasing trend of index values over time. This trend suggests that the vasodilatory endothelial functionality responds to the RH condition. However, the Reflective and Di-Amp indices were not able to show such a trend due to their high variability in index values. The rest of the short-term VED indices during the PRH stage have shown distinguishable variations in subjects with DM and CVD as well with in preclinical stages (HRF and PDM) compared to healthy subjects. Statistical analysis verified the significance of graphical plots for many of the short-term VED indices in Tables from 7.19 to 7.26 and ANOVA plots are depicted in Fig. 7.37 to 7.40.

According to summarized ANOVA results heat map of Short-term VED indices shown in Fig.7.41, RI, Di-Area, CT, CTR, AI and BSEVR VED indices have shown dominant significance comparing preclinical (HRF and PDM) and clinical (DM and CVD) stages with healthy category in both physiological parameters (PAT and PPG). In addition, Di-Amp and DT VED indices have shown significance exclusively for PAT whereas SI and PA VED indices have demonstrates statistical significance solely in PPG.

CHAPTER 9

CONCLUSION

This research study, titled on "Development of a system to analyze physiological parameters that are influenced by endothelial dysfunction to assess risks of diabetes" focused on early detection of vasodilatory endothelial dysfunction (VED) to facilitate timely intervention to prevent the risk of developing diabetes and severe cardiovascular diseases. The following key conclusions were derived from this research study.

9.1 Key Conclusions

1. A custom-made device was developed to detect VED non-invasively by analyzing the physiological parameters of digital body temperature (DBT), peripheral arterial tonometry (PAT), photoplethysmography (PPG) and peripheral bio-impedance (PBI).
2. The device was validated against the non-invasive gold standard, flow-mediated dilation (FMD) procedure, through a preliminary study involving a comparative analysis between healthy individuals and patients with cardiovascular disease (CVD), thereby demonstrating its reliability in assessing endothelial function.
3. The device demonstrated the capability of detecting VED in distinguish test subject categories in the clinical study, including individuals with cardiovascular disease (CVD), diabetes mellitus (DM), prediabetes (PDM), and healthy individuals with CVD risk factors (HRF).
4. The VED indices calculated from DBT, PAT, PPG and PBI parameters with long-term and short-term variations have shown statistical significance associated with the presence of VED in clinical stage test subject categories (DM and CVD) as well as preclinical stage test subject categories (HRF and PDM) in contrast to healthy control.
5. The findings have revealed that VED can be identified at preclinical stages, particularly in individuals with PDM and HRF, highlighting its value for early detection.
6. The current study has demonstrated the feasibility of using PBI as a novel biomarker to detect the presence of VED along with other well-established physiological parameters.
7. The study confirms the overall feasibility of non-invasive VED detection as an early diagnostic approach for assessing diabetes and cardiovascular disease risk

and strong potential for incorporating VED assessment into preventive health-care strategies to reduce the progression of these conditions.

9.2 Limitations and Future Work

The main limitation was the inability to detect short-term variations in PBI due to the time lag created by bipolar electrode configuration where the injection and reception of test currents were from the same pair of electrodes. Therefore, it is recommended to implement the tetrapolar electrode configuration to measure PBI variations.

The sample size of PDM subjects was low compared to other subject categories because such a condition is often undiagnosed. To improve the identification of PDM participants, it is proposed to broaden the inclusion criteria by incorporating additional measures, such as screening through oral glucose tolerance test (OGTT) and consulting healthcare professionals for a detailed interpretation of medical history.

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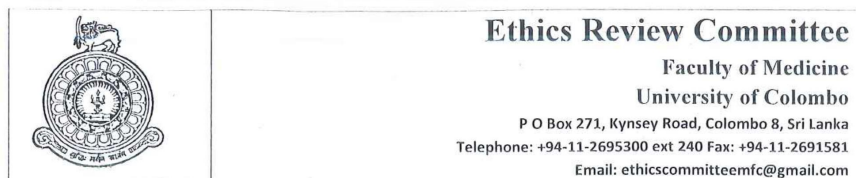
APPENDIX A

ETHICAL APPROVAL DOCUMENTS

The data collection process involves human test subjects where the sensors of custom-made devices are expected to be connected non-invasively. Therefore, the ethical approval application was submitted to the Ethical Review Committee in the Faculty of Medicine attached to the University of Colombo before commencing any experimental trials. After addressing the comments of several major and minor revisions, ethical approval was granted under protocol No: EC/18/038 version 3.2 for the current study to perform data acquisition using the custom-made device of human test subjects. All documents related to the ethical approval are annexed in Appendix A. Furthermore, the ethical approval documents obtained from the University of Colombo were also forwarded to the Ethical Review Committee of the University of Moratuwa and obtained ethical clearance from them as well.

Amid the COVID-19 (New Coronavirus) outbreak in Sri Lanka, investigators and test subjects had to adhere to specific safety guidelines issued by the government [110]. Henceforth, the wearing of face masks was mandatory for both investigators and participants throughout the data collection process. Investigators utilized surgical gloves to minimize the risk of virus transmission during the sensor attachment to the test subject. Before and following the data collection, the forearms of the test subjects as well as all the sensors underwent thorough sanitization. Furthermore, fresh BIA electrodes were employed for each participant to minimize the potential risk of contaminating the COVID-19 virus.

1. Ethical approval letter



REFERENCE: EC-18-038

18th October 2018.

Dr. Anjula De Silva,
Department of Electronic and Telecommunication Engineering,
Faculty of Engineering,
University of Moratuwa.

Dear Dr. De Silva,

RE : Protocol No EC-18-038

Title : Analysis of physiological parameters associated with vasodilatory endothelial dysfunction to assess risks of cardiovascular disease

**Investigators : Dr. Anjula De Silva
Prof. Saroj Jayasinghe
Mr. Kasun Samarawickrama
Mr. Nipun Perera
Dr. Mahen Wijesuriya
Dr. Chamari Warnapura**

Thank you for submitting the above research proposal, which was considered by the Ethics Review Committee, at its meeting on 18.10.2018. Approval is granted to proceed.

This approval relates to the following:

- Research Proposal (Version 3.2)
- Information sheets (Version 3.1)
- Consent forms (Version 2.0)
- Data collection forms (Version 3.0)

The following members of the ERC were present at the meeting:

Dr. Nilakshi Samaranayake, Mrs. Oshadee De Silva, Dr. Dinithi Fernando, Prof. Priyadarshani Galappatthy, Prof. Ariarane Gnanathanan, Dr. Sharmila Jayasena, Dr. Naazima Kamardeen, Prof. Kantha Lankathilake, Dr. Chandani Liyanage, Dr. Nishani Lucas, Dr. Kamal Perera, Dr. Priyanga Ranasinghe, Dr. Gayani Ranaweera, Dr. Dulani Samaranayake, Dr. S. Sivaganesh & Mrs. Nirmali Wickremesinghe



Ethics Review Committee

Faculty of Medicine

University of Colombo

P O Box 271, Kynsey Road, Colombo 8, Sri Lanka

Telephone: +94-11-2695300 ext 240 Fax: +94-11-2691581

Email: ethicscommitteemfc@gmail.com

You are asked to note the following.

- This approval is valid for one year from the date of issue of this letter, and the committee requires that you furnish a final report once the study is concluded.
- If the study is continued for a period beyond one year, you are required to furnish an annual progress report for the year and an application for the extension of approval by a further year. The ERC will issue such extension after consideration of the progress report and any other information it may require from you for this purpose.
- Progress reports and final reports should be submitted in the recommended template, which can be downloaded from the ERC web page of the Faculty of Medicine, University of Colombo website.
- In similar manner, you are required to furnish a progress report and an application for the extension of approval for each subsequent year as long as the study is continued. If no such application is made or extension of approval given, the ethics clearance lapses automatically once the current year of approval is finished.
- If the progress report and/or the final report is/are delayed more than one month beyond the due date (which is the final date of ethics approval in force), approval for the study will lapse and you will be required to furnish a new application should you wish to resume or continue the study.
- If a PI has three or more research proposals in which the progress reports and/or final reports have lapsed in this manner, no further applications for ethics review shall be entertained from such PI.

This approval relates to the ethical content of this study only, and you are responsible for the following:

- Negotiating individual arrangements with the heads of service departments in those situations where the use of their resources is involved.
- If appropriate, informing the study sponsor that the membership and procedures of the Faculty of Medicine, University of Colombo Ethics Review Committee comply with appropriate guidelines of the Forum of Ethics Review Committees in Sri Lanka (FERCSL).

Yours sincerely,

Dr. Enoka Corea
Chairperson
Ethics Review Committee
Faculty of Medicine
University of Colombo

Ethics Review Committee
Faculty of Medicine
University of Colombo
Kynsey Road
Colombo 8

2. Information sheet

Analysis of physiological parameters associated with vasodilatory endothelial dysfunction to assess risks of cardiovascular disease

(Version 3.1, 30 September 2018)

INFORMATION SHEET

This information sheet is intended for the participants recruited from the National Hospital of Sri Lanka, Colombo, Diabetes Association of Sri Lanka and elsewhere. The research study is a collaborative effort from the Faculty of Medicine, University of Colombo and Faculty of Engineering, University of Moratuwa. Following are the main personnel involved:

Principal Investigator: Dr. Anjula De Silva (Senior Lecturer, Biomedical Engineering, Faculty of Engineering, University of Moratuwa)

Co-investigators:

Prof. Saroj Jayasinghe (Faculty of Medicine, University of Colombo)

Mr. Kasun Samarawickrama (Faculty of Engineering, University of Moratuwa)

Mr. Nipun Perera (Faculty of Engineering, University of Moratuwa)

1.1 Introduction

The objective of this research study is to assess the risk of developing cardiovascular disease with the association of vasodilatory endothelial dysfunction. All the necessary information of the research and the procedure of data collection from you will be described in this document. You have the freedom to read it carefully and even take advice from another person before participating (even on another day).

Feel free to ask any questions from us, we will take time to explain. Even if you have questions later, feel free to contact the investigators through provided contact details.

1.2 The research study

Cardiovascular diseases have been found as the leading cause of hospital deaths in Sri Lanka. Current treatment modalities prevent the disease condition momentary. It is a consistent finding that vasodilatory endothelial dysfunction acts as an early indicator of developing atherosclerosis. Early detection of vasodilatory endothelial dysfunction can be used to make clinical interventions in order to prevent developing cardiovascular diseases at all. In this research, developed system to assess vasodilatory endothelial dysfunction will be used to obtain four non-invasive measurements namely impedance, temperature, oxygen saturation and arterial pressure from the fingertips and skin over wrist area. In order to stimulate endothelial function, reactive hyperaemia condition will be obtained by occlusion of upper arm by pressure cuff. Developed system acquired data will be validated by flow mediated dilation measurement obtained from ultrasound machine and flowrates obtained from Doppler machine.

Prior to the study every volunteer participant will be tested for fasting blood sugar, full blood count, HbA1c, C-reactive protein, lipid profile and serum creatinine. All these tests will be conducted for you free of charge.

With the quantitative information provided by the system, clinicians can identify vasodilatory endothelial dysfunction and assess the risk levels of developing cardiovascular disease in future.

1.3 Type of Research Intervention

You will be involved in a completely non-invasive procedure where only five measurements will be taken from finger tips and skin over wrist area from both arms as follows.

1. Impedance – skin over wrist area
2. Arterial pressure – index finger
3. Temperature – mid finger
4. Oxygen saturation – ring finger
5. Flow mediated dilation – ultrasound scan over radial artery

All the measurements will be acquired while you are in a comfortable sitting position. Once the sensors are connected as mention above and measurements will be obtained before and after occlusion of upper arm.

1.4 Participant selection

We involve participants who are within the age range of 18-60 years with cardiovascular diseases/diabetes/ pre-diabetic / healthy but having cardiovascular risk factors (positive cardiovascular disease family history, physical inactive, obesity etc.) and healthy.

1.5 Voluntary Participation

Your participation in this study is voluntary. You are free to not participate at all or to withdraw from the study at any time despite consenting to take part earlier. There will be no loss of medical care or any other available treatment for your illness or condition to which you are otherwise entitled. If you decide not to participate or withdraw from the study, you may do so at any time.

University students can refuse and withdraw from this research study anytime. Furthermore, there is no enforcement to participate by academic staff and no impact on academic performance.

1.6 Procedure

A day or week prior to the study small volume of blood (8 ml) will be obtained to test following (free of charge).

- Fasting blood sugar: 2 ml
- Full blood count + HbA1c: 2ml
- C-reactive protein + Lipid profile + Serum Creatinine: 4 ml

The study day, till the end of procedure, you will have to lie in comfortable sitting position.

- Sensors of the developed vasodilatory endothelial dysfunction assessment system will be connected to fingertips and wrist.
- Ultrasound probe will be used to scan radial artery.
- Pressure cuff will be connected to upper arm to stimulate the endothelial function.
- Thereafter, measurements will be taken from both developed system and ultrasound while you are at rest, reactive hyperaemia phase and post reactive hyperaemia phase.

1.7 Duration

You must rest for 30 minutes before the scan and hands should be clean. It will take around 20 minutes to acquire all the measurements from developed system and ultrasound scan. And also, you can recover immediately after the acquisition since it is non-invasive.

1.8 Side Effects

There are no side effects from the measuring system or from the ultrasound scan to the human body. Since, the data collection process is completely non-invasive, the patient can immediately recover after the data collection process.

1.9 Risks, hazards and discomforts

No major risks are involved with this research.

1.10 Potential Benefits

There may not be any direct or immediate benefit for you, but your participation is likely to help us find the answer to the research question. Future generations are likely to benefit if the research is successful.

1.11 Reimbursements

You will not be paid any sum of money for participating in this study.

1.12 Confidentiality

Your identity will be strictly confidential and will not be shared or reported in any format whatsoever. This information will be only accessible by the above-mentioned investigators for a period of 2 years. Your data will be referred only by a unique code not attached to your name.

1.13 Sharing the Results

Your identity will never be shared. At the end of the research we will publish results to educate other interested people.

1.14 Right to Refuse or Withdraw

You do not have to take part in this research study if you do not wish to do so. You may also withdraw at any time you choose to even while collecting data (with no penalty or effect on medical care or loss of benefits). Please notify the investigator as soon as you decide to withdraw your consent.

1.15 Clarifications

If you have any questions about any of the tests / procedures or information, please feel free to ask any of the persons listed below. You may ask at any time; before, while and after the study has started. You may also contact investigators for further clarifications through following contact details:

Dr. Anjula De Silva (077-7291737)

Prof. Saroj Jayasinghe (071-8619331)

This project has been approved by the Ethics Review Committee, Faculty of Medicine, University of Colombo. You may contact the committee if you wish to seek clarifications, record any concerns or make complaints about the study by calling 0112695300 extension 240 (between 9am and 4pm) or by sending an email to info.ethics@med.cmb.ac.lk

3. Questionnaire

Serial No:

Analysis of physiological parameters associated with vasodilatory endothelial dysfunction to assess risks of cardiovascular disease (Version 3.0)

Case Record Form

Date: Location:

Name:

Age: Height (cm): Weight (kg):

Sex: Male ☐ Female ☐ BMI (kg/m²):

Co-morbidities	Yes/No	Duration
Diabetes mellitus (Type I)		
Diabetes mellitus (Type II)		
Pre-diabetes		
Atherosclerosis		
Hypertension		
Myocardial Infraction		
Stroke		
Peripheral Vascular Disease		
Other cardiovascular disease		
Renal failure		
Liver cirrhosis		
Spinal cord or Finger Injury		

Family History	Yes/No
Diabetes mellitus (Type I)	
Diabetes mellitus (Type II)	
Atherosclerosis	
Hypertension	
Myocardial Infraction	
Stroke	
Peripheral Vascular Disease	
Other cardiovascular disease	

Physical Activity	Yes/No	Daily	Weekly
Sports			
Walking / Running			
Heavy worker			
Light worker			

Habits	Yes/No	Daily	Weekly	Monthly
Smoking				
Alcohol Consumption				
Vitamin Supplements				
Betel eating				
Sweets/ Sugar consumption				
Medical Drugs				

Please specify the disease/s of using medical drugs

.....
.....

Meal	Yes/No	Home	Outside
Breakfast			
Lunch			
Dinner			
Tea + snacks			
Extra meal			

4. Consent form

Analysis of physiological parameters associated with vasodilatory endothelial dysfunction to assess risks of cardiovascular disease

(Version 03, 310 August 2018)

CONSENT FORM

Part A - To be filled by the participant

The participant should complete the whole of this sheet herself/himself.

- | | |
|--|--------|
| 1. Have you read the information sheet? (Please keep a copy for yourself) | YES/NO |
| 2. Have you had an opportunity to discuss this study and ask any questions? | YES/NO |
| 3. Have you had satisfactory answers to all your questions? | YES/NO |
| 4. Have you received enough information about the study? | YES/NO |
| 5. Who explained the study to you? | |
| 6. Do you understand that you are free to withdraw from the study at any time, without having to give a reason and without affecting your future medical care? | YES/NO |
| 7. Information held by the investigators relating to your participation in this study may be examined by other research assistants. All personal details will be treated as STRICTLY CONFIDENTIAL. Do you give your permission for these individuals to have access to your records? | YES/NO |
| 8. Have you had sufficient time to come to your decision? | YES/NO |
| 9. Do you agree to take part in this study? | YES/NO |

Participant's signature: Date.....

Name (BLOCK CAPITALS):
.....

Part B - To be filled by the investigator

I have explained the study to the above volunteer and she has indicated her willingness to take part.

Signature of investigator: Date.....

Name (BLOCK CAPITALS):
.....