Non-invasive technique for Endothelial dysfunction assessment using a CBP-RPPG model in healthy and non-healthy subjects

Von der Fakultät für Ingenieurwissenschaften
Abteilung Elektrotechnik und Informationstechnik
der Universität Duisburg-Essen

Zur Erlangung des akademischen Grades

Doktor der Ingenieurwissenschaften (Dr.-Ing.)

genehmigte Dissertation

von

Vahid Sohani
aus
Teheran

Gutachter: Prof. Dr. -Ing. Axel Hunger
Gutachter: Prof. Dr. -Ing. Klaus Solbach
Gutachter: Prof. Dr. Aini Hussain
Gutachter: Prof. Dr. Jasmy bin yunus

Tag der mundlichen Prufung: 28.02.2017

ACKNOWLEDGEMENT

First and foremost I express my utmost gratitude to God who helps me to finish this work. Next, I would like to express my sincere gratitude to my supervisory team: Prof. Dr. Mohd Alauddin Mohd Ali, Prof. Dr. Edmond Zahedi, Prof. Dr.-Ing Axel Hunger and Dr. Gan Kok Beng for their excellent supervisions especially in giving important technical advice, invaluable guidance, many fruitful discussions and providing constant support and cooperation towards making this research progressive over the years. Further appreciation goes to the medical co-supervisors Prof. Dr. Oteh Maskon for his kind support and cooperation; from data collection to discussion and providing valuable advices.

My appreciation continues to all colleagues and friends involved in this work for their help and cooperation during data acquisition plus the fruitful and endless discussions on many important issues. I also would like to take this opportunity to thank the Ministry of Science, Technology and Innovation, Malaysia that supports this work through the Science Fund grant no. 03-01-02-SF0703.

Last but not least, this special appreciation is extended to my beloved spouse for her caring, understanding, patience, motivation and never-ending support and encouragement. Appreciation is also directed to my family who support me from the beginning to here.

ABSTRACT

The endothelial layer, which covers the internal surface of the arteries and arterioles, plays a vital role in maintaining adequate perfusion through constantly adjusting arterial diameter to metabolic demand. This explains why endothelial dysfunction is known as an early marker of cardiovascular diseases. Flow mediated dilation (FMD) is a well-known technique to assess non-invasively the endothelial function. During FMD, blood circulation of a large artery, such as the brachial, is blocked for five minutes and then suddenly released leading to reactive hyperemia due to increase in the diameter of the tested artery. Such a change in the artery diameter can be measured using high frequency ultrasound imaging. As the FMD-ultrasound technique is remarkably operator dependent and requires a very expensive setup, it has been limited to clinical research only. The main objective of the present study is to assess the endothelial performance via a forward model between central blood pressure (CBP) and radial Photoplethysmography (RPPG). To this end, CBP, RPPG and ultrasound images of the brachial artery are non-invasively and simultaneously recorded from 93 subjects. Ultrasound images are analyzed by an automatic edge-detection software providing a precise measurement of the arterial diameter. Using a threshold of 10% dilation, subjects are categorized into normal (FMD≥10%) and impaired endothelium groups (FMD<10%). Using system identification techniques, an individual CBP-RPPG model is estimated for each subject. The fitness of the individual model's output is used as a feature for discriminating normal from unhealthy subjects. The difference between the fitness during baseline (before blockage) and after cuff release is found to be a powerful criteria with results showing 86% and 96% of sensitivity and specificity when compared to FMD-ultrasound as reference. These results support the possibility of using this PPG-based technique to assess the endothelial performance non-invasively, hence opening the way towards a practical, low-cost alternative to perform mass-screening.

ÜBERSICHT

Die Endothelschicht, die die innere Oberfläche der Arterien und der Arteriolen bedeckt, spielt eine wesentliche Rolle bei der Aufrechterhaltung einer adäquaten Perfusion durch einen konstanten anpassenden arteriellen Durchmesser zur metabolischen Nachfrage. Dies erklärt, warum endotheliale Dysfunktion als frühzeitiger Marker von Herz-Kreislauf-Erkrankungen bekannt ist. Durchfluss-vermittelte Dilatation (FMD) ist in diesem Zusammenhang eine bekannte Technik, um die endotheliale Funktion nicht-invasiv zu bewerten. Bei FMD wird der Blutfluss einer großen Arterie getestet, indem sie zunächst für fünf Minuten blockiert und anschließend wieder geöffnet wird. Die dadurch verursachte reaktive Hyperämie durch die erweiterte Aterie kann mittels Ultraschall vermessen werden. Da die FMD-Ultraschalltechnik bemerkenswert bedienerabhängig ist und eine sehr aufwändige Einrichtung erfordert, wurde sie nur auf die klinische Forschung beschränkt. Das Ziel der vorliegenden Studie ist es, die Endotherialfunktion mittels einer Transferfunktion zwischen CBP und Photoplethysmo-graphie (RPPG) zu bewerten. Zu diesem Zweck sind CBP-, RPPG- und Ultraschallbilder der Arteria brachialis nicht-invasiv von 110 Probanden aufgenommen worden. Die Ultra-schallbilder werden durch die Verwendung einer automatischen Kantenerfassungssoftware analysiert. Bei einer Schwelle von 10% Dilatation werden die Probanden in normale (FMD> 10%) und beeinträchtigte Endothelgruppen eingestuft. Unter Verwendung von System-identifikationstechniken wird ein individuelles CBP-RPPG-Modell für jedes Individuum ermittelt. Die Fitness der einzelnen Modell-Resultate wird für die Unterscheidung von normalen von ungesunden Probanden verwendet. Der Unterschied zwischen der Fitness und der Fiteness in der Baseline (vor der Blockade) und nach der Öffnung ist signifikant verglichen mit dem FMD-Ultraschall als Referenz. Die Ergebnisse dieser Studie zeigen, dass die Untersuchung der Endothelfunktion durch das PPG-basierte Verfahrenauch nicht invasiv erfolgen kann und eine praktische und kostengünstigere Alternative darstellen.

CONTENTS

LIST OF TABLE

LIST OF ILLUSTRATIONS

LIST OF ABBREVIATION

α	False positive conclusion (Type I)
β	False negative conclusion (Type II)
AC	Alternating current (amplitude change)
Ach	Acetycholine
AHA	American Heart Association
ANOVA	Analysis of variance
ARX	Auto Regressive with exogenous input
ARMAX	Auto Regressive with Moving Average exogenous input
ASCII	American Standard Code for Information Interchange
AUC	Area under the curve
BA	Brachial artery
BMI	Body mass index
BP	Blood pressure
CAD	Coronary artery disease
CBP	Central Blood Pressure
CI	Confidence interval
CR	Coefficient of correlation
CRP	C-reactive protein
CoV	Coefficient of variation
CVD	Cardiovascular diseases
DC	Direct current (steady state)
DSP	Digital signal processing
ECG	Electrocardiograph
ED	Endothelial dysfunction
EDRF	Endothelium derived relaxing factor
ELAM	Endothelial leucocyte adhesion molecule
ET-1	Endothelin-1
EF	Endothelial function
FFT	Fast Fourier Transform

FN	False negative
FMD	Flow mediated dilation
FP	False positive
FPE	Final prediction error
GTN	Glyceryl trinitrate
GUI	Graphical user interface
Hb	Hemoglobin
HbO_2	Oxyhemoglobin
HF	High frequency
HUKM	Hospital University Kebangsaan Malaysia
ID	Identification number
LED	Light emitting diode
LF	Low frequency
LP	Low pass
LPF	Low pass filter
LTV	Linear time variant
NO	Nitric oxide
p-value	Probability of rejecting the null hypothesis
PPG	Photoplethysmography
PTT	Pulse transit time
PWA	Pulse wave analysis
PWV	Pulse wave analysis
R	Correlation coefficient
RMSE	Root mean square error
ROC	Receiver operating characteristic
RPPG	Radial Photoplethysmography
SD	Standard deviation
SE	Standard error
SEM	Standard error of mean
SI	Stiffness index
Sn	Sensitivity
Sp	Specificity
Std.	Standard

TN	True negative
TP	True positive
UKM	University Kebangsaan Malaysia
US	Ultra sound
V	Loss function
WHO	World Health Organization

CHAPTER I

INTRODUCTION

1.1 INTRODUCTION

CV disease plays an important role on human's mortality and morbidity to what extent that it is globally known as the world largest killer (Finegold et al. 2013; Go et al. 2014; Nowbar et al. 2014). The silent nature of CV diseases makes it more complicated compared to other chronic and death-leading diseases. This matter usually leads to late detection so that in many cases, CV abnormalities can only be controlled but not fully treated. Short-breathing, sweating, obesity and thoracic pain could be addressed as the most prevalent symptoms of CV diseases.

Nowadays, the possibility of capturing CV abnormalities has been significantly increased via high-tech technologies. Such achievement can be known as a result of contribution of other related disciplines. For instance, CV engineering -in both invasive and noninvasive aspects- could provide novel approaches for early detection of CV abnormalities (Abdolrazaghi et al. 2010; Haverich & Wilhelmi 2011; Nugent & Edelman 2003). Obviously, engineering-based technologies can enhance the reliability and accuracy of diagnostic techniques while improving the quality of therapy. However, there are still considerable cases in which CV diseases cannot be easily detected.

The CV system is consisted of the heart, vasculature and blood which cover human bodies from head to toe via closed network of vessels. Such a ubiquitous system circulates blood across the body from individual's birth to death. It is responsible for supporting the entire body with nutrition and oxygen while removing generated by-products and deoxygenated blood along each cardiac cycle.

Many factors could affect the process of blood circulation and CV performance. As such, physical activity, eating style, smoking, excessive alcohol consumption and mental stress can be mentioned (Harvey 2006). Surprisingly, genetics also play an incredible role on existing and progressing of CV (Go et al. 2014). Aging is another natural factor that gradually change the performance of CV system along individual's lifetime (North & Sinclair 2012). In this process, the vascular stiffness is gradually increased leading to local blockage and further abnormalities (Higashi et al. 2012).

Unquestionably, vascular abnormalities play a significant role on CVD (Custodis et al. 2010). As such, endothelial disorders can be mentioned (Taimeh et al. 2013). Endothelial layer is a thin layer of cells which are located across the interior surface of vessels. Blood clotting, inflammation, vasodilatation and vasoconstriction can be mentioned as some of vital features which are handled by the endothelial layer (Sandoo et al. 2010). In addition, the process of vascular tuning is mainly handled by this layer along each cardiac cycle (Lerman & Zeiher 2005). Like other CV disease, the endothelial-related abnormalities do not have any significant symptom(s) and thus, cannot be detected in early stages. This is a very general abnormality particularly among elderly people. Such abnormality is scientifically known as the e*ndothelial dysfunction*. Once it occurs, the physiological process of vascular tuning fails and disturbs the circulation causing further CV abnormalities to arise (Solomon et al. 2003).

The endothelial activity can be clinically evaluated via High Sensitive C-Reactive Protein test (HSCRP) which represents the value of "C protein" in blood sample. However, this parameter is not exclusively used for assessing the endothelial performance. In other words, inappropriate value of C protein in blood sample may show malfunctioning of other organs (e.g. liver, kidney) rather than the ED (Recio-Mayoral et al. 2011). Therefore, the HSCRP test is not known as an exclusive test to assess the endothelial performance.

Alternatively, the endothelial activity can be evaluated via ultrasound imaging. In this technique, the endothelial layer is physiologically stimulated by blocking the blood circulation in a large artery (e.g. radial artery). This technique is known as the flow mediated dilation (FMD) in which blood circulation in an artery is fully occluded

via a pressure cuff. Soon after five minutes, the pressure cuff is suddenly released resulting in retrieving blood circulation while providing a state of reactive hyperemia. The reaction of the functional endothelial cells to such a physiological stimulus is secretion of chemical substance of Nitride Oxide (NO). Once NO is released, vasodilatation occurred causing vessel size to increase. The endothelial activity along the FMD stimulation can be captured via ultrasound imaging. In this regard, the variation between brachial diameters in baseline and after cuff release shows the current performance of the endothelial layer (Corretti et al. 2002; Harris et al. 2010). This approach is known as FMD-Ultrasound (FMD-US) technique. The FMD-US technique has been well established and known as the most attended technique to assess the endothelial performance non-invasively.

Interestingly, Photoplethysmography (PPG) has also been attended as a potential tool to assess the endothelial performance non-invasively. In fact, it is a well-known technique in which variations of blood flow over each cardiac cycle can be easily detected via a cost-effective optical system (Webster 1997). The PPG signal has been widely used in clinical applications over last two decades (Allen 2007). It is also known as a vital signal and can be known as inseparable part of today's patient monitoring systems. One of the recent work in this field has been done by Rosmina Jaafar (Jaafar 2009). Results of this research have confirmed the suitability of PPG for ED assessment. In fact, it has shown that the endothelial performance can be also assessed via PPG. In this research, endothelial layer is physiologically stimulated -using the FMD test- across the peripheral artery while endothelial reaction is assessed from subject's index finger. Truly, implementation of this technique is considerably cost-effective, less operator dependent and simpler compared to the FMD-US technique. However, the main limitation of this technique is the possibility of capturing both endothelial and non-endothelial factors due to a physiological phenomenon known as the auto-regulation. Another limitation of this work is to measure the vessel diameter manually. In addition, occlusion of arterial blood circulation is done via a typical pressure cuff which is not match with the guideline of the FMD test (Corretti et al. 2002).

1.2 PROBLEM STATEMENT

Interestingly, there is a good consensus considering the ED as an early marker of many CV abnormalities such as atherosclerosis, hypertension and diabetics (Bonetti et al. 2003; Lerman & Zeiher 2005; Stam et al. 2006). In past (and even somewhere now) the ED is indirectly derived from other CVDs such as hypertension and diabetics (Calles-Escandon & Cipolla 2001; Schalkwijk & Stehouwer 2005). In other words, the ED is detected when an abnormality has already occurred whereas an early detection of ED may help in controlling disease at initial stages and thus, prevent from further CV abnormalities.

Recently, FMD-US technique has been well established. In fact, it is the most attended technique to assess the endothelial performance non-invasively. Using this technique, ED or endothelial abnormalities can be faithfully detected in early stages. However, implementation of this method requires an expensive system setup (e.g. ultrasound machine, high frequency transducer, etc.). In addition, this technique should be done by an experienced and skilled operator who has adequate knowledge about human physiology and system setup. Moreover, an additional vessel-edge-detection program is needed in order to trace the endothelial functionality based on ultrasound images. As conclusion, main limitations of the FMD-US technique can be summarized as below (Corretti et al. 2002):

1) Expensive system setup (mainly ultrasound and high frequency transducer)

2) Highly operator dependent

3) Need to an additional software in order to analysis vessel diameter

Referring to Rosmina Jaafar's work explained above, assessing the endothelial performance from a small artery (index finger) may reflect both endothelial and non-endothelial factors. Accordingly, another problem of using the recent PPG system would be:

4) Assessment of endothelial performance in conjunction with effect(s) of autoregulation in a small artery

Thus, reliability of the PPG technique -in conjunction with FMD-US- still needs further investigation(s). Therefore, the next step towards establishing PPG technique is to investigate the possibility of assessing the so-called pure endothelial performance (i.e. excluding endothelial non-factors) via a novel approach. Such technique is also supposed to be cost-effective and operator independent. Hence, there is a need for a novel and reliable approach to facilitate endothelial evaluation preferably with a cost-effective and operator independent system.

1.3 RESEARCH OBJECTIVES

The main objective of this research is to assess the pure endothelial performance via PPG. In this regard, parametric modelling is considered not only to assess the ED but also to trace the endothelial performance before (baseline) and after conducting the FMD stimulus.

It has been known that the aorta is coupled to the heart and thus, central (aortic) blood pressure (CBP) is not simply affected compared to BP in following arteries. From the other side, obtaining a PPG signal is considerably easy and cost-effective, as mentioned above. Since this research aims to investigate the pure endothelial performance (i.e. in a large artery), radial site (wrist) is considered to obtain the PPG signal. Accordingly, a forward model between CBP and the radial photoplethysmogram (RPPG) [CBP $\rightarrow$ RPPG] is considered to assess the endothelial performance among both healthy and un-healthy groups, as shown in Figure 1.1. The secondary objectives of this research are as follows:

1. To estimate a dynamic CBP $\rightarrow$ RPPG model for each subject at rest
2. To investigate the effect of the FMD test on a model (i.e. the goodness of a model before and after applying the FMD stimulus)
3. To propose a reliable approach to identify the ED
4. To compare results of the proposed technique and the FMD-US technique

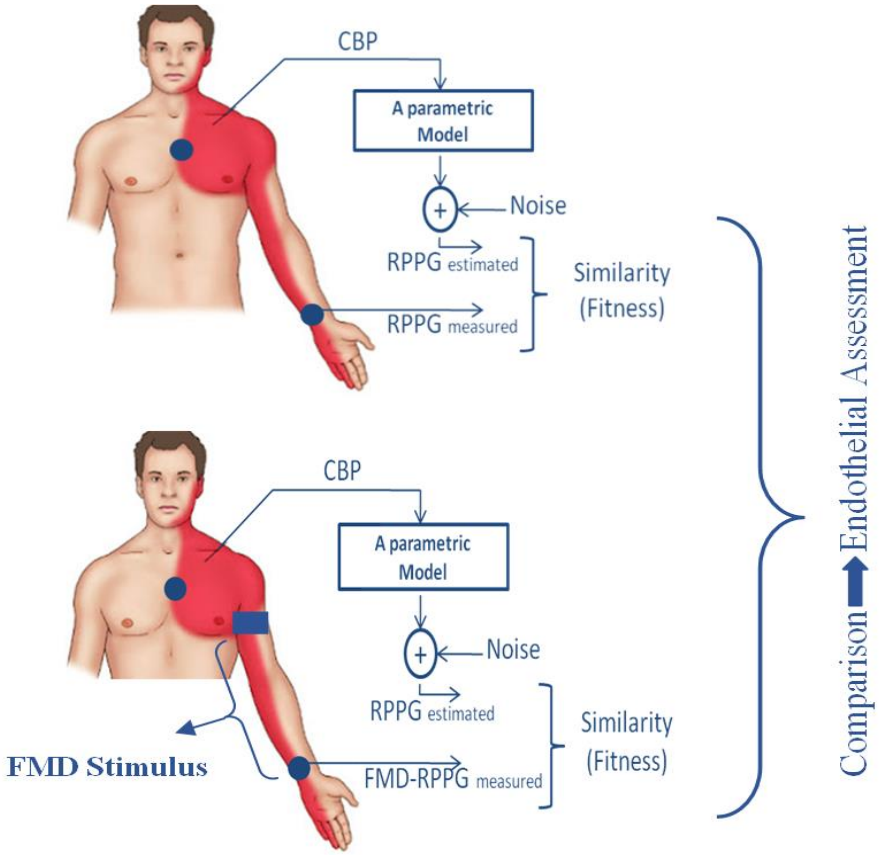


Figure 1.1 Research in one view: endothelial assessment by comparing goodness of CBP→RPPG model in baseline (top) and after the FMD stimulus (bottom)

1.4 RESEARCH SCOPE

Specifically, this research aims to improve the ED assessment via the PPG technique. Such improvement possesses two aspects; (i) taking a large artery to assess endothelial functionality in order to avoid capturing non-endothelial effects (e.g. auto-regulation) and (ii) analyzing the endothelial response based on a parametric model -between CBP and RPPG (CBP→RPPG)- in order to better trace the dynamic reaction of the endothelium. To this end, data were collected among both healthy and risky subjects in the HUKM. In general, subjects with stroke or myocardial infarction are excluded from this research. Instead, subjects between 30-60 years old with and without CVD are considered and participants. However, utilizing an in-use ultrasound machine can be mentioned as a big limitation of this work. Accordingly, the sample size and unbalancing between numbers of each group seems to be an unavoidable limitation in

this study. Regardless of such limitations, investigation of suitability of the proposed technique -in conjunction with FMD-US technique- among both healthy and non-healthy groups can be mentioned as the objective of this work.

1.5 HYPOTHESIS OF THE RESEARCH

In this research, we propose an alternative approach for evaluating the endothelial activity via CBP $\rightarrow$ RPPG model. This model is supposed to have a stable behavior under normal circumstances (baseline). In contrast, the goodness of this model is supposed to be affected (i.e. parametric changes) during the FMD stimulus. In this regard, the hypothesis of this research can be addressed as below:

- Goodness of CBP $\rightarrow$ RPPG model (i.e. fitness curve) can be used -as a criterion- to assess the endothelial performance
- After the FMD stimulation, goodness of CBP $\rightarrow$ RPPG model is significantly varied among healthy subjects whereas it is not significantly changed among unhealthy subjects
- There is a significant variation between values of height of peak of healthy and unhealthy subjects
- There is no significant variation between values of height of peak among male and female subjects
- The proposed model has similar result as the FMD-US technique

1.6 THESIS ORGANIZATION

Chapter I (Introduction) conveys the general concept of this research. It gives an overview of this investigation including: importance of this research, problem statement, proposed method and research objectives.

Chapter II (Literature Review) describes the human CV system and its constructive portions. Then, the role of endothelial layer on blood circulation and CV performance is studied. Afterwards, clinical importance of endothelial layer and symptom(s) of ED are reviewed. Then, some potential techniques to assess the

endothelial functionality are introduced. After that, current techniques to access the endothelial performance are described. Furthermore, the nature of PPG is explained and its clinical usage in today's healthcare industry is reviewed.

Chapter III (Methodology) covers the details of data acquisition: from protocol to hardware configuration and system setup. The proposed technique is explained in details including signal processing and system identification (modelling). This section is divided into two parts: (i) procedure of estimating a dynamic model between the CBP and RPPG and (ii) endothelial assessment via this model. Statistical analysis is then performed.

Chapter IV (Results and discussion) include results of the proposed technique explained in chapter three. The results are verified in conjunction with the FMD-US technique (as a reference technique). Then, the results are discussed and statistically analyzed to find out values of sensitivity, specificity and accuracy of the proposed technique. At the end of this chapter, advantages and disadvantages of this technique are discussed.

Chapter V (Conclusion and future work) summarizes the proposed technique and its results in conjunction with the FMD-US technique. In addition, limitations of the proposed methodology are explained and some suggestions are given in order to improve the quality of future works.

CHAPTER II

LITERATURE REVIEW

2.1 HUMAN CARDIOVASCULAR SYSTEM

The CV system is an asympathetic organ which circulates blood through our bodies. This ubiquitous system is the human's longest organ which plays a vital role on organic interactions along different metabolic conditions. The heart is always beating to supply entire organs by essential substances and fresh oxygen while removing deoxygenated blood, urea and by-products along each cardiac cycle. This process is known as systemic and pulmonary circulations respectively. Figure 2.1 shows the direction of pulmonary (red) and systemic (blue) blood circulations.

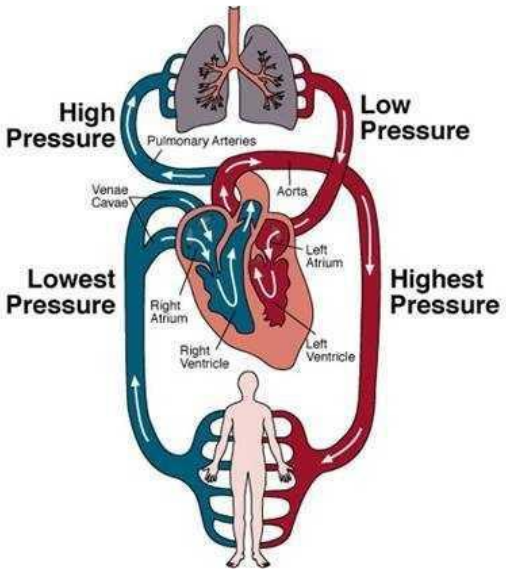


Figure 2.1 Direction of pulmonary (blue) and systemic (red) blood circulations

Source: Whittemore 2009

The CV system consists of three components; the heart, blood and vascular bed. In general, the heart can be considered as a muscular pump to propagate the blood within a closed network of vessel. The heart is continuously beating from birth to death, even at rest. The electrocardiography (ECG) is a well-known technique to represent electrical activity of the heart by placing several electrodes across the chest (Goldberger 2012). The general action of heart can be faithfully assessed by the ECG signal. It is always known as a vital sign and has a wide range of application in today's healthcare industry.

The blood is a limited volume of hemodynamic fluid which travels across our bodies to transfer nutrients, oxygen and humoral agents while returning generated by-products and urea to the heart. It is frequently purified along each breathing cycle to get fresh oxygen. The hemodynamic properties of blood (i.e. blood pressure and flow) are widely used in cardiovascular engineering and bio physics**.**

The vascular bed is an asymmetric T-shape elastic tube which covers our body from toe to head (Reinhold et al. 2012). It can be studied as the vital bio-transit path which facilitates blood circulation across our bodies. The vascular bed is consisted of artery, vein and capillary with similar functionality (streaming blood) but different structural characteristics. The arterial network is responsible for forwarding fresh blood from the heart to the entire organs while venous network is transfers deoxygenated blood from entire organs to the heart. Besides, the capillary facilitates blood circulation between artery and vein. In fact, capillary connects the arterial network to the vein system and thus, plays an important role on human microcirculation process. Vessels are geographically dissimilar in length, width and geometrical configurations (i.e. tapering, branching and thickening) and thus, have a different impedance (Mazumdar 2015; P. Segers 2010). Compare to artery and vein, the capillary has a lower conduit diameter with a thicker layer of cell. It facilitates exchanging of water, oxygen, carbon dioxide, nutrients and waste chemical substances between blood and surrounding tissues (Daneshfard et al. 2014). The index finger and auricle are two popular sites for capturing the microcirculation. Figure 2.2 shows the capillary in conjunction with an artery and vein.

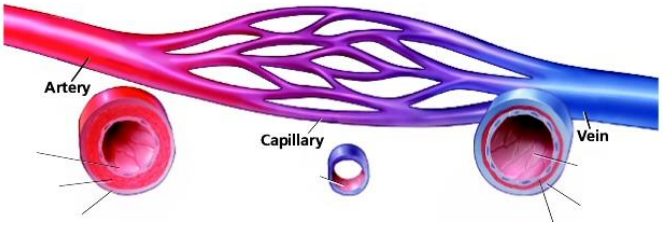


Figure 2.2 The capillary, a connection path between artery and vein

Source: Whittemore 2009

Since the vascular network is coupled to the heart, even small abnormality in the vasculature, particularly in coronary vessels, disturbs the cardiac performance and may lead to sudden heart attacks (Murthy et al. 2012). As such, the atherosclerosis (i.e. caused by endothelial dysfunction) can be mentioned in which fatty materials are gradually accumulated together and slowly decreased the vessel conduit (Libby et al. 2002). Another prevalent abnormality of the vessel is the vascular stiffness -known as arteriosclerosis- which naturally increases along the human's lifetime (Lee & Oh 2010).

2.2 ENDOTHELIAL LAYER

The endothelial layer is a thin layer of cell which entirely paves the interior surface of our vascular tree (Reinhold et al. 2012). This layer is also known as the innermost layer of vessel which is in direct and permanent touch with blood. There are various biological interactions which are mainly or partly handled by this multifunctional layer in both pulmonary and systemic circulations. The vascular tuning, coagulation, fibrinolysis, leukocyte adherence, platelet interaction and vascular permeability are mainly done by the Endothelial cells (Dignat-George & Boulanger 2011).

It has been demonstrated that endothelial layer has a network-like functionality meaning that a local endothelial performance represents the functionality of the endothelial performance across the entire vasculature (Celermajer 1997). The endothelial functionality is controlled by autonomous system and can be affected by local metabolic conditions (Bouïs et al. 2001). The endothelial performance is one of

the most reliable indicators to predict CV events (Kuvin et al. 2001). Figure 2.3 illustrates the pathology of this vital layer.

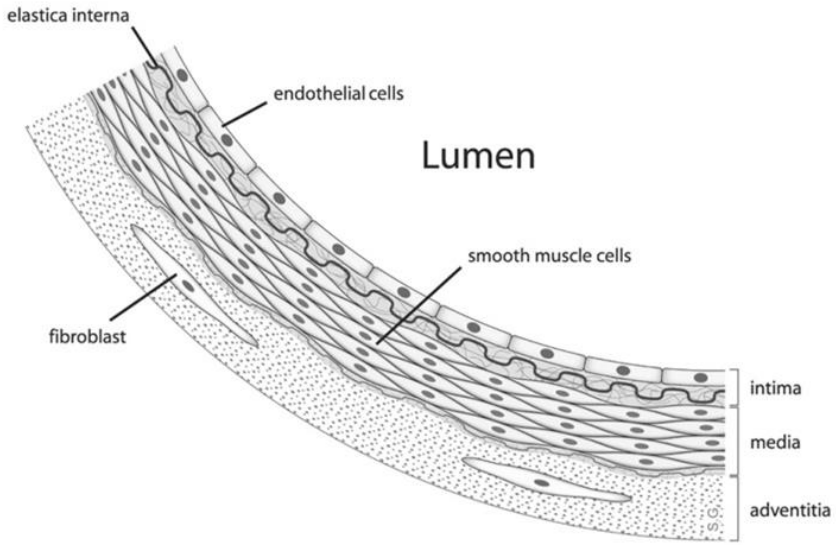


Figure 2.3 The human's innermost layer of vessel

Source: Félétou 2011

2.2.1 Role of Endothelial Layer on Blood Circulation Process

It is clear now that the density of blood alters frequently along each cardiac cycle mainly due to current metabolic condition. In addition, it may vary following changes in local interactions or even external stimulus. Therefore, the intravenous diameter should be accordingly adjusted in order to retrieve blood transferring and prevent from any size-related disturbance on blood circulation. Such a complex action is mainly handled by the endothelial cells (layer). In fact, the endothelial layer plays a homeostasis role to regulate blood flow along different metabolic conditions.

As quoted earlier the endothelial cells are located across the innermost layer of vessels and thus can affect blood circulation from hemodynamic viewpoint (e.g. blood pressure and flow) by changing the resistance of blood stream (vessel). This matter has been attended mostly in CV engineering and hemodynamics. For instance, Pries et al. proposed a model-based technique to measure the flow resistance in small vessels in-

vitro (Pries & Secomb 2005). In this work, the vessel diameter and hematocrit were studied. However, obtained in-vitro results were not similar to an observation which was invasively acquired from rats. They reported that the flow resistance in small vessels is considerably higher than what they measured in-vitro. After peer investigation, the endothelial layer was introduced as the main cause of such discrepancy and thus, the role of endothelial layer in vascular resistance, blood pressure and blood flow was endorsed again.

The endothelial layer has been also studied from permeability viewpoint. It is demonstrated that the endothelial layer acts as a sieve to pass nutritious particles into surrounding organs while transmitting by-products from surrounding organs into the blood circulation (Curry & Adamson 2012; Mehta & Malik 2006). In fact, the endothelial cells operate as a gate to pass favorite substances into surrounding organs and vice versa, then the waste substances get to the circulatory system. This process is mostly done in capillaries which are the thinnest component of vascular bed in conjunction with our autonomous system. Figure 2.4 shows this feature via a graphical illustration.

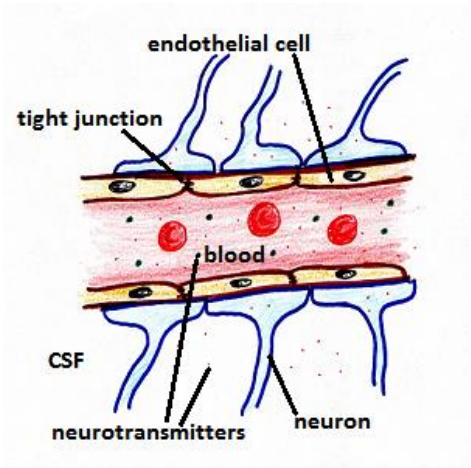


Figure 2.4 The endothelial cells as a transmitter

Source: Anon 2012

2.3 ENDOTHELIAL DYSFUNCTION

As quoted earlier, there are several interactions which are mainly or partly handled by the endothelial cells but the endothelium is normally known as a vascular tuner along different metabolic conditions. Although this matter is still being investigated but it was already discovered that the Nitride Oxide (NO) plays an important role on adjustment of vascular conduit (Tousoulis et al. 2012). This chemical substance is also known as an endothelium-dependent vasodilator. In fact, the process of vasodilatation and vasoconstriction are mainly handled by releasing sufficient volume of NO in conjunction with other chemical substances. The endothelial dysfunction is normally referred to the unbalancing between vasodilators and vasoconstrictors which are released by endothelial cells. An inappropriate secretion of NO may affect vascular tuning and lead to further CV abnormalities. The endothelial dysfunction has been captured as a root of many CV diseases such as hypertension and diabetics (De Vriese et al. 2000; Landmesser & Drexler 2007). This matter motivates scientist to investigate the possibility of endothelial assessment -as a reliable indicator- to predict CV events. The endothelial layer is shown in Figure 2.15.

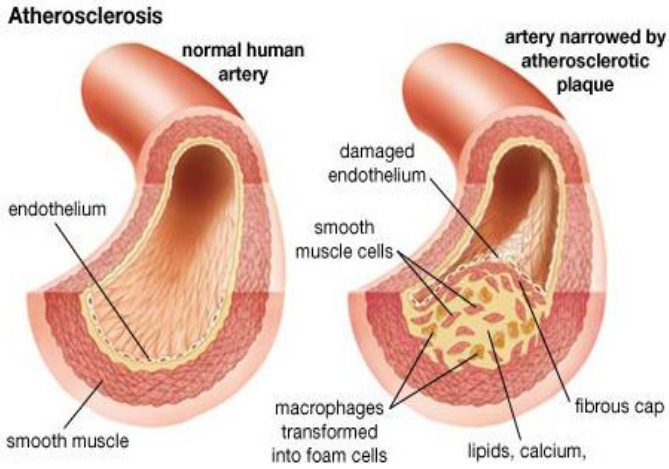


Figure 2.5 The Endothelial layer: normal (left) impaired (right)

Source: Anon 2012

2.3.1 Symptoms of Endothelial Dysfunction

Investigations have shown that ED does not reveal any symptom(s) in early stages. For instance, Al Suwaidi et al. conducted a cross-sectional study to investigate the possibility of detecting the ED at the beginning (Al Suwaidi et al. 2000). In this research, the endothelial functionality was examined among three groups, namely people with normal endothelium as well as those with mild and severe ED. During a long-term follow-up research which involved 157 subjects, only ten cardiac events were observed among those people with severe ED whereas no significant symptom(s) were observed among this group at the beginning. Thus, they concluded in their report that the ED cannot be detected at the beginning.

Nowadays, the silent nature of ED is known as a real threat and as such, several teams have investigated the possibility of ED assessment via extra clinical approaches (e.g. sleep and depression). In a very strange but interesting work, Sherwood et al. showed that there is an association between ED and depressive symptomatology (Sherwood et al. 2005). They measured the endothelial activity among 143 subjects in total and then reported that the endothelial reaction is reduced among those people with certain depression compared to others. They then used the antidepressant drugs and observed a slight improvement on endothelial reaction after administration of such drugs. The association between ED and depression has also been addressed by Mausbach et al. in another similar work (Mausbach et al. 2012).

In another research by Kohler et al. the endothelial performance was examined on 79 subjects which included 64 patients -who suffered from sleep apnea- and 15 healthy subjects (Kohler et al. 2008). Results of this investigation showed that there is a poor endothelial activity among patients with sleep apnea compared to others. Thus, it was concluded that the endothelial functionality may have inadequate activity among those people with sleep apnea.

2.3.2 Clinical Importance of Endothelial Dysfunction

The ED has been remarkably addressed as an initial marker of CV disorders. As an evidence, Perticone et al. reported that the ED occurred prior to hypertension (Perticone

et al. 2001). In this research, the endothelial cells was pharmacologically examined by injecting some chemical stimulant (e.g. sodium nitroprusside and acetylcholine) among a total of 225 untreated hypertensive patients. The endothelial reaction was assessed via dose-response curve to intra-arterial infusions. A very poor endothelial response was observed among these patients that led to a conclusion that the endothelial cells was already impaired among hypertensive patients. Therefore, they suggested that endothelial dysfunction can be used as an indicator of ongoing CV abnormalities among hypertensive patients.

In another similar study by Budhiraja et al. ED was introduced as one of the main factors of increasing blood pressure (Budhiraja et al. 2004). They reported that an inadequate secretion of endothelium-derived vasodilators (i.e. NO) may lead to unbalancing of bioavailability of NO so that high blood pressure could be consequently occurred.

The role of ED in diabetes has been also investigated (Sena et al. 2013). Although such association was already discovered but the issue of causality was still not fully understood (Caballero 2003). This matter was recently investigated by Balletshofer et al. in which they evaluated the endothelial functionality among a group of healthy individuals with at least one first-degree relative with certain type II diabetes (Balletshofer et al. 2000; Tesauro et al. 2007). Although they did not reveal any significant symptom(s) at the beginning but results confirmed the existence of ED among this group. Thus, the potential value of endothelial dysfunction assessment -as an early marker of diabetes- was endorsed again. Similarly, Schalkwijk and his co-worker reported that in type I diabetes, the ED occurred prior to diabetic micro-angiography whereas in type II diabetes, the endothelial cells became impaired as a consecutive result of diabetes (Schalkwijk & Stehouwer 2005).

Hyperglycemia is another prevalent abnormality in which glucose is accumulated within vessels. This abnormality is known as an initial stage of diabetes. Interestingly, there is a meaningful relationship between hyperglycemia and the ED (Bakker et al. 2009). Such relationship relies on the finding that an impaired vaso-regulation in presence of hyperglycemia is most probably caused by the ED. Therefore,

endothelial disorder may be considered as an early stage of hyperglycemia which in return, may lead to diabetes.

Atherosclerosis is a well-known chronic abnormality in which fatty particles are accumulated together and gradually formed a vascular blockage (Sanz & Fayad 2008). The association between ED and atherosclerosis was first investigated by Ludmer et al. (Ludmer et al. 1986). In this research, the acetylcholine was used to induce the endothelial cells. It was injected into a total of eighteen subjects which included; eight patients with advanced coronary disease, four healthy and six patients with mild coronary atherosclerosis while the endothelial reaction was assessed via angiography. Although acetylcholine was supposed to induce the endothelial cells to release the NO and dilate vessel diameter but a paradoxical reaction was observed among atherosclerotic patients in which acetylcholine led to vessel constriction instead. They concluded that the endothelial cells are already impaired among atherosclerotic patients. Therefore, the ED can be considered as an early sign of atherosclerosis.

The ED is even known as one of the principle factors of forming the atherosclerosis. In this regard, Bonetti et al. studied the role of ED on fat accumulation within the vessel (Bonetti et al. 2003). They described that the main cause of atherosclerosis is due to unbalance bioavailability of the NO as a potent substance of vascular tuning leading to ED as a reliable marker for detecting atherosclerosis in the early stage. ED is later captured as a prior disorder of atherosclerosis (Davignon & Ganz 2004). The relationship between the ED and atherosclerosis has been particularly attended over last decades. In fact, it is a very common abnormality among elderly people. A fatty bulk disturbs blood circulation by narrowing the vascular diameter causing the blood pressure and blood flow to increase (Insull 2009). Therefore, it is highly considered as a reliable indicator of atherosclerosis in preclinical stages (Mudau et al. 2012). Figure 2.6 illustrates the atherosclerosis in a vessel.

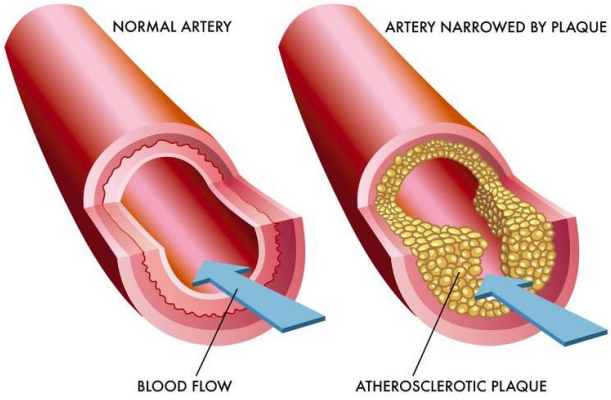


Figure 2.6 A normal vessel (left) versus an atherosclerotic vessel (right)

Source: Atherosclerosis 2016

The ED has been captured as a vascular disorder which most probably occurred before vascular abnormalities. Therefore, the clinical importance of ED has been particularly attended as a reliable indicator of vascular malfunctioning in preclinical stages (Puddu et al. 2000).

The association between ED, diabetes and atherosclerosis was also addressed by Järvisalo et al. in which endothelial performance was assessed via FMD technique (Järvisalo et al. 2004). The test was done on 75 children including 45 type I diabetes and 30 healthy. They noticed a lower peak of FMD and an increased in carotid intima-media thickness among diabetics compared to others. They also highlighted that the intima-media thickness was even larger among the 16 children who had certain ED as well as diabetes. They, then, suggested that ED in diabetic children may increase the chance of atherosclerosis. As a conclusion, the clinical importance of ED -to detect CV abnormalities in early stages- has been remarkably addressed in many studies (Meigs et al. 2004; Meigs et al. 2006; Rossi et al. 2005).

2.4 INTRODUCTION OF POTENTIAL TOOLS FOR ENDOTHELIAL ASSESSMENT

Referring to chapter I, the main objective of this research is to assess the ED non-invasively via a dynamic model between central blood pressure (CBP) and radial

photoplethysmogram (RPPG). In general, the proposed technique is consisted of two aspects: PPG and system identification (SI). Hence, these methods are firstly introduced in the following sections. Reasons of taking CBP -as model input- and current techniques to access it are then explained in order to better understand the proposed model. Finally, currently available methods to assess ED -including PPG and SI- are explained.

2.4.1 Photoplethysmography

Photoplethysmography (PPG) is an opto-electrical system for capturing blood volume change in small vessels. Basically, PPG probe is consisted of a light source (e.g. LED) for emitting light as well as a photodetector for collecting the light beams. These two components can be placed either in the same side (reflection mode) or opposite side (transmission mode) as can be seen in Figure 2.7 (Webster 1997). Basically, emitted beams can be passed, reflected or absorbed at the time of hitting to the skin. However, only passed beams can be collected by the photodetector. In fact, the passed beams reflect the blood flow change over each cardiac cycle. Such variation can be seen by representation of an obtained data through an electrical signal known as PPG signal.

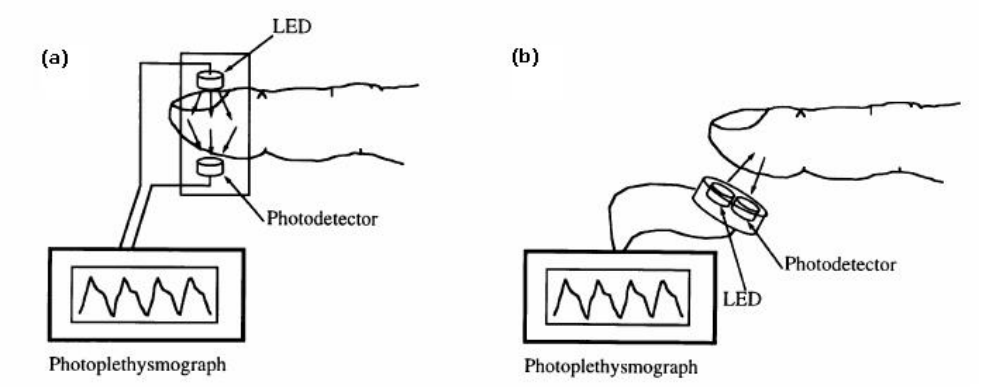


Figure 2.7 Configuration of a typical PPG probe in transmission (a) and reflection mode (b)

Source: Webster 1997

Simple configuration of PPG sensors makes this technology considerably cost-effective, easy to use, portable and operator independent compare to other vascular

screening technologies. Therefore, it has been widely used over last decades mainly for monitoring of autonomous functions, vital signs and blood oxygen saturation (i.e. SpO_2) (Allen 2007).

a) Role of PPG in Cardiovascular Engineering

Indeed, the PPG can be considered as a pioneer technology to access blood oxygen saturation (SpO_2) (Bagha & Shaw 2011). In this technique, the SpO_2 signal could be obtained by illuminating light beams across an auricle while wavelength was quickly changed between red and infra red. The amplitude of collected light was significantly varied due to dissimilar behavior of illuminated wavelengths to SpO_2 floated in the blood. Such a variation represented the degree of oxygen saturation in target vessel. This technique has been gradually developed and well established in today's clinical practices.

PPG was also considered for obtaining heart rate which is known as a reliable vital sign particularly in ambulatory medicine. However, a pure PPG signal was affected by some unwanted distortion mainly caused by respiratory movement. Such unavoidable noise may hide some significant information in a PPG signal. In 2005, Foo et al. improved the quality of PPG signal by removing the respiratory noise via an algorithm-based digital filtering (Foo et al. 2005). The accuracy of PPG-derived heart rate was then reinvestigated by Yu et al. (Yu et al. 2006). In this work, both ECG and PPG signals were simultaneously recorded among 158 accident patients for seven seconds. Using standard signal processing techniques, hear rate was calculated from each signal independently. Obtained heart rates were then compared to each other resulting in an excellent match between ECG-derived and PPG-derived heart rates with over 92 percent of similarity. Thus, suitability of the PPG technique -for obtaining the heart rate- was endorsed again.

PPG has been also considered as a potential tool to predict vascular risks. In this regard, Kalaivani Chellappan has proposed a model based approach to predict physiological age of vessels through empirical data modeling (Chellappan 2009). In this work, an electrical lumped model was used in order to model arterial blood flow and the left ventricular pressure. In fact, electrical elements were used to represent vessel

resistance, compliance and blood inertia. Such parameters were then validated using actual ageing, effect of exercise and CV risks. This approach was proposed based on similarity between components of PPG waveform and the above mentioned parameters. Accordingly, PPG was introduced as a practical approach to obtain vascular parameters non-invasively.

Respiratory rate is another vital sign that can be reliably obtained via PPG (Nilsson et al. 2000). In this regard, Leonard et al. proposed an automated algorithm for determination of respiratory rate based on PPG signal (Leonard et al. 2006). A comparison analysis was done between the PPG-based method and the ECG technique. Results showed a reliable accuracy in the PPG approach. Since then, this concept has been improved and well established in the healthcare industry as a reliable approach to obtain the respiratory rate (Sahni 2012).

Cardiac output can be also accessed via PPG. This parameter is scientifically known as the stroke volume which indicates the volume of blood rushed out from the heart towards the arterial network (Allen 2007). The stroke volume was previously calculated using Doppler-pulse technology (Pemberton et al. 2005). In this technique, stroke volume was calculated by multiplying values of aortic diameter and aortic velocity obtained via Doppler-ultrasonography. Irrespective of costly setup, further investigations showed that the stroke volume -obtained by the Doppler technique- is not reproducible and may vary from time to time (van Geldorp et al. 2011). Alternatively, PPG was used as a potential tool to access the stroke volume (Pollonini et al. 2015). In this research, data (i.e. ECG and PPG) were simultaneously obtained among fifteen healthy young subjects in baseline and during an incremental cycling protocol. In fact, the exercise was used to improve the blood supplement inside the aorta. Using linear models, hemodynamic variables (e.g. heart rate, pulse transit time, etc.) were calculated and compared to baseline values confirming the suitability of PPG for stroke volume assessment.

b) Advantages and Limitations

PPG is remarkably cost-effective compare to other sensors particularly in the domain of cardiology. In addition, it is really operator independent and very easy to use. Moreover, this system is portable, noninvasive and safe. As quoted earlier, this technique is widely used for representation of vital signs. Nowadays, this technology is widely used in daily clinical monitoring and ambulatory diagnosis. However, this technique is so sensitive to motion artifacts as explained earlier. Therefore, appropriate adjustment of a PPG probe -over site of interest- is highly required. Besides, the issue of environmental light(s) around the PPG sensor should be taken into account as it may disturb the quality of PPG signal. In this regard, data should be preferably collected in a twilight atmosphere (Allen 2007).

2.4.2 System Identification

The system identification is a mathematical technique to identify the relationship between system input(s) and output(s). Basically, this toolbox provides a reliable way for analysis dynamic systems while studying the role of associated parameters in system performance. In fact, in complex systems, the association between involved parameters and system performance is usually unclear. The human's biological system is one the most complex systems in which internal interactions are not fully discovered whereas overall role of each organ is more or less known. For instance, the vital role of endothelial cells in vascular tuning has been known whereas its fundamental function to secrete sufficient volume of NO is still being investigated. This technique was implemented in the Matlab "system identification" toolbox by Ljung (Ljung 1999). Since then, it has been improved and updated (Ljung 2010). A basic schematic of a typical model is illustrated in Figure 2.8.

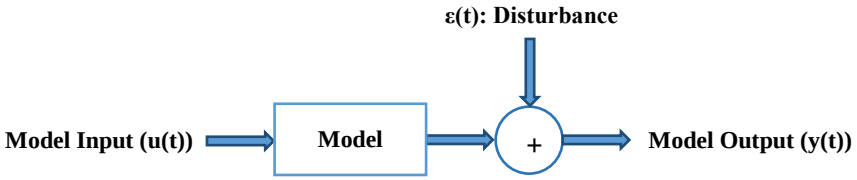


Figure 2.8 Schematic view of a typical model

Considering *u(t)* as model input and *y(t)* as model output, the mathematical relationship between parameters of a model can be expressed via equation 2.1:

$$y(t) = \sum_{k=0}^{n_a} a(k) \times u(t-k-n_k) + \sum_{k=1}^{n_b} b(k) \times y(t-k) + \varepsilon(t) \qquad (2.1)$$

where n_a is the order of denominator (number of poles), n_b is the order of numerator (number of zeros plus one), n_k is the input-output delay and *e(t)* is the residual noise.

a) Criterion of a Reliable Model

According to the theory of the system identification, the goodness of a model is normally evaluated in terms of the following criteria (Ljung 1999):

Model Validity: In general, model validity indicates how valid an estimated model is. In other words, it refers to the degree of similarity between measured and estimated data. Such comparison is normally discussed via the parameter of fitness expressed in terms of percentage. In Matlab, a value of fitness is computed by the following equation (Ljung 1999):

$$Fitness = 100 \times \left(1 - \frac{\sqrt{\sum_{t=1}^{N} \left(y(t) - \hat{y}(t) \right)^2}}{\sqrt{\sum_{t=1}^{N} \left(y(t) - \bar{y} \right)^2}} \right) \qquad (2.2)$$

where, *y(t)* represents a measured data at time *(t)*, $\hat{y}$ (t) represents an estimated data at time *(t)*, $\bar{y}$ represents mean value of initial data and *N* represents number of samples.

There is no certain value -for fitness- to evaluate the suitability of an estimated model. But higher fitness value is obviously desirable.

Reproducibility: The term "reproducibility" simply refers to the sustainability of results along different times of try. This parameter refers to the constancy of model behavior along different sets of similar data. Although results may not be exactly same for each data but the issue of constancy should be met among all obtained values of fitness in order to ensure the stability of a model.

Final prediction error (FPE): the final prediction error is another factor for evaluating models. In Matlab, it is calculated using the following equation:

$$\mathrm{FPE} = V \times \left(\frac{1 + \frac{p}{N}}{1 - \frac{p}{N}} \right) \tag{2.3}$$

where p is the number of estimated parameters and N is the number of samples. The V is known as the loss function which is defined as:

$$V = \det\left(\frac{1}{N} \sum_{k=1}^{N} \varepsilon(k, \theta_N)(\varepsilon(k, \theta_N))^T \right) \tag{2.4}$$

where θ_N addressing the vector of estimated parameters from the N data samples, ε is the residual error and *det()* is the matrix determinant operator.

b) Role of System Identification in Cardiovascular Engineering

Truly, system identification can be known as an applicant tool for discovering unknown parameters in CV engineering and hemodynamics. In a pioneer work by Pauca, an algorithm was proposed for non-invasive estimation of arterial pressure waveform from radial blood flow changes (Pauca et al. 2001). In this work, arterial pressure waveform and radial blood flow changes were simultaneously recorded and their relationship was then studied via system identification. Using this model, radial flow waveform could be estimated based on radial pressure waveform. This model was then inversed to facilitate

the estimation of pressure waveform from the volume change signal. This technique was then validated and improved (Bogert & van Lieshout 2005).

In continue using system identification techniques to explore CV characteristics, Xiao et al. proposed a novel approach for assessing neural cardiovascular regulation (Xiao et al. 2005). Using this method, effective parameters on the regulatory of cardiovascular system were characterized via system identification. Heart rate, peripheral resistance and sympathetic reaction were identified as most effective factors on neurology of cardiovascular operation. This concept was later improved by Jalali et al. offering analysis of correlation between arterial blood pressure and heart rate waveforms as well as durability in the performance of sympathetic nerves system (Jalali et al. 2011).

The role of system identification was highlighted again when Voss et al. discovered some potential causes of variation in heart rate through a model based study (Voss et al. 2009). In this study, association between heart rate, blood pressure and lung volume was investigated and two autonomic factors were identified as main causes of abnormality; heart rate baroreflex and respiratory arrhythmia. In addition, effects of respiratory (i.e. mechanical movement) were introduced as a kinetic factor which may improve the trend of abnormality. In order to evaluate this finding, a pharmacological method was considered to neutralize effect of the autonomic factors. This concept was then implemented among fourteen healthy subjects resulting in deactivating autonomic effects and improves the stability of heart rate and blood pressure. Therefore, they endorsed the great power of system identification in cardiovascular engineering by declaring that: "*system identification is an inverse modeling technique that provides a means for creating a closed-loop model of cardiovascular regulation for an individual subject without altering the underlying physiological control mechanisms*".

Possibility of employing system identification technique in clinical practices was then endorsed by Lowe et al. (Lowe et al. 2009). Similar to previous work, data were simultaneously recorded among thirty nine patients who were suffering from coronary heart diseases. Then, a parametric model was individually estimated for the first twenty subjects. Generalized model was then computed by averaging among

confidents of all individual models. This model was then applied to another nineteen subjects in order to estimate their CBP waveform. Results were then compared with measured data (invasively acquired). Results showed a very convinced similarity between model-derived and measured CBP waveforms with the root mean squared error of 2.2 ± 0.9 mmHg only.

2.4.3 Estimation of Central Blood Pressure via System Identification

a) Clinical Importance of Central Blood Pressure (CBP)

The fresh blood is originally rushed out from the left ventricle into the aorta which is the first and widest arterial segment in human bodies. In fact, the aorta is coupled to the heart and form a unique configuration for passing blood from the heart to faraway limbs (Levick 2013). Blood pressure within the aorta is known as the aortic blood pressure (ABP) or central blood pressure (CBP). It is then exponentially declined within the aorta and then passed through following small (McEniery et al. 2014).

In early of last decade, clinical importance of central (aortic) stiffness was highlighted as a potential tool of early detection of cardiovascular abnormalities (Safar 2000). This concept was later confirmed by Laurent et al. in which aortic stiffness was computed by measuring the carotid/femoral pulse wave velocity among 1980 hypertensive patients (Laurent et al. 2001). They found a relationship between aortic stiffness and hypertension prevalence and thus, introduced the aortic stiffness as an independent predictor for CV mortality among hypertensive patients.

The CBP has also been used for early diagnosis of renal diseases. For instance, Safar et al. (Safar et al. 2002) studied the relationship between central waveform and death-leading renal diseases among 180 certain renal patients who were undergoing hemodialysis. In this study, brachial BP, carotid/brachial ratio and aortic wave velocity were frequently recorded and documented along ten years. After precise analysis among all recorded data, they concluded that both CPB signals and amplification ratio between central and peripheral waveforms are two independent predictors of CV diseases among renal patients.

Investigations have shown that there are some fundamental differences between CBP and brachial pressure waveforms. For instance, there is a significant difference between the systolic pressures of the aorta and brachial artery, although diastolic and mean arterial pressures are relatively similar (McEniery, Hall, Qasem, Wilkinson, Cockcroft, et al. 2005). Such a discrepancy is occurred mainly due to wave reflection caused by increasing vascular stiffness moving away from the heart (Hirata et al. 2006).

This concept was later challenged by Ohte et al. as they reported that the diastolic pressure varied from aorta to brachial artery (Ohte et al. 2007). In this research, CBP and brachial BP of 82 subjects -who were suspected with coronary artery diseases- were simultaneously recorded during cardiac catheterization. After analysis, they revealed that there is a significant variation between bottom peak of central and brachial pressure signals. They found that the central diastolic value is lower than brachial diastolic value mainly due to the issue of wave reflection and vessel compliance. However, they soon published another work confirming the amplification ratio between central and brachial arterial pressure (Ohte et al. 2007). This matter was later endorsed by several other researchers (McEniery et al. 2014; Segers et al. 2009). Figure 2.9 illustrates the amplification ratio in upper limb arterial network; from aorta to radial.

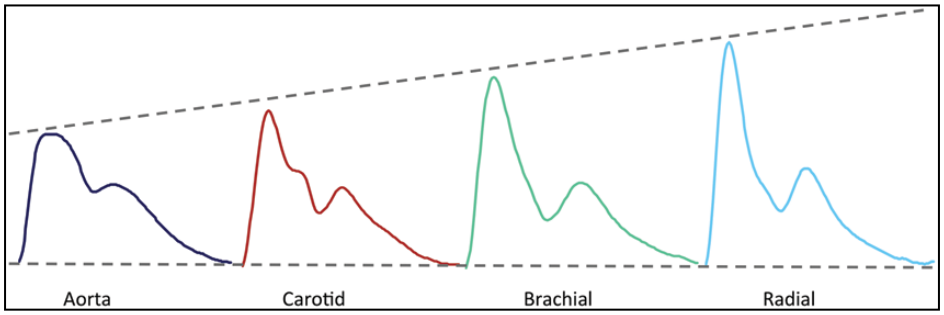


Figure 2.9 Amplification of a typical pressure pulse in upper limb

Source: McEniery et al. 2014

The CBP has also been introduced as a potential parameter for representing cardiac output. For instance, Roman et al. investigated the possibility of detecting hypertrophy (and vascular abnormalities) via CBP and peripheral blood pressure waveform (Roman et al. 2007). In this statistical work, the CBP and PBP were simultaneously recorded

among 3520 subjects. After five years follow-up study and statistical analysis, they revealed that hypertrophy can be more reliably predicted via CBP rather than brachial blood pressure signal. They reported the hazard ratios of CBP-based detection and PBP-based detection for 1.15 and 1.10, respectively. Afterwards, they investigated the possibility of detecting geometrical abnormalities of the heart via CBP signal. They observed a significant difference in CBP shape among hypertrophic patients compare to healthy subjects. Thus, it was concluded that systolic central pressure is more precise to identify the left ventricular hypertrophy rather than peripheral pulse (Roman et al. 2010).

Over last decades, a global interest has increased to discover other aspects of the CBP waveform as a prospective indicator of CV abnormalities. In this regard, Pini et al. have shown that the CBP can be used for early detection of CV abnormalities among elderly people (Pini et al. 2008). In this research, 398 subjects including 173 normotensive and 225 untreated hypertensive were selected to participate in a long-term study. During eight years of study, brachial, central and carotid blood pressure signals were frequently measured while CV abnormalities and death cases were also documented. After doing multivariate analysis, they endorsed that the CBP could be a better choice for predicting cardiovascular events.

As explained, it has been demonstrated that the CBP conveys significant physiological information that can be employed for early detection of CV abnormalities as well as assessing cardiac output. Therefore, the CBP has come to the center of attention since last decade to the extent that many believe it may be a better choice -compared to brachial pressure- for prediction of cardiovascular events (McEniery et al. 2014; Williams & Lacy 2010). In practice, however, measuring the central blood pressure is not easy. Possible approaches to access CBP waveform are explained below.

b) Practical Techniques to Access the CBP

CBP can be obtained through both invasive and non-invasive approaches. The heart catheterization is the most reliable way to obtain CBP waveform. In this approach, the central pressure can be directly measured by a thin flexible catheter during a tough invasive operation. Figure 2.10 shows an overview of this technique for measuring the

central pressure. Although this technique is highly precise but it is usually used for patients who are suffering from coronary heart disease. Moreover, this technique is considerably risky and complex. These limitations make it unsuitable for daily usage in clinics. Thus, global interest has been motivated to replace the catheter-based techniques with non-invasive approaches. In this regard, scientist has proposed several methods to estimate the CBP waveform from distal pressure signal (e.g. radial, brachial or carotid). Such techniques are listed in Table 2.1.

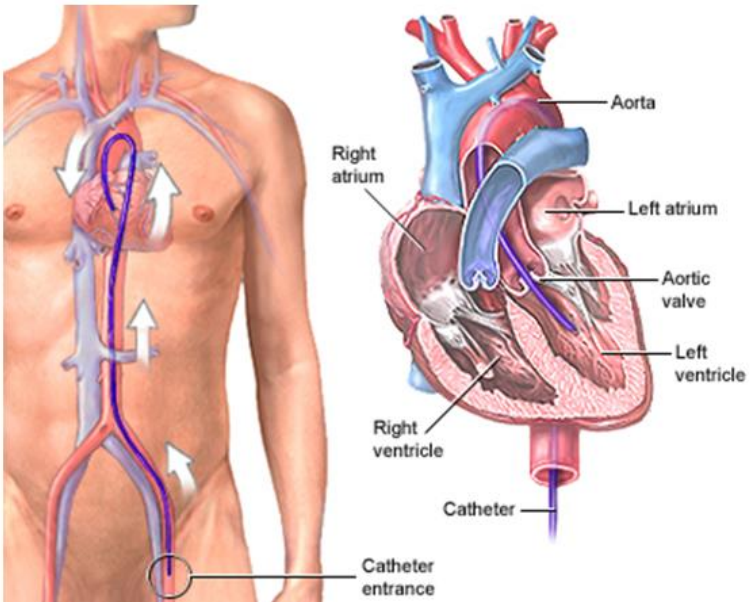


Figure 2.10 Cardiac catheterization from the femoral artery

Source: Anon 2016

Table 2.1 Non-invasive techniques to access CBP waveform

Methodology	Target Site	Method of Estimating CBP	Commercially available apparatus

Radial Tonometry	Wrist	- General Transfer Function - Late Systolic Shoulder - Algorithm	BPro, SphygmoCor SphygmoCor HEM9000AI
Brachial Cuff Pulse Volume Plethysmography	Upper Arm	- General Transfer Function	Vicorder, Centron CBP301 XCEL, ARCsolver
Supra-systolic Brachial Cuff Pulse Volume Plethysmography	Upper Arm	- Late Systolic Wave Amplitude - Algorithm	Arteriograph Cardioscopell

Carotid pressure had been particularly considered as a surrogate site for obtaining the central pressure mainly due to similar characteristics and close vicinity to the aorta (Segers et al. 2005). Although carotid blood pressure has been widely used in CV researches (e.g. to calculate pulse wave velocity) but investigations show that it cannot be considered as a fair surrogate because measuring the carotid blood pressure is not easy particularly among obese subjects (McEniery et al. 2014). Furthermore, there is a small amplification between carotid and aortic pressures so that the carotid pressure cannot reliably represent the aortic pressure (McEniery, Hall, Qasem, Wilkinson & Cockcroft 2005; Nichols et al. 2011; Van Bortel et al. 2001).

The global attention was then focused on estimating the CBP waveform from the radial pressure pulse. It can be obtained by placing a high sensitive piezoelectric sensor over the radial artery for converting the pulsatile blood flow to an electrical signal. For the first time, Chen et al. proposed a way in which the aortic blood pressure can be mathematically derived from the radial pressure pulse (Chen et al. 1997). It this work, the central aortic pressure and radial pressure pulse were simultaneously recorded among 20 patients. Then, an individual parametric model between central and radial pressure pulse was estimated via system identification. The generalized transfer function was then computed by averaging among coefficients of all individual models. Using this technique, CBP could be accurately estimated from radial pressure pulse with overall error of less than 0.2 ± 3.8 mmHg. This approach was then used in clinical studies as a commercial apparatus named SphygmoCor (Pauca et al. 2001). Since then,

the algorithm has been developed and validated (Ding et al. 2011; Sharman et al. 2006). This approach has been well established in today's medicine to that extent that it has been refereed as a reference technique in similar proposed studies (Hickson et al. 2009; Stea et al. 2014).

Referring to Table 2.1, there are two techniques to access CBP waveforms non-invasively; brachial cuff pulse volume plethysmography and supra-systolic brachial cuff volume plethysmography. Although the same approach is used in this technique but input of models are different from each other. For instance, model input of the first technique is brachial plethysmography whereas model input of the second technique is supra-systolic volume waveform.

2.5 METHODS TO ASSESS ENDOTHELIAL FUNCTION

In general, endothelial assessment can be done via clinical, invasive and non-invasive approaches. There are fundamental diversities among these approaches so that each method can be used in a particular situation. However, ED assessment in early stages can be known as the common goal of all proposed approaches.

2.5.1 Clinical Tests

According to the pathology of vascular system, ED leads to inflammation in human body (Karsan & Dauphinee 2010; Van den Oever et al. 2010). Such inflammations can be then accessed via some clinical tests. C-Reactive Protein (CRP) test can be mentioned as the well-known experiment of such tests. High Sensitive CRP (HSCRP) is also another test of this approach in which inflammation or its related chemical factors are measured based on a certain volume of blood sample. This test is normally done via high-tech laboratory devices. As a result, HSCRP test is more reliable rather than normal CRP test. However, ED cannot be exclusively accessed via clinical tests as inflammation factors may belong to other abnormalities (e.g. liver or kidney diseases, etc.) rather than ED. In other words, there is no way to confirm whether inflammations in blood samples are caused by ED or not. Therefore, clinical tests are normally used in conjunction with other examinations to detect the ED. Moreover, this approach may not

be able to detect ED in early stages. Therefore, this technique is not known as an exclusive test to assess the endothelial functionality (Devaraj et al. 2011).

2.5.2 Invasive Methods

In general, invasive approach refers to those techniques in which the assessment of ED is done invasively. As such, epicardial vasoreactivity and Doppler wires can be mentioned. Table 2.2 contains the most popular endothelial assessment techniques which are explained below.

Table 2.2 Comparison of the most popular techniques to assess the endothelial performance

Methodology	Site of interest	Advantages	Disadvantages
Coronary epicardial vasoreactivity	Epicardial marcovascular conduit arteries	Direct assessment to coronary vessels	Invasive, Expensive, Limited to coronary patients, challenging for reproducibility
Doppler wires	Coronary microvascular	Direct assessment to coronary vessels	Invasive, Expensive, Limited to coronary patients, challenging for reproducibility
FMD	Brachial artery, Femoral artery	Easy assessment, well correlate with invasive epicardial vascular function	Need standardization, highly operator dependent
Plethysmography	Peripheral artery	Easy assessment, stimulation by drug injection	Time consuming
EndoPAT	Finger microcirculation	Easy assessment, well correlate with invasive approaches	Highly sensitive, possibility of capturing non-Endothelial derived changes

FMD: Flow-mediated dilatation. PAT: peripheral arterial tonometry.

Source: Flammer et al. 2012

The epicardial endothelial assessment is a pioneer suggestion to assess the endothelial performance. This technique was first proposed by Ludmer et al. aiming to stimulate the endothelial layer chemically (Ludmer et al. 1986). In this approach, the endothelial

cell is stimulated by injection of acetylcholine while the endothelial reaction is captured either by US or coronary angiography operation. In respond to this stimulation, the intact endothelial cell show proper reaction by dilating vessel conduit (vasodilatation) whereas impaired endothelial cell react inversely to constrict the vessel diameter (vasoconstriction). This test can be also done by other chemical substances such as Salbutamol and Serotonin (Puri et al. 2012). This technique was highly attended in 1980s to that extent that many researchers took this approach as a gold standard technique to assess endothelial performance (Anderson 1999).

However, this approach was then challenged mainly due to administration of exogenous vasodilator substance. In fact, this technique represented the endothelial-independent reaction and thus could not be expanded to the endothelial-dependent dilatation. Later, this technique was modified by using both endothelial-dependent and independent substances (Drexler & Zeiher 1991). However, the epicardial endothelial assessment technique was invasive and thus could not be recognized as a suitable approach particularly for healthy subjects.

Functionally of endothelial layer was also assesses via an elastic-thin Doppler wire. Using this technique, endothelial cells were stimulated by infusion of chemical substances (e.g. nitroglycerin) while velocity of blood flow -along the site of interest- was measured using the Doppler wire as shown in Figure 2.11 (Anderson 1999). According to the human physiology, infusion of a vasodilator substance makes the blood vessel larger. In such case, blood flow velocity is supposed to be varied (decreased) compare to baseline and vice versa. However, this approach needed a special system setup and an experienced operator. Moreover, such operation was found risky as there is a possibility of cutting vessel walls. Furthermore, this technique could not be used for early assessment of ED (i.e. limited to coronary patients only).

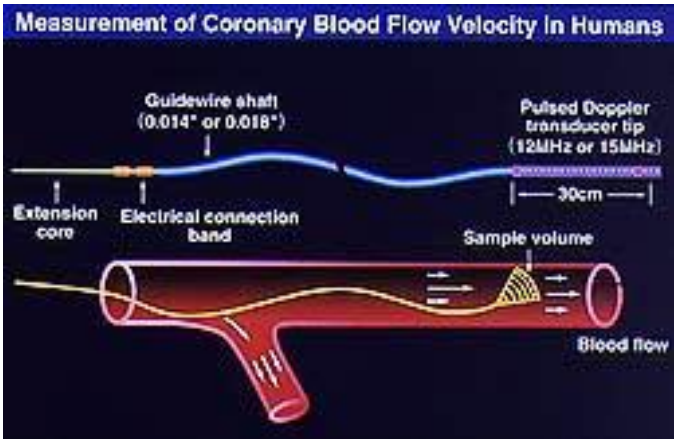


Figure 2.11 Endothelial dysfunction assessment via a Doppler wire

Source: Shimokawa 2016

In another less invasive approach, possibility of endothelial assessment using the concept of pulse wave analysis was investigated by Hayward et al (Hayward et al. 2002). In this work, the glyceryl trinitrate was used to stimulate the endothelial layer. Then, the parameter of augmentation index was used to trace the vascular reaction before and after administration of glyceryl trinitrate among eleven subjects. It was found at the end that the augmentation index is significantly varied (up to 6%) after stimulating the endothelial layer. Therefore, the pulse wave analysis was introduced as a potential technique to assess the endothelial functionality. Capability of such technique was later endorsed by other groups (Lind et al. 2003; Wilkinson et al. 2002). For instance, possibility of assessing the endothelial performance via pulse wave analysis was investigated by Jadhav et al. (Jadhav & Kadam 2004). In this work, pulse wave velocity was measured among totally 102 subjects. In addition, endothelial performance was captured via ultrasonography. Results showed an excellent correlation between these two techniques. As a conclusion of this work, pulse wave velocity was introduced as a potential parameter to assess the endothelial dysfunction non-invasively.

In another similar approach, endothelial performance was evaluated using a ratio in blood flow before and after injection of acetylcholine (i.e. chemical vasodilator). In this technique, blood was taken from the coronary artery. In healthy group, an increase in blood flow was observed as a result of endothelial-derived vasodilatation. However,

no change in blood flow (vessel diameter) was observed among non-healthy subjects. Using this approach, the endothelial functionality is normal if the blood flow become at least two times higher after administration of acetylcholine (Camici & Crea 2007).

In a comprehensive work by McEniery et al. (McEniery et al. 2006) the association between ED and aortic pulse wave velocity, augmentation index and central pulse pressure was investigated among 89 subjects. In this work, the endothelial cells was stimulated by sublingual nitroglycerin and inhaled albuterol. Values of the mentioned parameters were compared before and after the stimulation. In addition, the endothelial performance was assessed via ultrasound imaging. It was observed that the endothelial performance is significantly and inversely correlated with aortic pulse wave velocity, augmentation index and central pressure pulse.

However, invasive operation can be highlighted as a common disadvantage of all mentioned endothelial assessment techniques which are discussed above. Besides, the coronary artery is mostly used in these techniques. Such disadvantages limit these techniques to patients who are suffering from coronary diseases (near to do angiography or open heart surgery). In other words, invasive techniques cannot offer a possible way for examining the endothelial layer among healthy subjects. Therefore, the global interest has been motivated to propose non-invasive approaches to evaluate the endothelial activity.

2.5.3 Non-invasive Methods

Compare to invasive approach, non-invasive approach has been particularly attended over last two decades mainly due to possibility of mass-screening. Indeed, it relies on the fact that non-invasive techniques can be used generally whereas invasive techniques are limited to coronary patients only. However, in non-invasive approaches, coronary arteries cannot be used to assess the endothelial performance as there is a limitation to access such arteries. Thus, large arteries (e.g. brachial, femoral) are normally considered to assess the endothelial functionality. Since late 1990s, it was a dilemma that whether a local endothelial reaction can be considered as the so-called systemic endothelial functionality or not. In other words, endothelial layer has the same performance through the vascular network or not. Interestingly, such hypothesis has been investigated and

confirmed by many researchers meaning that local endothelial functionality can be extended to entire endothelial layer in vasculature (Jambrik et al. 2004; Matsuzawa et al. 2013). At present, the peripheral artery has been mainly attended -as a target site- to assess the endothelial functionality.

Referring to Table 2.2, there are three non-invasive methods to assess the endothelial dysfunction; FMD, plethysmograph and endoPAT. It should be noted that such categorization is based on endothelial assessment technique and not stimulating the endothelial cells. For instance, in plethysmography approach, endothelial cells are stimulated invasively using some vasodilator substances but in fact, assessment of endothelial functionality is done via plethysmography non-invasively. These approaches are explained below.

a) FMD-Ultrasound

FMD was first proposed by Celermajer et al. (Celermajer et al. 1992) in which they proposed a physiological way to stimulate endothelial cells non-invasively while endothelial reaction is captured via ultrasound (US) imaging. This technique has been established and used in today's medicine. In fact, this technique has been more attended compared to other non-invasive techniques. Accordingly, it is used as the reference technique in this research in order to evaluate current performance of endothelium. Therefore, this technique is explained in details in the following sections.

Flow Mediated Dilatation (FMD)

FMD is a non-invasive technique to stimulate endothelial cells (layer) via a physiological excitation (Corretti et al. 2002). Using this technique, blood circulation -in a large artery- is blocked for around five minutes. Such occlusion creates the phase of shear stress across the vessel wall. As blood circulation is retrieved again, a density of blood is immediately rushed into the block site. It provides a state of reactive hyperemia in which the endothelial cells secrets the NO leading to vasodilatation (increasing the vessel diameter). The activity of the endothelial layer can be captured via US imaging.

A) Procedure of the FMD test

The FMD test starts with measuring individual's blood pressure. Value of blood pressure is then used to apply an appropriate pressure over an artery of interest for occluding the blood circulation. After measuring blood pressure in normal situation (baseline), subject is asked to lie down on a bed and being in supine position. Then, a few minutes should be passed till blood circulation becomes stable again. In this phase, the current diameter of brachial artery is continuously captured for around three minutes via a high frequency US system. Obtained values of diameter are then averaged and considered as a baseline diameter.

Afterwards, the brachial blood circulation is fully occluded by applying additional 50 *mmHg* to value of pressure obtained in the baseline. Such occlusion can be done via the rapid cuff inflator apparatus. In case of blood occlusion (i.e. ischemia), the phenomenon of shear stress is created. Results would be a constant stress toward the vessel wall. Soon after five minutes, the pressure cuff is suddenly deflated resulting in the state of reactive hyperemia. In this situation, the normal endothelial layer is supposed to respond to such physiological stimulus by releasing an adequate volume of NO. The physical evidence of such secretion is widening the vessel conduit. The consequence of physiological steps during the FMD stimulus is illustrated in Figure 2.12.

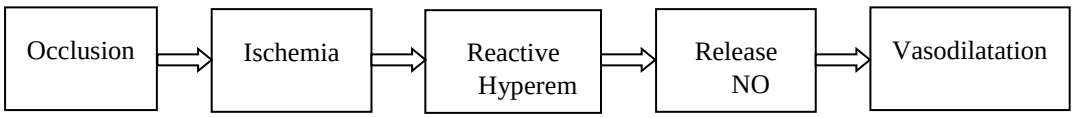


Figure 2.12 Physiological conditions during the FMD test

In order to be able to follow the endothelial reaction after releasing the pressure cuff (release mode), the vessel diameter is continuously captured for another five minutes. Finally, the actual functionality of endothelium can be assessed by comparing the brachial diameter before (baseline) and after (release) the occlusion. The normal endothelial reaction is supposed to dilate the brachial artery for at least 10% greater than baseline diameter as can be seen in Figure 2.13.

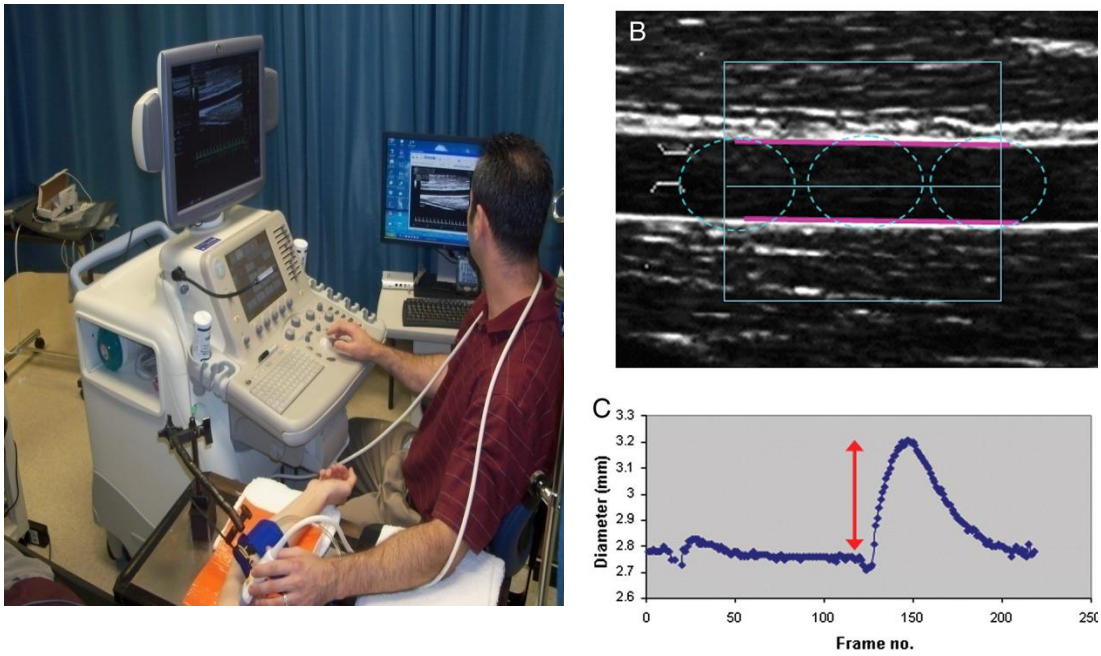


Figure 2.13 a) FMD-US practice b) Tracing the brachial diameter c) Representation of variations in vessel diameter through a curve

B) The FMD Setup

The FMD test requires special setup for conducting an appropriate stimulus over individual's artery. The setup is quite expensive mainly consist of a high frequency ultrasound system, rapid cuff inflator and additional edge detection software for measuring the brachial diameter during the FMD test. The system setup and role of each device in the FMD test is explained in the session below.

i) Rapid Cuff Inflator

Although brachial blockage can be done by a normal pressure cuff, but an instant occlusion is desirable in the FMD test. In fact, it can guarantee the efficiency of the shear stress and provide a better situation for exciting the endothelial cells. An instant occlusion has been addressed as a key factor in the FMD test (Betik et al. 2004; Dyson et al. 2006; Harris et al. 2010). It can be done via the rapid cuff inflator which is consisted of an air compressor, relay and a pressure cuff with possibility of quick inflation and deflation (i.e. one third of a second). A favorite pressure can be given to

the rapid cuff inflator so that once the switch key (relay) is pressed, the given pressure is applied to a site of interest along one third of a second.

ii) Sphygmomanometer

According to the FMD guideline, an additional 50 *mmHg* should be applied to the brachial artery for an appropriate occlusion (Corretti et al. 2002). Thus, primary measurement of the brachial blood pressure plays an important role in accuracy of the FMD test. Blood pressure can be obtained via an ordinary Sphygmomanometer apparatus.

iii) Ultrasound Machine

The ultrasound machine is another essential part of the FMD-US analysis. It provides a visual assessment to the endothelial activity during the FMD test so that the endothelial reaction can be faithfully assessed. Irrespective of the sonographer's skill, frequency of US transducer plays a key role on the resolution of ultrasonic images. Hence, a high frequency transducer -preferably above 10 MHz- is highly advised to get a high quality images.

iv) Edge Detection Software

The main part of the FMD analysis is to measure the variation of brachial size before (baseline) and after (cuff release) conducting the FMD stimulus. In fact, size of brachial vessel should be incessantly traced preferably by an automatic software. Such measurement should be done pixel by pixel using a precise algorithm. Only a few programs were found commercially available -at the time of doing this research- to detect the vessel wall automatically.

v) Operator

After releasing the pressure cuff, there is a short time to capture the endothelial reaction. Such time can be known as a gold moment in the FMD test. Thus, an experienced and trained sonographer is essentially needed for capturing such gold moment. In fact, FMD operator should be well familiar with the FMD test as well as physiological changes during the stimulus. It means that a good FMD-US test can be done only by a trained operator. Such difficulty is however considered as one of the disadvantaged of the FMD-US technique as mentioned in Table 2.2.

Difficulties of the FMD Test in Real Clinical Practice

The FMD-US technique has been known as a reliable and reproducible approach to assess the endothelial functionality (Charakida et al. 2013). Indeed, this technique is well established and known as the most attended technique to assess the ED non-invasively. However, this technique has also some limitations which are explained below.

A) Expense of Equipment

In order to implement this test, the FMD lab should be unavoidably equipped with essential elements which are addressed above as the FMD setup. In practice, providing the FMD setup is remarkably costly. The most expensive part of the FMD setup is a high frequency ultrasound machine which is usually limited in numbers in clinical centers. Fasting overnight should be also taken into account meaning that the best time of conducting the test is the morning (Corretti et al. 2002). The issue of fasting can be considered as a common requirement for other clinical tests (e.g. cardiac ultrasonography). So that, the peak time of using the ultrasound machine is mornings for both patient and healthy (client) groups. In such situation, priority of usage should be reasonably given to patients rather than those who want to checkup their endothelial performance. Such difficulty limits the possibility of voluntary endothelial checkup for healthy subjects. Thus, the FMD-US technique cannot be considered as a routine technique for assessing the endothelial performance particularly among non-healthy subjects.

B) Skilled Operator

Essential role of FMD operator can be considered as another limitation of this approach. As quoted earlier, capturing the endothelial activity should be done right after releasing the pressure cuff. Any insouciance or awkward mistakes -caused by lack of experience- may lead to lose the gold moment for capturing the endothelial activity. Therefore, this technique is highly operator dependent to that extent that it cannot be done without an experienced operator.

b) PPG

In general, plethysmography is an established technology to measure fluctuations of blood in an organ or whole body. Photoplethysmography is also a plethysmography-based technology in which blood volume change is accessed optically (Shi 2009). Such technology is well established and widely used in today's healthcare industry. As such, oxygen saturation signal can be mentioned which is obtained using a finger PPG sensor. Interestingly, PPG has been considered as a potential tool for detecting vascular abnormalities as it represents the blood volume change along each cardiac cycle. For instance, possibility of detecting atherosclerosis -as an indicator of ED- was first investigated by Barnes et al. (Barnes et al. 1977) in which the PPG signal was taken from supraorbital and frontal sites among 78 patients who were undergoing arteriography. Statistical analysis showed abnormal shapes of PPG waveforms among those with certain vascular occlusion confirmed by arteriography. Thus, it was reported that the PPG approach could detect the vascular blockage with over 93% of similarity with the gold standard technique. Therefore, the Photoplethysmography was introduced as an alternative method for detecting occlusive abnormalities in vessels.

In another similar work, Lund et al. (Lund 1986) studied the possibility of examining the endothelial performance via digital pulse Plethysmography. In this research, Nitroglycerin was used -as a chemical exciter of the endothelial cells- to stimulate the endothelial layer. Comparison between signals obtained before (baseline) and after the perfusion showed a significant difference in notch area of the PPG signal. Thus, it was concluded that the PPG signal could be a potential tool to capture the endothelial reaction. This concept was then reinvestigated by several different teams resulting in recognition of using the PPG-based approach for assessing the endothelial

functionality. (Chowienczyk et al. 1999; Ruiz-Vega et al. 1997). As such, Millasseau et al. proposed a PPG-based technique to assess the vascular stiffness (Millasseau et al. 2002).

Cardiac output has been also used to represent the vascular stiffness in the aorta. This parameter is scientifically known as the stroke volume which indicates the volume of blood which is rushed out from the heart to the following arterial network (Allen 2007). Stroke volume was previously calculated using Doppler-pulse technology (Huntsman et al. 1983). In this technique, it was calculated by multiplying values of aortic diameter and aortic velocity obtained via Doppler-ultrasonography. Irrespective of its costly setup, further investigations showed that the stroke volume which is obtained by this technique is not reproducible and may vary from time to time (Labovitz et al. 1985). PPG was then considered as a potential approach to assess the stroke volume. Such concept was first proposed by Romano & Pistolesi in which they suggested a technical way for calculating the stroke volume via PPG signals (Romano & Pistolesi 2002). This approach was then improved and compared with invasive approaches. Results showed a faithful agreement between these two methods with over 94% of similarity. The accuracy and suitability of the PPG approach has been investigated in several later studies (Butter et al. 2004; Whinnett et al. 2006).

PPG was also used to compute the pulse transit time in order to capture vascular occlusion (endothelial dysfunction) (Nitzan et al. 2002). In this research, ECG signal plus two PPG signals obtained from subject's finger and toe were simultaneously recorded among totally forty four healthy men. Using standard signal processing techniques, the time delay between ECG and pulse arrival time -in finger and toe- was computed. Results showed that such time delay is shorter among elderly people whereas it is longer among young subjects. This conclusion was in a good agreement with previous finding. Thus, the PPG-based approach was considered as a reliable technique to obtain the pulse transit time used for vascular assessment.

c) endoPAT

endoPAT is a well-known commercially available device to assess the endothelial dysfunction non-invasively (Itamar 2009). Using this device, endothelial cells are stimulated using the FMD test while endothelial reaction is captured from subject's

index finger. In fact, an arm is used as a reference while the other arm is used as the so-called experimental arm. As mentioned earlier, releasing the pressure cuff creates the phase of reactive hyperemia in which NO is secreted. Accordingly, vascular diameter is increased so that blood pressure and blood flow are varied. Such variations are then captured using the endoPAT device which indicates the endothelial response. In other words, characteristics of blood flow signal before (baseline) and after stimulating the endothelial cells are used -rather than vessel diameter- to represent the actual endothelial functionality (Faizi et al. 2009).

Investigations have shown that results of endoPAT are reproducible (Tierney et al. 2009). Moreover, it is operator independent and thus can be easily performed even by users. Furthermore, no ultrasound machine is needed in this approach. Therefore, its system setup is highly cost-effective compared to FMD-US. In addition, it does not require any additional program to analysis the test (i.e. vascular changes). However, comparison between endoPAT and FMD-US technique has shown that there is a disparity between these two techniques. For instance, Takasa and his co-worker (Takase & Higashimura 2013) examined the endothelial functionality of 47 hypertensive patients via FMD-US and endoPAT system. Using FMD-US technique, endothelial reaction was assessed via ultrasonography whereas in endoPAT, it was examined based on finger PPG signals. Comparison between results has shown that endoPAT is not able to measure NO-related dilation (i.e. which is a basic substance of endothelial reaction) and hence may not be reliable.

In another study by Moerland et al. (Moerland et al. 2012) the accuracy of endoPAT system was evaluated in conjunction with conventional techniques (e.g. FMD-US, angiography, etc.) among both healthy and unhealthy groups (different population). In this study, non-invasive measurements were repeated in order to investigate the accuracy and reproducibility of endoPAT concerning evaluation of endothelial changes. It was observed that effects of robust interventions could not be detected among healthy subjects. Moreover, it was also observed that endothelial function assessed by EndoPAT could be physiologically different from endothelial function which is assessed by conventional techniques. Accordingly, it was concluded that the EndoPAT might not be suitable choice to assess endothelial dysfunction.

In another study by Arrebola-Moreno et al. (Arrebola-Moreno et al. 2012) accuracy of endoPAT system to capture the so-called pure endothelial factors has been investigated. It has shown in this study that assessment of endothelial functionality from subject's index finger may reflect endothelial factors as well as non-endothelial factors. In fact, non-endothelial factors refer to the phenomenon of autoregulation in human vascular system. Such physiological interaction is the tendency for blood flow to remain constant in case of changes in local arterial pressure. According to physiology of human vascular system, this phenomenon is occurred only in small arteries (e.g. finger artery, auricle, etc.) (Stouffer 2016). In fact, stimulation of brachial artery comprises both endothelial reaction and the autoregulation. Therefore, an index finger PPG signal reflects endothelial and non-endothelial factors.

Regardless of limitations of the endoPAT system, plethysmography was found as a potential approach to be used to assess the endothelial dysfunction. As explained, this approach is considerably cost-effective, operator independent and easy to use compare to the FMD-US technique. Therefore, improvement of such approach has been particularly attended over last decade. As such, doctoral research of Rosmina Jaafar can be mentioned (Jaafar 2009). In this study, PPG was used to access finger blood volume change along each cardiac cycle. It was done using the Dolphin PPG system. FMD-US technique was also used -as the reference technique- to assess the endothelial dysfunction. In addition, subject's index finger PPG signal -from both left and right fingers- were simultaneously recorded to analysis the endothelial reaction. This experiment was performed among 145 subjects including 68 healthy subjects and 77 subjects with CV risk factors. Changes in DC component of finger PPG signal before (baseline) and after releasing the pressure cuff was used to represent the actual activity of endothelium. Goodness of this technique was then evaluated in conjunction with results obtained from FMD-US technique. Accordingly, sensitivity and specificity of the proposed technique were obtained 74% and 60% respectively. Also, the proposed technique could go one step forward and provide a practical way to replace the ultrasonography with PPG. Moreover, it was observed that the proposed technique is more repeatable compare to the FMD-US technique.

However, recent researches have shown that this approach has some conceptual and technical limitations. For instance, blood occlusion across the brachial artery was done via a typical blood pressure device meaning that inflammation and deflation of the pressure cuff was done manually. On the other side, such physiological stimulus need be done very quickly i.e. less than a second (Corretti et al. 2002). In fact, such quick time could apply (create) appropriate shear stress towards the vessel wall so that the endothelial cells can be appropriately stimulated. In other words, manual (long) deflation of the pressure cuff may moderate the endothelial reaction.

Another technical limitation of this study was to measure the vessel diameter manually. Since the FMD-US technique is based on variations of the vessel diameter, the cross sectional area of a target vessel need to be measured continuously and completely. However, such a measurement was done discretely and randomly in this work. In fact, automated measurement of vessel diameter during the FMD test is an essential part of this approach (Corretti et al. 2002). In other words, accuracy of studies in which ultrasound images were measured manually has been considerably challenged (Atkinson & Batterham 2013; Bots et al. 2005). Differences between automated measurement (left) and manual measurement (right) can be seen in Figure 2.14.

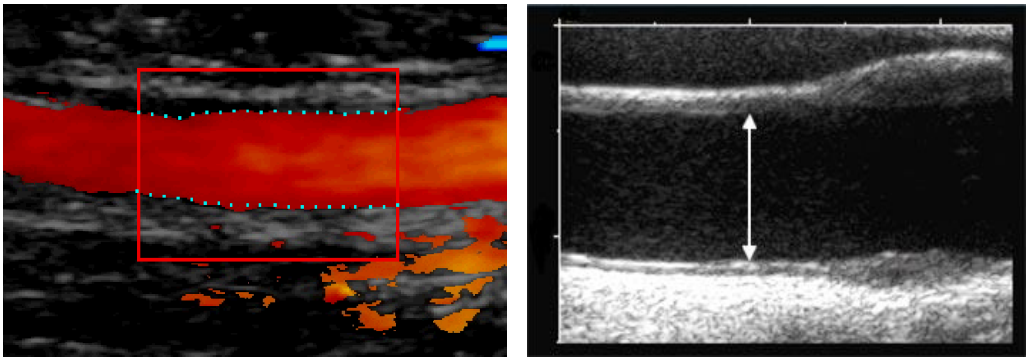


Figure 2.14 Measurement of vascular diameter using an automated program (left) and manual measurement

As for conceptual problem of this work, conducting endothelial stimulation in a large artery and capturing the endothelial response from a different vascular segment can be mentioned. In other words, endothelial stimulation was truly done across a large (peripheral) artery but the response was captured in a small artery in which arterial

resistance is different (i.e. higher) from the stimulated segment. In fact, it is demonstrated that vascular resistance in small arteries is considerably higher compare to large arteries (Bagher & Segal 2011; Muiesan et al. 2012). Thus, pathological characteristics of large arteries (e.g. peripheral, brachial, femoral, etc.) are different from small arteries (e.g. radial). Moreover, characteristics of microcirculation are even different from small arteries (Boulpaep 2012). Accordingly, the process of blood regulation in microcirculation is different from large arteries meaning that there is an additional parameter involved in vascular tuning rather than endothelial related factors. This parameter is known as autoregulation which refers to non-endothelial factors (Fung 2013).

Possibility of capturing non-endothelial related factors in small arteries has been investigated by Nohrina et al. (Nohria et al. 2006). In this study, effects of NO -as the main driver of endothelial reaction- on amplitude volume pulse of nineteen healthy subjects were investigated. In this regards, PPG signals were simultaneously recorded from peripheral and radial arteries before (baseline) and after infusion of a vasodilator substance. Pulses were then analyzed and compared to each other using typical signal processing techniques. Although NO-related effects (i.e. endothelial reaction) were supposed to be observed through both pulses but it was observed that the endothelial response in brachial artery is considerably lower compared to what is captured from peripheral artery. Regulation of vasomotor in brachial artery was introduced as such phenomenon. Accordingly, it was concluded that there is some additional factors -rather than endothelial regulation- in small arteries which facilitate the regulation of vascular tone.

Referring to the Jaafar's work discussed above, there is a possibility of capturing non-endothelial factors in subject's index finger due to effects of autoregulation (Jaafar 2009). In other words, assessment of endothelial dysfunction from a small artery whereas the FMD test (stimulation) was occurred in a larger vascular segment may lead to capturing both endothelial and non-endothelial factors. As can be seen in Figure 2.15 (a), the FMD test was performed across subject's peripheral artery while (b) endothelial response was simultaneously assessed from brachial and finger arteries.

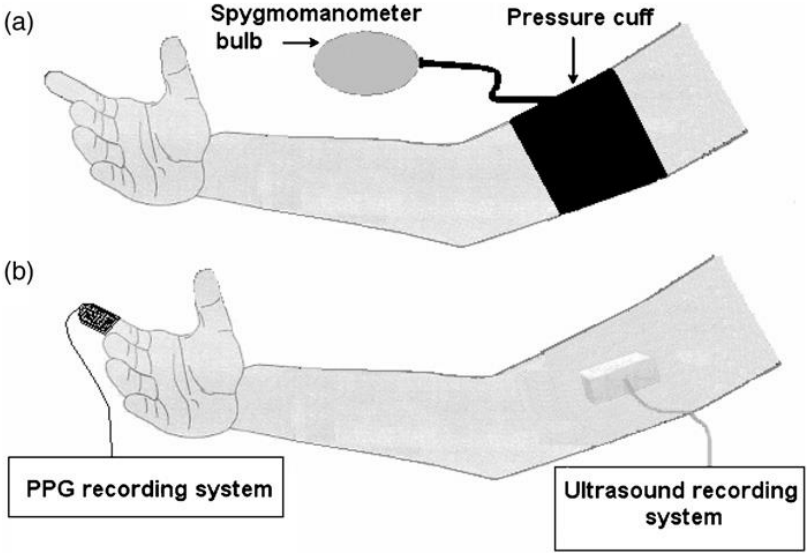


Figure 2.15 Sites for getting FMD response (a) and endothelial response (b)

Source: Zahedi et al. 2008

Figure 2.16 illustrates endothelial response from brachial (dotted) and finger (solid) arteries. Referring to human physiology, endothelial response in peripheral artery -in which the FMD test was performed- is supposed to occurred earlier compare to finger artery which is away from the stimulated segment (peripheral artery). However, endothelial peak captured in finger artery is occurred faster than peripheral artery. It may rely on the fact that endothelial response in subject's finger artery is augmented due to effects of autoregulation explained above. In fact, both endothelial and non-endothelial factors contribute on blood tuning in finger artery so that the diameter of vessel in this segment is backed to normal situation very quickly. However, such process takes longer time in peripheral artery as there are only endothelial-related factors. Therefore, capturing endothelial response from the brachial artery may exclude effects of autoregulation and thus, reflect the so-called pure endothelial response.

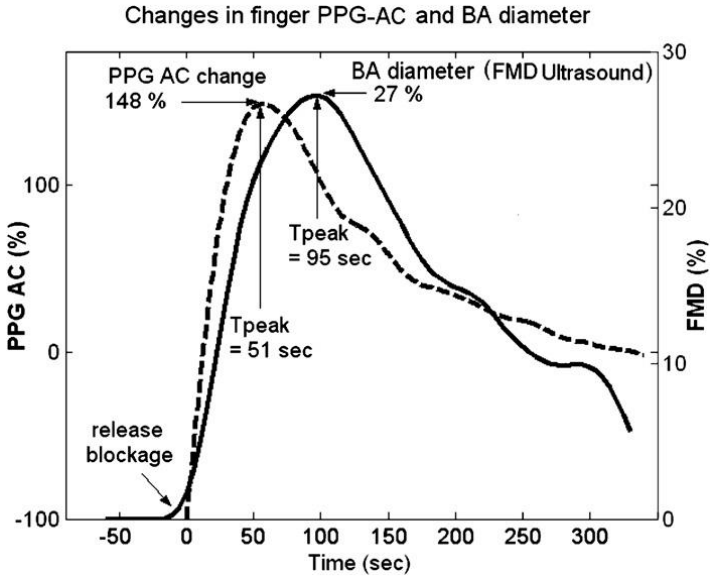


Figure 2.16 Assessment of endothelial reaction of a healthy subject from finger (dotted) and peripheral (solid) arteries

Source: Zahedi et al. 2008

As conclusion, PPG sensor is remarkably cost-effective compare to other sensors particularly in the domain of cardiology. In addition, it is operator independent and very easy to use. Moreover, this system is portable, noninvasive and safe. As quoted earlier, this technique is widely used for representation of vital signs. Nowadays, this technology is widely used in daily clinical monitoring and ambulatory diagnosis. However, this technique is so sensitive to motion artifacts. Thus, subject's cooperation is highly needed to get an optimal signal. Besides, environmental lighting should be taken into account as it may affect the quality of PPG signals. In this regard, data collection in a twilight environment is advised. As discussed, PPG has been known as a potential tool to assess endothelial dysfunction. Although recent researches have moved towards optimization of this approach but further improvements are still needed. For instance, utilizing PPG approach to assess pure endothelial reaction (i.e. excluding from non-endothelial factors) remains to be investigated.

2.6 CHAPTER SUMMARY

In this chapter, the human cardiovascular system and its main components are briefly explained. Endothelial cells, its vital role on human circulatory system and its

mechanism are then described. Accordingly, ED and its symptoms are reviewed. Afterwards, PPG and system identification are introduced as some potential tools to assess endothelial performance non-invasively. Applications of these techniques in cardiovascular engineering are then reviewed. Clinical importance of CBP waveform is then explained as it is a basis of CBP→RPPG models in this study. Possible techniques for obtaining CBP waveforms are then reviewed. Afterwards, most practical techniques to assess the endothelial performance are introduced. Such techniques are classified into clinical, invasive and non-invasive approaches. Advantage(s) and limitation(s) of each approach are then reviewed and discussed. FMD-US technique is then explained as the most established technique to assess ED non-invasively. Limitations of this technique are then explained and other non-invasive techniques are reviewed. Afterwards, PPG-based approaches to assess the ED are discussed. In addition, latest PPG-based approach to assess the ED (Rosmina Jaafar's work (Jaafar 2009)) is explained and its advantages and limitations are discussed. Possibility of capturing both endothelial and "non-endothelial" factors is introduced as the main limitation of this work. Effects of autoregulation in finger artery are mentioned as the main reason of such limitation. Afterwards, it is concluded that this work could take a considerable step towards establishing PPG-based techniques in order to assess the endothelial dysfunction in early stages. However, further investigations are still needed in order to capture the so-called pure endothelial response. Current research aims to investigate this issue by capturing endothelial response from brachial arteries and analysis endothelial response using a different approach (i.e. parametric modeling) and upgraded system setup (e.g. higher frequency transducer, an automated vessel-edge-detection software, rapid cuff inflator, etc.).

CHAPTER III

METHODOLOGY

3.1 RESEARCH STRUCTURE

The main objective of this study is to propose a non-invasive way to assess the ED based on a forward model between CBP and RPPG. To this end, a pilot study was designed in order to investigate the suitability of the CBP-RPPG model as a proposed criterion of assessing the endothelial performance. In fact, the pilot study was done to ensure the goodness of this model among different subjects along different times. In addition, it provided a practical way for primary evaluation of system setup, research protocols and data acquisition. The pilot study was done among eight healthy men who were participated in this research voluntarily. All subjects were fully informed about the research restriction and demands and signed the consent form before participating. On sampling day, both CBP and RPPG signals were simultaneously recorded for three minutes under normal condition (baseline). Data were then analyzed and stability of the CBP-RPPG model was confirmed. In addition, potential difficulties and problems were recorded to be avoided in the main data collection. For instance, it was understood that battery of PPG sensor need to be charged every day because low battery may lead to collect low quality signals. Moreover, there is a need to manage the timing of data collection via an automated recording system. Accordingly, a Labview program was designed in order to stop collecting data automatically after a given certain time. In addition, it was understood that using normal wires for data collection may lead to collect noisy data. This matter was solved when coaxial cables were used. Also, it was understood that there is a need to follow a protocol in order to manage data concerning subject number-name, date and time in order to avoid any confusion in the main data collection. Furthermore, a lot of experiences concerning sensors attachment, software-

hardware troubleshooting and collecting information at the time of data sampling were obtained.

Research proposal was then revised based on the experience obtained from the pilot study. Head of the cardiology department, Hospital Universiti Kebangsaan Malaysia (HUKM) was introduced as a medical partner of this research. In addition, a sonography room in this ward was considered as a venue of data collection because it was equipped with a high frequency ultrasound machine, bed and other basic equipment. Besides, an experienced and skilled sonographer was appointed -by the medical partner- to conduct the FMD-US test. Due to limited number of ultrasound machines in the ward and presence of numerous inpatients, only two hours per day was allocated to this research for data collection; from 7 to 9 am. Following research protocol, the blood test must be done after the FMD-US test in order to get information about the general health condition of the participants at the time of giving data. Therefore, each subject was asked to attend the sampling session only once. All necessary documents including research proposal, consent form, patient information sheet and a poster (to find volunteers) were then submitted to ethic committee of the HUKM. The research was then approved allowing data collection at department of cardiology. The ethics approval can be seen in Appendix A.

As mentioned above, the FMD-US test is also used -as a gold standard technique- to assess the endothelial performance in conjunction with the proposed technique. Thus, this research is a cross-sectional analysis to assess the endothelial dysfunction via both approaches. Moreover, it is a single-blind study as the functionality of the endothelial layer is not known at the beginning.

3.2 SUBJECT POPULATION

In this research, no gender limitation was considered because the ED is a prevalent vascular disorder among both males and females (Skaug et al. 2013). The ED is mostly observed among middle-age and elderly groups (Seals et al. 2011). Accordingly, favorite age range in this study was considered between 30-70 years old. All participants were grouped into either healthy or unhealthy groups. The healthy group consisted of those subjects who were free from CV risk factors. Conversely, the unhealthy group

consisted of those subjects with certain CV disease(s). The whole members of unhealthy group were selected by a cardiologist of HUKM among HUKM in-patients.

3.2.1 Sample Size

Ideally, number of participants in clinical researches should be preferably as many as possible. However, it is normally very difficult to conduct a research with numerous subjects mainly due associated limitations such as the cost and time. Therefore, the sample size should be realistically determined to ensure research quality while considering unavoidable limitations. The sample size of this study was calculated via the following equation:

$$sn \pm z_{1-\frac{\alpha}{2}} \sqrt{\frac{sn(1-sn)}{n_a}} \tag{3.1}$$

$$sp \pm z_{1-\frac{\alpha}{2}} \sqrt{\frac{sp(1-sp)}{n_n}} \tag{3.2}$$

in which *Sn* and *Sp* are sensitivity and specificity respectively, *z* is the probability of distribution, n_n is the number of healthy subjects, n_a is the number of non-healthy subjects and α is the type *I* error.

In these equations, by consideringαequal to 5 %, under controlled conditions, the proposed PPG technique is expected to have a sensitivity of (90±6)% and specificity of (90±6)% with equal number of healthy and unhealthy subjects. However, the FMD-US test is known as a very tough maneuver in clinical practices. In this regard, the sample size of sixty was acknowledged for FMD-based studies (Corretti et al. 2002).

3.2.2 Inclusion Criteria

Inclusion criteria can be considered as an inseparable part of clinical researches. In fact, it clarifies favorite group of study based on research objectives. In other words, it explains desired sample group and proffered conditions of conducting a study. In this

research, the inclusion criteria were separately determined for healthy and non-healthy as can be seen in Table 2.1.

Table 3.1 Inclusion Criterion of healthy and unhealthy groups

Inclusion Criterion	Healthy Group	• Male and female adults between 30-60 years old • Non-smoker, non-hypertensive, non-diabetics, non-dyslipidaemiaand free of CV heredity and risk factors
	Unhealthy Group	• Male and female adults between 30-60 years old • Adults *with* certain CV abnormalities like; diabetes, hypertension, atherosclerosis or dyslipidaemia • Adults with first-degree family disorders (i.e. those with relatives who are suffering from CV disease (heredity))

3.2.3 Exclusion Criteria

In contrast to the inclusion criterion, the exclusion criterion explains conditions or a specific group of people that cannot be considered in a study. Such filtering is designed based on research objectives. In this research, restrictions were considered for healthy group. Details of exclusion criterion for both healthy and unhealthy groups are shown in Table 2.2.

Table 3.2 Exclusion Criterion of healthy and unhealthy groups

Exclusion Criterion	Healthy Group	-
	Unhealthy Group	• Those with stroke or myocardial infarction history • Those with CV diseases and eGFR • Those who underwent vascular surgery or renovascular (perwtaneous or surgery) • Those with systemic inflammatory diseases • Those who had the heart or lungs transplantation • Those who have been administrated vasodilator including Calcium channel blockers nitrates, beta or alpha blockers

3.3 DATA ACQUISITION

A poster was designed and advertised in a notice board of UKM and HUKM in order to motivate people to take part in this research. As mentioned before, this poster was ethically and contently approved by ethical committee of the HUKM. Candidates were then selected based on the inclusion and exclusion criteria which are mentioned above. A general description of this study plus its procedure, demands and consent form were given to each participant in written form prior to data collection. They were asked to read the description and express their agreement -to participate to this study-by signing the consent form (Appendix B).

Data collection was done at the department of Cardiology, HUKM. Data were recorded under the same environmental condition by applying the same procedure to all subjects. The sampling room was temperature controlled and free of noise during all recording sessions. Ultrasound images were acquired by the sonographer who was responsible for the sector of ultrasound cardiology in the department of Cardiology, PPUKM. Data collection form can be seen in Appendix C.

The Labview program which was previously used for pre-data collection was upgraded and used to manage data monitoring and storing. Three windows were designed in this program to facilitate signal monitoring so that each signal can be individually displayed in a specific window. A "record" button was also designed in the Labview graphical panel in order to start data storing at the same time. In addition, a built-in timer was designed to stop data saving automatically after thirteen minutes (length of data collection). Figure 3.1 illustrates the graphical panel of the used Labview program.

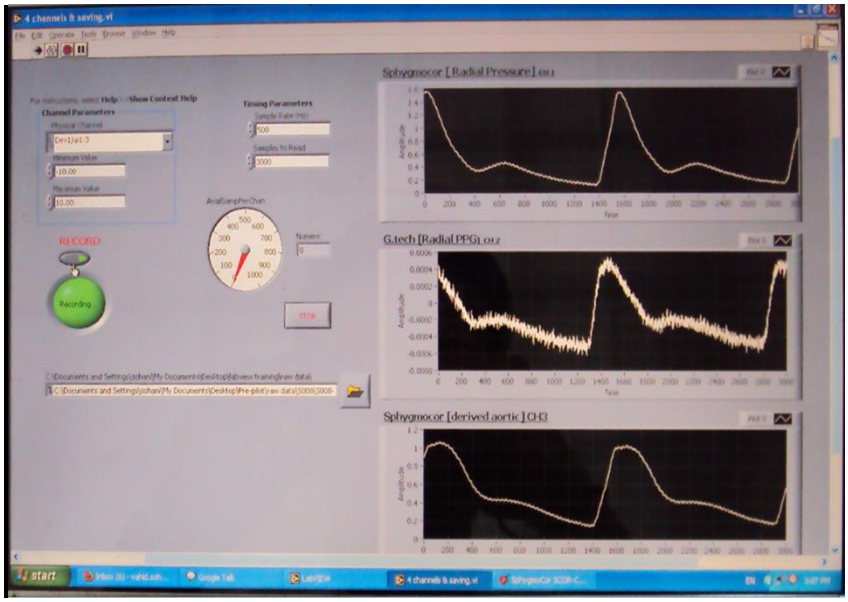


Figure 3.1 A screen shot of the Labview program used in the data collection

3.3.1 Subject Preparation and Sampling Atmosphere

Following the FMD-US guideline, all subjects were asked fast overnight and refrain from any drug administration and doing physical activity at least three days before data collection (Corretti et al. 2002). At the beginning of each sampling session, the subject was shortly asked about these notes in order to ensure that all restrictions are rightly followed and he/she is in a good health condition not only for the data collection but also for giving blood.

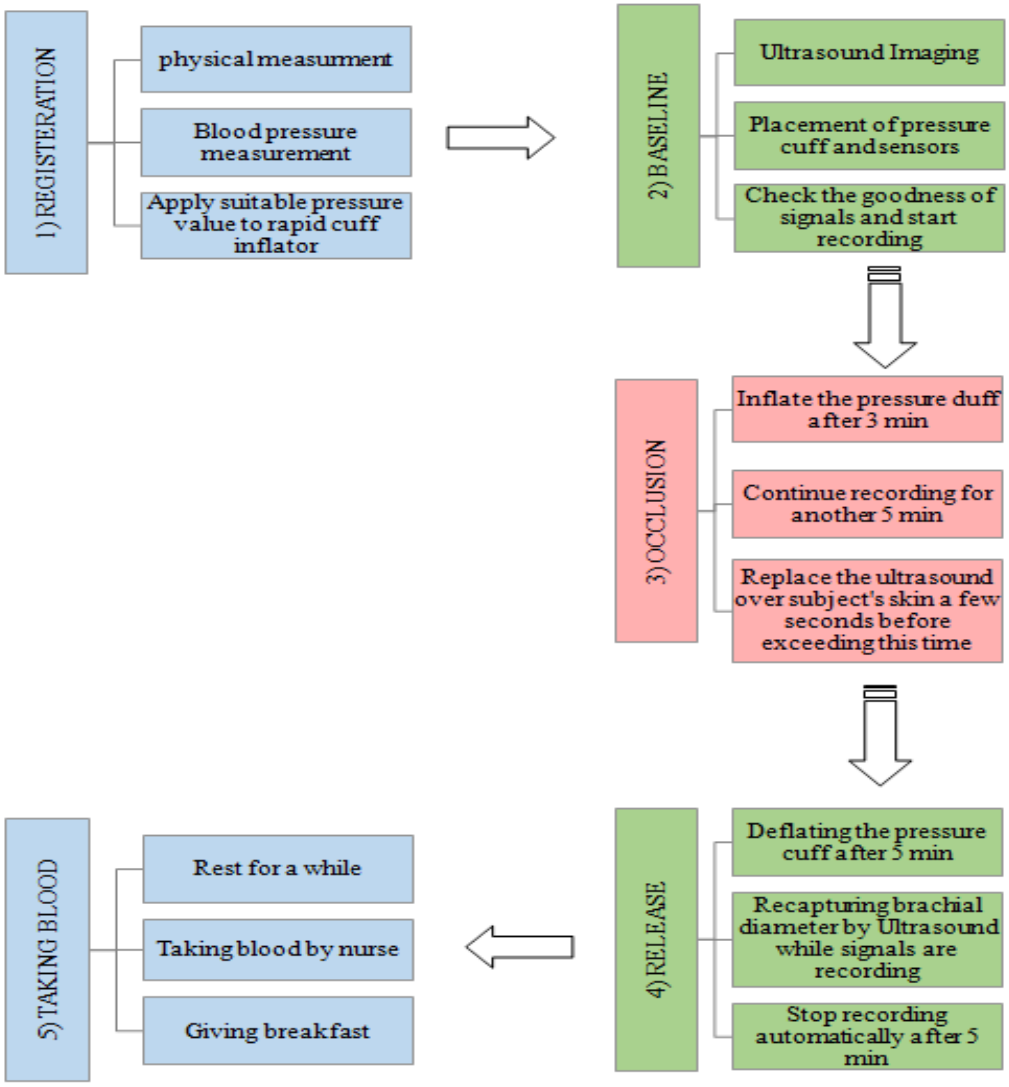
Subject was then asked to go to another room for measuring subject's height and weight. These parameters were measured for obtaining the body mass index (BMI). After completing this part, the subject was asked to lie on a bed in supine position. Ten minutes was intentionally spent to ensure that subject's blood circulation is stable. Blood pressure was then taken from both left and right arms by a digital sphygmomanometer (Omron, T8 Japan). These values were then averaged and considered as the blood pressure values.

In order to do the FMD test, the blood circulation in brachial artery must be fully blocked. To this end, an additional 50 *mmHg* pressure should be applied to the brachial artery via an inflating pressure cuff (Corretti et al. 2002). Therefore, an appropriate

pressure value (i.e. systolic pressure value plus 50 mmHg) was given to the rapid cuff inflator machine to be applied over the brachial site. Afterwards, an adjustable pressure cuff was wrapped around the right upper arm. In addition, the PPG sensor and applanation tonometry probe were located over the right and left radial arteries (wrists) respectively. Hence, the CBP, RPPG and radial pressure pulse (RPP) were obtained (displayed) on their own channel as can be seen in Figure 3.1.The stability and goodness of these signals were visually evaluated by operator.

After locating the sensors, ECG electrodes were placed across subject's chest in order to record the ECG signal along the ultrasonography. In favor of obtaining high resolution images, all subjects were asked to strictly avoid from any movement, arm shaking and even talking. The brachial diameter was then continuously captured for three minutes by the *vivid i* ultrasound machine accompanied by a 10 *MHz* transducer. All ultrasound images were collected by a skilled sonographer and saved in DICOM format under subject's name. The convention of data saving is explained in section 3.3.3.After obtaining brachial images, a few minutes were intentionally passed to make sure that the blood circulation is become stable again. Once all signals were found stable, the record bottom in the Labview program was pressed resulting in simultaneous data storing in the laptop.

As quoted earlier, data were recorded under normal condition (baseline) in the first three minutes. Soon after this time, a foot switch of the rapid cuff inflator was pressed resulting in sudden blood occlusion across the brachial artery. This phase (occlusion mode) was continued for five minutes to ensure that the FMD stimulus is perfectly performed. Before exceeding this time (i.e. after 4′:30″of blockage), the transducer was placed over subject's right arm in order to be ready to trace the brachial diameter (Endothelial reaction) immediately after releasing the pressure cuff. Sharp after five minutes, the pressure cuff was deflated by re-pressing the foot switch of rapid cuff inflator so that a large density of blood was suddenly rushed into the brachial artery while the brachial diameter was continuously capturing via ultrasound. Data recording was continued for another five minutes (release mode). The main steps of data collection are shown in flowchart 3.1.



Flowchart 3.1 Steps of data collection

Figure 3.2 shows the time chart of data collection. As can be seen in this chart, data were continuously recorded from thirteen minutes including baseline (3 minutes), blockage (5 minutes) and cuff release (another 5 minutes). A digital clock was used to follow the time chart.

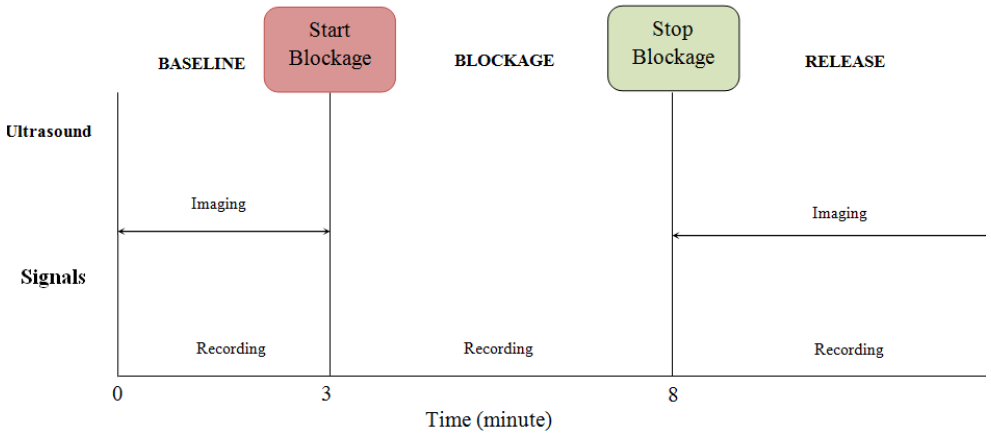


Figure 3.2 Time chart of data collection

Figure 3.3 shows the sampling environment, system setup and a subject during the data collection.

Figure 3.3 Subject and setup during data collection

After finishing the FMD-US test, blood was taken by an authorized nurse in the ward. A new pack of syringe and needle was used for each particular subject. Obtained blood sample was then shared into five small tubes in order to send to laboratory office of the PPUKM for different blood analysis (e.g. fasting lipids, liver check). After taking blood, a light sweet breakfast was given to each subject.

3.3.2 Device and Sensor

a) Pulse Oximeter Sensor

In this study, the g.tech plethysmographic pulse sensor -from g.tech company, Austria- was used to obtain blood volume change over the brachial artery (RPPG signal). In fact, it is a photoelectric pulse sensor that represents the variation of blood flow along each cardiac cycle. Figure 3.4 illustrates the used pulse sensor in this study.

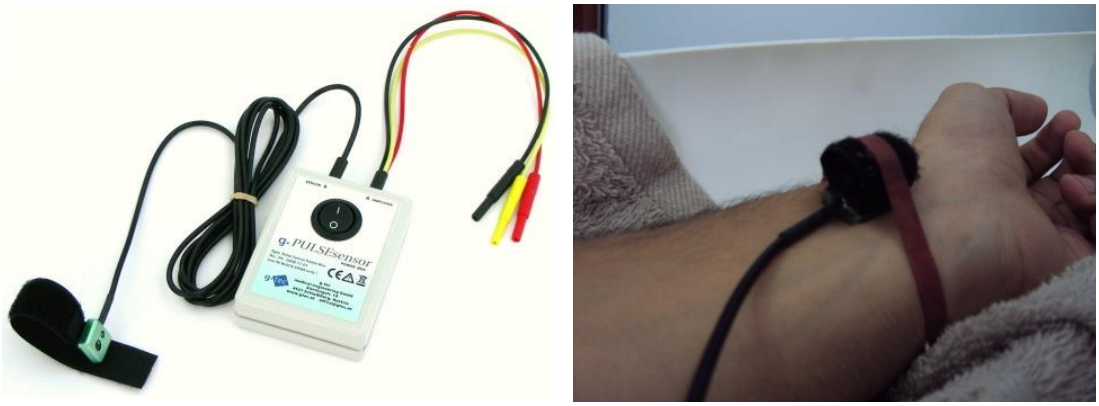


Figure 3.4 The g.tech sensor (left) and its probe over the brachial artery (right)

This sensor works with a 9 *v* battery. In order to avoid battery-related inconvenience during data collection, two chargeable batteries were used intermittently. At the beginning of each session, a full charged battery was put in the sensor while the other one was charging. These batteries were replaced at the beginning of each session. The probe was fixed over the brachial artery by an elastic strap as shown in Figure 3.4. Output of this sensor was a voltage signal with alternation between ±1 *mV*. The goodness and stability of the RPPG signal -obtained by this sensor- was visually evaluated by an operator on site before start recording.

b) The SphygmoCor

There are several devices available in the healthcare market for estimating the central blood pressure (CBP) non-invasively. Among them, the SphygmoCor has been mostly attended due to convinced degree of accuracy and validation papers (Ding et al. 2011;

Sharman et al. 2006). In fact, the technology of the SphygmoCor is based on a general transfer function in which the CBP waveform can be estimated from the RPP signal non-invasively. The theory of this concept was discussed in section 2.7.1. This device is consisted of three main components: a hardware box, an applanation tonometry and a software program.

The SphygmoCor box contains the hardware components and external ports for exporting data (signals). The external ports are located at the back of the box. Using RS 232 cable the ports can be connected to a computer in which the SphygmoCor driver is already installed in. In fact, this software is a windows-based interfacing system between the SphygmoCor and a computer to facilitate Real-Time signal monitoring and storing. The SphygmoCor box also has a drawer for isolating a female plug -which is installed in- from environmental disturbances. The applanation tonometry is a piezoelectric sensor which converts the pulsatile stroke into an electrical signal (pressure pulse). It's connected to the female plug via a cable. The mentioned components of the SphygmoCor are shown in Figure 3.5.

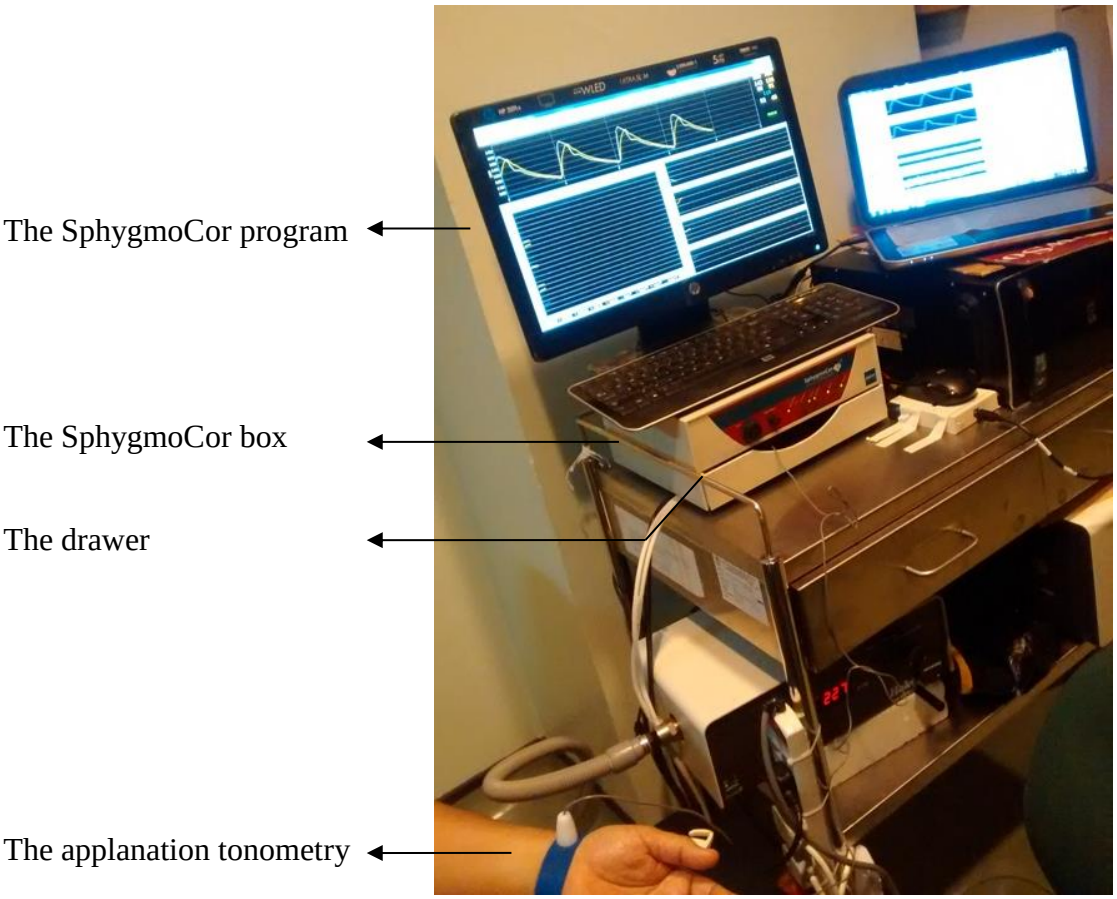


Figure 3.5 The SghygmoCor device and its components

The SphygmoCor device can operate in two different modes;

1. Pulse Wave Velocity (PWV): in this mode, either carotid-ECG or radial/femoral-ECG can be simultaneously recorded for pulse wave analysis. In this mode, the CBP cannot be estimated.

2. Pulse Wave Monitoring (PWM): in which, both CBP and RPP can be accessed in arbitrary unit.

In this study, the SphygmoCor was worked in the PWM mode so that both CBP and RPP were simultaneously obtained. These signals (voltages) were collected from the external ports of the SphygmoCor which are located at the back of this device. The

signals were then connected to the data acquisition unit via two similar cables provided by the manufacturer. Ports of the SphygmoCor are shown in Figure 3.6.

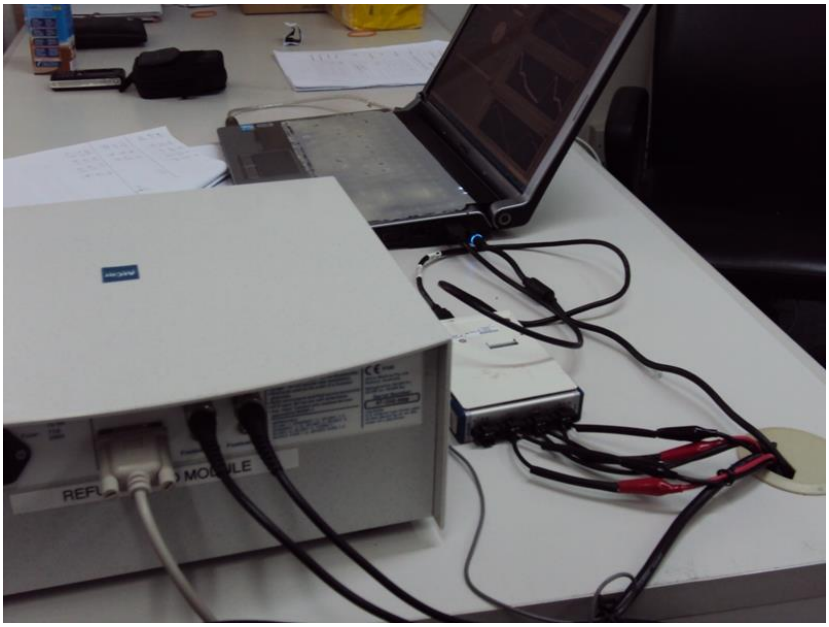


Figure 3.6 External ports of the SphygmoCor

c) Data Acquisition Unit

In this study, NI 9239 from National Instrument, USA was used to manage simultaneous data collection. It was accomplished by the Labview program to display and store signals in a laptop. The connection between data acquisition and laptop was done via type B USB cable. As can be seen in Figure 3.7 the NI 9232 has four channels to collect analogue signals (voltage) between ±10 *V*. In fact, it is an analogue to digital converter which facilitates data transmission into the laptop with same sampling frequency. The default sampling value for the NI 9239 is 1600 *Hz* but in this study, it was increased to 2000 *Hz* in order to simplify further calculations. It means that two thousand samples were transferred from the data acquisition unit to the laptop in each single second.

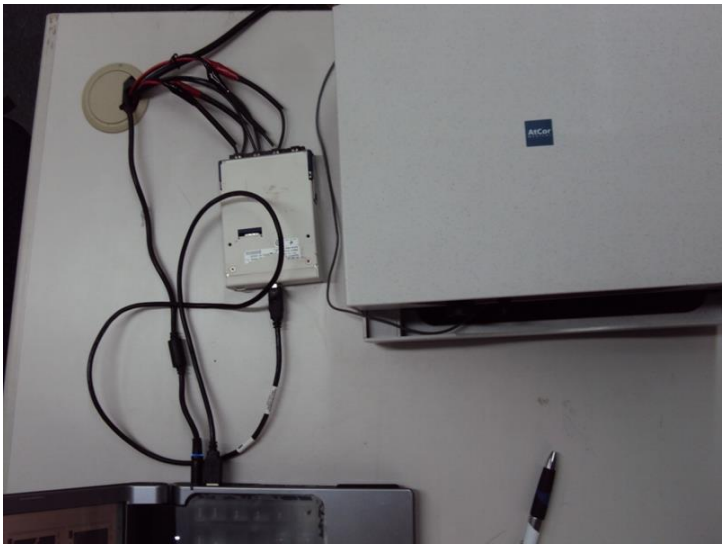


Figure 3.7 The NI 9239 data acquisition unit

d) Static Blood Pressure Measurement

As mentioned earlier, a value of systolic blood pressure plays an important role in the FMD test for blocking the brachial artery. In fact, this value indicates an appropriate pressure value that need to be applied across the brachial artery to be fully occluded. Therefore, precise measurement of blood pressure should be highly taken into account in the FMD analyses. In this research, the Omron T8 -from Japan- was used as a digital sphygmomanometer to measure subject's blood pressure and heart rate. As usual, it was done by wrapping an inflatable pressure cuff across the brachial artery. Systolic, diastolic and heart rate were then obtained and documented. This device is shown in Figure 3.8.

Figure 3.8 The Omron T8 blood pressure device

e) Ultrasound Imaging of the Artery

In order to capture the endothelial reaction during the FMD test, an ultrasound machine with preferably high frequency transducer is needed. In this research, the *GE vivid i* ultrasound machine with a 10 *MHz* transducer was used. This machine has been used in department of cardiology, HUKM hospital for daily cardiovascular imaging. In order to visualize the vascular border, the top of the transducer should be covered by some disposable gel. Despite, the transducer should be correctly placed over the subject's skin in order to obtain a high resolution image. The common mistake is to locate the transducer in perpendicular position to an artery but it is a worst case indeed. However, the relationship between angle of ultrasound probe and blood stream can be mathematically expressed via equation 3.3:

$$f_d = 2 f_0 \frac{v}{c} \cos(\alpha) \tag{3.3}$$

in which f_d is the reflected ultrasound, f_0 is the transmitted frequency, v is the blood velocity, c is the sound velocity in tissue and α is the insonation angle between the ultrasound beam and the velocity vector (Harris et al. 2010). According to this equation, if the ultrasound probe is vertically located over the skin, no image can be captured. However, an optimal range of α is between $60° < \alpha < 90°$ (Thijssen et al. 2011).

The vivid *i* ultrasound machine provides a way of recording the ECG signal simultaneously. To this end, the ECG electrodes should be placed across subject's chest. Figure 3.9. shows a typical ultrasound image in conjunction with the ECG signal.

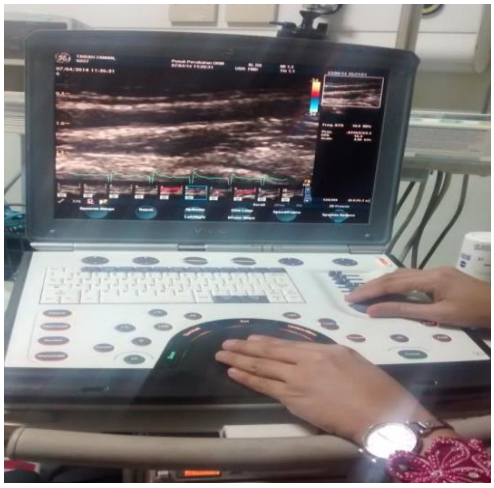



Figure 3.9 The vivid i ultrasound machine

f) Rapid Cuff Inflator

One of the key issues in the FMD-US studies is a possibility of sudden circulatory occlusion across the brachial site. It can be done by a typical Sphygmomanometer. However, it may take a few seconds which affects the efficiency of the FMD stimulus so that sudden occlusion is desirable (Corretti et al. 2002). In this study, the rapid cuff inflator E20 from Hokanson company, USA was used. It facilitates a rapid occlusion (and compensation) over the brachial artery in one third of a second. This device is consisted of an air compressor, rapid cuff inflator, a plastic tube, a pressure cuff and a foot switch as can be seen in Figure 3.10.

In order to use the rapid cuff inflator, a favorite pressure value should be firstly given to the device. It can be simply done by rounding a circular lever. Possible range of pressure is 0-300 *mmHg* in which every single 1 *mmHg* can be seen via a digital display. Secondly, the pressure cuff should be wrapped across subject's brachial artery. Once the foot switch is pressed, the compressed air is suddenly (i.e. 0.3s) flowed from the tank to the pressure cuff resulting in a rapid cuff inflation and sudden brachial occlusion. The cuff deflation can be similarly done by re-pressing the foot switch.

Figure 3.10 The E20 rapid cuff inflator

g) Hardware Configuration

In this study, the ultrasound machine and the rapid cuff inflator were independently used meaning that they were not physically connected to other devices. The configuration between pulse sensor, SphygmoCor, data acquisition unit and computer is explained below.

As mentioned above, the SphygmoCor was connected to the computer via RS 232 cable. The CBP and RPP signals were collected from two ports located at the back of the SphygmoCor as can be seen in Figure 3.6. They were connected to the first and second channels of the NI 9239 via two separate coaxial cables. On the other side, the output of the PPG sensor was connected to the third channel of the NI 9239 via a similar coaxial cable. The NI 9239 unit was then connected to a laptop via type B USB cable. The Labview program was used on this laptop so that all signals were displayed and stored. The setup configuration is illustrated in Figure 3.11.

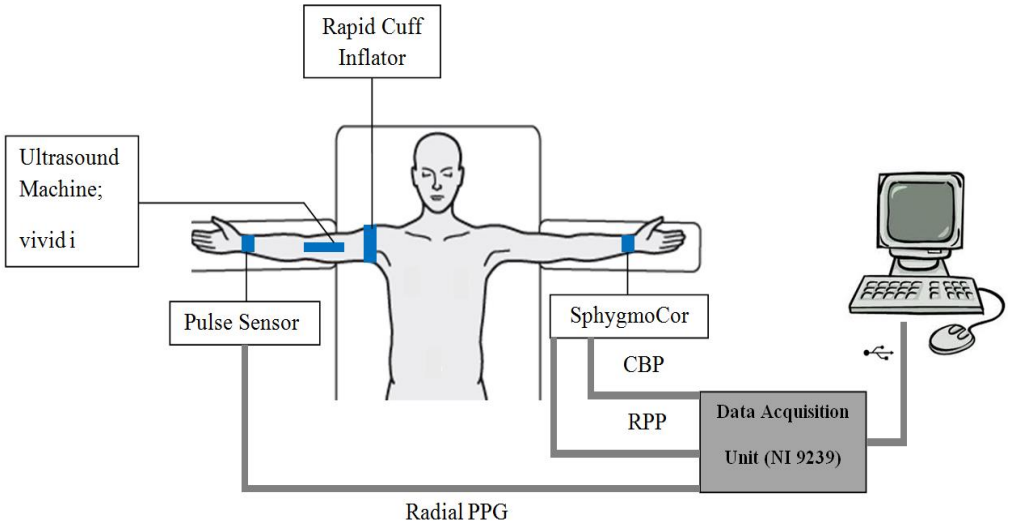


Figure 3.11 The setup configuration

3.3.3 Data files: Naming Convention

A convention was used to facilitate data identification after completing data collection. In this regard, an exclusive identification number (ID) was given to each participant so that all information were saved into an excel file under subject's ID and name. In the excel file, all subjects were listed in first column while all parameters such as; age, gender, blood pressure and clinical parameters where listed in another columns. Then, information of each subject was written in front subject's ID and under parameter's name. A screenshot of this file is shown in Figure 3.12.

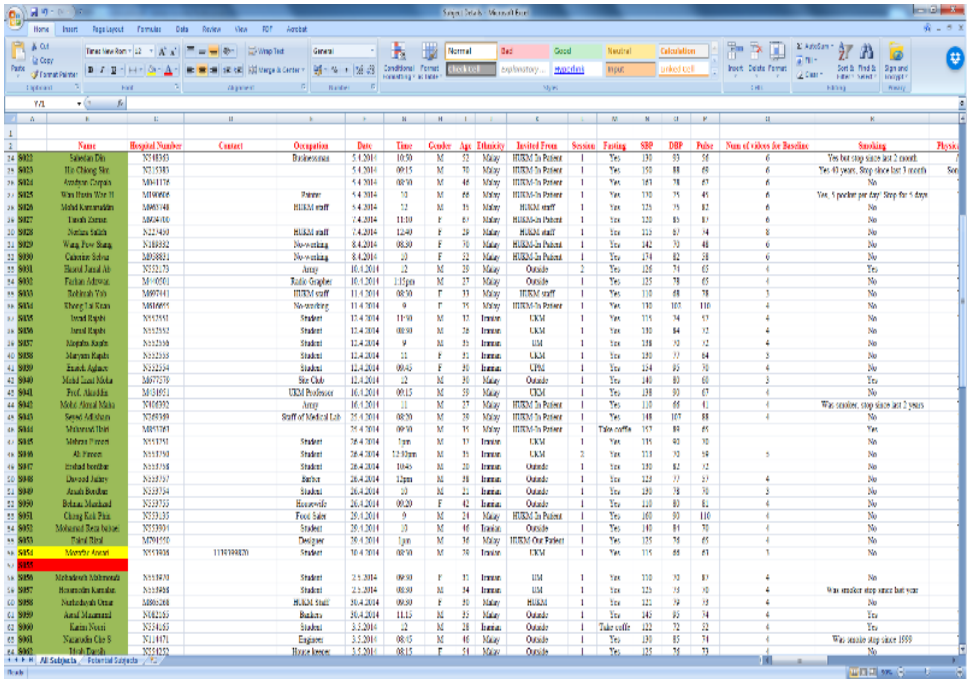


Figure 3.12 A screenshot of the excel file

Another file was considered for documenting results of Matlab processing. In this file, each sheet was allocated to one participant. The Matlab code was written in a way that result of each analytical section was automatically imported to subject's own sheet. In this study, two types of data were collected; ultrasound images and signals. The procedure of data saving for each of them is separately explained below.

a) Ultrasound Images

As quoted earlier, the vivid *i* ultrasound machine was used in this research. Before ultrasound imaging, a folder was created -in the device- under subject's ID. This folder was then addressed as a saving location so that ultrasound images of each participant were accordingly saved into subject's own folder in DICOM format. At the end of data collection, all images were transferred from the ultrasound machine to a hard disk via USB cable.

b) Signals

As mentioned above, three signals were recorded in this research: CBP, RBP and RPPG. A single text file was used to store data (signals) of each particular subject. Each text file was consisted of three columns in which the CBP was saved in the first column while the RPP and RPPG signals were saved into the second and third columns respectively. The text file was named by subject's ID followed by session number. For example, data of subject 87 in session one was saved as: S087session1.txt. At the time of data collection, subject's folder was manually addressed so that signals were directly saved into subject' own folder. Figure 3.13 shows a screenshot of typical text file including three columns.

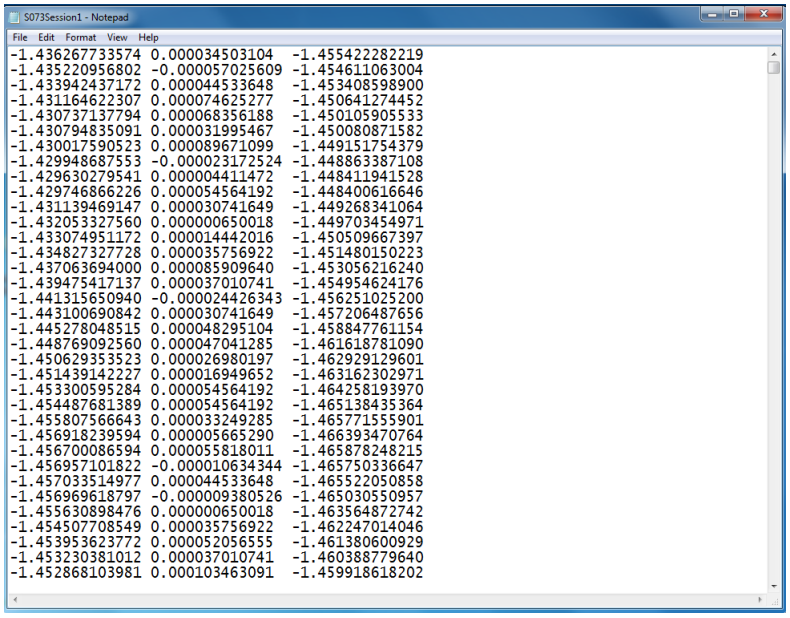


Figure 3.13 Structure of data (signals) storing in text format

3.3.4 Clinical Tests

In order to access subject's clinical parameters at time of data collection blood test was considered. It addition, it provided a reliable way for realizing risk factors. Details of blood taking and types of analysis are explained below.

a) Blood Test

Blood was taken shortly after finishing the FMD-ultrasound test when subject was still fasted. It was done by a nurse -in the department of cardiology- who was on-duty on sampling day. All blood takers were authorized and worked at this ward as a nurse. Around 3 *cc* of blood was taken from each participant using a new syringe and needle. Blood sample was then shared into five small tubes for different analysis. Four of these tubes were immediately send to HUKM laboratory for analysis of fasting lipids, blood urea serum electrolytes and liver panel. A single tube was send to a private laboratory for high sensitive C-reactive protein test (HS-CRP) which did not perform in laboratory of the HUKM hospital at the time of this research.

b) HS-CRP

The C-reactive protein is normally generated by liver if there is an inflammation in body(Du Clos 2000). The high sensitive C-reactive protein test is widely used to predict cardiovascular disease (e.g. atherosclerosis, heart attack) (Morrow & Ridker 2000). The normal range of HSCRP test is between 1 to 3 mg/L so that higher values are considered as high risk. However, result of this test is normally interpreted in conjunction with other risk factors such as levels of cholesterol, LDL-C, triglycerides, glucose, smoking, high blood pressure and diabetes.

3.4 MEASUREMENT OF VESSEL DIAMETER (ULTRASOUND IMAGES)

As quoted earlier, the FMD-US is a well-known technique to assess the endothelial performance. Using this technique the endothelial reaction -to the FMD stimulus- can be captured via ultrasound imaging. In fact, the variation in brachial diameter before (baseline) and after releasing the cuff represents the endothelial performance (Corretti et al. 2002). Therefore, measuring the diameter of brachial artery plays an important role on accuracy of the FMD-US technique. Such a measurement should be continuously done by an automated system.

In this research, the Cardiovascular Suite program -from the Quipu company- was used to measure the vessel border automatically (Quipu 2014). In fact, it is an

algorithm based program to trace the brachial diameter which is induced either by the FMD test or drug perfusion (e.g. nitroglycerine). In this study, no drug was used to stimulate the brachial vessel so that only the FMD-studio program was used. As can be seen in Figure 3.14 this program has five small windows which are explained in Appendix G.

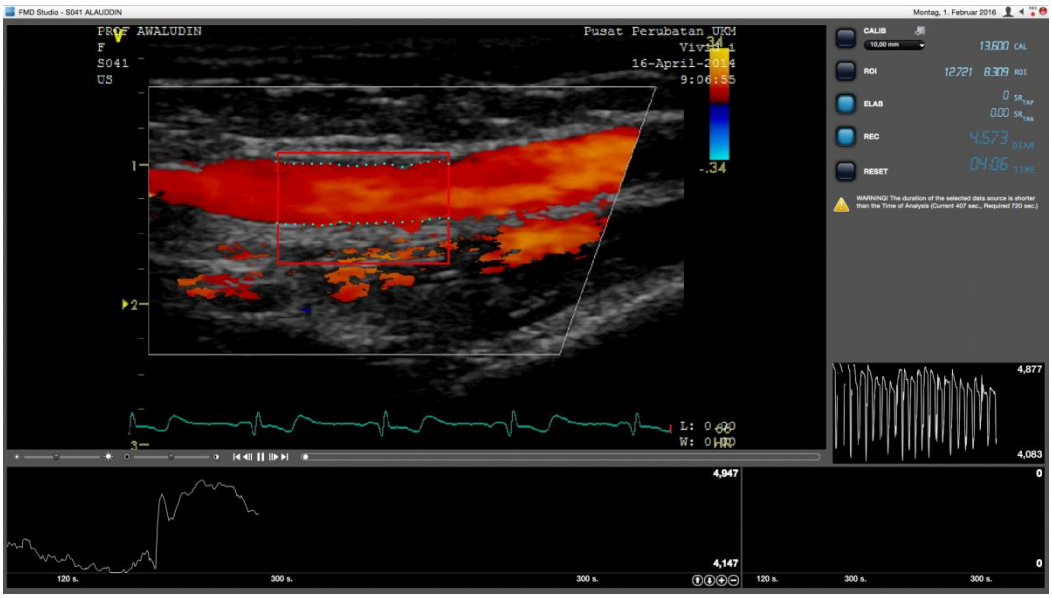


Figure 3.14 Measuring the vessel diameter by cardiovascular suite program

Using this program, brachial diameters were continuously measured from the beginning of the experiment (baseline) to the end (after cuff release). Figure 3.15 shows a screenshot of a typical result box.

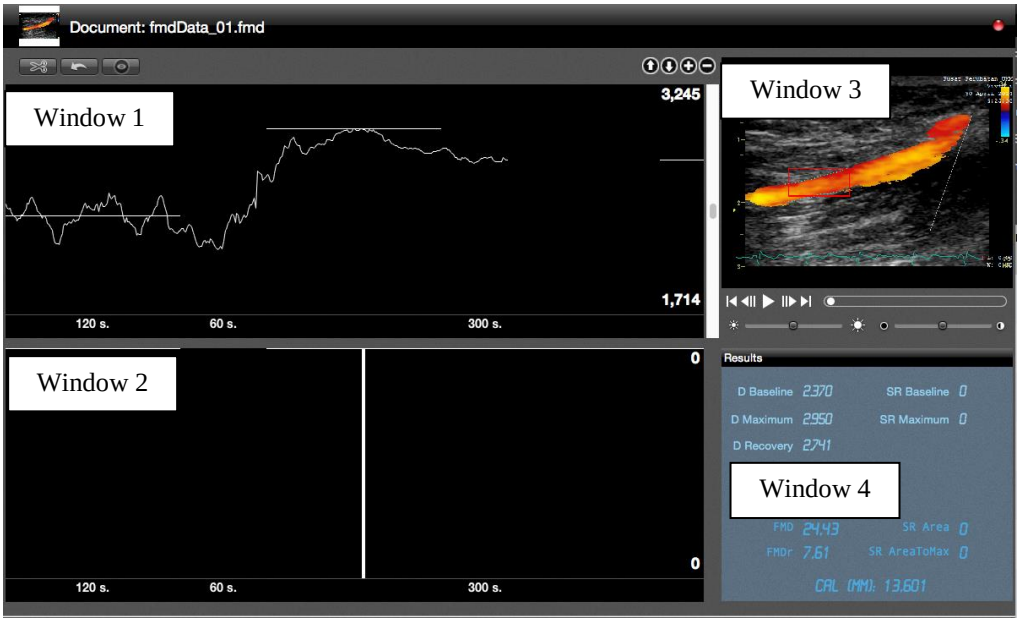


Figure 3.15 A typical result window

Result box is consisted of four windows which are explained below.

Window 1: This window shows the variation in diameter of brachial artery before and after applying the FMD stimulus. The curve is plotted in terms of the time (second) and values of diameter (millimeter). However, assessment to the exact value of the time and diameter for a specific point is only possible by moving a mouse across the curve while the shift key is pressed. Highest (peak) and lowest values of curves were obtained manually and then imported to an excel file.

Window 2: This window represents result of the Doppler analysis. As quoted earlier, this analysis was not considered in this research and hence, this window remains empty in Figure 4.22.

Window 3: This window contains a video which show the user operation (i.e. selection of ROI, calibration, etc.) during the FMD-Ultrasound analysis. It can be played any time after conducting analysis (off-line analysis).

Window 4: This window shows some basic parameters and their values after finishing the analysis. There are three parameters on the left up side of this window: D

$_{Baseline}$, $D_{Maximum}$ and $D_{recovery}$. The $D_{Baseline}$ indicates the average of brachial diameter in baseline while $D_{Maximum}$ and $D_{recovery}$ showing the maximum diameter during occlusion and recovery time (after release the pressure cuff) respectively. There are another two parameters on the left down side of this window; FMD and FMD showing the rate of brachial dilatation during occlusion and cuff release respectively.

3.5 SIGNAL PROCESSING

In general, signal processing is an analytical approach to prepare data (signals) for further analysis. In fact, it provides a practical way for applying some mathematical features on raw data; from waveform generation to design filter and spectral analysis. In this research, all signals were processed before start modeling (system identification). It was done by the Matlab software (version R2012a). The used Matlab code in this research can be seen in appendix D. Steps of signal processing are explained in following sections.

3.5.1 Distinguishing and Detrending

As mentioned before (Figure 3.13) raw signals were initially saved in different columns of a single text file. Thus, first step of data processing was to distinguish data. It was simply done by Matlab so that signals were separated from each other. At the end of this session, each signal was named individually (i.e. raw CBP, raw RPP and raw RPPG). All signals were then detrended in order to remove the linear trend.

3.5.2 Filtering

Raw data is always obtained with some unwanted effects known as noise. Such effects should be removed in order to make signal ready for further analysis. This step of signal processing is known as filtering. In fact, filtering can be considered as one of the key features of signal processing to reveal so-called real data rather than noise. The Matlab software is mostly used to conduct the filtering. However, realizing type -and preferably source- of noise can improve the quality of noise removal.

In this study, subjects' breathing, hand movement and physical contact pressure between sensors and subject's skin can be mentioned as potential sources of noise. It was observed that there is no important information above the frequency of 50 *Hz*. Therefore a low pass filter was designed based on the characteristics of signals. The signal processing toolbox of the Matlab was used for such analysis. The optimal cut-off frequency was set to 50 *Hz* so that higher frequencies were automatically filtered. The goodness of this filter was evaluated by comparing the raw and filtered signals using frequency responses. This filter was then applied to all signals resulting in removing noise while keeping important information of signals. Figure 3.16 illustrates two typical segments of CBP and RPPG signals before and after filtering.

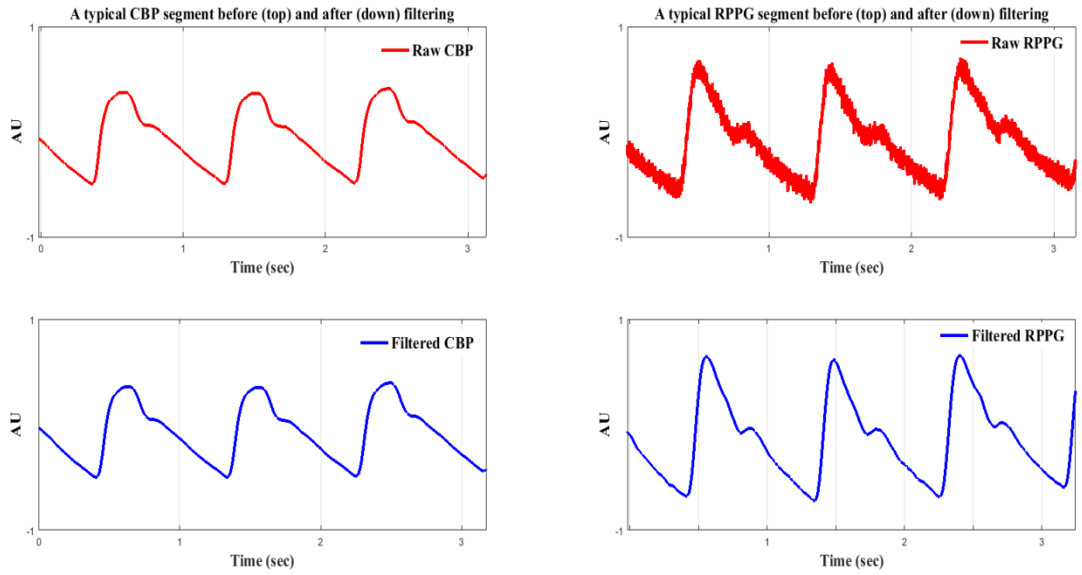


Figure 3.16 A typical segment of CBP (left) and RPPG (right) before and after filtering

3.5.3 Resampling

Referring to characteristics of the NI 9239 device, the default sampling frequency of this device is 1600 *Hz*. At the time of data collection, this value was rounded-up to *2000* Hz. However, frequency analysis (i.e. using Fast Fourier Transform (FFT)) showed that the highest frequency of recorded RPPG signals is around 50 *Hz*. It was also observed that the highest frequency rate of PPG is between 45-50 *Hz* in former referred works (Webster 2002). Considering the Nyquist frequency in which sampling frequency

should be at least two times bigger than the higher frequency, signals were down-sampled to 100 *Hz* to avoid unnecessary analysis over useless data. The Nyquist equation is as follows:

$$f_s \geq f_c \tag{3.4}$$

in which f_s is the sampling frequency and f_c is the highest frequency contained in the signal.

3.5.4 Segmentation

Results of the pilot study showed that the goodness of the CBP-RPPG model is good if whole signal is fragmented into several segments. In other words, poor suitability may obtain in case of using whole CBP and RPPG signals as model input and output respectively. In fact, characteristics (e.g. amplitude, etc.) of model input and output should be preferably similar to each other in order to get a reliable model output. Accordingly, variations between peak values of signals can be moderated if short time-series data are chosen as model input and output. In other words, long time-series data may be too different from each other resulting in low quality of estimated model output. Moreover, the endothelial reaction cannot be closely traced along the FMD test as it occurs immediately after releasing the pressure cuff. Thus, each signal was entirely fragmented into several segments with the same length of 3 seconds. It was done via a "for" loop in Matlab as can be seen in Appendix D. To this end, each signal was first separated into three parts: baseline, occlusion and release data. In this regard, the first three minutes of each signal was cut and considered as baseline data. The second and third five minutes were similarly cut and considered as occlusion and release data respectively. Figure 3.17 illustrates a typical raw data in which the phases of data collection are mentioned. Although data were continuously collected for thirteen minutes but as can be seen in this Figure, no data were obtained for RPPG signal during the blockage as there was no blood circulation during the blockage.

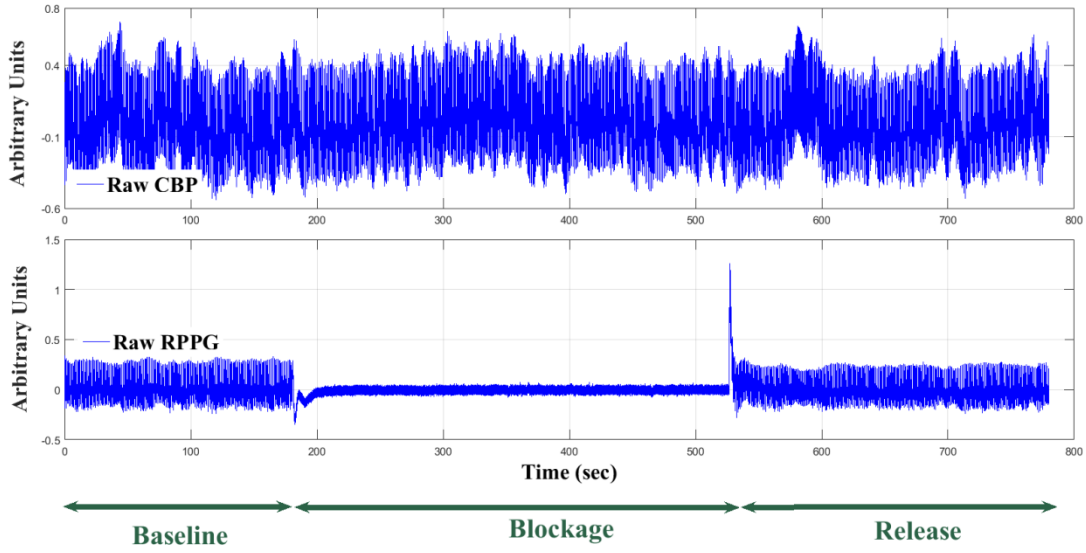


Figure 3.17 Representation of a typical raw data; CBP (top) and RPPG (bottom) waveforms including the phases of data collection (green arrows)

Data (i.e. baseline and release) were then fragmented into several segments for further analysis. Figure 3.18 illustrates graphical representation of this step. It should be noted that number of segments are not exactly same among all subjects because heart rate of subjects were different from each other.

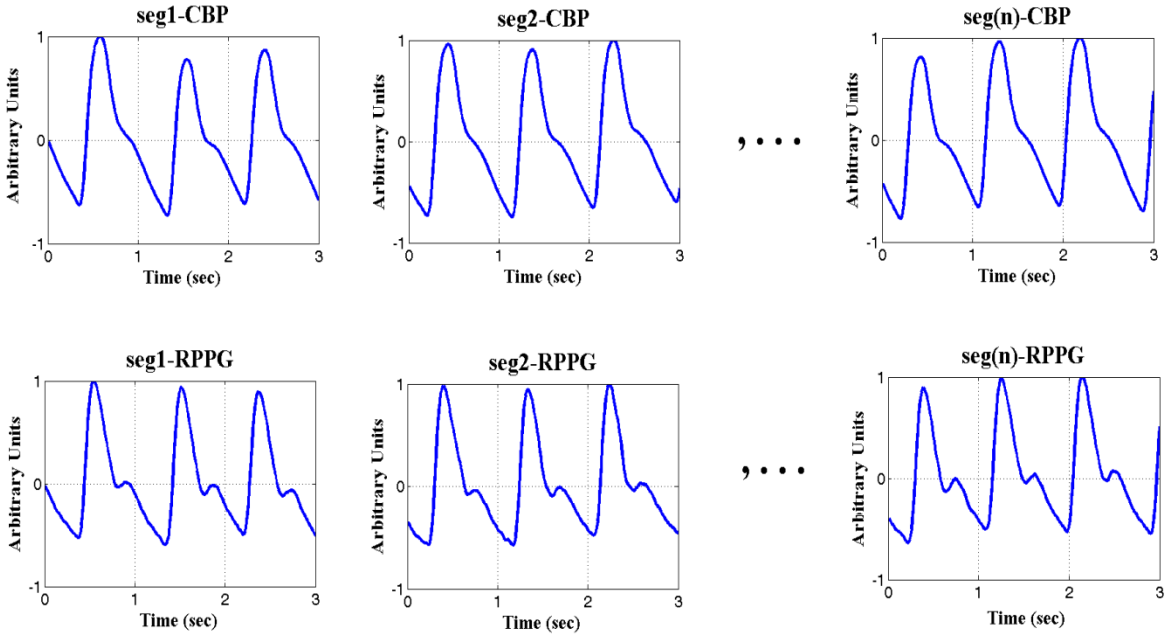


Figure 3.18 Segmentation of the baseline data

3.5.5 Normalization

According to the theory of the Matlab system identification toolbox, the amplitude of a given input-output pair plays a key role on suitability of the final outcome (Ljung 1999). It means that signals with similar amplitude can produce a better model. However, amplitude of signals may not be close to each other. In such case, signals can be justified in order to be mapped to a certain value of amplitude. In this research, amplitudes of CBP and RPPG signals were not close to each other as they obtained via different sensors with different technology. Thus, all segments were individually normalized to "1"for ensuring that maximum value in each segment is equal to value 1. It was simply done by dividing each segment to its maximum value so that the maximum value in each segment is 1. Figure 3.19 shows two atypical segments before and after normalization.

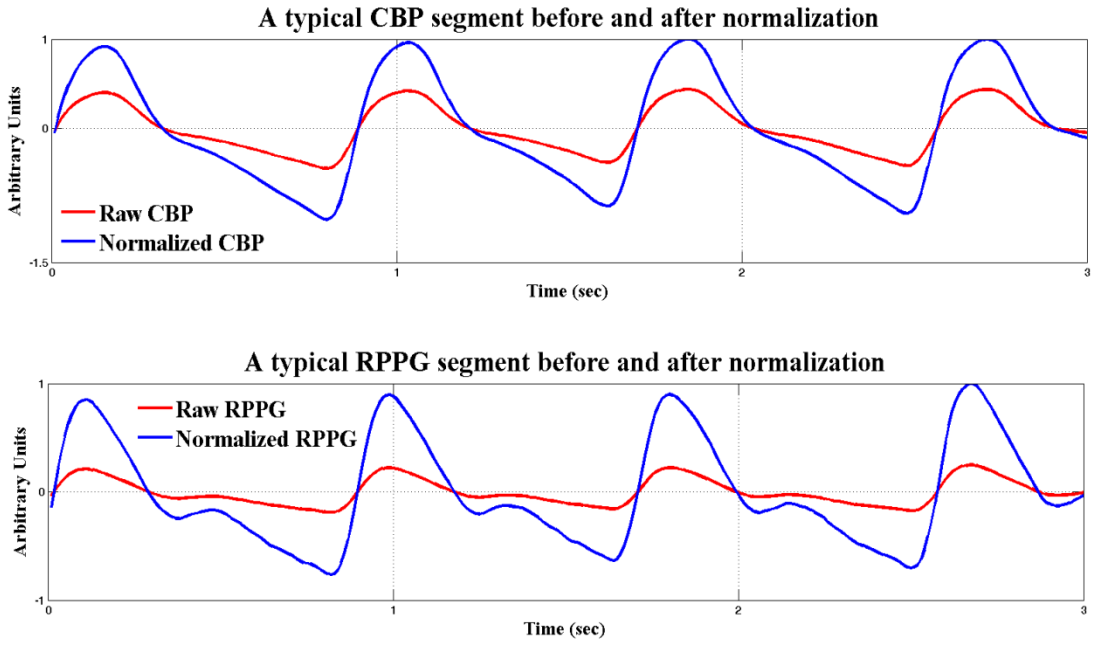


Figure 3.19 Normalization of a typical CBP (top) and RPPG (bottom) segments

3.5.6 Synchronization

Obviously, there is a very short time delay between the central and radial pressure pulse waveforms (Takazawa et al. 2007). In other words, the central pressure pulse needs a few milliseconds to reach to the radial artery. However, such a physiological time delay

is not taken into account in the generalized transfer function used in the SphygmoCore device (Pauca et al. 2001). It means that the estimation of the CBP waveform from the RPP signal is not a function of time. Since the SphygmoCor device was used in this study for obtaining the CBP waveform, time delays between CBP and RPPG waveforms were observed (Figure 3.22 (top)). Such time is definitely not same among all participants meaning that it is an exclusive value for each subject due to different physiological and metabolic conditions. Therefore, it is not possible to propose a generalized transfer function. Same issue was reported in similar works -including the SphygmoCor- and synchronization is suggested as the solution (Ding et al. 2011; Guelen et al. 2008). Accordingly, CBP and RPPG segments are aligned with respect to time. Figure 3.20 shows a typical CBP-RPPG segment before and after synchronization.

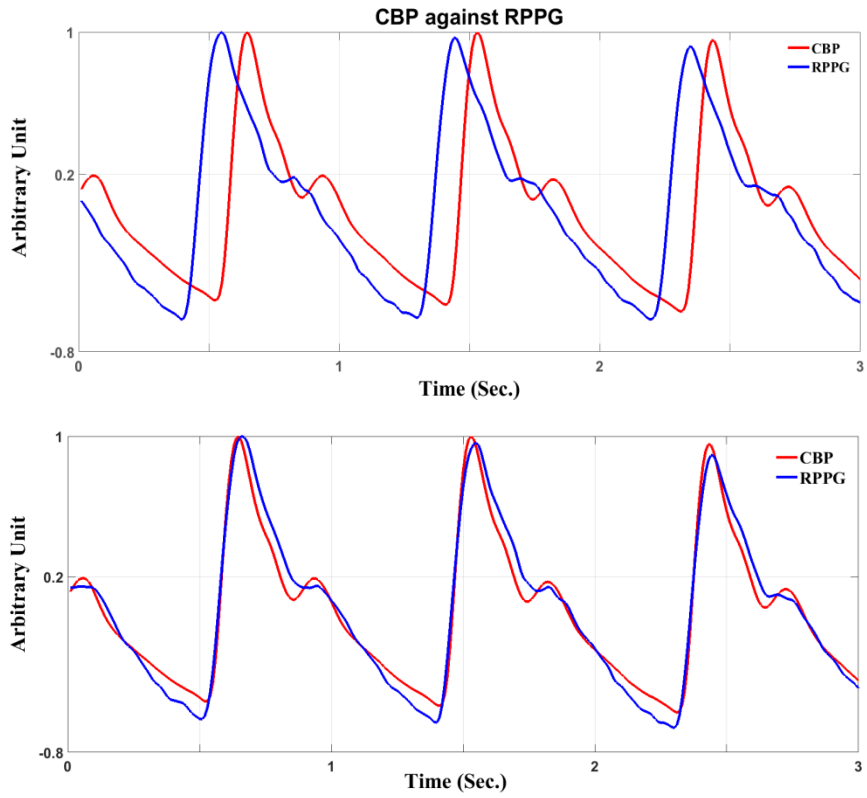


Figure 3.20 A typical pair of CBP-RPPG before (top) and after (down) synchronization

3.6 SYSTEM IDENTIFICATION

As quoted earlier, the main objective of this research is to assess endothelial functionality using a forward model between CBP and RPPG waveforms. In fact, such model is estimated using baseline data and then used as a robust model while endothelial cells are stimulated. Variation(s) of the model is then considered as the endothelial response. To this end, a CBP→RPPG model should be estimated for each subject. In this research, the Matlab system identification toolbox was used to identify a linear parametric model between CBP and RPPG. The analytical steps and interpretation of model behavior before and after doing the FMD test are explained in following parts.

3.6.1 Creation of Time-series Data

Referring to Figure 2.8, each model should have at least one input and one output. In fact, the model is the mathematical expression of relation between system input and output. According, CBP and RPPG are defined as model input and output respectively. As explained earlier, each set of data is fragmented into several segments with the same length of 3 sec. Segments were then used to create the so-called time-series data. To this end, each CBP segment is considered as model input while its corresponding RPPG segment is considered as model output. Figure 3.21 illustrates the protocol which is used to create the time-series data.

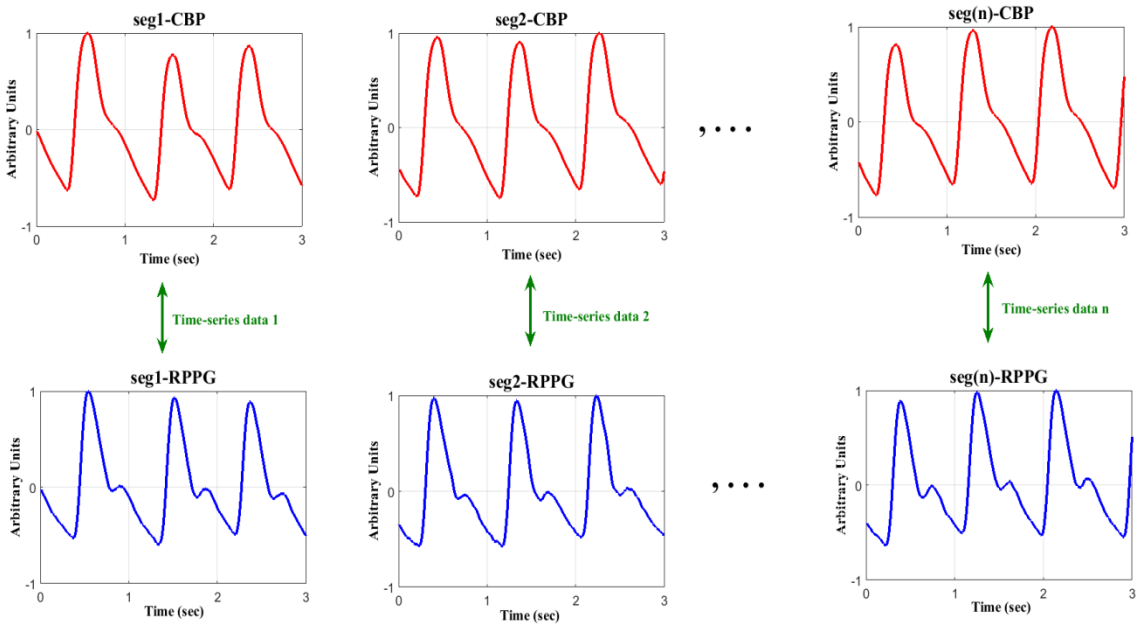


Figure 3.21 Creation of time-series data using RPPG segment (y1, output) and its corresponding CBP segment (u1, input)

A typical time-series data and its constructive segments is also shown in Figure 3.22.

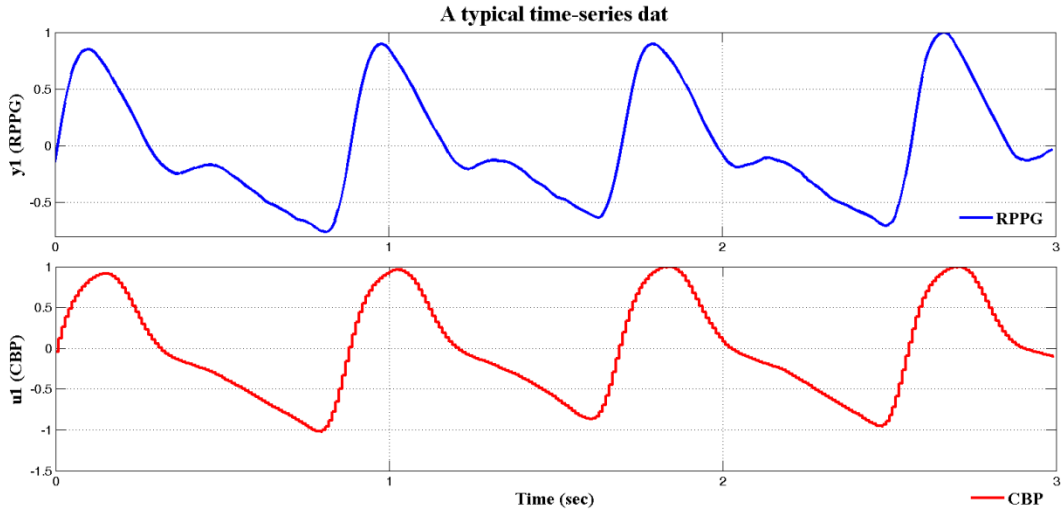


Figure 3.22 Representation of a typical time-series: RPPG as model output (y1) and its corresponding CBP as model input (u1)

3.6.2 Model Determination

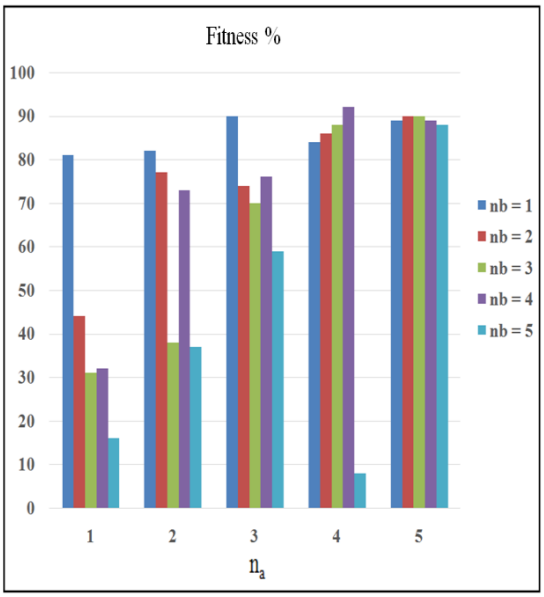
One of the key parameters in system modeling is the type of a model which is used to describe relations between model input and output (Ljung 1999). In this regard, a primary analysis may help to identify the best suited model structure concerning data, model and effective factors on model output. In this research, several model structures were investigated over data obtained from the pilot study. In addition, model structures in similar studies were studied. Auto-regressive with exogenous (ARX) model was previously used -in a similar study by our team- and showed a convinced stability and accuracy (Shariati & Zahedi 2005). Therefore, it is used to estimate a forward model between the CBP and the RPPG waveforms in this study. In general, the mathematical relation between model input *CBP(t)* and output *RPPG(t*) in ARX model is defined by the following equation:

$$RPPG(t) = \sum_{k=0}^{n_a} a(k) \times CBP(t-k-n_k) + \sum_{k=1}^{n_b} b(k) \times RPPG(t-k) + \varepsilon(t) \qquad (3.5)$$

where, the n_a is the order of denominator (number of poles), the n_b is the order of numerator (number of zeros), the n_k is the time delay between model input and output, the *a(k)* and *b(k)* are coefficients of numerator and denominator respectively and *ε(t)* is the residual noise (Ljung 1999) By convention and following the theory of system identification, the ARX model is shown as ARX [n_a n_b n_k] in this study (Ljung 1999).

3.6.3 Model Order

After determination of model structure, the order of a polynomial equation should be specified. As mentioned earlier, constructive data were synchronized to each other so that the physiological lag time between CBP and RPPG was removed (i.e. n_k=0). In order to determine the number of poles and zeros (n_a and n_b), different model orders from ARX [1 1 0] to ARX [5 5 0] were investigated. Referring to section 2.7.2, results were then compared to each other based on fitness and the FPE. As can be seen in Figure 3.23, models with n_a and n_b below 3 were found unstable because of low fitness value and high FPEs. The ARX [4 4 0] model had highest fitness value while having the lowest FPE value. It was also observed that there is no significant improvement concerning model quality for higher model orders (i.e. ARX [5 5 0]) Therefore, the model ARX [4 4 0] was selected this study.



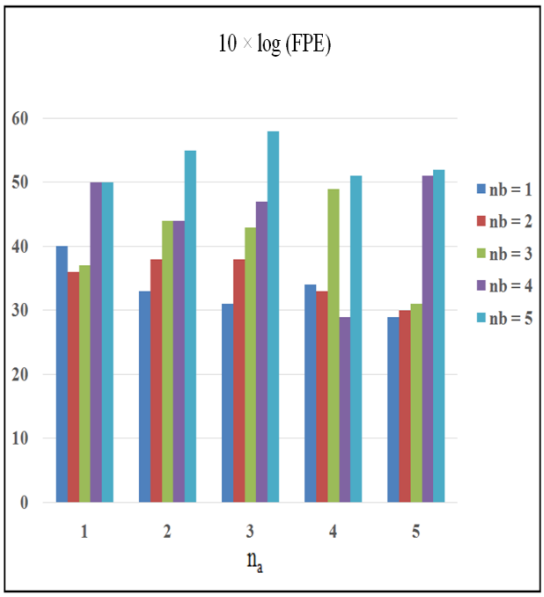


Figure 3.23 Goodness of models in terms of fitness (left) and FPE (right)

However, it should be noted that, different model orders may work better for some specific segments. In other words, the ARX [4 4 0] generally works well for all data (segments) whereas different models may predict system output more precisely. This issue is shown in Figure 3.24. As can be seen, goodness of different model orders against a specific segment has been investigated. Compare to ARX [4 4 0] model, the ARX [5 5 0] has a better performance although the ARX [4 4 0] has a convinced performance too (Figure 3.26 (up)). In contrast, the ARX [5 5 0] model does not work well for another segment whereas the ARX [4 4 0] does (Figure 3.26 (bottom)).

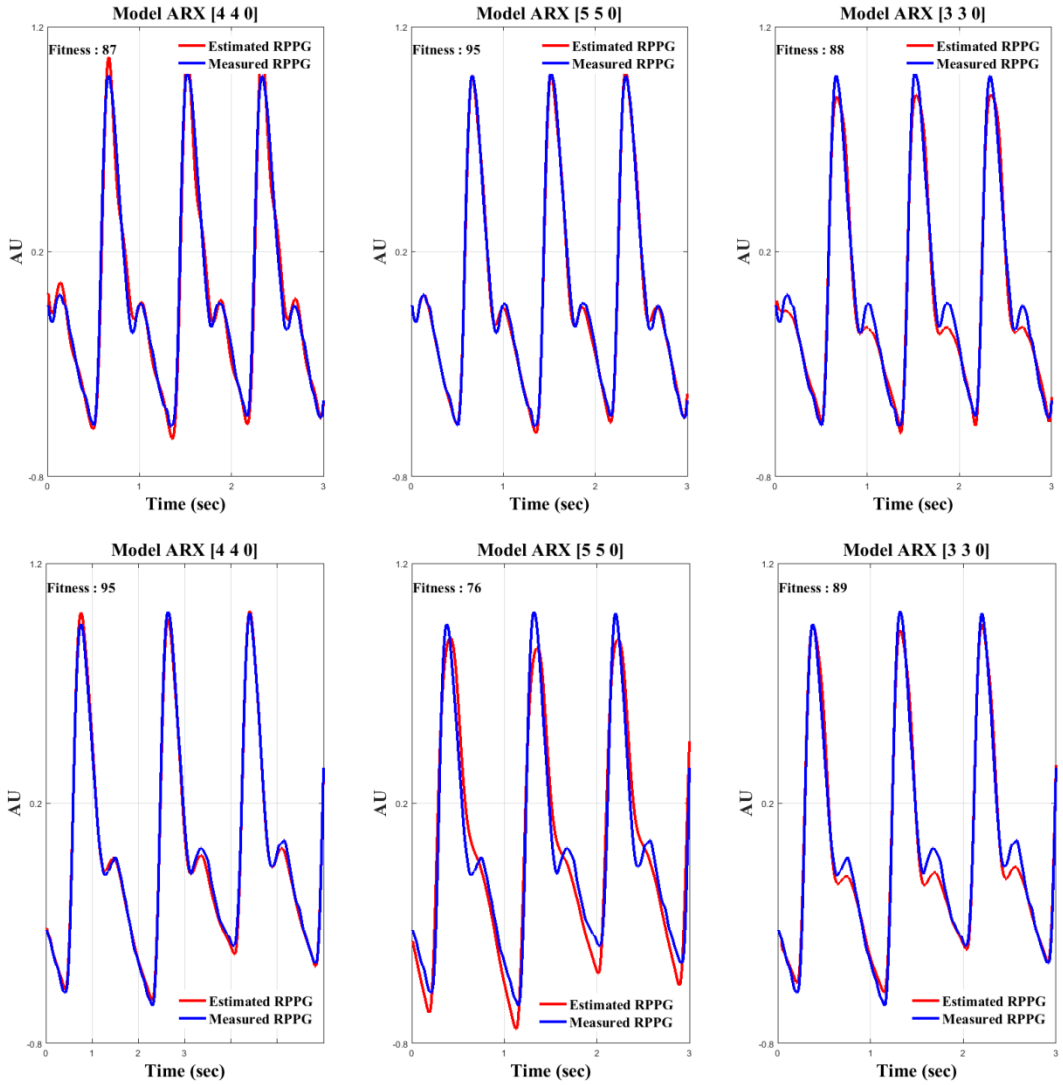


Figure 3.24 Goodness of different model orders against two typical segments

3.6.4 Interpretation of Pole-Zero

The pole-zero analysis is normally done in system modeling to ensure the stability of a proposed model. In this regard, pole(s) and zero(s) are drawn in the unit circle to find out how far they are from the circle. A proposed model can be considered as a stable model if its poles and zeros are located inside the unit circle. Figure 3.25 illustrates the pole-zero diagram of the ARX [4 4 0] model. As can be seen in this figure, all poles and zeros are located inside the unit circle confirming the stability of a proposed model.

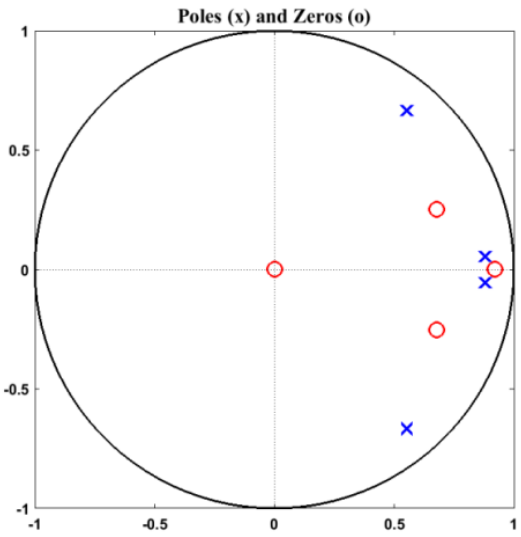


Figure 3.25 Pole-zero diagram of a typical ARX [4 4 0] model

3.6.5 Model and Segments in Baseline Mode

As quoted earlier, data was continuously recorded along three different modes; baseline, blockage and cuff release. CBP-RPPG models were initially estimated over baseline data to ensure that the model is estimated under normal condition (i.e. without stimulus). An estimated model was then used -as a criterion- to represent the endothelial performance after conducting the FMD stimulus. The whole steps of system modeling were done by system identification toolbox of the Matlab as can be seen in Appendix D. The used steps are explained in the following parts.

a) Self-Model

Referring to section 3.5.4, same numbers of CBP and RPPG segments were obtained for each particular subject. Accordingly, individual models were estimated by considering CBP segment as model input and its corresponding RPPG segment as model output. In fact, self-models refer to a model between each CBP segment and its corresponding RPPG segment. This was done by "iddata" command in Matlab as can be seen in Appendix D. The same model structure and order was used for sets of data (i.e. all subjects and in all days). The focus of models was set to be *stability*.

The goodness of an estimated model was then evaluated via the parameter of fitness which was obtained by applying an estimated model to its constructive data (estimated RPPG versus measured RPPG). Afterwards, model-derived and measured RPPG waveforms were compared to each other using the "compare" command in the Matlab as can be seen in Figure 3.26. Fitness value and coefficients of all estimated models were then saved into the excel file. As quoted earlier, the process of exporting data from Matlab to the excel file was automatically done by "xlswrite" command in the Matlab. Data of each particular subject were saved into subject's sheet. At the end of this step, several forward CBP $\rightarrow$RPPG models were obtained for each subject.

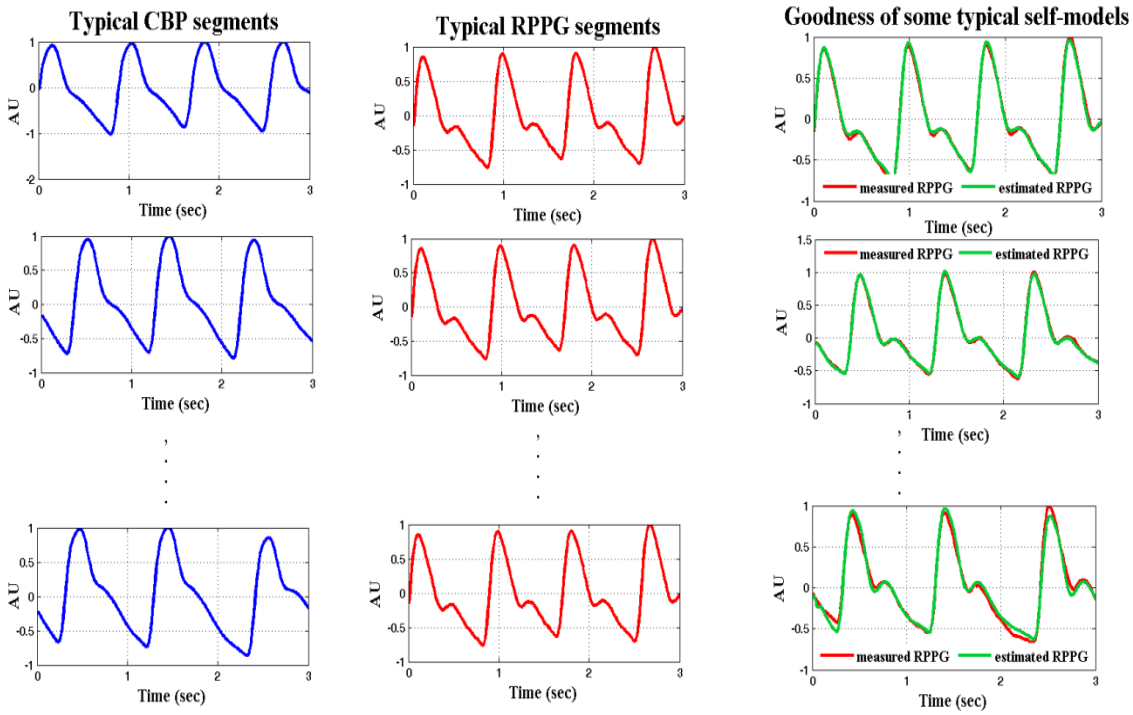


Figure 3.26 Representation of self-modeling

b) Computation of Average model

Outcome of the last section was obtaining several self-models for each set of data. Fitness values of such models were mostly found high. However, a few of self-models in each data set were found unstable mainly due to the effect of motion artifacts (e.g. moving or shaking during data collection). In this regard, the threshold value of 70% was considered in order to identify unstable models (Ljung 2010). Accordingly, those unstable models were discarded and only stable self-models with fitness value of higher than 70% were considered for further analysis.

However, it was not possible to trace the endothelial functionality via several self-models. Hence, an average model was considered to be used as a single model for assessing the endothelial performance. It was done by getting average among coefficients of stable self-models explained above (i.e. fitness $\geq$ 70%). An averaged model was exclusively computed for each particular subject. It was then applied to whole segments in baseline and results (fitness values) were saved into the excel file. As a summary, a single CBP $\rightarrow$ RPPG model was computed for each subject in order to assess the endothelial activity. Figure 3.27 illustrates the algorithmic chart of such processing. Figure 3.28 shows graphical view of the modeling.

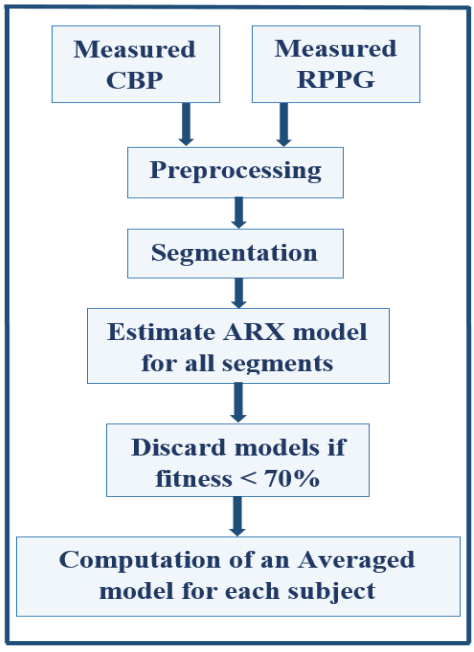


Figure 3.27 Algorithm of computational steps

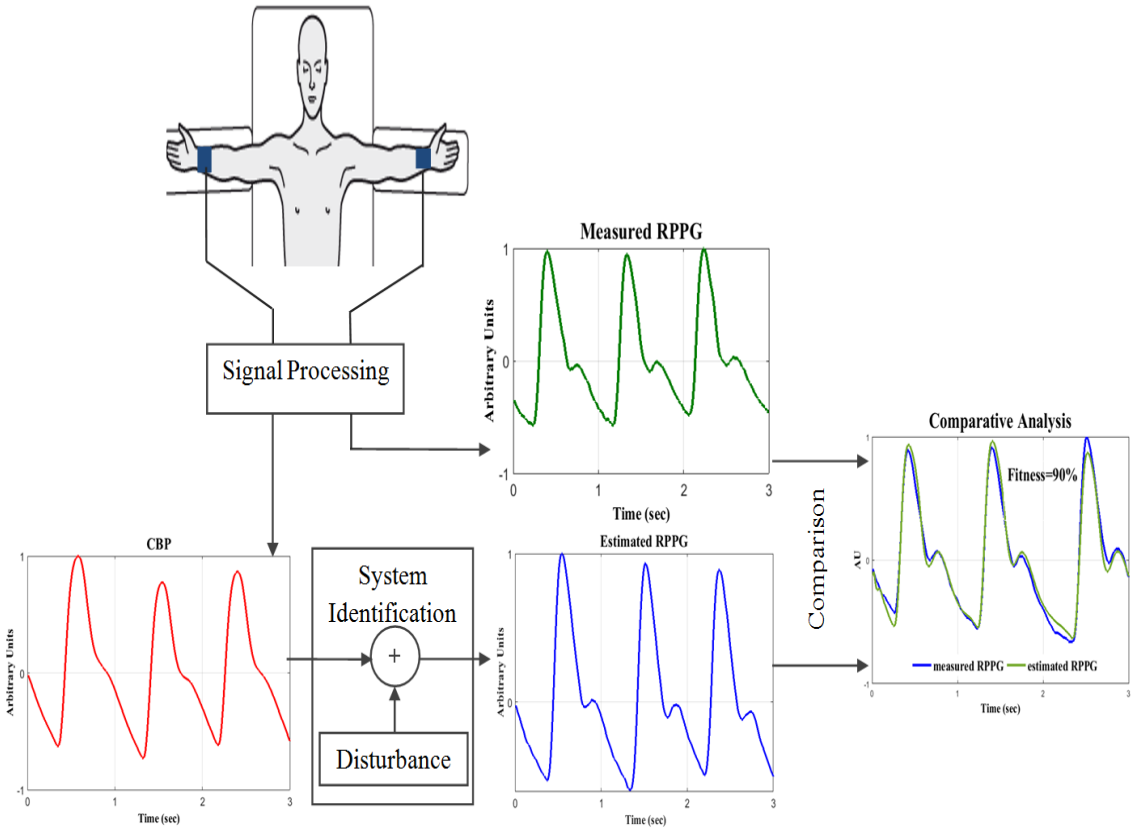


Figure 3.28 Graphical representation of modeling

Referring to research hypothesis, this model is supposed to have a stable behavior during the baseline where there is no stimulation. Goodness of a typical average model during the baseline is shown in Figure 3.29.

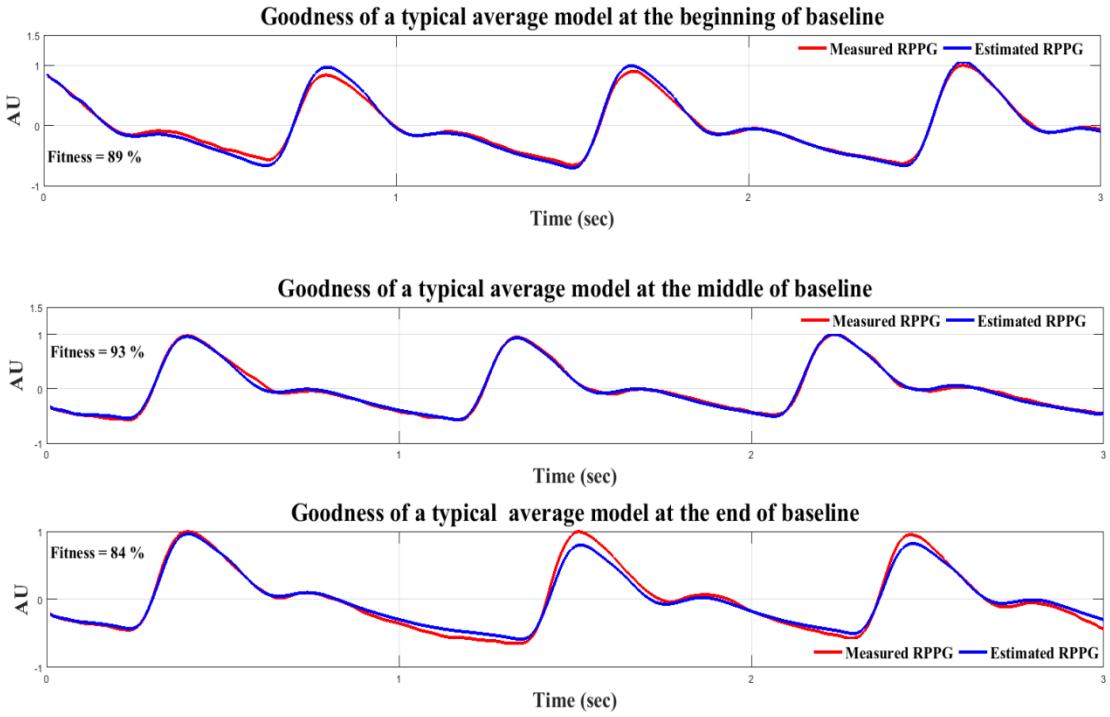


Figure 3.29 Goodness of a typical average model at the beginning (top), middle (middle) and end (button) of the baseline

3.6.6 Average Model in Release Mode

The main objective of this study is to assess the endothelial dysfunction based on the CBP-RPPG model. The procedure of preparing baseline data and obtaining an average model were explained above. In the following sections, steps of preparing release data and procedure of assessing the endothelial reaction via average models are explained.

As a first step, the last five minutes of each signal were separated and considered as release data. It was then fragmented into several segments with the same length of three seconds. Each segment was then normalized and synchronized (exactly like the processing procedure in section 3). Afterwards, a time series data were created by addressing the CBP segment as model input and its corresponding RPPG segment as model output.

Average models -which are obtained in baseline- were then used to assess the endothelial performance after releasing the pressure cuff. In this regard, average model was applied over release data (i.e. release segments) and fitness values were saved and

considered as endothelial response. These data were then plotted in order to capture the Endothelial-related changes across the brachial artery. Figure 3.30 illustrates the goodness of a typical model -of a healthy subject- before and after releasing the pressure cuff. As can be seen, for a healthy subject, fitness values are significantly dropped. In contrast, for an unhealthy subject, fitness values are not considerably changed, as can be seen in Figure 3.31.

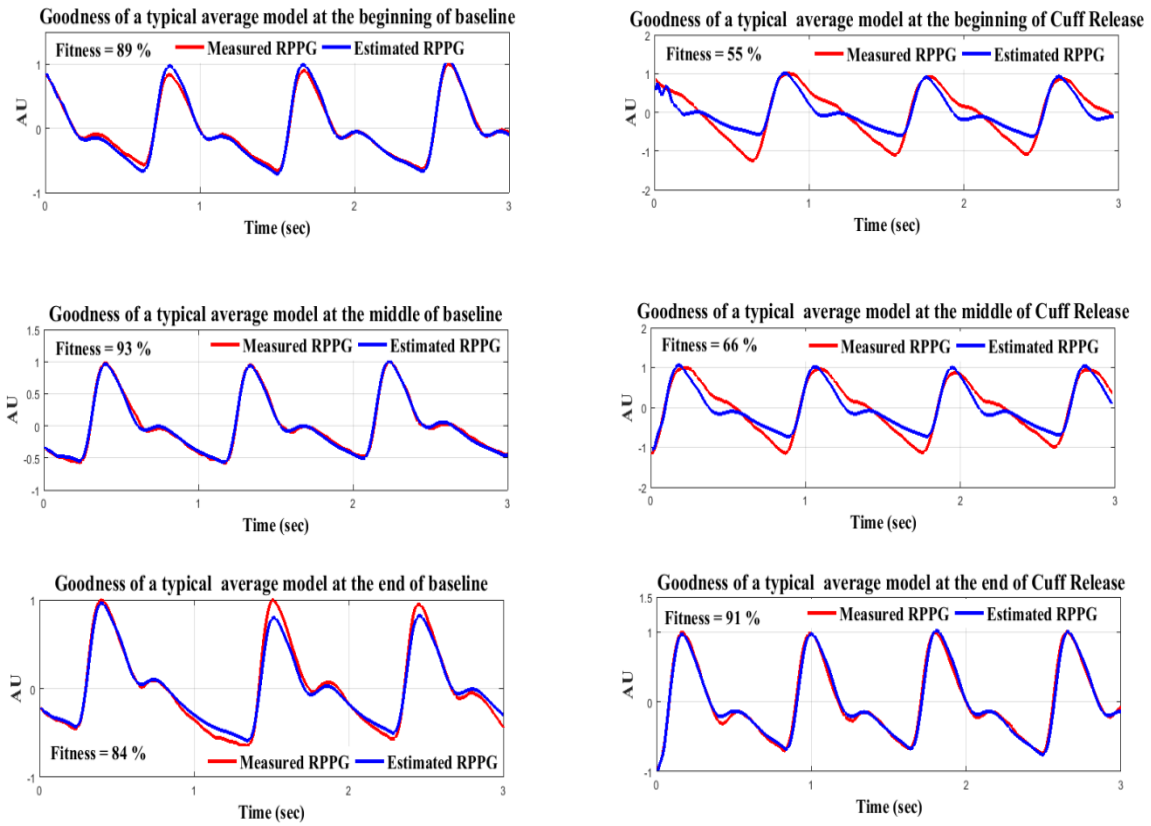


Figure 3.30 Goodness of a typical average model of a healthy subject at the beginning (top), middle (middle) and end (bottom) of baseline (left) and release (right) data

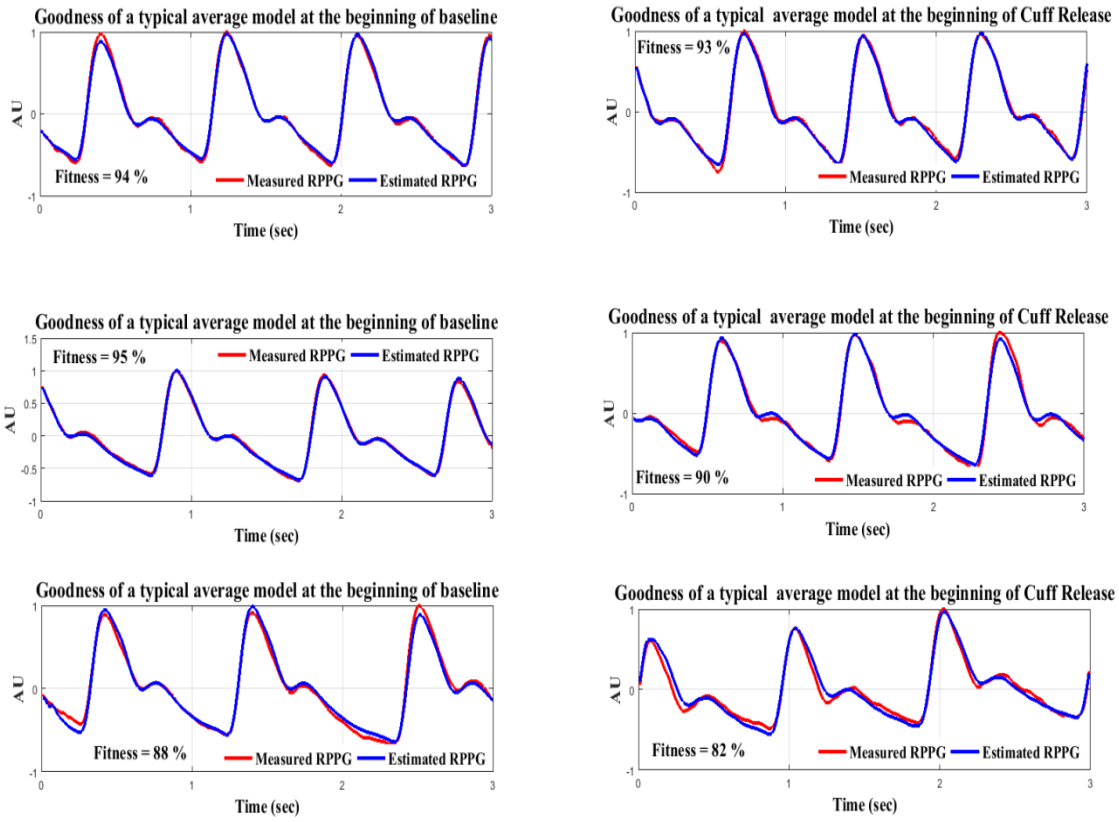


Figure 3.31 Goodness of a typical average model of an unhealthy subject at the beginning (top), middle (middle) and end (bottom) of baseline (left) and release (right) data

a) Golay Filter

Graphical representation of fitness values -obtained in release mode- showed several uneven small lines while illustrating a meaningful trend in overall view. It was observed among all fitness curves. In general, two types of changes were observed among all curves in release mode; small changes (uneven small lines) and an overall trend. According to the theory of the FMD-US test, such small changes in vessel diameter are not considered in calculation of vessel dilatation (final FMD percentage) (Corretti et al. 2002). In other words, FMD value is obtained by comparing values of vessel diameter in baseline and release conditions. Hence, the overall trend of fitness curve was considered as an indicator of the endothelial performance. In order to capture the overall trend of fitness curves, all fitness values were smoothed by the Golay filter (Schafer 2011). Using this filter, the mentioned small uneven lines were ignored while an overall trend was better appeared (Luo, Ying, He, et al. 2005). The Golay filter was applied to

whole data (baseline and release) by the "sgolayfilt" command in Matlab. The details of using this code can be seen in appendix D. Figure 3.32 shows a typical fitness curve before and after applying the Golay filter.

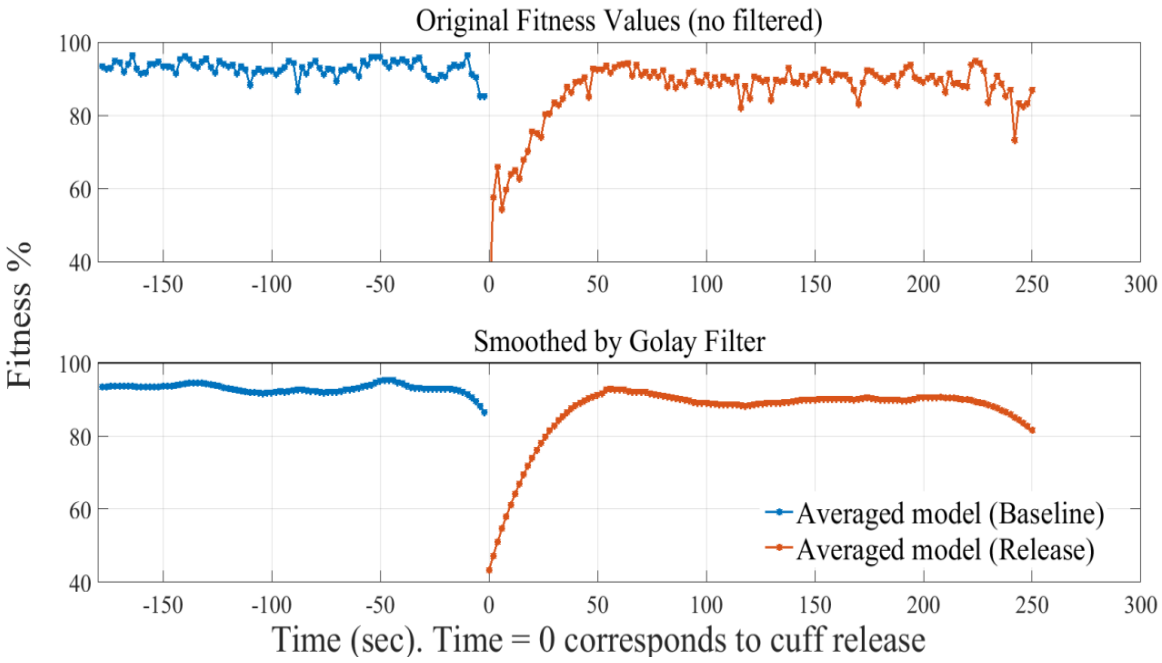


Figure 3.32 A typical fitness curve before (top panel) and after (down panel) smoothing by the Golay filter

3.6.7 Data Analysis after Cuff Release

Initial analysis showed that fitness curves are quite stable in baseline whereas there is a peak-alike trend in release data. This matter can be seen in Figure 3.33. In fact, when the pressure cuff is released, fitness values were remarkably dropped and then slowly become better (higher). Hence, release data were separated into five windows with the same length of one minute. Such strategy provided a practical way for processing data step by step in order to capture the endothelial-related vasodilatation in the brachial vessel. Figure below illustrates those windows.

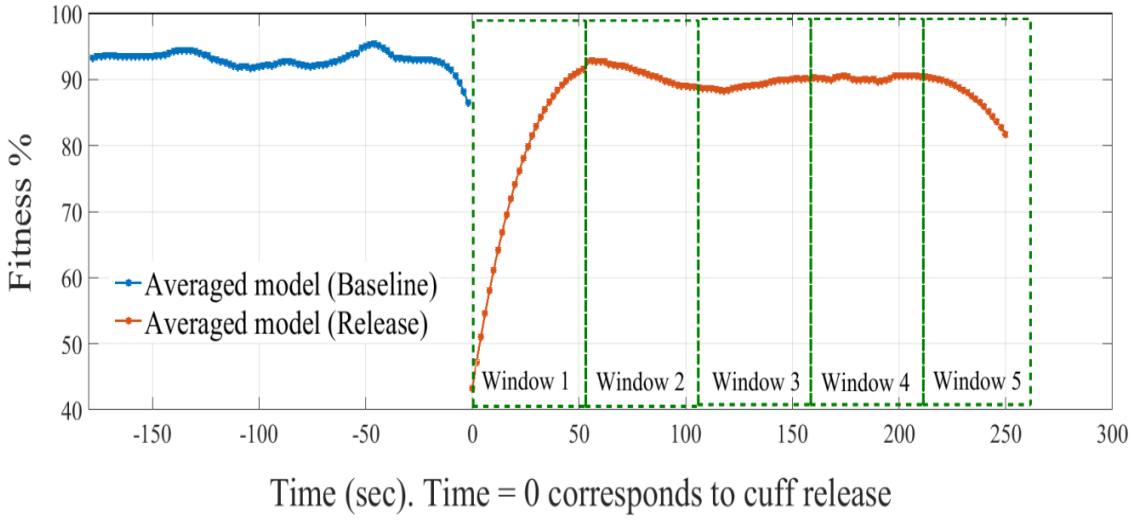


Figure 3.33 A typical fitness curve with analytical windows

3.6.8 Endothelial Dysfunction Assessment

As mentioned above, the main objective of separating fitness curves into several windows was to implement different analytical strategies to detect changes in each window individually. Initial investigation -among fitness curves of healthy subjects- showed that fitness values drops immediately after releasing the pressure cuff and then gradually tends to mean fitness value of the baseline. However, such an ascending trend was not observed among non-healthy subjects. Hence, rise time, slope, mean fitness value and height of peak were considered to be analyzed in the first window. However, only stability and mean fitness value were considered among window 2 to window 5 because a peak-alike trends was observed only in the first window. Details of computing these parameters are explained below.

Rise Time: This parameter can be considered as one of the most important features in signal processing. In fact, it indicates how fast a peak can take place. In the first window, rise time was calculated by subtracting time values of maximum and minimum points of a peak. Figure 3.34 illustrates the rise time (red line) in window 1.

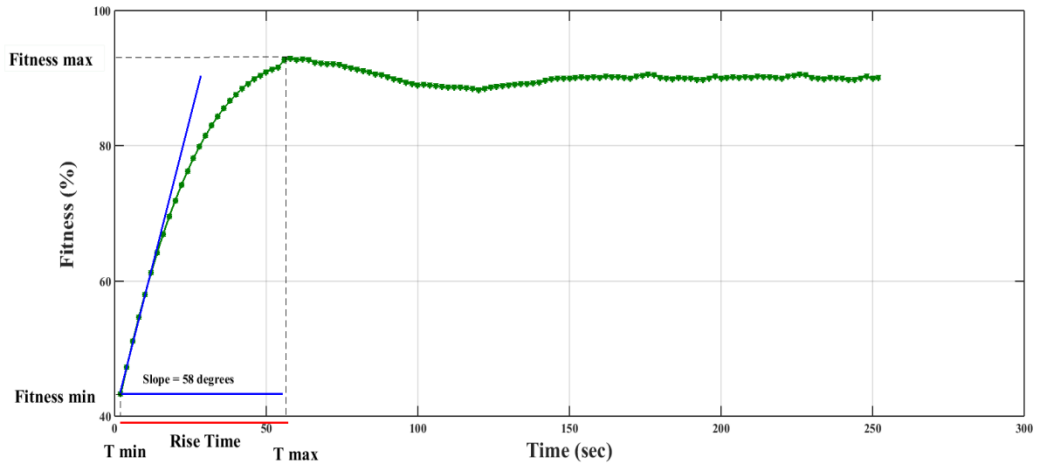


Figure 3.34 A typical fitness curve with considered parameters in window 1

Slope: The slope of a peak was calculated by dividing the subtraction of critical points (Δy) over subtraction of their corresponding time values (Δx) as can be seen in the Figure above (blue color). The inverse tangent of obtained value was then computed to represent the slope in degrees scale. This approach can be mathematically expressed via the following equation:

$$\text{Slope}_{\text{degree}} = \tan^{-1}\left(\frac{Fitness_{\max} - Fitness_{\min}}{t_{\max} - t_{\min}}\right) \tag{3.6}$$

where $Fitness_{max}$ shows a highest (maximum) fitness value of a peak, $Fitness_{min}$ shows a lowest (minimum) fitness value of a peak and t_{max} and t_{min} show time values corresponding to maximum and minimum values of a peak respectively (blue line in Figure 3.31). Such a calculation was done using "atand" command in Matlab as can be seen in Appendix D. In this research, values of slop were computed across all fitness values (i.e. every two points) in the first minutes. These values were then averaged and a single value was used as the slope of curve in the first window.

Mean fitness value: Mean fitness value was used as a potential parameter to detect changes in window 1. It was simply computed by averaging among fitness values of window 1. The "mean" command in Matlab was used for this step of analysis among all windows.

Height of peak: In addition to the parameters explained above, height of peaks was also taken into account as a potential indicator of the endothelial reaction. In fact, this parameter was considered because it was found dissimilar among healthy subjects. The height of peak was calculated by subtracting maximum fitness value from minimum fitness value as can be seen in Figure 3.35.

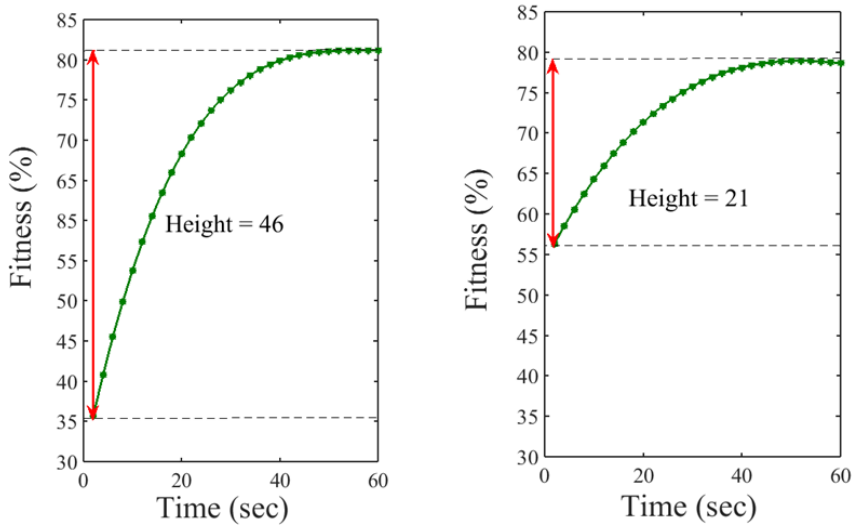


Figure 3.35 Representation of different values of height of peak

Stability: Irrespective of the first window (ascending trend), the stability was found a potential parameter to ensure the goodness of fitness curve in other windows. According to the hypothesis of this research explained in chapter II, vessel diameter should be increased after releasing the pressure cuff -endothelial reaction- and then slowly backed to its normal condition (vessel size in baseline). In this regard, it is very important to follow the trend of fitness curve to ensure it tends to mean fitness value. However, such trend was not linear -particularly after window 3- meaning that it varies toward a mean fitness value. In order to find a valid range of such variation, degree of variation in baseline was considered. It relies on a fact that in baseline, no stimulus is applied and thus fitness values have a convinced stability despite having high values of fitness. For each particular subject, range of variation in own baseline was computed by subtracting highest fitness value from lowest fitness value. Result was considered as a criterion of stability among windows 2 to 5. A typical range of stability in baseline is graphically shown in Figure 3.36.

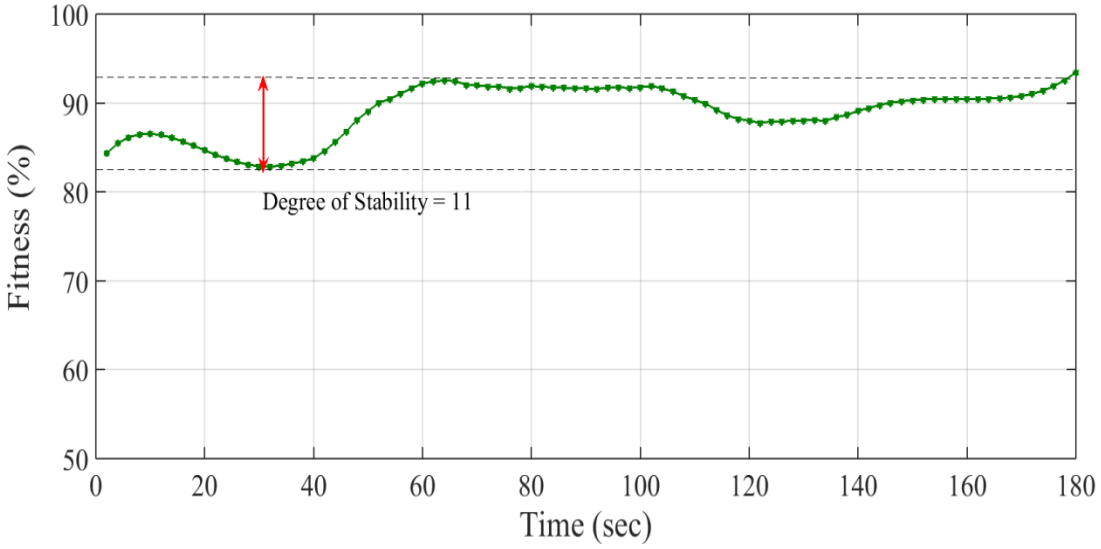


Figure 3.36 Variations of a typical fitness curve in baseline

3.7 STATISTICAL ANALYSIS

Nowadays, statistics is widely used in different parts of science including clinical researches. In fact, statistical analysis provides a mathematical interpretation in which research outcome can be better presented. Statistical analysis can be considered as an inseparable part of clinical researches. However, this field is considerably progressed to that extent that the biostatistics itself is now established as a special category of the statistics. It contains many definitions, parameters, phrases and formulas. Obviously, only some of them are used in clinical researches based on research objectives. In this research, the sensitivity, specificity and receiver operating characteristics (ROC) are used to interpret results of the proposed approach in conjunction with results of the FMD-US technique (as a gold standard). Definition of basic statistical parameters can be seen in Appendix F. Other phrases are described here.

Standard deviation (SD): Standard deviation can be considered as a complementary parameter of the variance to show how spread out data is. It is the square root of the variance which is computed by the following equation:

$$SD = \sqrt{\frac{1}{n}\sum_{i=1}^{n} (x_i - \bar{x})^2} \tag{3.7}$$

where n is a number of data, x_i is a set of data and $\bar{x}$ is the mean.

Coefficient of variation (CV): The coefficient of variation is a statistical parameter used for indicating dispersion of a distribution. This parameter is obtained by the following equation:

$$CV = \frac{SD}{\bar{x}} \tag{3.8}$$

where SD and $\bar{x}$ representing the standard deviation and variance respectively.

Null hypothesis (H_0): Null hypothesis refers to a hypothesis claiming if a proposed technique is applied among different groups, same outcome is achieved. Although probability of happening such hypothesis is not high in clinical researches but it should be either disapproved or nullified by evidence obtained during a research. Rejection of H_0 shows that the null hypothesis is not plausible so that research hypotheses can be accepted. In contrast, if evidence(s) is failed to reject the H_0 then it can be concluded that the null hypothesis is plausible and thus research hypotheses should be rejected. The null hypothesis is this research is that there is no significant difference between endothelial reaction of healthy and unhealthy subjects based on the proposed PPG technique.

Statistical Error: Statistical error is used to show a difference between retained and true values. The statistical error is usually expressed in terms of type including type I error and type II error. In general, type I error -which is also known as alpha (α)- refers to a wrong rejection of a true null hypothesis (false positive). Besides, type II error -which is also known as beta (β)- indicates the failure to reject a false null hypothesis (false negative). Table 3.1 shows the relationship between truth/falseness of the null hypothesis.

Table 3.1 Statistical errors

	H_0 true	H_0 false
Accept H_0	Correct!	Type II error
Reject H_0	Type I error	Correct!

3.7.1 Statistical Tests

In clinical researches, it is always important to find out the level of significance between two (or even more) sets of data. The analysis of variance (ANOVA) can be mentioned as one of the most popular statistical approaches to conduct such analysis. The parameter of p-value is used in this analysis to represent the statistical significance between two sets of data. Using the ANOVA test, a p-value is compared with a given type I error (α). In this regard, if a p-value obtained less than a type I error, then it can be concluded that there is a significant difference between two sets of data. However, an obtained p-value of greater than type I error can confirm insignificant difference between data.

In this study, the ANOVA test is used to analysis the effect of proposed technique among healthy and non-healthy groups. In fact, it provides a practical way for ensuring the performance of the PPG technique -to assess the endothelial dysfunction- over different subjects so that the suitability of the proposed technique among healthy and non-healthy groups can be analyzed. To this end, the level of confidence was set to 95% for confirming the quality of a proposed technique. Thus, a value of 0.05 was considered as a threshold value of testing the means between healthy and non-healthy groups. Therefore, a value of difference of less than 0.05 ($p< 0.05$) was considered as a significant difference.

Sensitivity (Sn): One of the key factors in clinical research is the ability of a proposed method to detect abnormality which is already identified and confirmed by an alternative method (i.e. Gold standard). Such analysis is expressed via the parameter of sensitivity referring to the proportion of positives that are correctly detected by a gold standard technique (Indrayan 2012). In other words, the sensitivity of a test refers to the proportion of people who are considered as patient by an established method. This is called positive detection in biostatistics. Conversely, the percentage of incorrect detection by a proposed technique -compare to gold standard- is known as false positive detection (e.g. healthy people who are identified as a patient incorrectly). The sensitivity is obtained via the following equation:

$$\text{Sensitivity}\ (\%) = 100 \times \frac{\text{True Positives}}{(\text{True Positives}) + (\text{False Negatives})} \tag{3.9}$$

For example, if method B is considered as a proposal technique to detect a specific disease while method A is known as an establish method for identifying that disease, then the percentage of true positive detections -which correctly identify those people with that disease- is known as sensitivity.

Specificity (Sp): In contrast to the sensitivity, specificity focuses on true negative detections. In other words, the specificity relates to the percentage of healthy people who are confirmed to have no disease (instead of capturing those with disease). Such detection is known as the true negative while incorrect detection is considered as false negative. In the above example, the percentage of correct detections to identify healthy people -rather than patients- is considered as the specificity. The specificity is obtained via the following equation:

$$\text{Specificity}\ (\%) = 100 \times \frac{\text{True Negatives}}{(\text{True Negatives}) + (\text{False Positives})} \tag{3.10}$$

Accuracy**:** Accuracy can be known as one of the most popular parameters in biostatistics for indicating the degree of conformity between an established approach and a proposed technique. It can be obtained via the following equation:

$$\text{Accuracy}\ (\%) = 100 \times \frac{\text{TN} + \text{TP}}{(\text{Total sample population})} \tag{3.11}$$

where *TN* and *TP* show true negative and rue positive respectively.

Contingency Table: This table is widely used in clinical research to give an overall view about accuracy of a proposed technique. It is consisted of totally four cells as shown in Figure 3.37.

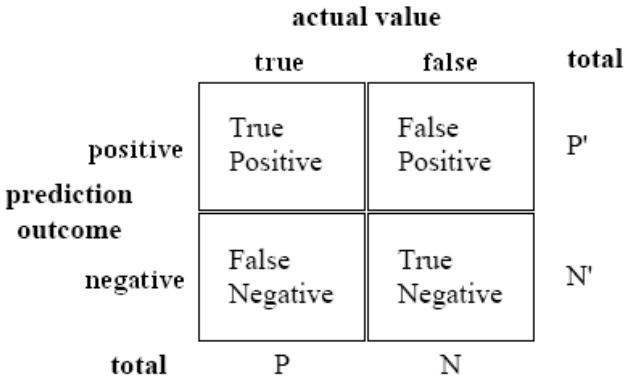


Figure 3.37 The contingency table

3.7.2 Receiver operating characteristic (ROC)

The receiver operating characteristics (ROC) is a graphical curve -known as the ROC curve- which is used to evaluate the accuracy of a diagnostic test (Indrayan 2012). The ROC curve is plotted in terms of the true positive (sensitivity) against the false positive (specificity). Thus, each point on the ROC curve shows a pair of sensitivity-specificity corresponding to a particular value of threshold. Although the range of both sensitivity and specificity are normally considered between 0 to 100 but in the ROC curve, such range is mostly considered between zeros to one. In this case, the horizontal axis is marked by the "1-specificity" as can be seen in Figure 3.38. The ROC curve is normally explained in term of the area under the curve (AUC). In fact, the AUC is a measure of how well a method can distinguish healthy and non-healthy subjects. The highest value of the AUC is "1" which is usually expressed as a percentage (0-100%). Ideally, the convexity of the ROC curve should tend to the value one. In other words, proximity to value 1 represents the degree of the accuracy of a used method.

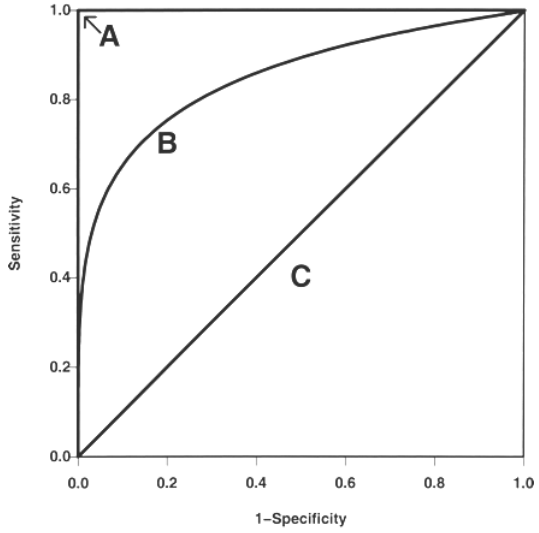


Figure 3.38 Representation of three hypothetical ROC curves showing the diagnostic accuracy of the gold standard (lines A; AUC=1), a typical ROC curve (curve B; AUC=0.85) and a diagonal line corresponding to random chance (line C; AUC=0.5).

Source: Zou et al. 2007

In this study, the FMD-US technique was used -as an established technique- to assess the endothelial performance. Hence, results of the proposed PPG technique were compared with values of FMD obtained from the FMD-US technique. The ROC curve was used to show such comparison. To this end, heights of peaks were calculated -as explained above- and put into a matrix. In addition, values of FMDs were similarly put in the second column of that matrix. In order to be able to use the binary classifier system (ROC), the value of 10% was used as the threshold value (Corretti et al. 2002). It means all FMD values which are greater than the threshold are considered as one whereas those values which are less than the threshold value are considered as zero. Then, both FMD and peak values were graphically classified via the ROC curve. It was done by the "perfcurve" command in Matlab as can be seen in Appendix D. Result of the classification was then plotted like the ROC curve in Figure 4.25.

3.8 CHAPTER SUMMARY

This chapter has begun by giving an overall view about whole study. In this regard, the role of pilot study and its effect on improving main research were explained. Details of

research proposal were then reviewed. The FMD-US technique was mentioned as an established approach to assess the endothelial performance non-invasively. The procedure of data collection -from protocol to system setup and configuration- was then explained. Besides, procedure of taking blood -for blood test- to access individual's clinical parameters at the time of data collection was discussed. Afterwards, whole analytical steps were explained in details. After preparing signals via standard signal processing techniques, the parametric model ARX [4 4 0] was used as a potential model structure for estimating a forward model between CBP and RPPG waveforms. It was then mentioned that this model was exclusively estimated for each particular subject in order to be used as a criterion to assess the endothelial performance. It was then explained that goodness of a CBP $\rightarrow$ RPPG model before (baseline) and after releasing the pressure cuff (cuff release) was followed via the fitness parameter. It was mentioned that fitness curve has a stable behavior during the baseline time whereas there is a peak-alike curve right after releasing the pressure cuff. The peak was then considered as a potential mark of the endothelial reaction. Thus, it was deeply analyzed. Afterwards, the role of statistics in clinical research was reviewed. The ROC curve was then explained as an established tool for indicating the suitability in diagnostic techniques (binary classification).

CHAPTER IV

RESULTS AND DISCUSSION

4.1 ULTRASOUND MEASUREMENTS

As mentioned earlier, the FMD-US technique was used in this research -as an established technique- to assess the endothelial performance non-invasively. Using this technique, the endothelia-related vasodilatation can be observed during the FMD test. At present, borders of brachial artery (vessel walls) can be viewed via ultrasound imaging. Irrespective of its associated costs and operator dependency, this technique is the most attended technique to assess the ED non-invasively. However, the issue of measuring vessel diameter is different from obtaining vessel borders. In other words, an additional program is needed for measuring the distance between two sides of the brachial vessel. Indeed, such measurement plays an important role on accuracy of the FMD-US technique (Bots et al. 2005; Peretz et al. 2007; Thijssen et al. 2011). As quoted in the last chapter, the cardiovascular suite program was used for measuring the brachial diameter during in this research. The measurement was done off-line with exactly the same procedure among all subjects. Results of such measurement are discussed in the following sections.

4.1.1 Arterial Diameter in Baseline

The procedure of measuring vessel diameter was explained in details in section 3.6. Following the time chart of data collection (Figure 3.2) ultrasound images were continuously obtained for three minutes during the baseline. Using the cardiovascular suite program, brachial diameters were measured pixel by pixel. However, an averaged diameter was finally reported by the program as a brachial diameter in baseline. These values were obtained and imported in the excel file. Figure 4.1 shows values of brachial diameter in the baseline.

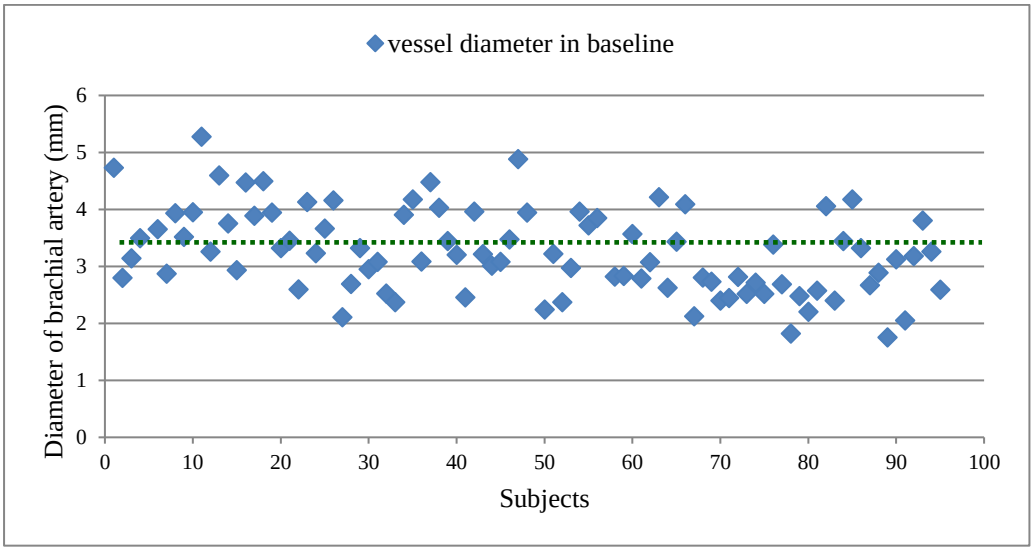


Figure 4.1 Brachial diameters in baseline

Obviously, diameter of brachial artery is not same among different subjects even in a specific group. As can be seen in the figure above values of brachial diameter are mostly between 2-4 mm. Interestingly, this range is in a good match with brachial diameters -of adult subjects- obtained in other studies (Atkinson & Batterham 2013; Thijssen et al. 2008).The mean value of brachial diameters in this research is about 3.2 *mm* as illustrated in the figure above (the green dotted line).

4.1.2 Arterial Diameter after Cuff Release

The same procedure and calibrating factor was used for measuring the brachial diameter during reactive hyperemia (after cuff release). A normal endothelium is supposed to react to the FMD stimulus by releasing the NO which leads to increasing the vessel diameter. This phenomenon is gradually took place until reaching to a maximal diameter (peak point). Afterwards, the vessel diameter is slowly decreased until back to the normal situation (baseline). Such trend can be viewed in the cardiovascular suite via a graph as shown in Figure 4.2. As can be seen in this figure, there are two horizontal lines in each graph. The lower line shows the mean value of brachial diameter in baseline and the higher line shows the mean value of brachial diameter after releasing the pressure cuff. The top picture shows a normal endothelial reaction (significant

changes in diameter) whereas the button picture shows insignificant changes in the brachial artery (an impaired endothelium).

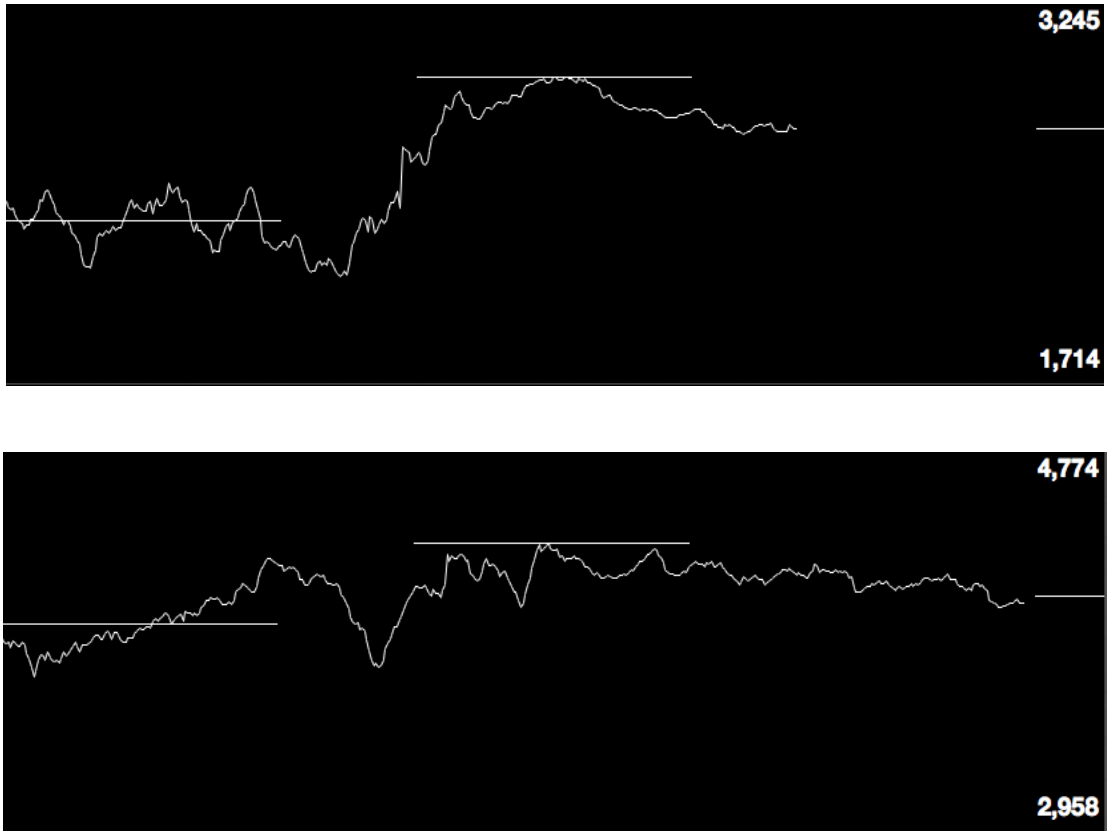


Figure 4.2 Variations of brachial diameter in healthy (top) and non-healthy (down) subject

However, there is no option to export constructive values of curves. In this regard, assessment to a specific point -in the curve- is only possible by putting a mouse over that point while the shift key is pressed. Using this program, final degree of vessel dilatation (FMD%) is automatically computed by dividing maximum value of vessel diameter over the minimum value in the baseline. All peak values were manually obtained and exported to the excel file. Figure 4.3 shows peak values of brachial diameter during the state of reactive hyperemia (after cuff release).

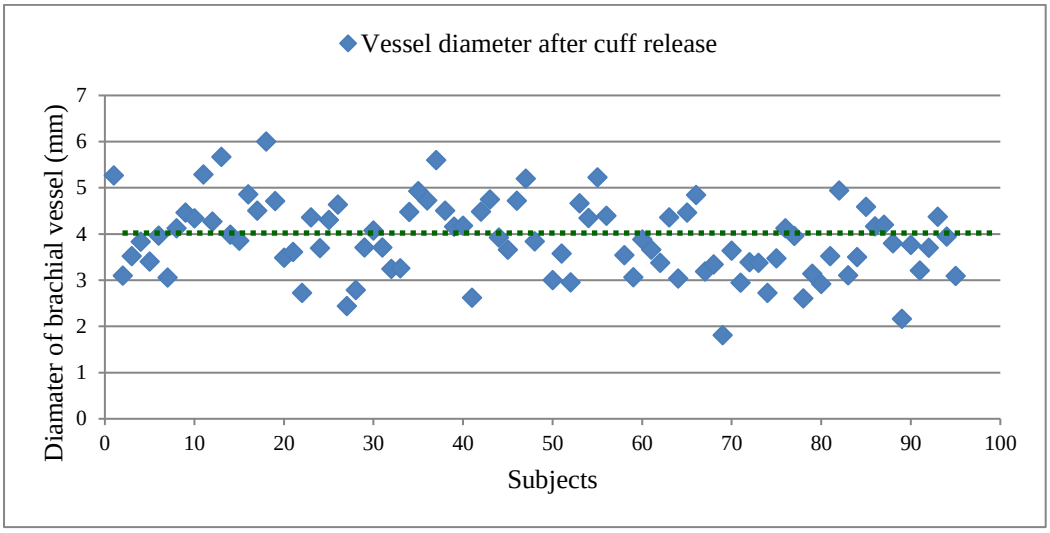


Figure 4.3 Representation of vessel diameter in brachial artery after cuff release

4.1.3 Computation of the FMD Index

Finding the variation rate in brachial diameter -during the FMD test- can be truly considered as the most important part of the FMD-US technique. This rate is expressed as the FMD% which is obtained via the following equation (Corretti et al. 2002):

$$FMD\% = \frac{\mathrm{D_{peak}} - \mathrm{D_{Baseline}}}{\mathrm{D_{Baseline}}} \tag{4.1}$$

where D_{peak} and $D_{Baseline}$ indicate mean value of diameter in release and baseline modes respectively. The same equation is used in the cardiovascular suite program so that a value of FMD% is automatically computed after conducting the measurement.

Since this research is about endothelial assessment, the functionality of the endothelial layer is considered for classifying subjects into healthy and non-healthy groups. In this regard, the FMD-US technique was used to assess the endothelial performance. Referring to the FMD-US technique, FMD values of equal or greater than 10% (FMD≥10%) are considered as normal (healthy endothelium) whereas values of less than such threshold (FMD<10%) are considered as abnormal (impaired endothelium) (Corretti et al. 2002). Thus, those with FMDs less than 10% were considered as non-healthy subjects whereas others (FMD≥10%) were considered as

healthy subjects. Figure 4.4 shows values of FMD% among all subjects. As can be seen, the green dotted line illustrates the threshold value of the FMD (FMD=10%) so that upper points indicate healthy subjects whereas lower points show non-healthy subjects. Thus, sixty three subjects were classified into healthy group whereas another thirty were put into non-healthy group.

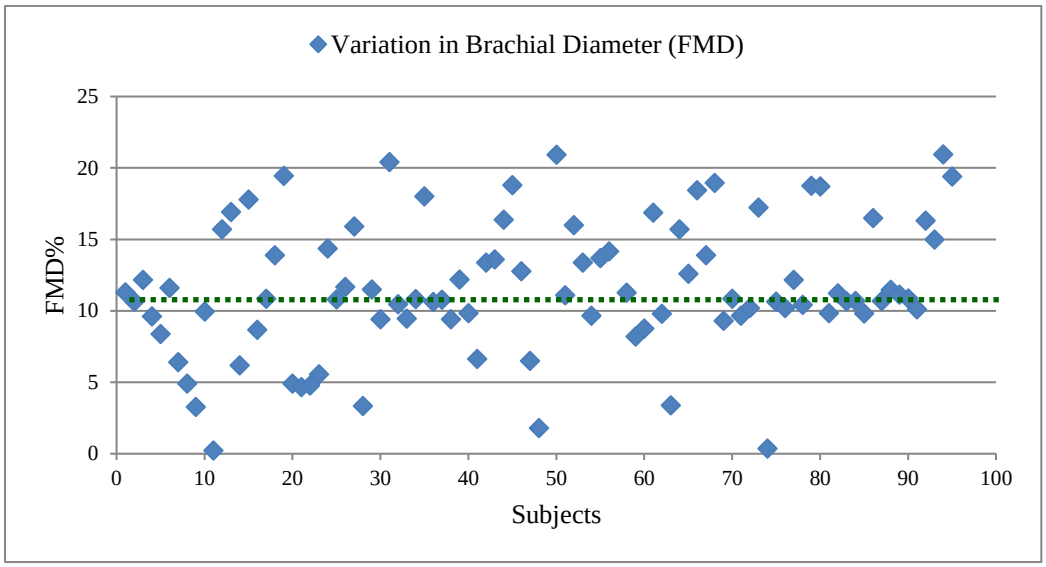


Figure 4.4 Categorization of subjects based on FMD values

4.2 DATA ANALYSIS IN BASELINE VIA CBP $\rightarrow$RPPG MODEL

In this study, CBP $\rightarrow$RPPG model is used as a criterion to represent the endothelial activity before (baseline) and after releasing the pressure cuff (cuff release). According to the research hypothesis, the goodness of such a model is supposed to be affected by the FMD stimulus. In this regard, the endothelial performance was evaluated during the baseline and after releasing the pressure cuff in order to trace the endothelial-related changes in brachial artery. Steps of model estimation were explained in details in the last chapter. Results are discussed in the following parts.

4.2.1 Fitness of Self-Model

As mentioned in section 3.5.5, several CBP $\rightarrow$RPPG models were obtained in the baseline. Models were then applied to its constructive data to ensure the suitability of

that model. Fitness values were then obtained and averaged resulting in a single fitness value in baseline for each subject. Figure 4.5 shows such values.

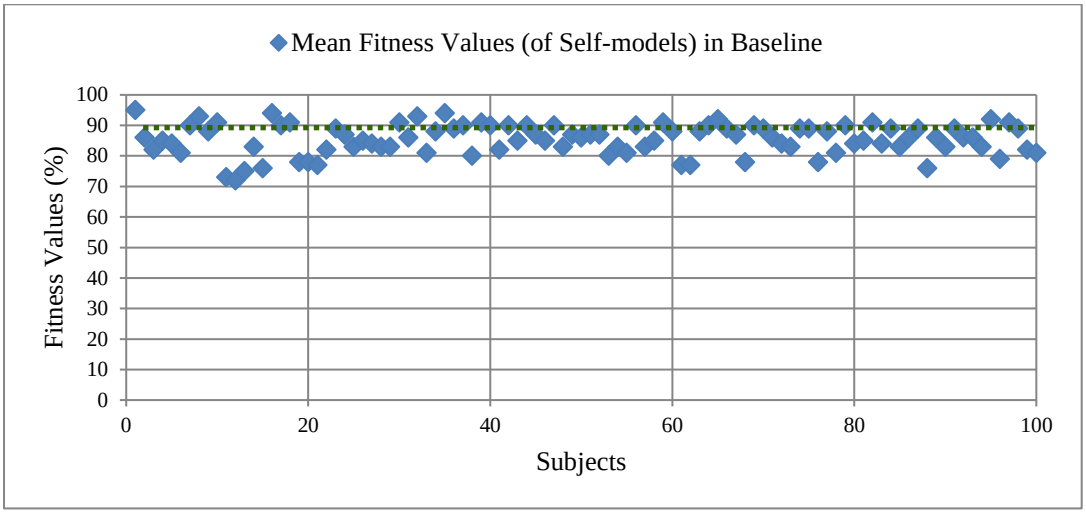


Figure 4.5 Mean fitness values of self-models in Baseline with mean of 86% (green dotted line)

Referring to the Figure above, highest and lowest fitness values were obtained 95% and 72% respectively. According to the theory of system identification, a fitness value of less than 70% shows an unsuitable model (Ljung 1999). Hence, the lowest fitness value of 72% -in this research- confirms the goodness of the ARX [4 4 0] model structure for this analysis. Mean fitness value of self-models in baseline was obtained around 86 as illustrated in the figure above. Moreover, no meaningful relationship was observed among fitness values. It means, the proposed method has the same effect on all subjects regardless of age, gender, nationality and etc.

4.2.2 Fitness of Average Model

An average model was computed by getting average among coefficients of all self-models as explained in section 3.5.5. Thus, a single average model was obtained for each particular subject. This model was then applied to all segments in Baseline. Figure 4.6 shows mean fitness values of all average models.

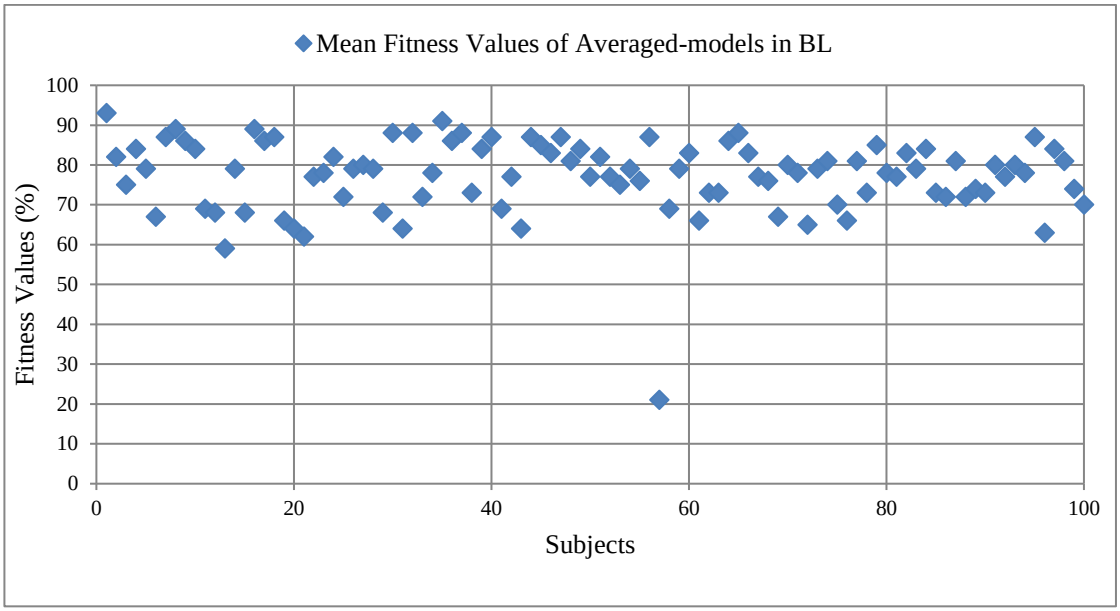


Figure 4.6 Mean fitness values of Average models in Baseline

As can be seen in the Figure above, most of mean fitness values were found greater than 70% with highest and lowest fitness values of 87% and 21% respectively. However, totally nineteen points (fitness values) were observed less than 70% including seventeen points between 60%-70% and only two points less than 60%. The effect of motion artifact can be mentioned as the main cause of obtaining such low values of fitness. Despite this, it should be noted that a single model structure (ARX [4 4 0]) was used among all data. It means that if the order of a model was exclusively estimated for each participant then a higher fitness values could be obtained. The idea of using different model order for each set of data (exclusive model estimation) is obviously not practical because the main objective of this method is to assess the endothelial performance rather than get high fitness values. Besides, a single criterion must be used among all subjects to examine the endothelial reaction. Therefore, the ARX [4 4 0] model structure was used and data which produced low fitness values were reconsidered in terms of the stability (Ljung 1999). Figure 4.7 shows three fitness curves with good (a), medium (b) and poor (c) fitness values as discussed above. Results confirmed that data with mean fitness value of 59% (Subject 14) can be still used as it has a good stability (Figure 4.7.b). In contrast, data which produced the lowest fitness value of 21% (subject 58) was found unstable and thus excluded from any further analysis (Figure 4.7.c).

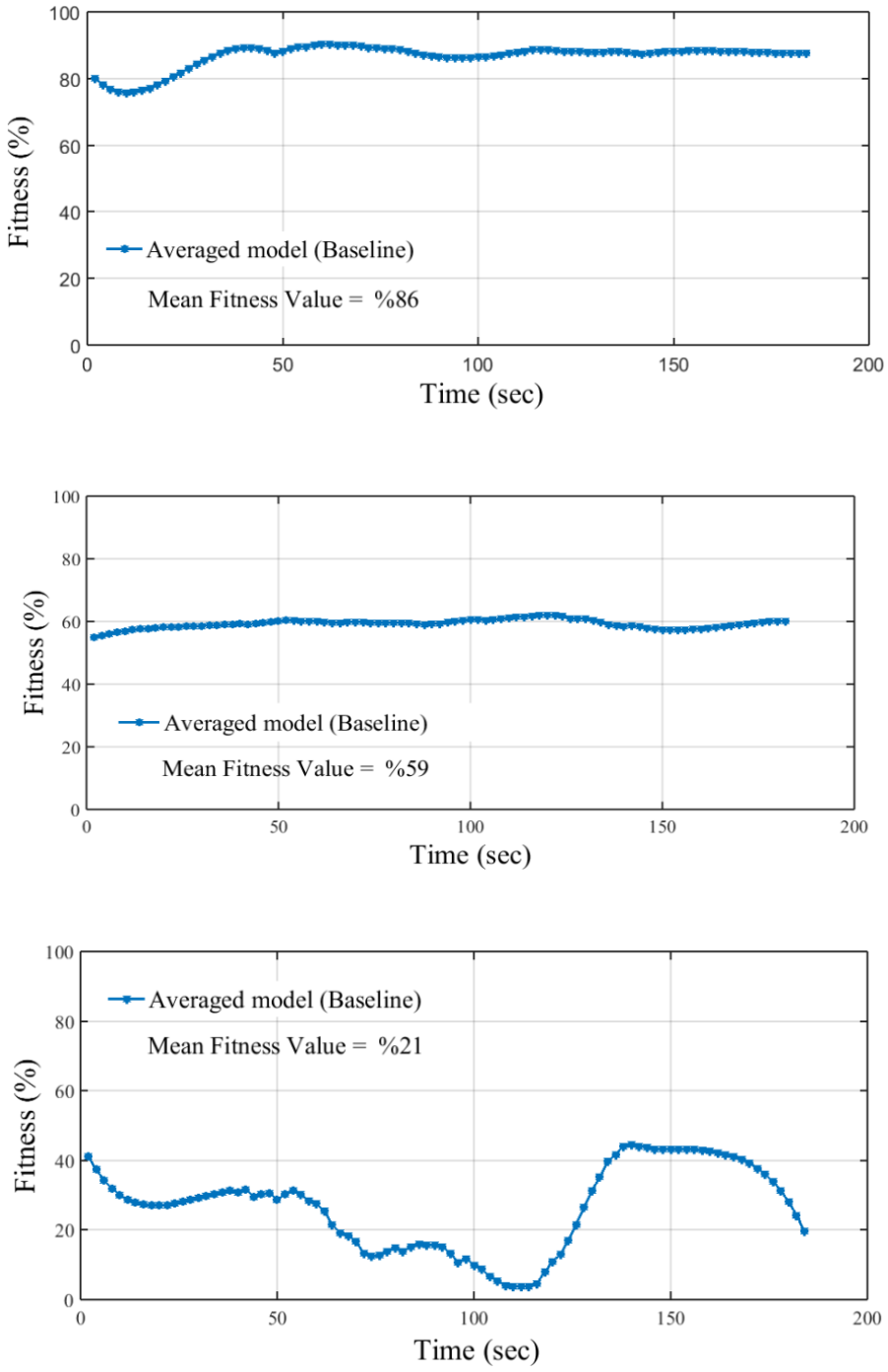
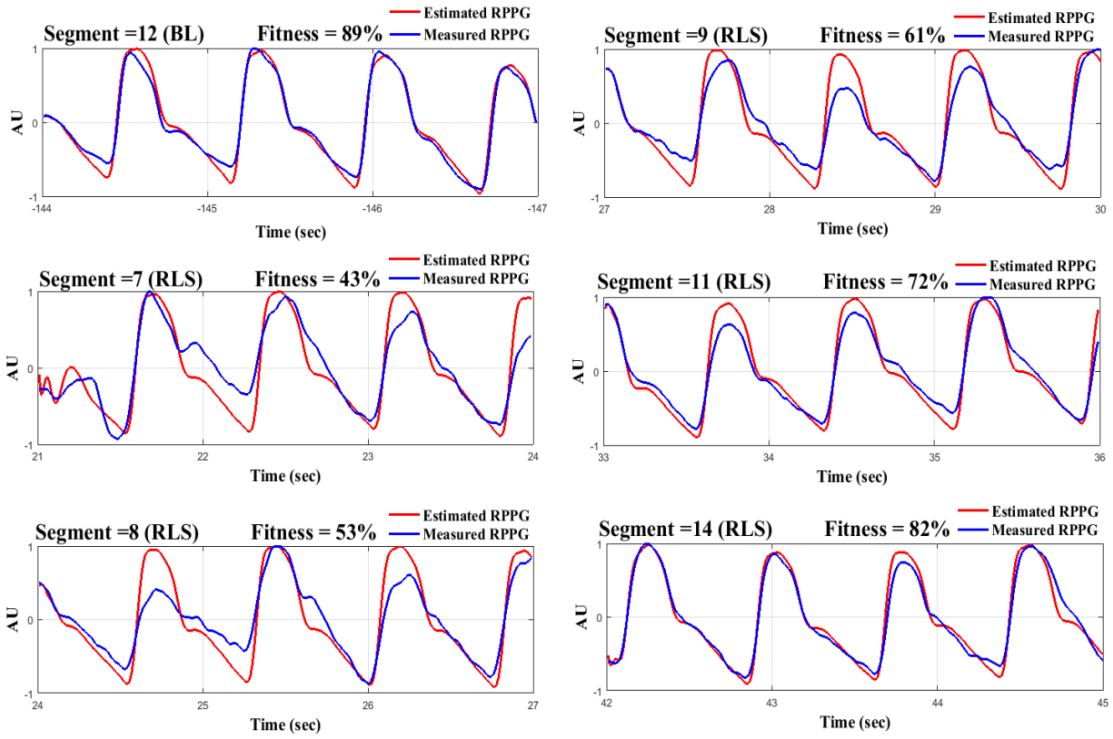


Figure 4.7 Three typical fitness curves with high (top), medium (middle) and poor (down) fitness values

4.3 DATA ANALYSIS AFTER CUFF RELEASE VIA CBP →PPG MODEL

The main objective of this research is to assess the endothelial performance via PPG model. To this end, an average model of a subject was exclusively used to reflect the

endothelial activity of that particular subject after conducting the FMD stimulus (cuff release). It provides a practical way to evaluate the endothelial performance based on its normal activity during the normal condition (baseline). Hence, the trend of fitness curves (fitness values) after releasing the pressure cuff was considered -as the criterion- to assess the endothelial activity. Figure 4.8 illustrates variations of fitness values in baseline and after releasing the pressure cuff. As can be seen in this Figure, average model has a stable behavior in the baseline (figure top left, fitness=89%). However, fitness values are considerably dropped after releasing the pressure cuff. Accordingly, fitness value drops to value 43% and gradually increase to value 82% which is very near to the fitness value which is obtained in baseline. According to the importance of such trend, fitness curves were smoothed in order to provide a better way for tracing the endothelial reaction (as explained in section 3.5.6). Results are discussed in the following parts.



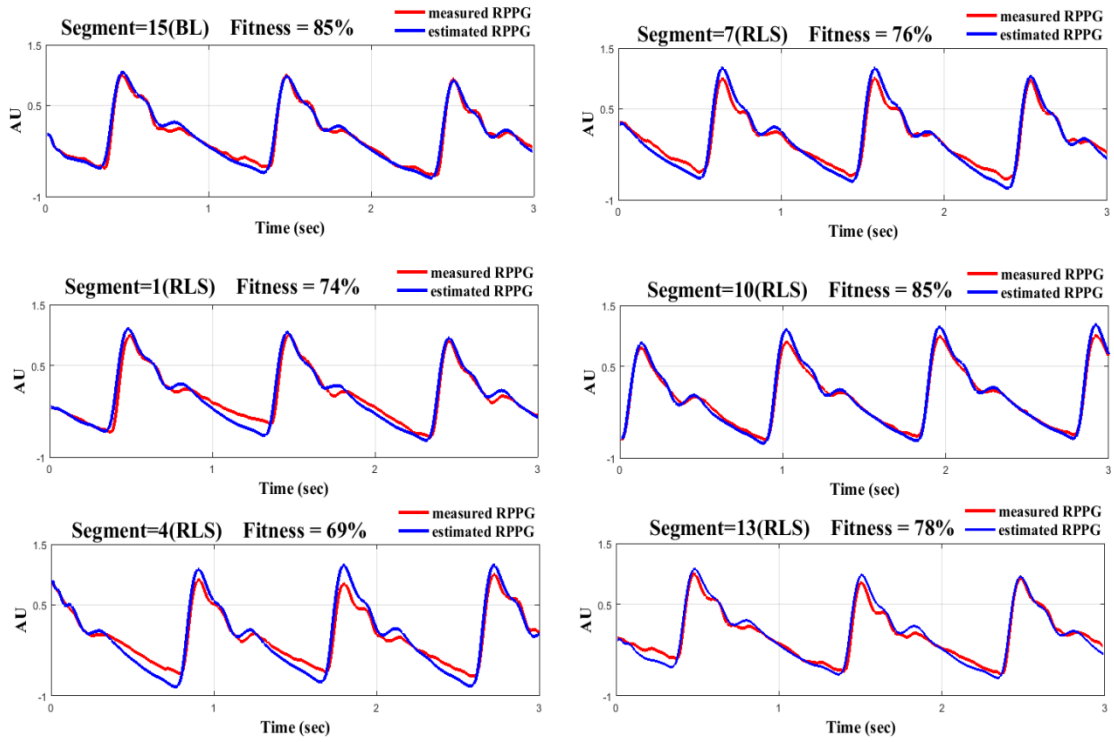


Figure 4.8 Representation of fitness changes of a typical healthy (top) and non-healthy (down) subject during the FMD stimulus

4.3.1 Results of Smoothing Fitness Curves

All fitness curves were smoothed via the Savitzky–Golay filter. Although primary (visual) comparison -between raw and filtered fitness curves- showed insignificant changes but it was investigated among all data via numerical analysis. In this regard, a filtered fitness curve was subtracted from its primary curve so that several values were obtained. By obtaining the mean value, a distance between each single value and the mean value was measured (variance). All variance values were then averaged to represent a single value for each set of data. In this regard, a high value of variance shows that there is a significant change between primary and filtered curves. In contrast, a low value of variance confirms an insignificant change in a curve before and after smoothing. Results are shown in Figure 4.9.

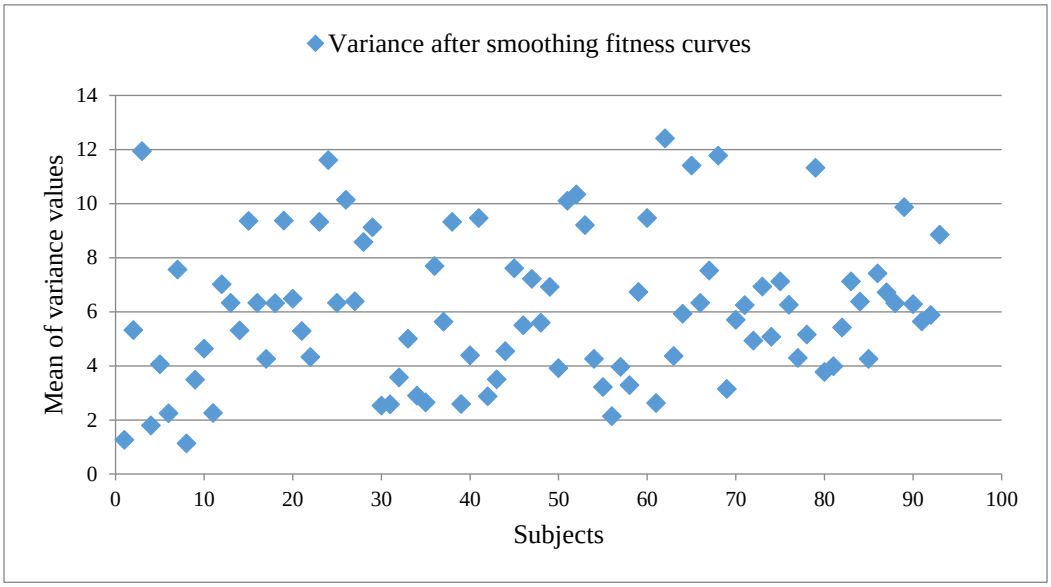


Figure 4.9 Comparison between primary and filtered curves via variance

As can be seen in Figure above, the maximum and minimum values of (mean) variance were obtained 12.5 and 1 respectively. Such range shows a confident degree of change caused by smoothing (Luo, Ying & Bai 2005). Figure 4.10 shows a typical fitness curve before and after applying the Golay filter. As can be seen in this figure, it is difficult to trace the trend of fitness curve after releasing the pressure cuff in the primary diagram (top). It also makes the analysis complex because a complicated algorithm may be needed for processing fitness curves. In fact, the Golay filter provides a better situation for capturing the endothelial-related changes (i.e. peak) as shown in the figure below (down).

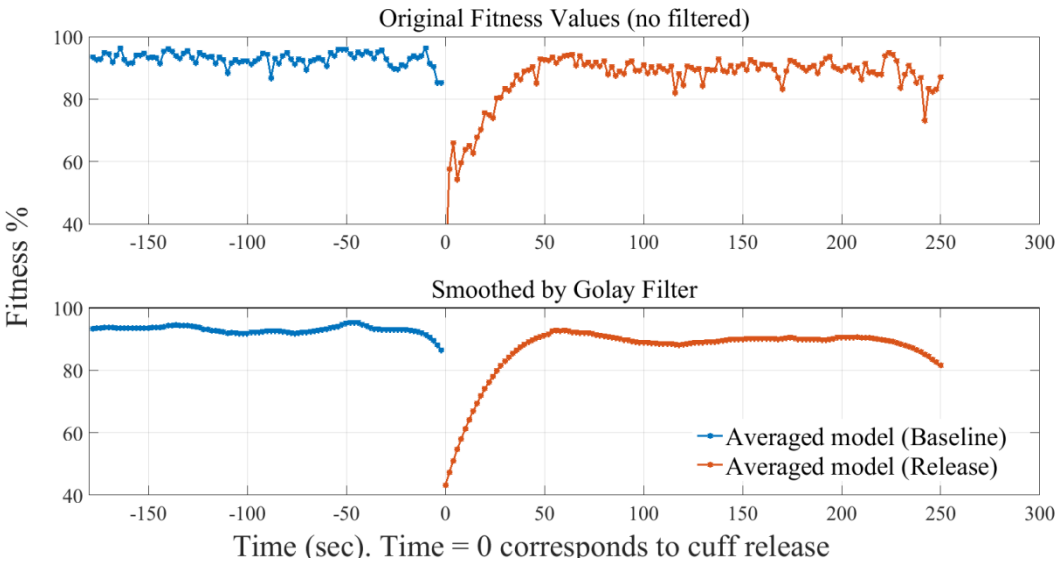


Figure 4.10 A typical fitness curve before (top) and after (down) applying the Golay filter

4.3.2 Analysis in Windows

In order to trace the endothelial activity after releasing the pressure cuff (the FMD stimulus), fitness curves -in release part- were fragmented into five windows as discussed in last chapter. Referring to the FMD-ultrasound technique, the first minute after releasing the pressure cuff can be considered as a "gold" time to capture the peak of Endothelial reaction (Corretti et al. 2002). After this time, vessel diameter is gradually backed to the normal situation (width) so that the endothelial reaction is accordingly reduced. As discussed in the last chapter, three parameters were considered in the first window: rise-time, slope and height of peak. Results are discussed in the following part.

Window 1: The first window was analyzed with the main aim of finding some reliable parameter to in order to distinguish healthy subjects from patients. Therefore it is very necessary to analyze data specifically over healthy and non-healthy groups. As mentioned earlier, subjects were classified into healthy and non-healthy group based on the FMD-US examination. Thus, data analysis was individually done over each group.

Rise Time: This parameter was used to analyze the ascending time in window 1. In fact, rise time represents the spending time between minimum and maximum

fitness values which are corresponding to the release and peak time respectively. In other words, the rise time indicates how fast the vessel diameter can reach to its maximum (widest) diameter. This test was separately done over healthy and non-healthy groups. Figure 4.11 shows rise-time values in first windows among healthy (blue) and non-healthy (red) groups.

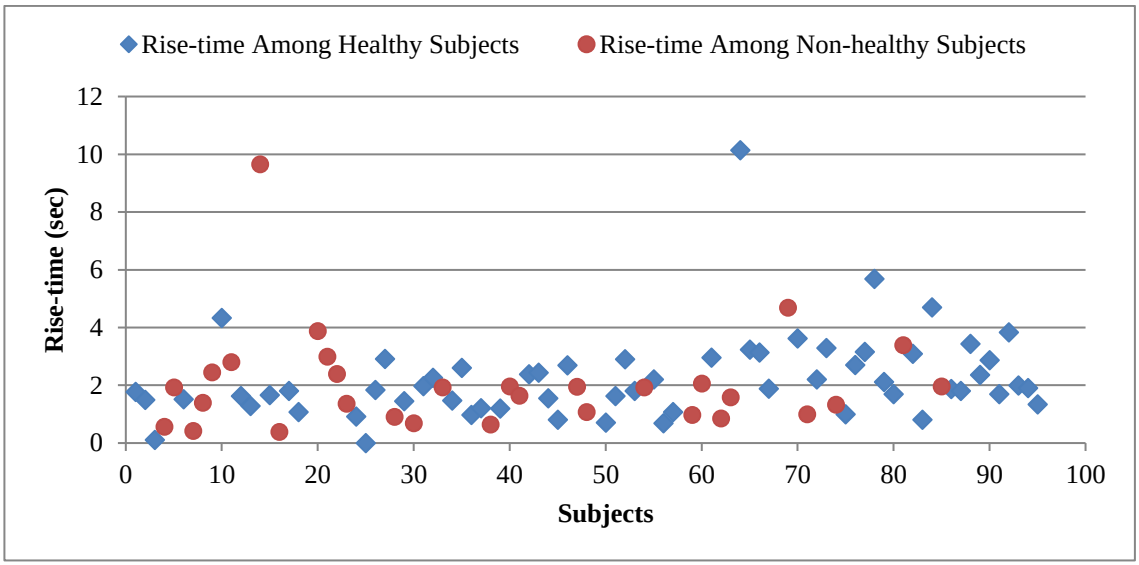


Figure 4.11 Rise time values among healthy (blue) and non-healthy (red) subjects

As can be seen in the figure above, rise time values are mostly less than 4sec among both healthy (blue) and non-healthy (red) subjects. In other words, there is meaningful relationship between values obtained among healthy and non-healthy groups. In fact, there is no exclusive range of rise time among healthy (or non-healthy) subjects. Therefore, the parameter of rise time cannot be used as a reliable parameter to identify those with ED.

Slope: Primary analysis showed that, the parameter of slope is varied from one subject to another. Thus, this parameter was investigated to find out whether it can be used -as a criterion- to detect patients or not. Slopes of fitness curves in the first window are shown in the Figure 4.12. As can be seen in this figure, these values are quite scattering compare to the rise time chart. Besides, there are no meaningful relationship between slope values obtained from healthy (blue) and non-healthy (red) groups. In other words, the endothelial activity cannot be evaluated via the parameter of slope.

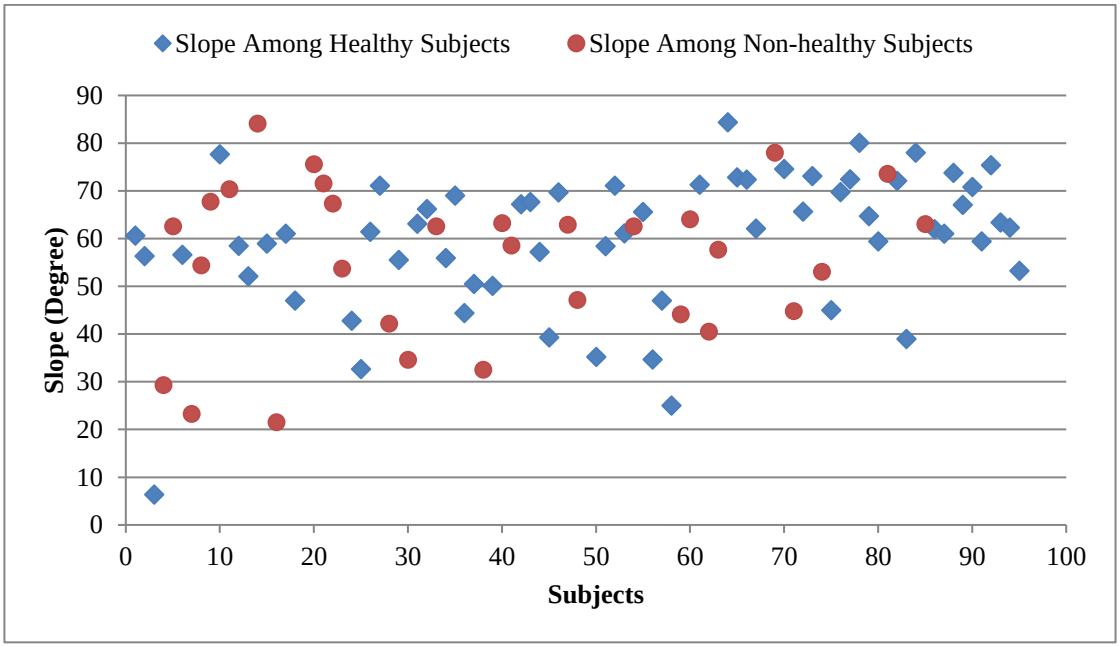


Figure 4.12 Slope Values among healthy (blue) and non-healthy (red) subject

Height of peak: This parameter refers to the difference of maximum and minimum values of fitness which are corresponding to the peak and starting times respectively. It has been widely used for analyzing the endothelial response in the FMD-US technique (Pyke & Tschakovsky 2007; Stoner & Sabatier 2012; Thijssen et al. 2009). In fact, the height of peak can be considered as a reliable indicator of vascular changes caused by the FMD stimulus. The association between height of peak -in FMD ultrasound technique- and ED is already established. Hence, a healthy endothelium represents a high peak whereas an impaired endothelium reflects a very short peak (Pyke & Tschakovsky 2007). Such difference is not only used to assess the ED but also used to indicate the degree of the endothelial performance.

In this study, a fitness curve was considered to assess the endothelial activity. As mentioned above, this curve has a peak-alike trend similar to the peak of reactive hyperemia in the FMD-US technique. Thus, the parameter of height of peak was considered. To this end, a difference between maximum and minimum values of fitness -in the first window- was considered as the height of peak. Figure 4.13 show heights of peaks among both healthy (blue) and non-healthy (red) subjects. As can be seen, heights of peaks of non-healthy subjects are mostly less than twenty whereas it is greater than

twenty among healthy subjects. Such a meaningful relationship was used as a reliable criterion to identify the endothelial dysfunction in this research (PPG-based technique). As a conclusion, the value of twenty was considered as the threshold in this work so that the endothelial performance can be evaluated via this approach. Using this classification, height of peak of less than twenty represents an impaired endothelium (Endothelial dysfunction) whereas higher values show a normal endothelium.

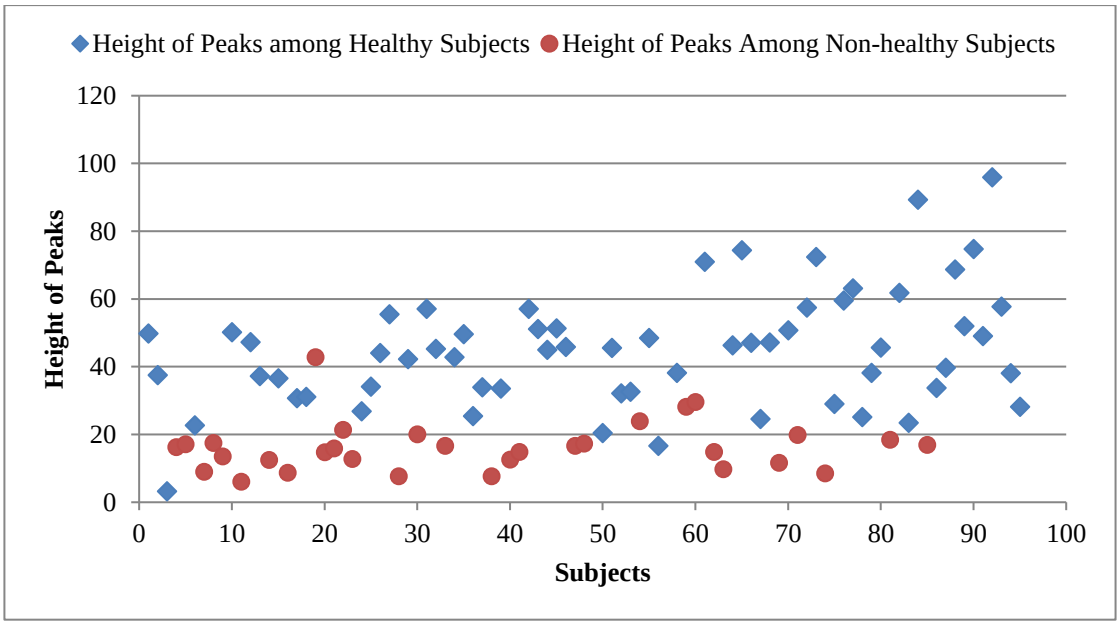


Figure 4.13 Height of peaks among healthy (blue) and non-healthy (red) subjects

In a separate analysis, the height of peak was obtained by subtracting a first value of fitness -after releasing the pressure cuff- from a mean value of fitness in baseline. However, results confirmed that there is no significant change between this approach and the one mentioned above. Figure 4.14 show differences (subtraction) between peak values obtained by the mentioned approaches. As can be seen in the chart below, results of subtraction are mostly around zero confirming that peak values can be faithfully obtained by subtracting the maximum from minimum fitness values in the first window.

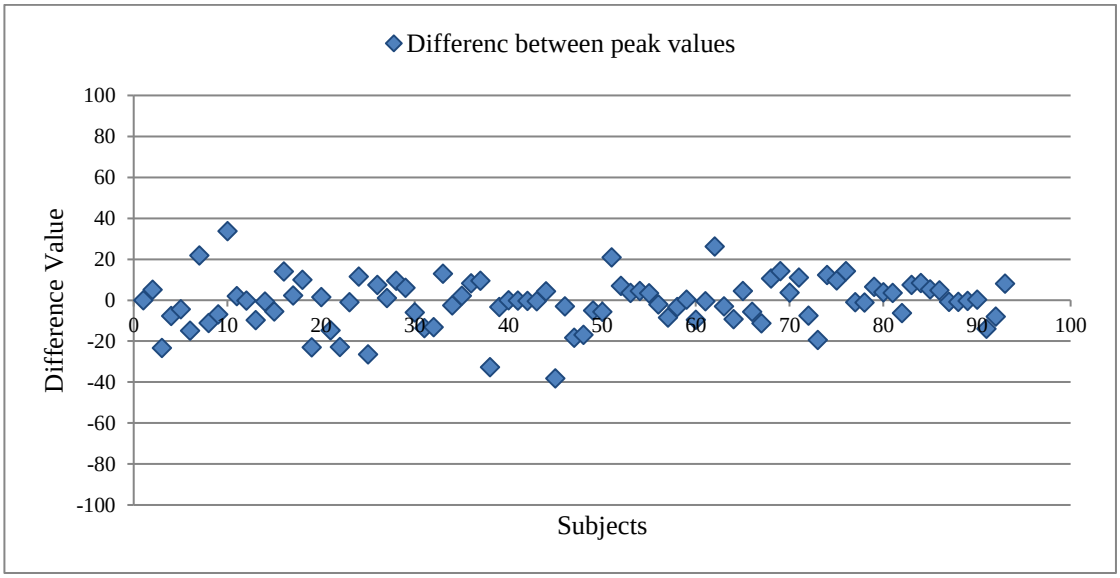


Figure 4.14 Differences (subtraction) between values of peaks obtained by different methods

Window 2-5: As mentioned in chapter III, the peak time occurred right after releasing the pressure cuff. Thus, fitness curve tends to its mean fitness value in the baseline. Such trend shows that the endothelial-related changes in the brachial vessel is declining and going to back to the normal (baseline) situation. Therefore, the test of stability was done over windows 2 to 5 to ensure that vessel diameter is decreased until reaching to the normal diameter. Procedure of conducting the analysis of stability was explained in section 3.5.7. As mentioned, outcome of this part was a single value -for each subject- showing the mean value of variation between each window and mean fitness value in the baseline. Results are shown in Figure 4.15.

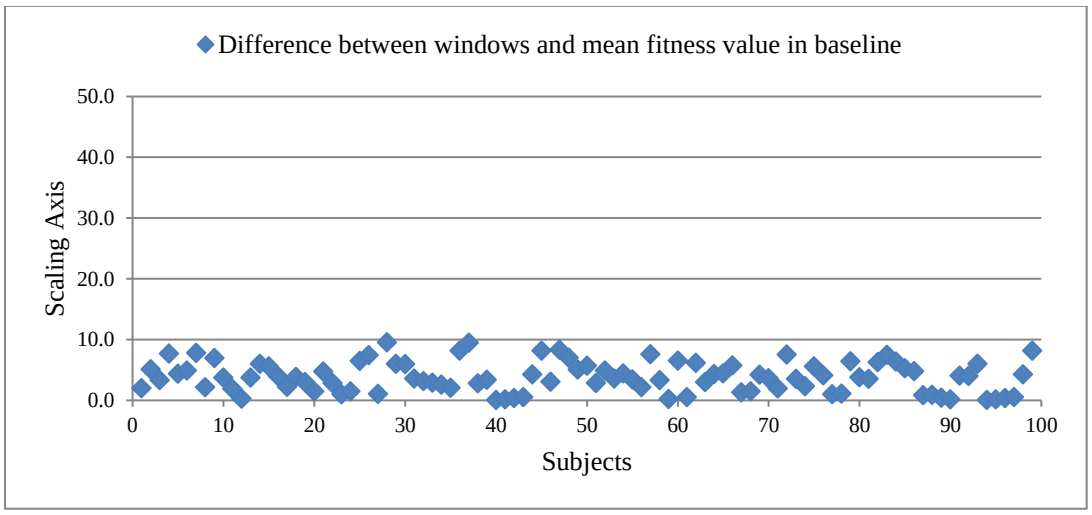


Figure 4.15 Representation of stability in last four minutes of fitness curves

4.4 Results in Conjunction with Ultrasound Images

This section contains results of comparison between FMD-US technique and the proposed PPG technique concerning the ED assessment. In fact, final outcome of both techniques is the binary classification of subjects based on endothelial activity. Accordingly, healthy refers to normal endothelial reaction whereas non-healthy refers to impaired endothelial reaction.

4.4.1 Healthy Group

As quoted earlier, results of the FMD-US technique showed normal endothelial reaction among sixty two subjects. Thus, these subjects were categorized into the healthy group including both male and female genders. Such a classification relies on the fact that the FMD-US test is not a gender-related practice. In other words, this approach is globally accepted as a practical way to assess the endothelial performance regardless of subject's gender, age, nationality, etc (Herrington et al. 2001). Results of the FMD-US technique were discussed above (section 4.1). Results of the proposed technique are discussed below in conjunction of the FMD-US technique.

a) Results of the PPG

In the proposed technique, the parameter of height of peak was considered to reflect the endothelial reaction as mentioned earlier. Interestingly, the PPG model showed a good capability for identifying those with normal endothelium (and ED). Figure 4.16 show values of heights of peaks among the healthy group. As can be seen, values of peak are mostly greater than the threshold value of twenty which is illustrated by the green dotted-line in this figure. Thus, sixty one subjects were re-recognized as healthy by the proposed technique whereas only two subjects were wrongly identified with having normal endothelium. Such comparison confirms the suitability of the proposed

technique to recognize those people with normal endothelium (healthy). In this regard, the correlation between the PPG technique and the FMD-US (as a reference) is approximately 96% in healthy group.

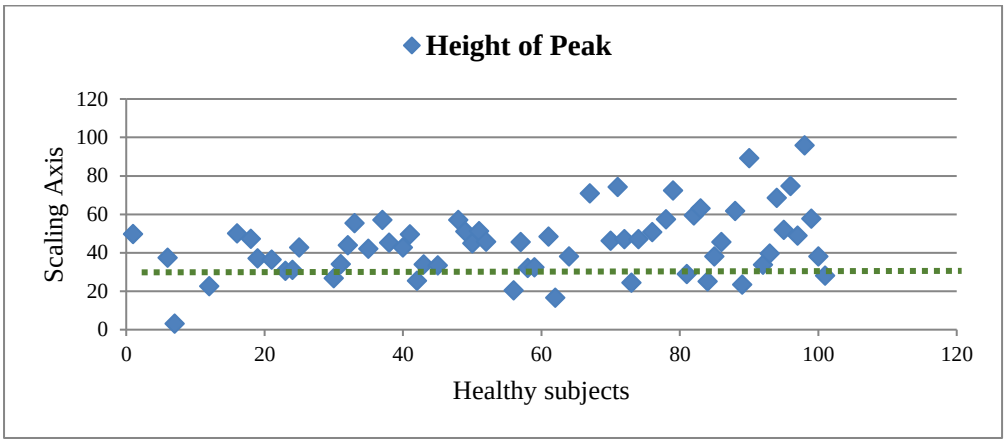


Figure 4.16 Assessment of the endothelial layer via the PPG model with threshold value of twenty (green dotted line)

Although the endothelial activity can be evaluated via the PPG model, values of peaks are varied as can be seen in the figure above. Such dispersion can be also observed among values of FMD as shown in Figure 4.17.

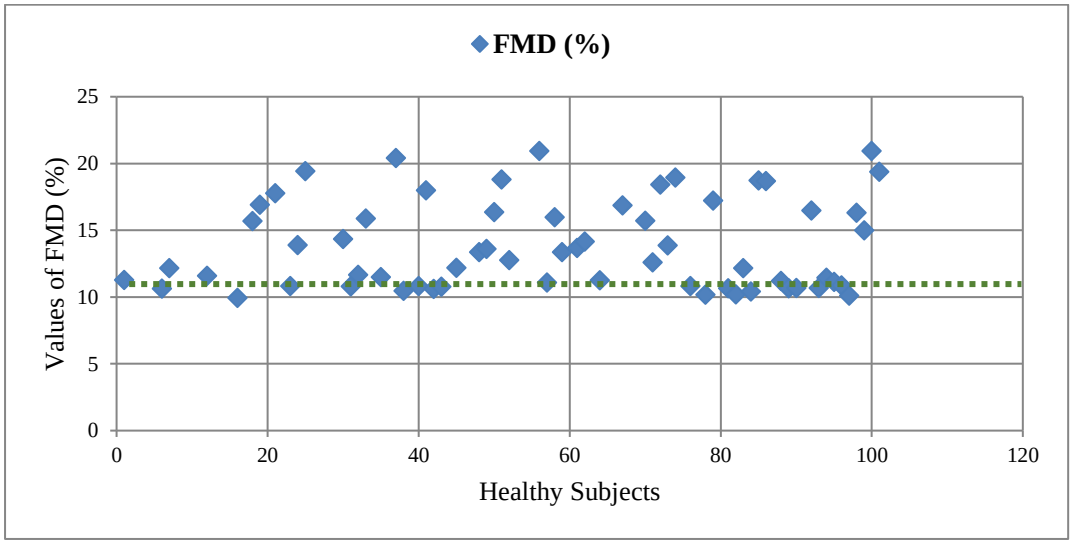


Figure 4.17 Values of FMD among healthy group with threshold value of ten as illustrated by the green dotted line

As can be seen in the figure above, all FMD values are greater than ten percent (threshold value) which confirm the normal activity of the endothelium in this group. The minimum and maximum values of FMD among this group were obtained 10% and 21% respectively. Thus, the range of FMD values can be considered between 10-20 percent. Similarly, values of peak -obtained from the proposed technique (figure 4.14)- are also varied from values 21 to 95. However, values of peak are mostly between 20 and 75 as can be seen in figure 4.15. In other words, there are only two values which are too high (greater than 75). Although the degree of dispersion is different between these two techniques, the issue of dispersion can be considered as a common issue in both techniques. This issue can be justified by explaining the relationship between physiological conditions of the brachial artery during the FMD test with focus on vessel diameter.

As quoted earlier, the brachial vessel is fully blocked via a pressure cuff in the FMD test. As the pressure cuff is released, a density of blood is suddenly rushed into the brachial artery. This time can be considered as a peak time in which the brachial vessel increases to its widest condition (maximum vessel diameter). Such dilatation is mainly caused by the NO which is secreted from the endothelial cells. However, a volume of the released NO (bioavailability of NO) is not the same among all people (Tousoulis et al. 2012). Moreover, the effectiveness of the NO may be different from one person to another as it is a function of current metabolic and physiological conditions (Green et al. 2014). Therefore, a degree of vasodilatation could be dissimilar among different groups. In other words, there is no certain value (or range) for the FMD to assess the endothelial performance (Peretz et al. 2007).

Results of the PPG model showed a meaningful relationship between endothelial-related changes in vessel diameter and fitness values. In this regard, lowest fitness value is obtained just a few seconds after releasing the pressure cuff (peak time). Fitness values are then become higher while the brachial diameter is recovering to its normal condition (diameter). In other words, there is an inverse relationship between brachial diameter and fitness values meaning that low fitness values represent vasodilatation while high fitness values indicate no changes in vessel diameter. Although, similar trend in fitness curves was observed among healthy group but a

difference between maximum and minimum values of fitness (height of peak) is not same among different subjects in this group. This phenomenon confirms that the proposed technique can reflect the endothelial performance similar to the FMD-US technique (Pyke & Tschakovsky 2007).

Figure 4.18 shows two fitness curves which produce maximum and minimum vales of peak. As can be seen, the trend of increasing fitness values -after releasing the pressure cuff- is similar. However, values of peak are significantly different from each other.

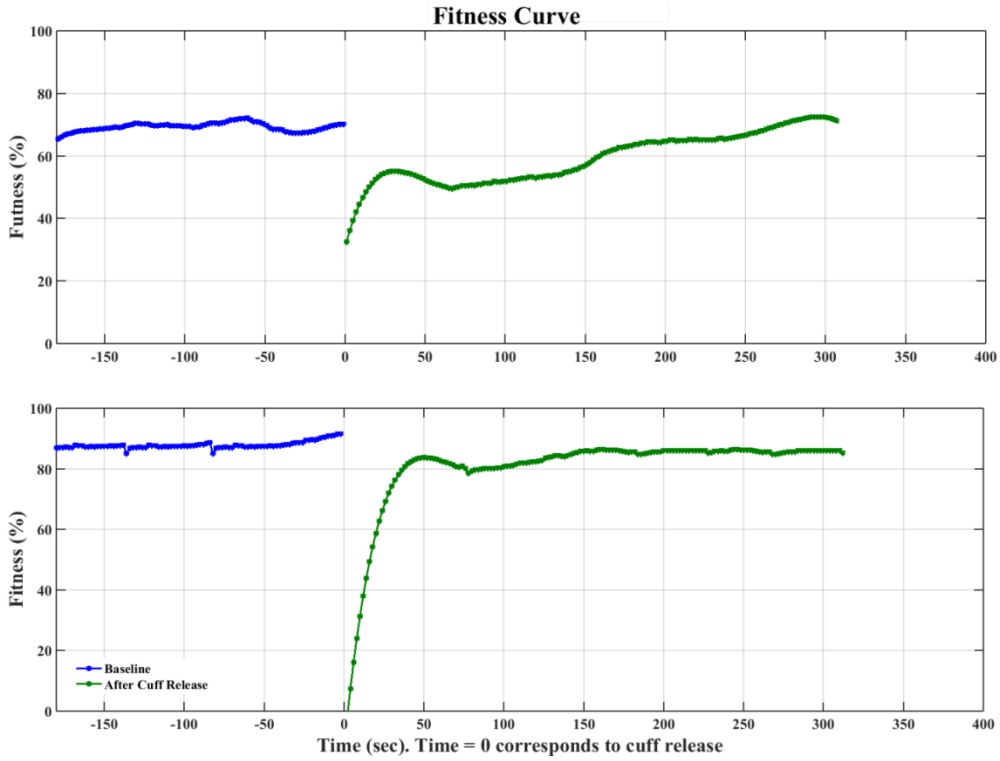


Figure 4.18 Two fitness curves with minimum (top) and maximum (down) peak values

4.4.2 Non-Healthy Group

The procedure of classifying subjects into healthy (non-healthy) group was explained above. Following this procedure, a FMD value of less than ten percent (FMD<10) was considered as an indicator of impaired endothelium. Thus, thirty subjects were identified with ED. These subjects were categorized under the non-healthy group. Values of FMD of non-healthy subjects are shown in Figure 4.19.

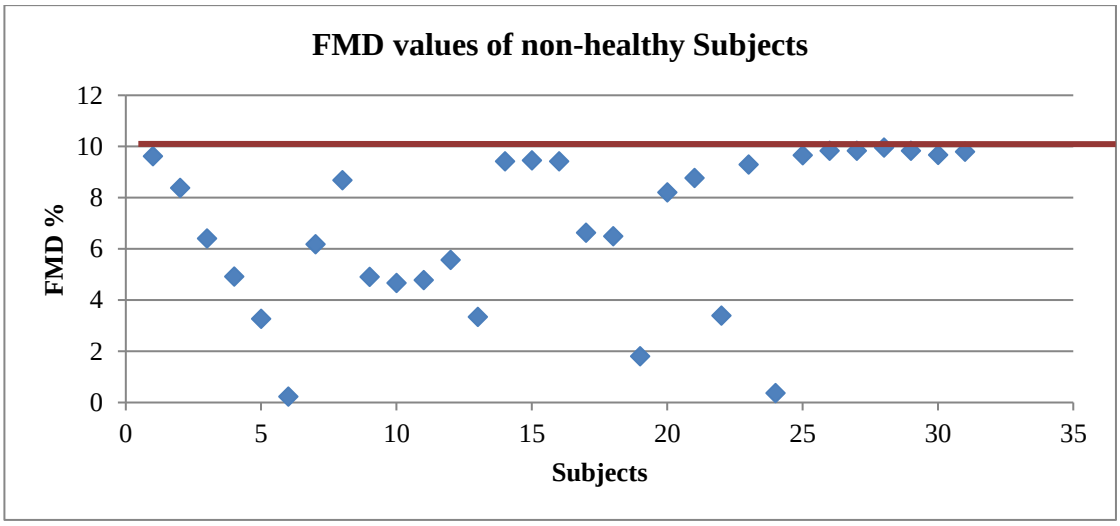


Figure 4.19 FMD values of non-healthy subjects

Likewise last session, the proposed PPG technique was used among non-healthy group to find out how well the PPG technique can recognize patients (those with confirmed ED).

a) Results of the PPG

To this end, the parameter of (height of) peak was calculated among non-healthy subjects with the same procedure as healthy group. So that, those with a peak value of less than twenty (the threshold value in the PPG technique) were identified as patient. Values of height of peak among non-healthy subjects -obtained in the PPG approach- are shown in Figure 4.20.

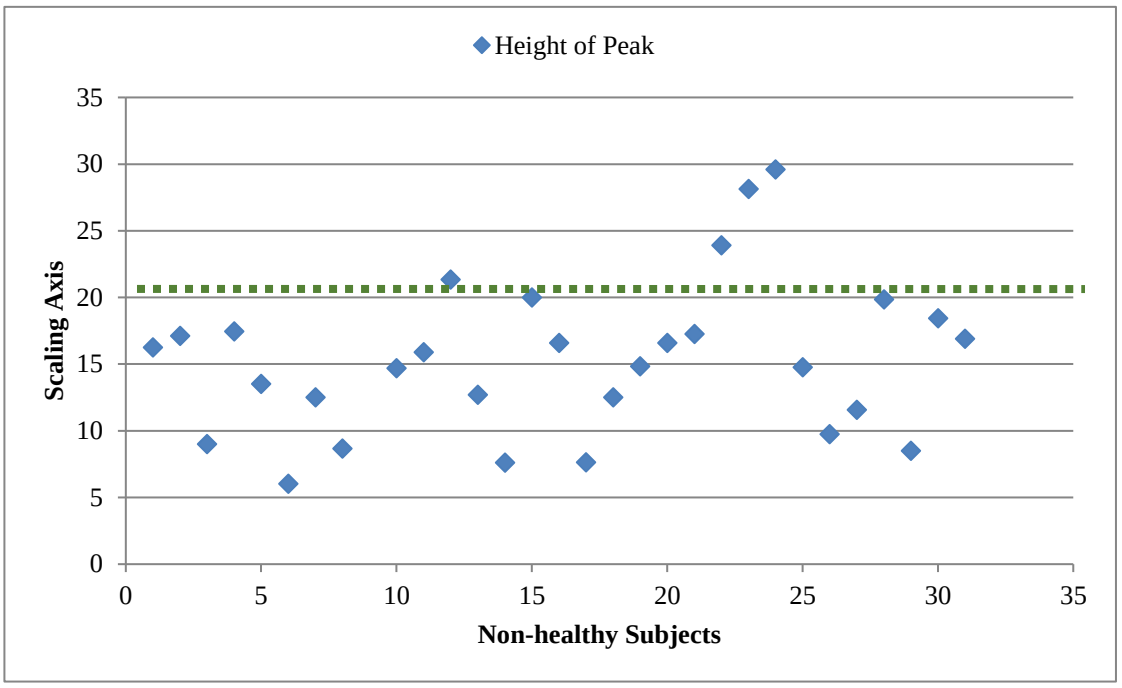


Figure 4.20 Values of height of peak among non-healthy subjects with threshold value of twenty (green dotted line)

Considering the total number of thirty subjects in the non-healthy group, the proposed PPG technique confirmed the ED of twenty six subjects in this group. In other words, only four subjects were wrongly detected as a healthy subject with normal endothelial reaction. As can be seen in the figure above, minimum and maximum values of height of peak are 6 and 29 respectively. The variation of peak values among the non-healthy group is considerably low compare to the healthy group. Reason can be explained by considering the endothelial reaction (fitness curves) in healthy and non-healthy groups. As can be seen in Figure 4.21, two types of fitness curve were observed among non-healthy subjects: curves with inadequate (poor) peaks and curves with even no peak.

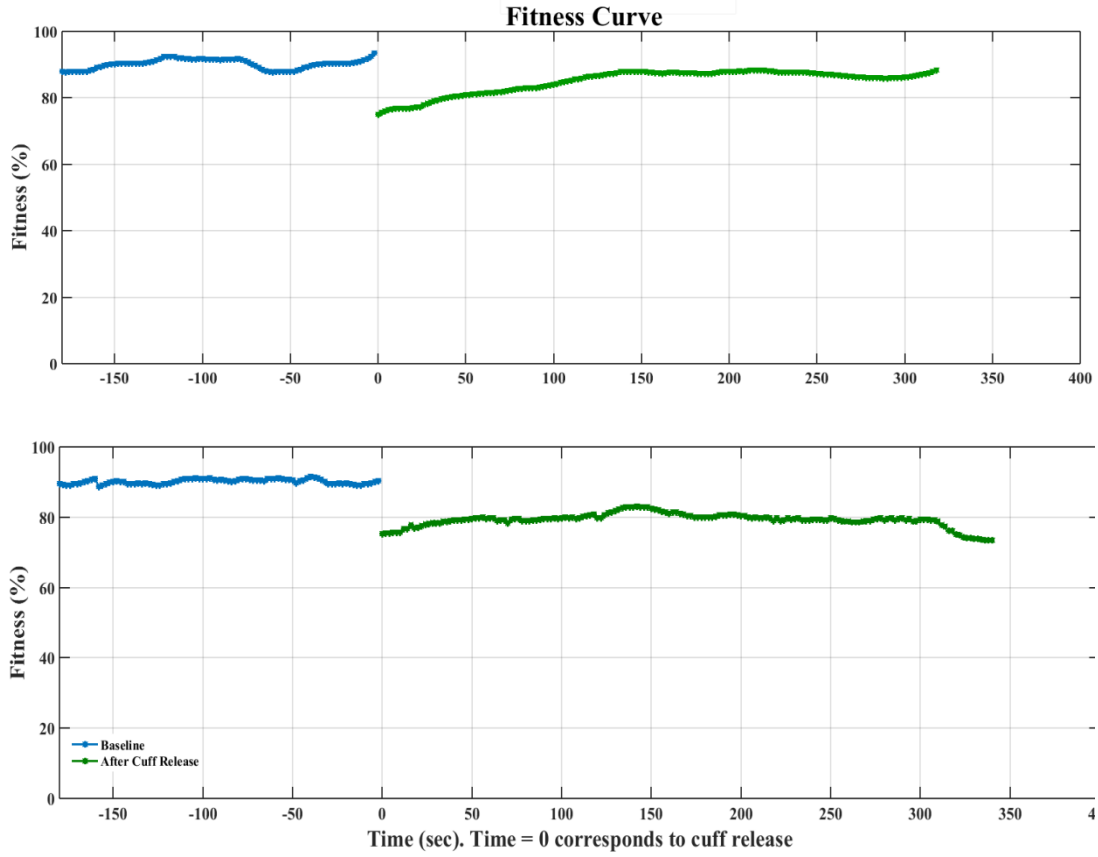


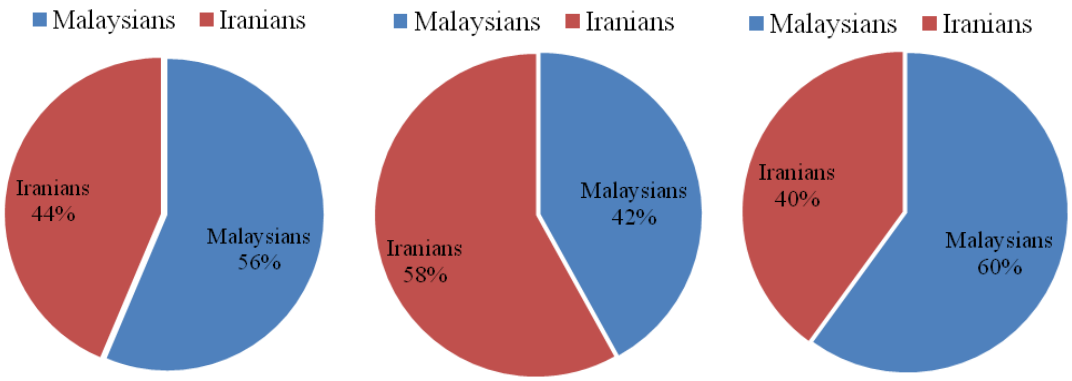
Figure 4.21 Two types of endothelial reaction among non-healthy group: poor reaction (top) and no reaction (down)

As explained earlier, a healthy endothelium is supposed to show a peak-alike response after releasing the pressure cuff. However, analysis among fitness curves of non-healthy subjects showed that the peak is either low or not even exists. In fact, a low peak curve (response) indicates an inadequate endothelial performance whereas curves with no peak show a certain ED meaning that the diameter of the brachial vessel is not changed before (baseline) and after (release) applying the FMD stimulus. This phenomenon can be also considered as a poor endothelial reaction versus insignificant endothelial reaction.

4.5 STATISTICAL ANALYSIS

4.5.1 Distribution of Data

As mentioned before, data of this research were collected at department of cardiology, hospital university Kebangsaan Malaysia (PPUKM) among two ethnic groups: Malaysians and Iranians. According to the main objective of this research (ED assessment) data were collected among those with and without CV abnormalities. To provide healthy groups, subjects were mostly invited from outside the hospital on voluntary basis as mentioned in chapter III. Although this group was supposed to be free of any CV abnormality, essential tests (i.e. clinical test) were done to confirm that they are free from any risk factor at the time of data collection. Besides, inpatients of the PPUKM hospital were considered as potential non-healthy subjects. Some of them were then selected -by our medical partner- based on research criteria. They were then asked to participate in this research voluntarily. Figure 4.22 shows data distribution in terms of ethnicity. In addition, data distribution in terms of gender can be seen in Figure 4.23.



a) All subjects b) Healthy subjects c) Non-healthy subjects

Figure 4.22 Data distribution in terms of ethnicity in total (a), healthy (b) and non-healthy (c) views

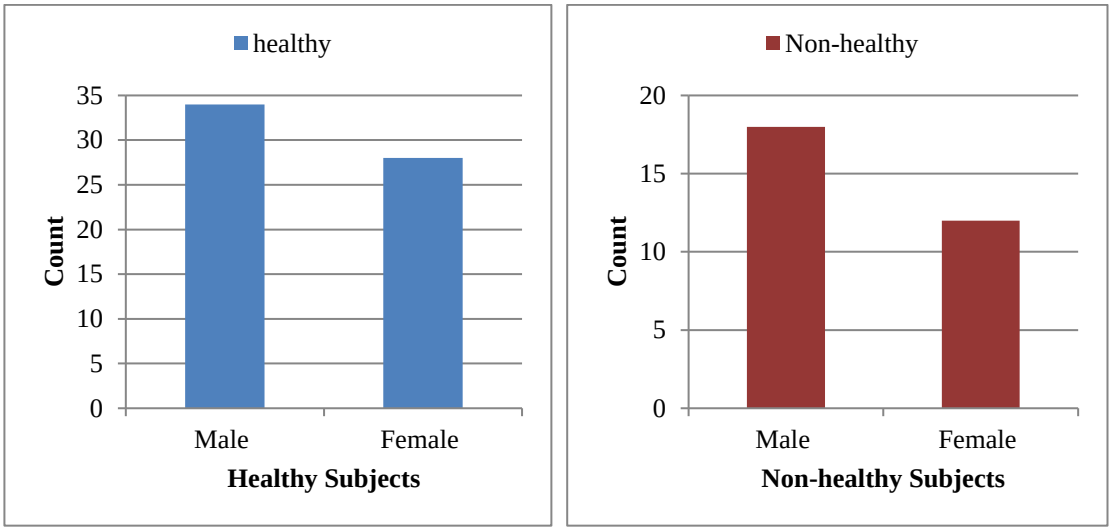


Figure 4.23 Data distribution in terms of gender

4.5.2 Statistical Analysis among Different Groups

a) Gender

In clinical researches, the parameter of gender should be taken into account in order to interpret results. In other words, the suitability of a proposed technique among both male and female subjects should be evaluated. In general, performance of the CV system is not same among male and female subjects. For instance, it is already demonstrated that the recovery time after conducting an endovascular repair is considerably longer in women rather than men (Grootenboer et al. 2013). However, there is no significant difference among male and female subjects in terms of FMD values (Hu et al. 2008; Sader & Celermajer 2002). In other words, the endothelial performance can be fairly evaluated via the FMD-US technique among both male and female subjects.

The goodness of the PPG technique to detect the ED among males and females was also investigated in this research. Results of this study showed that there is an insignificant difference between peak values among male and female subjects. Using the ANOVA test, the p-values are obtained 0.79 and 0.20 in healthy and non-healthy groups respectively. Since both of these values are greater than type I error ($\alpha = 0.05$), it can be concluded that the proposed PPG technique can be used to assess the

endothelial performance regardless of the issue of gender. Results of the ANOVA test are shown in Table 4.1.

Table 4.1 Gender-based ANOVA analysis in PPG technique

			α = 0.05				
Parameters	Count	Sum	Average	Variance	F	P-value	F crit
Healthy subjects							
Males	34	1507	44	243	0.06	0.79	3.99
Females	29	1318	45	350			
Non-healthy subjects							
Males	18	256	14	36	1.64	0.20	4.19
Females	12	203	16	25			

b) Aging

Aging can be truly considered as a critical parameter in CV performance (Dahlöf 2010). Thus, the effect of age was studied in this research to ensure the suitability of the PPG technique over different age groups. To this end, subjects were classified into tens groups. Such classification is shown in Figure 4.24.

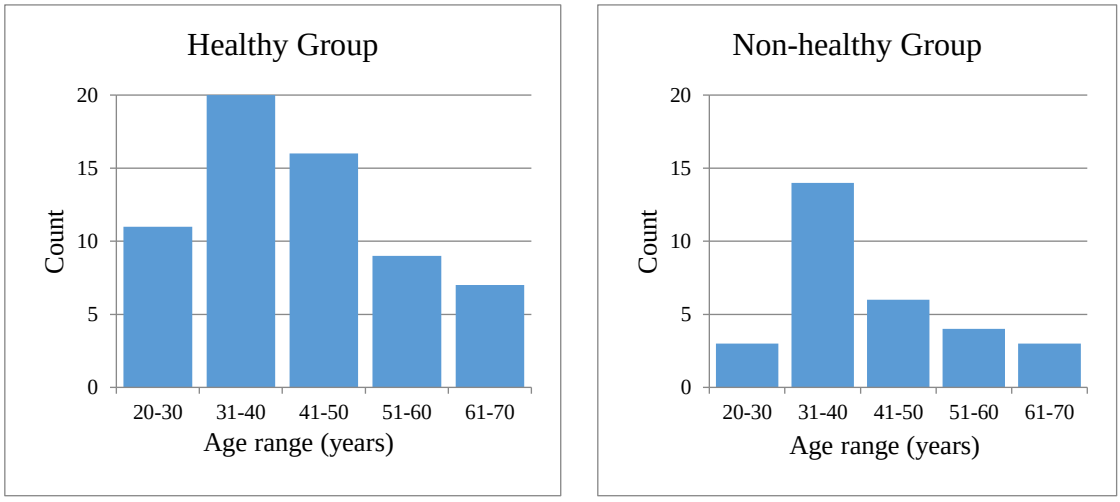


Figure 4.24 Data distribution among healthy (left) and non-healthy (right) groups in terms of age

The ANOVA test was done to ensure whether the PPG technique can be used over different age-groups or not. In this regard, an appropriate range of age should be considered for the classification. In general, the elderly age starts at 65 years old (Organization 2010). However, the association between the endothelial dysfunction and age was considered -in this research- in order to find an appropriate age for classification. Although there is no certain age for the ED, the range of 45-50 years old is mostly addressed in prior works as a potential age of starting the ED (Seals et al. 2011). Therefore, the age of 50 was considered as a potential age of starting the ED. Thus, subjects were classified into two groups of age: below 50 and above 50. Peak values were then considered in the analysis.

The ANOVA analysis showed an insignificant change in peak values among young and elderly subjects. Details of this analysis are shown in Table 4.2. in which values of statistical parameters (sum, average and variance) are rounded up for a better representation. The last three columns of this table contain source of variations (F, P-value and F crit) between so-called young and elderly groups. As can be seen in this table, both P-values are greater that type I error ($\alpha = 0.05$) confirming insignificant changes of peak values among healthy and non-healthy groups.

Table 4.2 Age-based ANOVA Analysis for the PPG Technique

			$\alpha = 0.05$				
Parameters	Count	Sum	Average	Variance	F	P-value	F crit
Healthy subjects							
Group < 50	47	2194	46	307	2.20	0.14	3.99
Group > 50	16	631	39	206			
Non-healthy subjects							
Group < 50	23	374	16	34	3.04	0.09	4.19
Group > 50	7	84	12	15			

c) Healthy and Non-Healthy:

As mentioned in chapter III, the parameter of height of peak was considered to assess the ED in this research. In this regard, the value of twenty was determined as the threshold value. Statistical analysis (ANOVA test) showed that there is a significant

difference between peak values of healthy and non-healthy subjects. Result of this analysis is shown in Table 4.3. As can be seen in this table, the obtained p-value is less than type I error (α=0.05) showing a significant difference between peak values in healthy and non-healthy groups. It confirms that the parameter of (height of) peak can be effectively employed to identify those with ED. Accordingly, the first hypothesis -in section 1.5- becomes true.

TABLE 4.3 Difference of peak values among healthy and non-healthy groups in the PPG technique

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Non-healthy	30	459,828	15,327601	32,954
Healthy	63	2826,24	44,860998	288,191

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	17725,8	2	17725,792	85,6932	9,1E-15	3,94569
Within Groups	18823,5	91	206,85178			
Total	36549,3	93				

4.5.3 Clinical Parameters

As mentioned in chapter III, blood was taken from all participants in order to assess clinical parameters at the time of data collection. Table 4.4 shows an overall view of clinical variables in this study. Considering the value of 0.05 for type I error (α=0.05), there is no significant difference between clinical parameters among healthy and non-healthy groups. It shows that clinical parameters (per se) cannot be used to assess the ED.

TABLE 4.4 Descriptive variables of the study

		Healthy				Non-Healthy				*ANOVA*
Clinical Variables	unit	Min	Max	Mean	SD	Min	Max	Mean	SD	*p*-value
Age	years	30	71	42	12	30	70	36	12	0.05<
Systolic BP	mmHg	105	134	114	11	105	190	125	18	0.05<
Diastolic BP	mmHg	60	105	75	9	55	113	75	11	0.05<
Pulse rate	BPM	40	110	70	12	48	110	63	12	0.05<
*Fasting blood glucose	mmol/L	3.8	8.4	4.65	1	4	9.7	5.8	1	0.05<
**Total cholesterol	mmol/L	2.53	5.16	3.78	1	3.29	7.96	5	1	0.05<

*Normal range of Fasting blood glucose: [5.6 - 6.9] mmol/L
**Normal range of total cholesterol: < 3.37mmol/L

4.5.4 Sensitivity and Specificity

As mentioned earlier, the main aim of conducting statistical analysis is to evaluate the suitability of the proposed technique among different people (Glantz 2005). In other words, it is very important to know whether a method is only restricted to a specific group or can be widely used over different people with dissimilar characteristics (e.g. gender, age, ethnicity, etc.). The basic parameters of statistics are explained in details in section 3.6. In the following parts, results of the statistical analysis are discussed. As mentioned above, the final outcome of the proposed technique is a binary detection of ED. In other words, final result would be either healthy (normal) or non-healthy (impaired) endothelium so that the proposed technique can be considered as a binary assessment of the endothelial layer. As shown in Table 4.5 (contingency table) totally six cases were detected wrongly by the PPG technique in this research.

TABLE 4.5 Statistical errors

	True	False
Positive	26	2
Negative	61	4

According to the table above, results of the binary detection can be summarized as below:

- True Positive: Totally 26 subjects were identically detected as non-healthy (those with ED) via both FMD-US and the PPG approaches.
- True Negative: Similarly, 61 subjects were identically identified as healthy (those with normal endothelium) via both techniques.
- False Positive: However, 2 subjects were detected as non-healthy via the PPG technique whereas results of FMD analysis confirmed that they are healthy.
- False Negative: In addition, there are 4 subjects who are identified as healthy via the PPG technique whereas results of the FMD analysis showed they are non-healthy.

Following equations 3.7 and 3.8, the sensitivity and specificity of the proposed technique are obtained 86% and 96% respectively. Using equation 3.9, accuracy is obtained for 63%.

As quoted earlier, the ROC curve is an established method for representing the suitability of a proposed method in terms of sensitivity and specificity. Referring to section 3.6, the area under curve (AUC) is known as the most important parameter in the ROC diagram. In fact, the AUC shows how well a method can perform as a binary classifier to detect abnormalities. Therefore, it can show the actuality of the proposed technique for detecting the ED among both healthy and non-healthy groups. Figure 4.25 illustrates the ROC diagram of the proposed technique. As can be seen, a value of AUC is mentioned in the middle of ROC diagram. Considering the maximum value of 100 for the AUC, an obtained value of 96% confirms that the proposed technique can be faithfully used -as a binary classification- to detect the ED among all people.

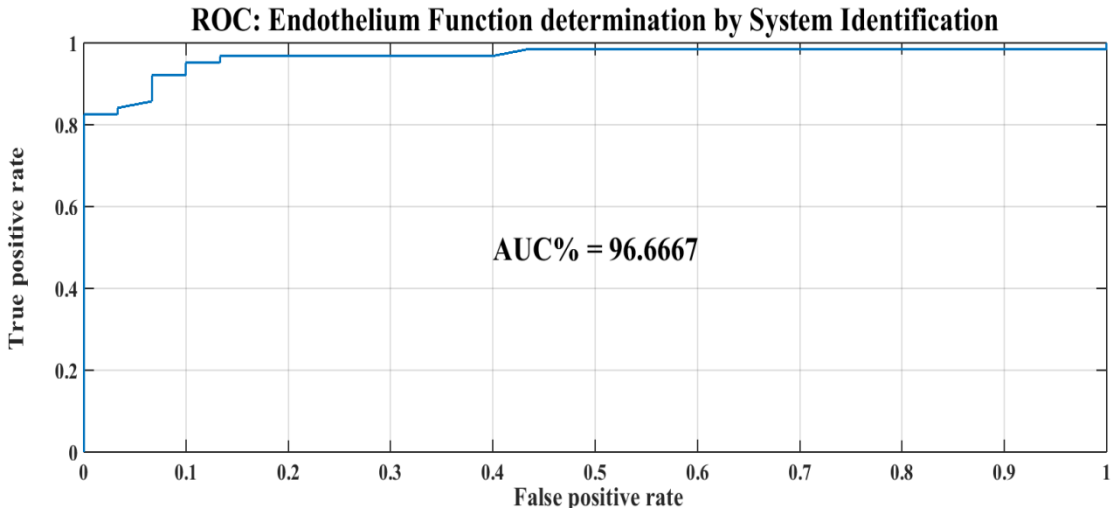


Figure 4.25 The ROC curve of this study

4.6 CHAPTER SUMMARY

Results of the ultrasound imaging and PPG technique have been elaborated in this chapter. It is begun with the results of the ultrasound imaging providing a precise assessment to the endothelial performance. Results were then discussed. Finally, subjects were classified into healthy and non-healthy groups based on the current activity of the endothelium obtained by the FMD-US.

Results of the PPG technique are then expressed in details. It is discussed separately over baseline and release data. At first, the goodness of the auto-regressive with exogenous model structure (ARX) for estimating a forward CBP $\rightarrow$ RPPG model is discussed. Results show that this model has a reliable performance for representing the endothelial reaction during the FMD test. Using this model, a peak-like trend is captured among fitness curves as a result of stimulating the brachial artery through the FMD test. Results of analysis the peak is then showed and discussed endorsing the suitability of using the "height of peak" for assessing the endothelial performance in the PPG technique.

Statistical analysis shows that values of peak are significantly different among healthy and non-healthy groups. It confirms that the -parameter of- height of peak can be effectively used to identify the ED. Results of the proposed technique are then

compared with results of the US imaging reflecting convinced values of sensitivity, specificity and accuracy of 86%, 96% and 93% respectively. The AUC is also obtained around 96%. Thus, endothelial stimulation (FMD test) is performed on a large artery (brachial) while endothelial reaction is assessed also from brachial artery (i.e. excluding from effects of the autoregulation). Therefore, it can be concluded that proposed technique is able to assess the so-called pure endothelial functionality without capturing non-endothelial factors.

CHAPTER VI

CONCLUSION AND FUTURE WORK

5.1 CONCLUSION

Endothelial dysfunction has been known as an early marker of atherosclerosis and further CV diseases. In fact, it has been demonstrated that early assessment of ED may lead to early detection of CV abnormalities and thus, control related diseases by adequate and on-time treatments. As explained, endothelial assessment can be done mainly via invasive and non-invasive approaches. The main limitation of invasive approach is that, it is limited to coronary patients due to invasive nature of such technique. In contrast, FMD-US has been introduced as an established and most attended technique to assess the endothelial performance non-invasively. However, this technique requires special system setup as well as an experienced sonographer. It is then mentioned that proposing a cost-effective and operator independent technique -to assess the endothelial performance- has been particularly attended over last decade. In this regard, previous researchers proposed the PPG technique to assess the endothelial functionality. For instance, in the latest work by Rosmina Jaafar, a PPG-based technique has been introduced to identify ED among both healthy and risk groups (Jaafar 2009). In this technique, endothelial layer is stimulated across subject's peripheral artery while endothelial reaction is assessed from subject's index PPG signal. This study has truly shown the possibility of detecting the ED via PPG. Results of this study have also shown good agreement with results of the FMD-US technique (i.e. reference technique). Accordingly, specificity and sensitivity of this technique were obtained 59% and 63% respectively. However, using this technique, both endothelial and non-endothelial factors can be captured. As discussed, it is mainly due to the issue of autoregulation in small arteries. In other words, in this technique, endothelial stimulation was truly done across subject's peripheral artery but endothelial reaction was captured from smaller artery in which the autoregulation moderates vascular tuning in addition to endothelial

mechanism. Therefore, non-endothelial factors (i.e. effects of autoregulation) were also captured from subject's index finger.

5.2 Research Finding and Research Hypothesis

In the current study, a new technique is investigated to assess the endothelial performance via PPG. Using the proposed technique, the endothelial performance can be evaluated by a forward model between the CBP and radial PPG [CBP $\rightarrow$ RPPG]. Goodness of such model before (baseline) and after releasing the pressure cuff is considered as a criterion to reflect the endothelial performance (i.e. fitness curves).

Refereeing to research hypothesis (chapter I), goodness of such model is supposed to vary among healthy subjects whereas it is supposed to fix (i.e. insignificant change) among unhealthy subjects. According to results (chapter IV), this matter has been approved. It was observed that, in healthy group, values of peak after releasing the pressure cuff (i.e. height of peaks) are significantly varied compared to baseline. In contrast, there is no significant variation after releasing the pressure cuff among unhealthy subjects. Such comparison was done via threshold value of twenty (height of peak) obtained in conjunction with FMD-US test. This issue was also examined via the statistical analysis. It has shown that there is a significant difference between peak values in healthy and non-healthy groups. This result confirms the suitability of the proposed technique to identify the ED.

Moreover, the proposed PPG technique is supposed to have similar results as FMD-US technique (research hypothesis, chapter I). Results also confirmed that there is a high agreement between these two techniques concerning ED assessment. As explained in chapter IV, in total, only six cases were wrongly detected using the proposed technique compared to the FMD-US technique. Therefore, it can be concluded that the proposed technique can identify ED as good as the FMD-US technique.

Furthermore, it was stated in research hypothesis (chapter I) that there is no difference among male and female subjects concerning height of the peak. This issue has been also proofed in chapter IV. It was shown that there is no significant variation

concerning peak values among male and female subjects. This matter was also examined via the statistical test.

Using the PPG technique, ultrasound device is not needed for capturing the endothelial activity so that the role of operator (sonographer) can be considerably reduced. In addition, associated costs (e.g. ultrasound machine, high frequency transducer, etc.) can be moderated. Furthermore, the PPG technique may provide a reliable way for the self-assessment of the endothelial performance by a user friendly device. However, the PPG sensor is remarkably sensitive to even small movements. Moreover, the issue of contact pressure between the PPG sensor and subject's skin should be highly taken into account before start recording data. These items can be addressed as critical limitations of the proposed technique. In this research, the PPG sensor was wrapped around the target site (brachial artery) and the quality of signals were visually checked -by operator in site- before start data collection. Besides, all participants were asked to avoid any movement during data collection. This helped to record optimal signals.

Another limitation of using the PPG sensor is the issue of temperature in a data collection room. PPG signals obtained from an uncontrolled room may not be reliable due to inadequate blood perfusion in target vessel. Therefore, temperature of a data collection room should be maintained between 28-32ºC to avoid any temperature-caused disturbance on subject's blood circulation. In this study, room temperature was kept in this range during all data collection sessions. It was done using an adjustable temperature control key in the data collection room.

Number of participants can be considered as a common limitation of clinical researches. While high number of participants can improve the quality of work, the sample size should be estimated realistically based on research objectives and limitations (Hulley et al. 2013). Another difficulty is the issue of variety in sample population. Usually, it is very difficult to find healthy subjects among elderly people. On the other side, young individuals are normally in a good health condition without any risk factor. This issue may lead to misbalancing on variety of data. Nevertheless, it is unavoidable particularly in FMD-based researches (Peretz et al. 2007).

5.3 Contribution

The main contribution of this work is to exclude effect(s) of autoregulation from the endothelial response in order to capture the pure endothelial reaction. In this regard, endothelial stimulation was done across subject's peripheral artery while endothelial response was assessed from subject's brachial artery. It was explained that the phenomenon of autoregulation is just in small arteries (e.g. finger, tow, auricle, etc.) and not the brachial segment.

Moreover, vessel diameters were automatically and continuously measured using the Cardiovascular Suit Program. Accordingly, variations of brachial changes were also obtained by comparing mean diameter of vessel diameter before and after releasing the pressure cuff. The experiment was also done via upgraded system setup including a high frequency transducer and rapid cuff inflator.

Furthermore, endothelial assessment was done via a forward model between CBP and RPPG rather than analyzing AC components of PPG signal. This approach provides a reliable way to trace the endothelial response from releasing the pressure cuff until end of experiment. In addition, it enhances the quality of analysis as even small changes can be identified. Accordingly, specificity and sensitivity of the proposed technique are obtained 96% and 86% respectively. Compare to the latest work (Rosmina Jaafar), such high values could be considered as higher reliability of the proposed technique.

5.4 SUGGESTIONS FOR FUTURE WORKS

The following issues were observed during data collection and data analysis which might be considered to improve the quality of final outcome in future works.

5.4.1. System Setup

Software of the SphygmoCor device needs the whole screen of a computer to be run and display the central and radial blood pressure waveforms in real-time. In addition, another computer is needed for displaying the radial PPG signal as well as storing data.

Therefore, two computers were essentially needed (used) in this research. Irrespective of its cost, using two computers require more system setup -which is time consuming- and operator's consideration during whole data collection time. This problem can be managed by customizing the software of the SphygmoCor so that its window can be minimized allowing PPG signals to be displayed in the same computer. However, such customization must be done by the manufacturer because the software cannot be adjusted by user. This matter was understood at the middle of data collection. Thus, it was not possible to transfer the SphygmoCor device to the manufacturer (in Australia) for solving this problem as it was an essential part of data collection.

Right placement of the PPG sensor over subject's skin was found very tough and time consuming. Solving this problem requires an automated system not only to place the PPG sensor right over the radial artery but also to apply an adequate pressure in order to obtain an optimal PPG signal. To the best of our knowledge, such system was not commercially available at the time of data collection and thus it can be even considered as a worth pursuing project for further PPG-based investigations. If this system cannot be used (find) in future works, getting help from assistant may mange the problem in a better way.

5.4.2 Sample Population

This research investigates the possibility of assessing the endothelial performance via the PPG approach. Results show that the proposed technique can be reliably employed -as a binary classifier- to detect the endothelial dysfunction. However, providing a reliable way to obtain the degree of endothelial dysfunction can be considered in future works. To this end, the sample size should be increased. In fact, more number of subjects can guarantee the reliability of a proposed technique. Besides, the sample population should be expanded to different nationalities including different ranges of age. In addition, it is good to balance the quantity of healthy and non-healthy subjects particularly from the age viewpoint. In this regard, the main issue would be providing healthy subjects among elderly people and non-healthy subjects among young people.

5.4.3 Procedure of Data Acquisition

As mentioned earlier, the flow mediated dilatation (FMD) test was used in this study for stimulating the endothelial layer (cells). Using this test, the brachial blood circulation is blocked for five minutes and then suddenly unblocked leading to the state of reactive hyperemia (vascular dilatation). However, it was observed that most of subjects were feeling discomfort during such long time blood occlusion over the brachial artery. Alternatively, the endothelial layer can be stimulated by injecting some pharmacological substances such as Nitroglycerin. However, the injection requires subject's agreement and an authorized person (e.g. medical doctor, nurse) as well as ethical agreement from a medical Centre.

In this research, the ultrasound imaging was used during data collection in order to capture the Endothelial activity before (baseline) and after applying the FMD stimulus (after cuff release). Ultrasound images were then analyzed offline using an automatic edge-detection software. However, there is a possibility of measuring the brachial diameter during the FMD test in case of using a modern ultrasound system accompanied by an edge detection software. In this regard, an ultrasound device should be connected to a computer in which the software is installed in. Such configuration and system setup should be done by an experienced FMD operator (sonographer). A synchronous measurement of the brachial diameter can guarantee a right assessment to the endothelial activity as well as reducing analysis time.

REFERENCES

Abdolrazaghi, M., Navidbakhsh, M. & Hassani, K. 2010. Mathematical modelling and electrical analog equivalent of the human cardiovascular system. *Cardiovascular Engineering* 10(2): 45-51.

Al Suwaidi, J., Hamasaki, S., Higano, S. T., Nishimura, R. A., Holmes, D. R. & Lerman, A. 2000. Long-term follow-up of patients with mild coronary artery disease and endothelial dysfunction. *Circulation* 101(9): 948-954.

Allen, J. 2007. Photoplethysmography and its application in clinical physiological measurement. *Physiological measurement* 28(3): R1.

Anderson, T. J. 1999. Assessment and treatment of endothelial dysfunction in humans. *Journal of the American College of Cardiology* 34(3): 631-638.

Anon. 2012. Endothelial cells as a transmitter. www.nhs.uk [

Anon. 2016. Cardiac Cathetrization from Femoral Artery. http://nursingcrib.com [

Arrebola-Moreno, A. L., Laclaustra, M. & Kaski, J. C. 2012. Noninvasive assessment of endothelial function in clinical practice. *Revista Española de Cardiología (English Edition)* 65(1): 80-90.

Atherosclerosis. 2016. Atherosclerosis. https://www.ndhealthfacts.org/ [

Atkinson, G. & Batterham, A. M. 2013. Allometric scaling of diameter change in the original flow-mediated dilation protocol. *Atherosclerosis* 226(2): 425-427.

Atkinson, G. & Batterham, A. M. 2013. The percentage flow-mediated dilation index: a large-sample investigation of its appropriateness, potential for bias and causal nexus in vascular medicine. *Vascular medicine*: 1358863X13508446.

Bagha, S. & Shaw, L. 2011. A real time analysis of PPG signal for measurement of SpO2 and pulse rate. *International journal of computer applications* 36(11): 45-50.

Bagher, P. & Segal, S. S. 2011. Regulation of blood flow in the microcirculation: role of conducted vasodilation. *Acta Physiologica* 202(3): 271-284.

Bakker, W., Eringa, E. C., Sipkema, P. & van Hinsbergh, V. W. 2009. Endothelial dysfunction and diabetes: roles of hyperglycemia, impaired insulin signaling and obesity. *Cell and tissue research* 335(1): 165-189.

Balletshofer, B. M., Rittig, K., Enderle, M. D., Volk, A., Maerker, E., Jacob, S., Matthaei, S., Rett, K. & Häring, H. U. 2000. Endothelial dysfunction is detectable in young normotensive first-degree relatives of subjects with type 2 diabetes in association with insulin resistance. *Circulation* 101(15): 1780-1784.

Barnes, R. W., Clayton, J. M., Bone, G. E., Slaymaker, E. E. & Reinertson, J. 1977. Supraorbital photoplethysmography. Simple, accurate screening for carotid occlusive disease. *Journal of Surgical Research* 22(4): 319-327.

Betik, A. C., Luckham, V. B. & Hughson, R. L. 2004. Flow-mediated dilation in human brachial artery after different circulatory occlusion conditions. *American Journal of Physiology-Heart and Circulatory Physiology* 286(1): H442-H448.

Bogert, L. W. & van Lieshout, J. J. 2005. Non-invasive pulsatile arterial pressure and stroke volume changes from the human finger. *Experimental physiology* 90(4): 437-446.

Bonetti, P. O., Lerman, L. O. & Lerman, A. 2003. Endothelial dysfunction a marker of atherosclerotic risk. *Arteriosclerosis, thrombosis, and vascular biology* 23(2): 168-175.

Bots, M. L., Westerink, J., Rabelink, T. J. & de Koning, E. J. 2005. Assessment of flow-mediated vasodilatation (FMD) of the brachial artery: effects of technical aspects of the FMD measurement on the FMD response. *European heart journal* 26(4): 363-368.

Bouïs, D., Hospers, G. A., Meijer, C., Molema, G. & Mulder, N. H. 2001. Endothelium in vitro: a review of human vascular endothelial cell lines for blood vessel-related research. *Angiogenesis* 4(2): 91-102.

Boulpaep, E. L. 2012. Arteries and veins. *Medical physiology* 2: 467-481.

Budhiraja, R., Tuder, R. M. & Hassoun, P. M. 2004. Endothelial dysfunction in pulmonary hypertension. *Circulation* 109(2): 159-165.

Butter, C., Stellbrink, C., Belalcazar, A., Villalta, D., Schlegl, M., Sinha, A., Cuesta, F. & Reister, C. 2004. Cardiac resynchronization therapy optimization by finger plethysmography. *Heart Rhythm* 1(5): 568-575.

Caballero, A. E. 2003. Endothelial dysfunction in obesity and insulin resistance: a road to diabetes and heart disease. *Obesity research* 11(11): 1278-1289.

Calles-Escandon, J. & Cipolla, M. 2001. Diabetes and endothelial dysfunction: a clinical perspective. *Endocrine reviews* 22(1): 36-52.

Camici, P. G. & Crea, F. 2007. Coronary microvascular dysfunction. *New England Journal of Medicine* 356(8): 830-840.

Celermajer, D. S. 1997. Endothelial dysfunction: does it matter? Is it reversible? *Journal of the American College of Cardiology* 30(2): 325-333.

Celermajer, D. S., Sorensen, K., Gooch, V., Sullivan, I., Lloyd, J., Deanfield, J. & Spiegelhalter, D. 1992. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. *The Lancet* 340(8828): 1111-1115.

Charakida, M., de Groot, E., Loukogeorgakis, S. P., Khan, T., Lüscher, T., Kastelein, J. J., Gasser, T. & Deanfield, J. E. 2013. Variability and reproducibility of flow-mediated dilatation in a multicentre clinical trial. *European heart journal* 34(45): 3501-3507.

Chellappan, K. 2009. Non-invasive vascular risk prediction by Photoplethysmography. Thesis PhD, Universiti Kebangsaan Malaysia.

Chen, C.-H., Nevo, E., Fetics, B., Pak, P. H., Yin, F. C., Maughan, W. L. & Kass, D. A. 1997. Estimation of central aortic pressure waveform by mathematical transformation of radial tonometry pressure validation of generalized transfer function. *Circulation* 95(7): 1827-1836.

Chowienczyk, P. J., Kelly, R. P., MacCallum, H., Millasseau, S. C., Andersson, T. L., Gosling, R. G., Ritter, J. M. & Änggård, E. E. 1999. Photoplethysmographic assessment of pulse wave reflection: blunted response to endothelium-dependent beta2-adrenergic vasodilation in type II diabetes mellitus. *Journal of the American College of Cardiology* 34(7): 2007-2014.

Corretti, M. C., Anderson, T. J., Benjamin, E. J., Celermajer, D., Charbonneau, F., Creager, M. A., Deanfield, J., Drexler, H., Gerhard-Herman, M. & Herrington, D. 2002. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. *Journal of the American College of Cardiology* 39(2): 257-265.

Curry, F. & Adamson, R. 2012. Endothelial glycocalyx: permeability barrier and mechanosensor. *Annals of biomedical engineering* 40(4): 828-839.

Custodis, F., Schirmer, S. H., Baumhäkel, M., Heusch, G., Böhm, M. & Laufs, U. 2010. Vascular pathophysiology in response to increased heart rate. *Journal of the American College of Cardiology* 56(24): 1973-1983.

Dahlöf, B. 2010. Cardiovascular disease risk factors: epidemiology and risk assessment. *The American journal of cardiology* 105(1): 3A-9A.

Daneshfard, B., Yarmohammadi, H. & Dalfardi, B. 2014. The origins of the theory of capillary circulation. *International journal of cardiology* 172(2): 491-492.

Davignon, J. & Ganz, P. 2004. Role of endothelial dysfunction in atherosclerosis. *Circulation* 109(23 suppl 1): III-27-III-32.

De Vriese, A. S., Verbeuren, T. J., Van de Voorde, J., Lameire, N. H. & Vanhoutte, P. M. 2000. Endothelial dysfunction in diabetes. *British journal of pharmacology* 130(5): 963-974.

Devaraj, S., Kumaresan, P. R. & Jialal, I. 2011. C-reactive protein induces release of both endothelial microparticles and circulating endothelial cells in vitro and in vivo: further evidence of endothelial dysfunction. *Clinical chemistry* 57(12): 1757-1761.

Dignat-George, F. & Boulanger, C. M. 2011. The many faces of endothelial microparticles. *Arteriosclerosis, thrombosis, and vascular biology* 31(1): 27-33.

Ding, F.-H., Fan, W.-X., Zhang, R.-Y., Zhang, Q., Li, Y. & Wang, J.-G. 2011. Validation of the noninvasive assessment of central blood pressure by the SphygmoCor and Omron devices against the invasive catheter measurement. *American journal of hypertension* 24(12): 1306-1311.

Drexler, H. & Zeiher, A. 1991. Endothelial function in human coronary arteries in vivo. Focus on hypercholesterolemia. *Hypertension* 18(4 Suppl): II90.

Du Clos, T. W. 2000. Function of C-reactive protein. *Annals of medicine* 32(4): 274-278.

Dyson, K. S., Shoemaker, J. K. & Hughson, R. L. 2006. Effect of acute sympathetic nervous system activation on flow-mediated dilation of brachial artery. *American Journal of Physiology-Heart and Circulatory Physiology* 290(4): H1446-H1453.

Faizi, A. K., Kornmo, D. W. & Agewall, S. 2009. Evaluation of endothelial function using finger plethysmography. *Clinical physiology and functional imaging* 29(5): 372-375.

Félétou, M. 2011. The endothelium, Part I: Multiple functions of the endothelial cells--focus on endothelium-derived vasoactive mediators. *Colloquium Series on Integrated Systems Physiology: From Molecule to Function*, hlm. 1-306.

Finegold, J. A., Asaria, P. & Francis, D. P. 2013. Mortality from ischaemic heart disease by country, region, and age: Statistics from World Health Organisation and United Nations. *International journal of cardiology* 168(2): 934-945.

Flammer, A. J., Anderson, T., Celermajer, D. S., Creager, M. A., Deanfield, J., Ganz, P., Hamburg, N. M., Lüscher, T. F., Shechter, M. & Taddei, S. 2012. The assessment of endothelial function from research into clinical practice. *Circulation* 126(6): 753-767.

Foo, J. Y. A., Leong, W.-Y., Wilson, S. J. & Homer, J. 2005. Adaptive algorithms to optimise photoplethysmographic signals in heart rate estimation. *IASTED International Conference*, hlm. 331-334.

Fung, Y.-c. 2013. *Biomechanics: circulation*. Springer Science & Business Media.

Glantz, S. A. 2005. Primer of biostatistics.

Go, A. S., Mozaffarian, D., Roger, V. L., Benjamin, E. J., Berry, J. D., Blaha, M. J., Dai, S., Ford, E. S., Fox, C. S. & Franco, S. 2014. Heart disease and stroke statistics--2014 update: a report from the American Heart Association. *Circulation* 129(3): e28.

Goldberger, A. L. 2012. *Clinical electrocardiography: a simplified approach*. Elsevier Health Sciences.

Green, D. J., Dawson, E. A., Groenewoud, H. M., Jones, H. & Thijssen, D. H. 2014. Is flow-mediated dilation nitric oxide mediated? A meta-analysis. *Hypertension* 63(2): 376-382.

Grootenboer, N., Hunink, M. M., Hendriks, J. M., van Sambeek, M. R. & Buth, J. 2013. Sex differences in 30-day and 5-year outcomes after endovascular repair of abdominal aortic aneurysms in the EUROSTAR study. *Journal of vascular surgery* 58(1): 42-49. e41.

Guelen, I., Westerhof, B. E., van der Sar, G. L., van Montfrans, G. A., Kiemeneij, F., Wesseling, K. H. & Bos, W. J. W. 2008. Validation of brachial artery pressure reconstruction from finger arterial pressure. *Journal of hypertension* 26(7): 1321-1327.

Harris, R. A., Nishiyama, S. K., Wray, D. W. & Richardson, R. S. 2010. Ultrasound assessment of flow-mediated dilation. *Hypertension* 55(5): 1075-1085.

Harvey, W. 2006. *The circulation of the blood*. Cosimo, Inc.

Haverich, A. & Wilhelmi, M. 2011. Heart and Cardiovascular Engineering. *Tissue Engineering*: 317-333.

Hayward, C. S., Kraidly, M., Webb, C. M. & Collins, P. 2002. Assessment of endothelial function using peripheral waveform analysis: a clinical application. *Journal of the American College of Cardiology* 40(3): 521-528.

Herrington, D. M., Fan, L., Drum, M., Riley, W. A., Pusser, B. E., Crouse, J. R., Burke, G. L., McBurnie, M. A., Morgan, T. M. & Espeland, M. A. 2001. Brachial flow-mediated vasodilator responses in population-based research: methods, reproducibility and effects of age, gender and baseline diameter. *Journal of cardiovascular risk* 8(5): 319-328.

Hickson, S. S., Butlin, M., Broad, J., Avolio, A. P., Wilkinson, I. B. & McEniery, C. M. 2009. Validity and repeatability of the Vicorder apparatus: a comparison with the SphygmoCor device. *Hypertension Research* 32(12): 1079-1085.

Higashi, Y., Kihara, Y. & Noma, K. 2012. Endothelial dysfunction and hypertension in aging. *Hypertension Research* 35(11): 1039-1047.

Hirata, K., Kawakami, M. & O'Rourke, M. F. 2006. Pulse wave analysis and pulse wave velocity. *Circulation Journal* 70(10): 1231-1239.

Hu, R., Wang, W. Q., Lau, C. P. & Tse, H. F. 2008. Gender Differences on Brachial Flow-mediated Dilation and Carotid Intima-media Thickness for Prediction of Spontaneous Cardiovascular Events. *Clinical cardiology* 31(11): 525-530.

Hulley, S. B., Cummings, S. R., Browner, W. S., Grady, D. G. & Newman, T. B. 2013. *Designing clinical research*. Lippincott Williams & Wilkins.

Huntsman, L., Stewart, D., Barnes, S., Franklin, S., Colocousis, J. & Hessel, E. 1983. Noninvasive Doppler determination of cardiac output in man. Clinical validation. *Circulation* 67(3): 593-602.

Indrayan, A. 2012. *Medical biostatistics*. CRC Press.

Insull, W. 2009. The pathology of atherosclerosis: plaque development and plaque responses to medical treatment. *The American journal of medicine* 122(1): S3-S14.

Itamar. 2009. endoPAT. http://www.itamar-medical.com/ [

Jaafar, R. 2009. Development of a noninvasive technique for endothelial dysfunction ssessment using photoplethysmography. Thesis PhD, Electrical engineering Universiti Kebangsaan Malaysia.

Jadhav, U. M. & Kadam, N. 2004. Non-invasive assessment of arterial stiffness by pulse-wave velocity correlates with endothelial dysfunction. *Indian heart journal* 57(3): 226-232.

Jalali, A., Ghaffari, A., Ghorbanian, P. & Nataraj, C. 2011. Identification of sympathetic and parasympathetic nerves function in cardiovascular regulation using ANFIS approximation. *Artificial Intelligence in Medicine* 52(1): 27-32.

Jambrik, Z., Venneri, L., Varga, A., Rigo, F., Borges, A. & Picano, E. 2004. Peripheral vascular endothelial function testing for the diagnosis of coronary artery disease. *American heart journal* 148(4): 684-689.

Järvisalo, M. J., Raitakari, M., Toikka, J. O., Putto-Laurila, A., Rontu, R., Laine, S., Lehtimäki, T., Rönnemaa, T., Viikari, J. & Raitakari, O. T. 2004. Endothelial dysfunction and increased arterial intima-media thickness in children with type 1 diabetes. *Circulation* 109(14): 1750-1755.

Karsan, A. & Dauphinee, S. 2010. *Endothelial dysfunction and inflammation*. Springer Basel AG.

Kohler, M., Craig, S., Nicoll, D., Leeson, P., Davies, R. J. & Stradling, J. R. 2008. Endothelial function and arterial stiffness in minimally symptomatic obstructive sleep apnea. *American journal of respiratory and critical care medicine* 178(9): 984-988.

Kuvin, J. T., Patel, A. R., Sliney, K. A., Pandian, N. G., Rand, W. M., Udelson, J. E. & Karas, R. H. 2001. Peripheral vascular endothelial function testing as a noninvasive indicator of coronary artery disease. *Journal of the American College of Cardiology* 38(7): 1843-1849.

Labovitz, A. J., Buckingham, T. A., Habermehl, K., Nelson, J., Kennedy, H. L. & Williams, G. A. 1985. The effects of sampling site on the two-dimensional echo-Doppler determination of cardiac output. *American heart journal* 109(2): 327-332.

Landmesser, U. & Drexler, H. 2007. Endothelial function and hypertension. *Current opinion in cardiology* 22(4): 316-320.

Laurent, S., Boutouyrie, P., Asmar, R., Gautier, I., Laloux, B., Guize, L., Ducimetiere, P. & Benetos, A. 2001. Aortic stiffness is an independent predictor of all-cause and cardiovascular mortality in hypertensive patients. *Hypertension* 37(5): 1236-1241.

Lee, H.-Y. & Oh, B.-H. 2010. Aging and arterial stiffness. *Circulation Journal* 74(11): 2257.

Leonard, P. A., Douglas, J. G., Grubb, N. R., Clifton, D., Addison, P. S. & Watson, J. N. 2006. A fully automated algorithm for the determination of respiratory rate from the photoplethysmogram. *Journal of clinical monitoring and computing* 20(1): 33-36.

Lerman, A. & Zeiher, A. M. 2005. Endothelial function cardiac events. *Circulation* 111(3): 363-368.

Levick, J. R. 2013. *An introduction to cardiovascular physiology*. Butterworth-Heinemann.

Libby, P., Ridker, P. M. & Maseri, A. 2002. Inflammation and atherosclerosis. *Circulation* 105(9): 1135-1143.

Lind, L., Pettersson, K. & Johansson, K. 2003. Analysis of endothelium-dependent vasodilation by use of the radial artery pulse wave obtained by applanation tonometry. *Clinical physiology and functional imaging* 23(1): 50-57.

Ljung, L. 1999. *System Identification: theory for the user*. Upper Saddle River, New Jersey 07458: Prentice Hall,.

Ljung, L. 2010. Perspectives on system identification. *Annual Reviews in Control* 34(1): 1-12.

Lowe, A., Harrison, W., El-Aklouk, E., Ruygrok, P. & Al-Jumaily, A. 2009. Non-invasive model-based estimation of aortic pulse pressure using suprasystolic brachial pressure waveforms. *Journal of biomechanics* 42(13): 2111-2115.

Ludmer, P. L., Selwyn, A. P., Shook, T. L., Wayne, R. R., Mudge, G. H., Alexander, R. W. & Ganz, P. 1986. Paradoxical vasoconstriction induced by acetylcholine in atherosclerotic coronary arteries. *New England Journal of Medicine* 315(17): 1046-1051.

Lund, F. 1986. Digital pulse plethysmography (DPG) in studies of the hemodynamic response to nitrates—a survey of recording methods and principles of analysis. *Acta pharmacologica et toxicologica* 59(S6): 79-96.

Luo, J., Ying, K. & Bai, J. 2005. Savitzky–Golay smoothing and differentiation filter for even number data. *Signal Processing* 85(7): 1429-1434.

Luo, J., Ying, K., He, P. & Bai, J. 2005. Properties of Savitzky–Golay digital differentiators. *Digital Signal Processing* 15(2): 122-136.

Matsuzawa, Y., Sugiyama, S., Sumida, H., Sugamura, K., Nozaki, T., Ohba, K., Matsubara, J., Kurokawa, H., Fujisue, K. & Konishi, M. 2013. Peripheral endothelial function and cardiovascular events in high-risk patients. *Journal of the American Heart Association* 2(6): e000426.

Mausbach, B. T., Chattillion, E., Roepke, S. K., Ziegler, M. G., Milic, M., von Känel, R., Dimsdale, J. E., Mills, P. J., Patterson, T. L. & Allison, M. A. 2012. A longitudinal analysis of the relations among stress, depressive symptoms, leisure satisfaction, and endothelial function in caregivers. *Health Psychology* 31(4): 433.

Mazumdar, J. 2015. *Biofluid mechanics*. World Scientific.

McEniery, C. M., Cockcroft, J. R., Roman, M. J., Franklin, S. S. & Wilkinson, I. B. 2014. Central blood pressure: current evidence and clinical importance. *European heart journal* 35(26): 1719-1725.

McEniery, C. M., Hall, I. R., Qasem, A., Wilkinson, I. B. & Cockcroft, J. R. 2005. Normal vascular aging: differential effects on wave reflection and aortic pulse wave velocity: the Anglo-Cardiff Collaborative Trial (ACCT). *Journal of the American College of Cardiology* 46(9): 1753-1760.

McEniery, C. M., Hall, I. R., Qasem, A., Wilkinson, I. B., Cockcroft, J. R. & Investigators, A. 2005. Normal vascular aging: differential effects on wave reflection and aortic pulse wave velocity: the Anglo-Cardiff Collaborative Trial (ACCT). *Journal of the American College of Cardiology* 46(9): 1753-1760.

McEniery, C. M., Wallace, S., Mackenzie, I. S., McDonnell, B., Newby, D. E., Cockcroft, J. R. & Wilkinson, I. B. 2006. Endothelial function is associated with pulse pressure, pulse wave velocity, and augmentation index in healthy humans. *Hypertension* 48(4): 602-608.

Mehta, D. & Malik, A. B. 2006. Signaling mechanisms regulating endothelial permeability. *Physiological reviews* 86(1): 279-367.

Meigs, J. B., Hu, F. B., Rifai, N. & Manson, J. E. 2004. Biomarkers of endothelial dysfunction and risk of type 2 diabetes mellitus. *JAMA* 291(16): 1978-1986.

Meigs, J. B., O'Donnell, C. J., Tofler, G. H., Benjamin, E. J., Fox, C. S., Lipinska, I., Nathan, D. M., Sullivan, L. M., D'Agostino, R. B. & Wilson, P. W. 2006. Hemostatic markers of endothelial dysfunction and risk of incident type 2 diabetes the Framingham Offspring Study. *Diabetes* 55(2): 530-537.

Millasseau, S., Kelly, R., Ritter, J. & Chowienczyk, P. 2002. Determination of age-related increases in large artery stiffness by digital pulse contour analysis. *Clinical Science* 103(4): 371-378.

Moerland, M., Kales, A., Schrier, L., Van Dongen, M., Bradnock, D. & Burggraaf, J. 2012. Evaluation of the EndoPAT as a tool to assess endothelial function. *International journal of vascular medicine* 2012:

Morrow, D. A. & Ridker, P. M. 2000. C-reactive protein, inflammation, and coronary risk. *Medical Clinics of North America* 84(1): 149-161.

Mudau, M., Genis, A., Lochner, A. & Strijdom, H. 2012. Endothelial dysfunction: the early predictor of atherosclerosis: review article. *Cardiovascular journal of Africa* 23(4): 222-231.

Muiesan, M. L., Salvetti, M., Rizzoni, D., Paini, A., Agabiti-Rosei, C., Aggiusti, C., Bertacchini, F., Stassaldi, D., Gavazzi, A. & Porteri, E. 2012. Pulsatile hemodynamics and microcirculation. *Hypertension*: HYPERTENSIONAHA. 111.00006.

Murthy, V. L., Naya, M., Foster, C. R., Gaber, M., Hainer, J., Klein, J., Dorbala, S., Blankstein, R. & Di Carli, M. F. 2012. Association between coronary vascular dysfunction and cardiac mortality in patients with and without diabetes mellitus. *Circulation*: CIRCULATIONAHA. 112.120402.

Nichols, W., O'Rourke, M. & Vlachopoulos, C. 2011. *McDonald's blood flow in arteries: theoretical, experimental and clinical principles*. CRC Press.

Nilsson, L., Johansson, A. & Kalman, S. 2000. Monitoring of respiratory rate in postoperative care using a new photoplethysmographic technique. *Journal of clinical monitoring and computing* 16(4): 309-315.

Nitzan, M., Khanokh, B. & Slovik, Y. 2002. The difference in pulse transit time to the toe and finger measured by photoplethysmography. *Physiological measurement* 23(1): 85.

Nohria, A., Gerhard-Herman, M., Creager, M. A., Hurley, S., Mitra, D. & Ganz, P. 2006. Role of nitric oxide in the regulation of digital pulse volume amplitude in humans. *Journal of applied physiology* 101(2): 545-548.

North, B. J. & Sinclair, D. A. 2012. The intersection between aging and cardiovascular disease. *Circulation research* 110(8): 1097-1108.

Nowbar, A. N., Howard, J. P., Finegold, J. A., Asaria, P. & Francis, D. P. 2014. 2014 Global geographic analysis of mortality from ischaemic heart disease by country, age and income: Statistics from World Health Organisation and United Nations. *International journal of cardiology* 174(2): 293-298.

Nugent, H. M. & Edelman, E. R. 2003. Tissue engineering therapy for cardiovascular disease. *Circulation research* 92(10): 1068-1078.

Ohte, N., Saeki, T., Miyabe, H., Sakata, S., Mukai, S., Hayano, J., Niki, K., Sugawara, M. & Kimura, G. 2007. Relationship between blood pressure obtained from the upper arm with a cuff-type sphygmomanometer and central blood pressure

measured with a catheter-tipped micromanometer. *Heart and vessels* 22(6): 410-415.

Organization, W. H. 2010. *World health statistics 2010*. World Health Organization.

P. Segers. 2010. *Vascular biomechanics and hemodynamics*.

Pauca, A. L., O'Rourke, M. F. & Kon, N. D. 2001. Prospective evaluation of a method for estimating ascending aortic pressure from the radial artery pressure waveform. *Hypertension* 38(4): 932-937.

Pemberton, J., Li, X., Kenny, A., Davies, C. H., Minette, M. S. & Sahn, D. J. 2005. Real-time 3-dimensional Doppler echocardiography for the assessment of stroke volume: an in vivo human study compared with standard 2-dimensional echocardiography. *Journal of the American Society of Echocardiography* 18(10): 1030-1036.

Peretz, A., Leotta, D. F., Sullivan, J. H., Trenga, C. A., Sands, F. N., Aulet, M. R., Paun, M., Gill, E. A. & Kaufman, J. D. 2007. Flow mediated dilation of the brachial artery: an investigation of methods requiring further standardization. *BMC cardiovascular disorders* 7(1): 11.

Perticone, F., Ceravolo, R., Pujia, A., Ventura, G., Iacopino, S., Scozzafava, A., Ferraro, A., Chello, M., Mastroroberto, P. & Verdecchia, P. 2001. Prognostic significance of endothelial dysfunction in hypertensive patients. *Circulation* 104(2): 191-196.

Pini, R., Cavallini, M. C., Palmieri, V., Marchionni, N., Di Bari, M., Devereux, R. B., Masotti, G. & Roman, M. J. 2008. Central but not brachial blood pressure predicts cardiovascular events in an unselected geriatric population: the ICARe Dicomano Study. *Journal of the American College of Cardiology* 51(25): 2432-2439.

Pollonini, L., Padhye, N., Re, R., Torricelli, A., Simpson, R. & Dacso, C. 2015. Pulse transit time measured by photoplethysmography improves the accuracy of heart rate as a surrogate measure of cardiac output, stroke volume and oxygen uptake in response to graded exercise. *Physiological measurement* 36(5): 911.

Pries, A. R. & Secomb, T. W. 2005. Microvascular blood viscosity in vivo and the endothelial surface layer. *American Journal of Physiology-Heart and Circulatory Physiology* 289(6): H2657-H2664.

Puddu, P., Puddu, G. M., Zaca, F. & Muscari, A. 2000. Endothelial dysfunction in hypertension. *Acta cardiologica* 55(4): 221-232.

Puri, R., Liew, G. Y., Nicholls, S. J., Nelson, A. J., Leong, D. P., Carbone, A., Copus, B., Wong, D. T., Beltrame, J. F. & Worthley, S. G. 2012. Coronary β2-adrenoreceptors mediate endothelium-dependent vasoreactivity in humans: novel insights from an in vivo intravascular ultrasound study. *European heart journal* 33(4): 495-504.

Pyke, K. E. & Tschakovsky, M. E. 2007. Peak vs. total reactive hyperemia: which determines the magnitude of flow-mediated dilation? *Journal of applied physiology* 102(4): 1510-1519.

Quipu. 2014. http://www.quipu.eu/cardiovascular-suite/fmd-studio/ [

Recio-Mayoral, A., Banerjee, D., Streather, C. & Kaski, J. C. 2011. Endothelial dysfunction, inflammation and atherosclerosis in chronic kidney disease–a cross-sectional study of predialysis, dialysis and kidney-transplantation patients. *Atherosclerosis* 216(2): 446-451.

Reinhold, H., Fajardo, L. & Hopewell, J. 2012. The vascular system. *Advances in radiation biology* 14: 177-226.

Roman, M. J., Devereux, R. B., Kizer, J. R., Lee, E. T., Galloway, J. M., Ali, T., Umans, J. G. & Howard, B. V. 2007. Central pressure more strongly relates to vascular disease and outcome than does brachial pressure the strong heart study. *Hypertension* 50(1): 197-203.

Roman, M. J., Okin, P. M., Kizer, J. R., Lee, E. T., Howard, B. V. & Devereux, R. B. 2010. Relations of central and brachial blood pressure to left ventricular hypertrophy and geometry: the Strong Heart Study. *Journal of hypertension* 28(2): 384-388.

Romano, S. M. & Pistolesi, M. 2002. Assessment of cardiac output from systemic arterial pressure in humans. *Critical care medicine* 30(8): 1834-1841.

Rossi, R., Cioni, E., Nuzzo, A., Origliani, G. & Modena, M. G. 2005. Endothelial-dependent vasodilation and incidence of type 2 diabetes in a population of healthy postmenopausal women. *Diabetes care* 28(3): 702-707.

Ruiz-Vega, H., Benitez, G., Carranza-Madrigal, J. & Huape-Arreola, M. S. 1997. Evaluation of vasodilator effects by means of changes of photoplethysmographic pulse waveshape. *Proceedings of the Western Pharmacology Society*, hlm. 73-74.

Sader, M. A. & Celermajer, D. S. 2002. Endothelial function, vascular reactivity and gender differences in the cardiovascular system. *Cardiovascular research* 53(3): 597-604.

Safar, M. 2000. Pulse pressure, arterial stiffness, and cardiovascular risk. *Current opinion in cardiology* 15(4): 258-263.

Safar, M. E., Blacher, J., Pannier, B., Guerin, A. P., Marchais, S. J., Guyonvarc'h, P.-M. & London, G. M. 2002. Central pulse pressure and mortality in end-stage renal disease. *Hypertension* 39(3): 735-738.

Sahni, R. 2012. Noninvasive monitoring by photoplethysmography. *Clinics in perinatology* 39(3): 573-583.

Sandoo, A., van Zanten, J. J. V., Metsios, G. S., Carroll, D. & Kitas, G. D. 2010. The endothelium and its role in regulating vascular tone. *The open cardiovascular medicine journal* 4: 302.

Sanz, J. & Fayad, Z. A. 2008. Imaging of atherosclerotic cardiovascular disease. *Nature* 451(7181): 953-957.

Schafer, R. W. 2011. What is a Savitzky-Golay filter?[lecture notes]. *Signal Processing Magazine, IEEE* 28(4): 111-117.

Schalkwijk, C. & Stehouwer, C. D. 2005. Vascular complications in diabetes mellitus: the role of endothelial dysfunction. *Clinical Science* 109: 143-159.

Seals, D., Jablonski, K. & Donato, A. 2011. Aging and vascular endothelial function in humans. *Clinical Science* 120: 357-375.

Segers, P., Mahieu, D., Kips, J., Rietzschel, E., De Buyzere, M., De Bacquer, D., Bekaert, S., De Backer, G., Gillebert, T. & Verdonck, P. 2009. Amplification of the pressure pulse in the upper limb in healthy, middle-aged men and women. *Hypertension* 54(2): 414-420.

Segers, P., Rietzschel, E., Heireman, S., De Buyzere, M., Gillebert, T., Verdonck, P. & Van Bortel, L. 2005. Carotid tonometry versus synthesized aorta pressure waves for the estimation of central systolic blood pressure and augmentation index. *American journal of hypertension* 18(9): 1168-1173.

Sena, C. M., Pereira, A. M. & Seiça, R. 2013. Endothelial dysfunction—a major mediator of diabetic vascular disease. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease* 1832(12): 2216-2231.

Shariati, N. H. & Zahedi, E. 2005. Comparison of selected parametric models for analysis of the photoplethysmographic signal. *Computers, Communications, & Signal Processing with Special Track on Biomedical Engineering, 2005. CCSP 2005. 1st International Conference on*, hlm. 169-172.

Sharman, J. E., Lim, R., Qasem, A. M., Coombes, J. S., Burgess, M. I., Franco, J., Garrahy, P., Wilkinson, I. B. & Marwick, T. H. 2006. Validation of a generalized transfer function to noninvasively derive central blood pressure during exercise. *Hypertension* 47(6): 1203-1208.

Sherwood, A., Hinderliter, A. L., Watkins, L. L., Waugh, R. A. & Blumenthal, J. A. 2005. Impaired endothelial function in coronary heart disease patients with depressive symptomatology. *Journal of the American College of Cardiology* 46(4): 656-659.

Shi, P. 2009. Photoplethysmography in noninvasive cardiovascular assessment. Thesis © Ping Shi.

Shimokawa, H. 2016. Endothelial Dysfunction Assessment via Doppler wires. http://www.j-circ.or.jp [

Skaug, E.-A., Aspenes, S. T., Oldervoll, L., Mørkedal, B., Vatten, L., Wisløff, U. & Ellingsen, Ø. 2013. Age and gender differences of endothelial function in 4739 healthy adults: the HUNT3 Fitness Study. *European journal of preventive cardiology* 20(4): 531-540.

Solomon, H., Man, J. & Jackson, G. 2003. Erectile dysfunction and the cardiovascular patient: endothelial dysfunction is the common denominator. *Heart* 89(3): 251-253.

Stam, F., van Guldener, C., Becker, A., Dekker, J. M., Heine, R. J., Bouter, L. M. & Stehouwer, C. D. 2006. Endothelial dysfunction contributes to renal function–associated cardiovascular mortality in a population with mild renal insufficiency: The Hoorn study. *Journal of the American Society of Nephrology* 17(2): 537-545.

Stea, F., Bozec, E., Millasseau, S., Khettab, H., Boutouyrie, P. & Laurent, S. 2014. Comparison of the Complior Analyse device with Sphygmocor and Complior SP for pulse wave velocity and central pressure assessment. *Journal of hypertension* 32(4): 873-880.

Stoner, L. & Sabatier, M. J. 2012. Use of ultrasound for non-invasive assessment of flow-mediated dilation. *Journal of atherosclerosis and thrombosis* 19(5): 407-421.

Stouffer, G. A. 2016. *Cardiovascular hemodynamics for the clinician*. John Wiley & Sons.

Taimeh, Z., Loughran, J., Birks, E. J. & Bolli, R. 2013. Vascular endothelial growth factor in heart failure. *Nature Reviews Cardiology* 10(9): 519-530.

Takase, B. & Higashimura, Y. 2013. Disparity between endopat measurement and brachial artery flow-mediated vasodilatation in hypertensive patients. *Journal of the American College of Cardiology* 61(10): E2100.

Takazawa, K., Kobayashi, H., Shindo, N., Tanaka, N. & Yamashina, A. 2007. Relationship between radial and central arterial pulse wave and evaluation of central aortic pressure using the radial arterial pulse wave. *Hypertension Research* 30(3): 219.

Tesauro, M., Rizza, S., Iantorno, M., Campia, U., Cardillo, C., Lauro, D., Leo, R., Turriziani, M., Cocciolillo, G. C. & Fusco, A. 2007. Vascular, metabolic, and inflammatory abnormalities in normoglycemic offspring of patients with type 2 diabetes mellitus. *Metabolism* 56(3): 413-419.

Thijssen, D. H., Black, M. A., Pyke, K. E., Padilla, J., Atkinson, G., Harris, R. A., Parker, B., Widlansky, M. E., Tschakovsky, M. E. & Green, D. J. 2011. Assessment of flow-mediated dilation in humans: a methodological and physiological guideline. *American Journal of Physiology-Heart and Circulatory Physiology* 300(1): H2-H12.

Thijssen, D. H., Bullens, L. M., van Bemmel, M. M., Dawson, E. A., Hopkins, N., Tinken, T. M., Black, M. A., Hopman, M. T., Cable, N. T. & Green, D. J. 2009. Does arterial shear explain the magnitude of flow-mediated dilation?: a comparison between young and older humans. *American Journal of Physiology-Heart and Circulatory Physiology* 296(1): H57-H64.

Thijssen, D. H., van Bemmel, M. M., Bullens, L. M., Dawson, E. A., Hopkins, N. D., Tinken, T. M., Black, M. A., Hopman, M. T., Cable, N. T. & Green, D. J. 2008. The impact of baseline diameter on flow-mediated dilation differs in young and older humans. *American Journal of Physiology-Heart and Circulatory Physiology* 295(4): H1594-H1598.

Tierney, E. S. S., Newburger, J. W., Gauvreau, K., Geva, J., Coogan, E., Colan, S. D. & de Ferranti, S. D. 2009. Endothelial pulse amplitude testing: feasibility and reproducibility in adolescents. *The Journal of pediatrics* 154(6): 901-905.

Tousoulis, D., Kampoli, A.-M., Tentolouris Nikolaos Papageorgiou, C. & Stefanadis, C. 2012. The role of nitric oxide on endothelial function. *Current vascular pharmacology* 10(1): 4-18.

Van Bortel, L. M., Balkestein, E. J., van der Heijden-Spek, J. J., Vanmolkot, F. H., Staessen, J. A., Kragten, J. A., Vredeveld, J. W., Safar, M. E., Boudier, H. A. S. & Hoeks, A. P. 2001. Non-invasive assessment of local arterial pulse pressure: comparison of applanation tonometry and echo-tracking. *Journal of hypertension* 19(6): 1037-1044.

Van den Oever, I. A., Raterman, H. G., Nurmohamed, M. T. & Simsek, S. 2010. Endothelial dysfunction, inflammation, and apoptosis in diabetes mellitus. *Mediators of inflammation* 2010:

van Geldorp, I. E., Delhaas, T., Hermans, B., Vernooy, K., Broers, B., Klimusina, J., Regoli, F., Faletra, F. F., Moccetti, T. & Gerritse, B. 2011. Comparison of a non-invasive arterial pulse contour technique and echo Doppler aorta velocity-time integral on stroke volume changes in optimization of cardiac resynchronization therapy. *Europace* 13(1): 87-95.

Voss, A., Schulz, S., Schroeder, R., Baumert, M. & Caminal, P. 2009. Methods derived from nonlinear dynamics for analysing heart rate variability. *Philosophical Transactions of the Royal Society of London A: Mathematical, Physical and Engineering Sciences* 367(1887): 277-296.

Webster, J. G. 1997. *Design of pulse oximeters*. CRC Press.

Webster, J. G. 2002. *Design of pulse oximeters*. CRC Press.

Whinnett, Z. I., Davies, J. E., Willson, K., Chow, A. W., Foale, R. A., Davies, D. W., Hughes, A. D., Francis, D. P. & Mayet, J. 2006. Determination of optimal atrioventricular delay for cardiac resynchronization therapy using acute non-invasive blood pressure. *Europace* 8(5): 358-366.

Whittemore, S. 2009. *The circulatory system*. Infobase Publishing.

Wilkinson, I. B., Hall, I. R., MacCallum, H., Mackenzie, I. S., McEniery, C. M., van der Arend, B. J., Shu, Y.-E., MacKay, L. S., Webb, D. J. & Cockcroft, J. R. 2002. Pulse-wave analysis clinical evaluation of a noninvasive, widely applicable method for assessing endothelial function. *Arteriosclerosis, thrombosis, and vascular biology* 22(1): 147-152.

Williams, B. & Lacy, P. S. 2010. Central haemodynamics and clinical outcomes: going beyond brachial blood pressure?, Eur Soc Cardiology.

Xiao, X., Mullen, T. J. & Mukkamala, R. 2005. System identification: a multi-signal approach for probing neural cardiovascular regulation. *Physiological measurement* 26(3): R41.

Yu, C., Liu, Z., McKenna, T., Reisner, A. T. & Reifman, J. 2006. A method for automatic identification of reliable heart rates calculated from ECG and PPG waveforms. *Journal of the American Medical Informatics Association* 13(3): 309-320.

Zahedi, E., Jaafar, R., Ali, M. M., Mohamed, A. & Maskon, O. 2008. Finger photoplethysmogram pulse amplitude changes induced by flow-mediated dilation. *Physiological measurement* 29(5): 625.

Zou, K. H., O'Malley, A. J. & Mauri, L. 2007. Receiver-operating characteristic analysis for evaluating diagnostic tests and predictive models. *Circulation* 115(5): 654-657.

APPENDICES

Appendix A

ETHICS OF THE RESEARCH

Ethics

UNIVERSITI KEBANGSAAN MALAYSIA
The National University of Malaysia

Pusat Perubatan UKM | *UKM Medical Centre*

Jawatankuasa Etika Penyelidikan UKM **UKM 1.5.3.5/244/FF-2013-409**

28 Oktober 2013

Profesor Madya Dr. Oteh Maskon
Jabatan Perubatan
Pusat Perubatan Universiti Kebangsaan Malaysia

Tuan,

Kelulusan Etika Menjalankan Penyelidikan di UKM

Tajuk : *"Noninvasive Technique For Endothelial Dysfunction Assessment Using A Radial PPG-CAP Modeling In Healthy And Non-Healthy Subjects"*

Kod Projek : **FF-2013-409**

Perkara yang tersebut di atas adalah dirujuk.

Sukacita dimaklumkan, Jawatankuasa Etika Penyelidikan UKM meluluskan permohonan penyelidikan tuan bagi tajuk diatas. Tempoh kelulusan kajian adalah dari **1 Oktober 2013 – 31 Januari 2014.** Sila kemukakan sebarang **Laporan Kesan Sampingan** sebaik sahaja penyelidikan tamat kepada Jawatankuasa Etika Penyelidikan UKM.

Sekian, terima kasih.

Yang benar,

Profesor Madya (K) Dato' Dr Fuad Ismail
Pengerusi
Jawatankuasa Etika Penyelidikan UKM
Universiti Kebangsaan Malaysia

s.k.
- **Ketua Jabatan Perubatan**
  Pusat Perubatan Universiti Kebangsaan Malaysia
- **Prof. Dr. Mohd Alauddin Mohad Ali**
  Pengarah
  Institut Angkasa Sains Angkasa
  Universiti Kebangsaan Malaysia
- **Prof. Madya Dr. Edmond Zahedi**
  Fakulti Kejuruteraan Dan Alam Bina
  Universiti Kebangsaan Malaysia
- **Dr. Kalaivani A/p Chellapan**
  Institut Angkasa Sains Angkasa
  Universiti Kebangsaan Malaysia

Sekretariat Penyelidikan Perubatan & Inovasi, Pusat Perubatan Universiti Kebangsaan Malaysia
Tingkat 1, Blok Klinikal, Jalan Yaacob Latif, Bandar Tun Razak, 56000 Cheras Kuala Lumpur. MALAYSIA.
Telefon: **+603-9145 5002/5003/5046/5047/5048/6718/7164** Faksimili: **+603-9145 6634**
E-mel: sppi@ppukm.ukm.edu.my Laman Web: http://www.ppukm.ukm.my

APPENDIX B

CONCENT FORM

1) English Version

I _____ (patients' name), bearing a postal address at _____
have agreed to take part in the study:

"Noninvasive technique for Endothelial dysfunction assessment using a radial PPG-CAP modeling in healthy and non-healthy subjects"

The nature and purpose of the study has been explained to me in detail by Dr./Mr./Ms._____ who is carrying out the study. I was able to ask him/her questions regarding all aspects of the study. In addition, he/she had given me a copy of the participant information leaflet.

My identity will not be disclosed and I can withdraw from the study at any time, and it will not affect my treatment.

Signature of patient:

I/C No:

Date:

Signature of doctor:	Name:
Date:	I/CNo:

Signature of witness:	Name:
Date:	I/CNo:

2) Malay Version

1) Malay Version

Saya, _____ (namapesakit), yang beralamatdi _____ telahbersetujuuntukmengambilbahagiandalampenyelidikan yang bertajuk:

"Tekniksecaratidakinvasifuntukpenilaiandisfungsiendotelialdenganmenggunakanpemodelan PPG-CAP radial bagisubjekdalamkeadaan yang sihatdantidaksihat"

Sayatelahdiberipenjelasansepenuhnyaolen Dr._____ mengenaikeadaandantujuannya.
Sayadibenarkanuntukbertanyabeliaumengenaisemuaaspekpenyelidikanitu. Beliau juga telahmemberisayasatusalinankertasmaklumatuntukpesakit.
Identitisayaakandirahsiakandansayaberhakmenarikdiripadabila-bila masa, danianyatidakakanmempengaruhirawatansaya.

Tandatanganpeserta:

No.K.P:

Tarikh:

Tandatanganpegawaiperubatan: Nama penuh:
Tarikh: No.K.P.:

Tandatangansaksi: Nama penuh:
Tarikh: No.K.P.:

APPENDIX C

DATA COLLECTION FORM

Pre-pilot (FKAB)- FMD excluded	
Pilot	
Principal sampling (PPUKM)	

Number

Subject

Sampling

Date/Time

Session Number

PERSONAL PROFILE

Full Name

Date of Birth

Age:

Gender

Male	
Female	

Nationality/Ethnicity

Land line	
Cell Phone	

Email	

Contact Number

Weight (kg)	
Height (cm)	
Waist Diameter (cm)	
Body Temperature	ºC
Occupation	

Kindly answer the following questions:

1. Have you felt any pain across your chest? If so, when? How long it does normally takes?

2. Do you attend the physical activities frequently? How often?

3. Are you smoker? If so, for how long?

4. Have you had any heart failure, cardiovascular abnormalities or diabetic in your first order family? (Father, Mother or Siblings)

5. How long normally you sleep during a day?

In order to get a reliable result, you must follow the protocol strictly which described to you earlier. Kindly tick the headline of the protocol (below) **if** you follow it **otherwise** please declares the violation.

- ☐ Fasted over night (at least 12h)
- ☐ Avoid any physical exercise over last three days
- ☐ Stop medications since three days ago
- ☐ Do not be in menstruation cycle
- ☐ Do not drink any beverage over night

MEDICAL PROFILE

Blood Pressure

Left Arm	
Systolic (mmHg)	
Diastolic (mmHg)	
Pulse (BPM)	

Right Arm	
Systolic (mmHg)	
Diastolic (mmHg)	
Pulse (BPM)	

Averaged	
Systolic (mmHg)	
Diastolic (mmHg)	
Pulse (BPM)	

PATIENTS BASELINE DEMOGRAPHY & RISK FACTORS

- ☐ Hypertension
- ☐ Diabetic Mellitus
- ☐ Dyslipidemia (LDL > 3.4 TC > 5.6)
- ☐ Active Smoker (up to 1 month)
- ☐ Positive family heredity for permanent heart disease

NOTES TO OPERATOR

Terms and conditions of sampling

1. Vitamin supplementation and antioxidants are not allowed since three days before sampling

2. Medication is not allowed since three days before sampling
3. Smoking (even smoke exposure) is not allowed since one day before sampling
4. Not food and drink is allowed since 12 hours before sampling
5. Female shouldn't be in menstrual cycle
6. No physical exercise is allowed since 12 hours before sampling

TO OPERATOR …

1. Have you strictly followed the protocol of sampling and FMD test?

2. Have you seen any abnormality during the sampling? If so, please explain in details.

Name Date & time

APPENDIX D

MATLAB CODE

% Reading Data

```
clear all; clc
Subject="Subject ID";
WD=cd;

if Subject<10
Data_Folder=['F:\vahid\PhD\Raw Data FMD-US at HUKM 2014\Renamed Data for
Matlab Analysis\S00' int2str(Subject) '\Session 1']
Filename=['S00' int2str(Subject) ' Session1.txt']

elseif Subject<100
Data_Folder=['F:\vahid\PhD\Raw Data FMD-US at HUKM 2014\Renamed Data for
Matlab Analysis\S0' int2str(Subject) '\Session 1']
Filename=['S0' int2str(Subject) ' Session1.txt']

elseif Subject>=100
Data_Folder=['F:\vahid\PhD\Raw Data FMD-US at HUKM 2014\Renamed Data for
Matlab Analysis\S' int2str(Subject) '\Session 1']
Filename=['S' int2str(Subject) ' Session1.txt']
end

cd(Data_Folder)
signal=load(Filename);
cd(WD)
 RawCBP=detrend(signal(:,1));              % Derived CBP
 RawRPressure=detrend(signal(:,2));        % Radial Pressure
 RawRPPG=detrend(signal(:,3));             % Radial PPG
 RawFPPG=detrend(signal(:,4));             % Finger PPG


SF=(max(RawRPressure)-min(RawRPressure))/(max(RawRPPG)-min(RawRPPG));
RawRPPG=RawRPPG*SF;
N=length(RawRPPG);
```

```
CBP_BL=detrend(RawCBP(1:t_BL*Fs));
RPressure_BL=detrend(RawRPressure(1:t_BL*Fs));
RPPG_BL=detrend(RawRPPG(1:t_BL*Fs));
FPPG_BL=detrend(RawFPPG(1:t_BL*Fs));

% Find&Cancel delay

S1=RPPG_BL;S2=CBP_BL;
C=xcorr(S1,S2);
[Value Idx] =max(C);
Delay_Signals_Baseline=length(S1)-Idx;

if Delay_Signals_Baseline> 0
S2=S2(1+Delay_Signals_Baseline:end);
S1=S1(1:end-Delay_Signals_Baseline);

else
S1=S1(1-Delay_Signals_Baseline:end);
S2=S2(1:end+Delay_Signals_Baseline);
end

CBP_BL=S2;
RPPG_BL=S1;

LPF=VS_2_LPF1;B=LPF.Numerator;

 CBP_BL=filter(B,1,CBP_BL);              % Filtered CBP
 RPressure_BL=filter(B,1,RPressure_BL);  % Filtered Radial pressure
 RPPG_BL=filter(B,1,RPPG_BL);            % Filtered radial PPG
 FPPG_BL=filter(B,1,FPPG_BL);            % Filtered finger PPG


CBP_BL=detrend(resample(CBP_BL,100,Fs));
RPressure_BL=detrend(resample(RPressure_BL,100,Fs));
RPPG_BL=detrend(resample(RPPG_BL,100,Fs));
FPPG_BL=detrend(resample(FPPG_BL,100,Fs));

% Definition of Input/Output and Segmentation

IN=CBP_BL;OUT=RPPG_BL;

disp('Calculating Baseline Fitness Values...')
```

```
seg=3; fs=100;
num_seg=floor(length(CBP_BL)/(seg*fs));
segments_IN=zeros(2*fs,num_seg);
segments_OUT=zeros(2*fs,num_seg);

SEGMENTS_IN_BL=cell(num_seg,1);
SEGMENTS_OUT_BL=cell(num_seg,1);

Fitness_idv=[];Fitness_avr=[];index=[];SF2=[];SF3=[];
fit=[];Coeff_A_BL=[];Coeff_B_BL=[];Just_Fitness=[];

for i=1:num_seg;
start=(i-1)*fs+1;
stop=start+(seg*fs)-1;
segments_IN(:,i)=detrend(IN(start:stop));
segments_OUT(:,i)=detrend(OUT(start:stop));
SF2=[SF2 ; max(segments_IN(:,i))];
SF3=[SF3 ; max(segments_OUT(:,i))];

segments_IN(:,i)=(segments_IN(:,i))/max(segments_IN(:,i));
segments_OUT(:,i)=(segments_OUT(:,i))/max(segments_OUT(:,i));

S1=segments_IN(:,i);S2=segments_OUT(:,i);

Find_Delay;Cancel_Delay;

SEGMENTS_IN_BL{i,1}=S1;
SEGMENTS_OUT_BL{i,1}=S2;

% Creation of time-series data in Baseline

DATA=iddata(SEGMENTS_OUT_BL{i,1},SEGMENTS_IN_BL{i,1},1/fs);
opt=arxOptions('focus','stability');
Model_each_Seg=arx(DATA,[4 4 0],opt);
[y_idv, fit_idv, x0_idv] = compare(DATA,Model_each_Seg);
Fitness_idv(i,1)=[fit_idv];

% Obtain model coefficients

[Coeff_A_BL(i,:),Coeff_B_BL(i,:)] = polydata(Model_each_Seg);
Fitness_idv(i,2)=Coeff_A_BL(i,1);
Fitness_idv(i,3)=Coeff_A_BL(i,2);
Fitness_idv(i,4)=Coeff_A_BL(i,3);
```

```
Fitness_idv(i,5)=Coeff_A_BL(i,4);
Fitness_idv(i,6)=Coeff_A_BL(i,5);

Fitness_idv(i,7)=Coeff_B_BL(i,1);
Fitness_idv(i,8)=Coeff_B_BL(i,2);
Fitness_idv(i,9)=Coeff_B_BL(i,3);
Fitness_idv(i,10)=Coeff_B_BL(i,4);
Just_Fitness(i,1)=Fitness_idv(i,1);

Reference=70;
index=find(Just_Fitness<Reference);
end

Fitness_idv(index,:)=[];
[row,column]=size(Fitness_idv);
for i=1:row;
Avr_A0=1;
Avr_A1=sum(Fitness_idv(:,3))/row;
Avr_A2=sum(Fitness_idv(:,4))/row;
Avr_A3=sum(Fitness_idv(:,5))/row;
Avr_A4=sum(Fitness_idv(:,6))/row;

Avr_B1=sum(Fitness_idv(:,7))/row;
Avr_B2=sum(Fitness_idv(:,8))/row;
Avr_B3=sum(Fitness_idv(:,9))/row;
Avr_B4=sum(Fitness_idv(:,10))/row;
```

% Definition of an Averaged model in BaseLine(BL)

```
A=[Avr_A0 Avr_A1 Avr_A2 Avr_A3 Avr_A4];
B=[Avr_B1 Avr_B2 Avr_B3 Avr_B4];
Model_Avr_BL=idpoly(A,B,[],[],[],0,1/fs);
end
```

% Apply the Averaged model to all segments

```
for i=1:num_seg;
%DATA=iddata(segments_OUT(:,i),segments_IN(:,i),1/fs);
DATA=iddata(SEGMENTS_OUT_BL{i,1},SEGMENTS_IN_BL{i,1},1/fs);
[y_avr, fit_avr(i,:), x0_avr] = compare(DATA,Model_Avr_BL);
Fitness_avr_BL=[fit_avr];
end
```

%Data Analysis in Release:

```
Number_Total_Segments_BL=num_seg;
Reference;
Number_Fitness_Below_Reference=length(index);
Number_segments_above_Reference=(num_seg)-(length(index));

CBP_RLS=detrend(RawCBP((t_RLS*Fs):end));
RPressure_RLS=detrend(RawRPressure((t_RLS*Fs):end));
RPPG_RLS=detrend(RawRPPG((t_RLS*Fs):end));
FPPG_RLS=detrend(RawFPPG((t_RLS*Fs):end));

S1=RPPG_RLS;S2=CBP_RLS;
C=xcorr(S1,S2);
[Value Idx] =max(C);
Delay_Signals_Release=length(S1)-Idx;

if Delay_Signals_Release> 0
S2=S2(1+Delay_Signals_Release:end);
S1=S1(1:end-Delay_Signals_Release);

else
S1=S1(1-Delay_Signals_Release:end);
S2=S2(1:end+Delay_Signals_Release);
end

RPPG_RLS=S1; CBP_RLS=S2;

LPF=VS_2_LPF1;B=LPF.Numerator;

 CBP_RLS=filter(B,1,CBP_RLS);              % Filtered CBP
 RPressure_RLS=filter(B,1,RPressure_RLS);  % Filtered Radial pressure
 RPPG_RLS=filter(B,1,RPPG_RLS);            % Filtered radial PPG
 FPPG_RLS=filter(B,1,FPPG_RLS);            % Filtered finger PPG

CBP_RLS=detrend(resample(CBP_RLS,100,Fs));
RPressure_RLS=detrend(resample(RPressure_RLS,100,Fs));
RPPG_RLS=detrend(resample(RPPG_RLS,100,Fs));
FPPG_RLS=detrend(resample(FPPG_RLS,100,Fs));
```

% Creation of time-series data in Release

```
IN=CBP_RLS;OUT=RPPG_RLS;
```

```
disp('Calculating Release Phase Fitness Values...')
Fitness=[];
Nb_Seg_IN=floor(length(IN)/(length_seg*fs));
IN_seg=zeros(2*fs,Nb_Seg_IN);
OUT_seg=zeros(2*fs,Nb_Seg_IN);

SEGMENTS_IN_RLS=cell(Nb_Seg_IN,1);
SEGMENTS_OUT_RLS=cell(Nb_Seg_IN,1);

Fitness_idv_RLS=[];Coeff_A_RLS=[];Coeff_B_RLS=[];
stop=0;

for i=1:Nb_Seg_IN;
start=stop+1;
stop=start+(length_seg*fs)-1;
IN_seg(:,i)=detrend(IN(start:stop));
OUT_seg(:,i)=detrend(OUT(start:stop));

IN_seg(:,i)=(IN_seg(:,i))/max(IN_seg(:,i));
OUT_seg(:,i)=(OUT_seg(:,i))/max(OUT_seg(:,i));

S1=IN_seg(:,i);S2=OUT_seg(:,i);
Find_Delay;Cancel_Delay;
SEGMENTS_IN_RLS{i,1}=S1;
SEGMENTS_OUT_RLS{i,1}=S2;

% Creation of time-series data in Release

DATA_RLS=iddata(SEGMENTS_OUT_RLS{i,1},SEGMENTS_IN_RLS{i,1},1/fs);
% Apply the averaged model (in BL) to all segments in RLS
[y_RLS, fit_RLS(i,:), x0_RLS] = compare(DATA_RLS,Model_Avr_BL);
Fitness_avr_RLS=[fit_RLS];
end

% Computation of Mean Values

[row1,column1]=size(Just_Fitness);
Mean_Self_Fitness_BL=sum(Just_Fitness(:,1))/row1;

[row2,column2]=size(Fitness_avr_BL);
Mean_Fitness_avr_BL=sum(Fitness_avr_BL(:,1))/row2;
```

```
[row3,column3]=size(Fitness_avr_RLS);
Mean_Fitness_avr_RLS=sum(Fitness_avr_RLS(:,1))/row3;

frame=round((Nb_Seg_IN)/2);
remainder = rem( frame,2);

if  remainder==0;
frame=(frame)+1;
end

frame=(frame)-10;
Filtered_Fitness_avr_RLS = sgolayfilt(Fitness_avr_RLS,4,frame);
frame_BL=round((num_seg)/2);
remainder = rem( frame_BL,2);

if  remainder==0;
frame_BL=(frame_BL)+1;
end

frame_BL=(frame_BL)-10;
Filtered_Fitness_avr_BL = sgolayfilt(Fitness_avr_BL,4,frame_BL);

% window1; t=0:1min

for i=1:30;
window1(i,1)=Filtered_Fitness_avr_RLS(i,1);
end

[row4,column4]=size(window1);
Mean_Fitness_avr_RLS_window1=sum(window1(:,1))/row4;

y2_1=max(window1);
y1_1=min(window1);
x2_1 = find(window1 == y2_1);
x1_1 = find(window1 == y1_1);
slop1=((y2_1)-(y1_1))/((x2_1)-(x1_1));
Degree_slop1=atand(slop1);
height1=(y2_1)-(y1_1);
length1=(x2_1)-(x1_1);

% window2; t=1:2min

for i=30:60;
```

```
window2(i,1)=Filtered_Fitness_avr_RLS(i,1);
end

[row5,column5]=size(window2);
Mean_Fitness_avr_RLS_window2=sum(window2(:,1))/row5;

if (max(Mean_Fitness_avr_RLS_window2)-
min(Mean_Fitness_avr_RLS_window2))<10
EP2_stability=1;
else EP2_stability=0;
end
y2_2=max(window2);
y1_2=min(window2);
x2_2 = find(window2 == y2_2);
x1_2 = find(window2 == y1_2);
slop2=((y2_2)-(y1_2))/((x2_2)-(x1_2));
Degree_slop2=atand(slop2);
height2=(y2_2)-(y1_2);
length2=(x2_2)-(x1_2);

% window3; t=2:3min

for i=60:90;
window3(i,1)=Filtered_Fitness_avr_RLS(i,1);
end
[row6,column6]=size(window3);
Mean_Fitness_avr_RLS_window3=sum(window3(:,1))/row6;

if  Mean_Fitness_avr_RLS_window2<Mean_Fitness_avr_RLS_window3
EP3_mean=1;
else EP3_mean=0;
end

if (max(Mean_Fitness_avr_RLS_window3)-
min(Mean_Fitness_avr_RLS_window3))<10
EP3_stability=1;
else EP3_stability=0;
end

% window4; t=3:4min

for i=90:120;
window4(i,1)=Filtered_Fitness_avr_RLS(i,1);
```

```
end

[row7,column7]=size(window4);
Mean_Fitness_avr_RLS_window4=sum(window4(:,1))/row7;

if  Mean_Fitness_avr_RLS_window3<Mean_Fitness_avr_RLS_window4
EP4_mean=1;
else EP4_mean=0;
end

if (max(Mean_Fitness_avr_RLS_window4)-
min(Mean_Fitness_avr_RLS_window4))<10
EP4_stability=1;
else EP4_stability=0;
end
```

% window5; t=4:5min

```
for i=120:Nb_Seg_IN;
window5(i,1)=Filtered_Fitness_avr_RLS(i,1);
end
[row8,column8]=size(window5);
Mean_Fitness_avr_RLS_window5=sum(window5(:,1))/row8;

if  Mean_Fitness_avr_RLS_window4<Mean_Fitness_avr_RLS_window5
EP5_mean=1;
else EP5_mean=0;
end

if (max(Mean_Fitness_avr_RLS_window5)-
min(Mean_Fitness_avr_RLS_window5))<10
EP5_stability=1;
else EP5_stability=0;
end
```

% Plot results from Main program

```
t1=[1:1:length(Fitness_avr_BL)]*length_seg;
t2=[length(Fitness_avr_BL):length(Fitness_avr_BL)+length(Fitness_avr_RLS)-
1]*length_seg;
t3=[1:1:length(Fitness_idv_RLS)]*length_seg;
```

```
figure, subplot(2,1,1);plot(t1-(t_BL),Fitness_avr_BL,'*-',t2-
(t_BL)+length_seg,Fitness_avr_RLS,'*-');grid on;
title('Original Fitness Values (no filtered)','FontSize',12);
subplot(2,1,2);plot(t1-(t_BL),Filtered_Fitness_avr_BL,'*-',t2-
(t_BL)+length_seg,Filtered_Fitness_avr_RLS,'*-');grid on;
axis([-180 300 0 140]);
line([-180 300],[100 100],'Color','black','LineWidth',1.5);
title(['Subject = ', num2str(Subject), '        Model ARX [4 4 0];        Before & After
Release (Filtered using sgolayfilt)'],'FontSize',12);
legend('Averaged model (Baseline)','Averaged model (Release)',4);
xlabel('Time (sec). Time = 0 corresponds to cuff release');ylabel('Fitness %')
text(-180, 135,['Gender : ',num2str(Gender)],'FontSize',12);
text(-140, 135,['Age : ',num2str(Age)],'FontSize',12);
text(-110, 135,['Nationality : ',num2str(Nationality)],'FontSize',12);
text(-40, 135,['Invited From : ',num2str(Invited_From)],'FontSize',12);
text(58, 135,['Smoking : ',num2str(Smoking)],'FontSize',12);
text(39, 106,['Family History : ',num2str(Family_History)],'FontSize',12);
text(-180, 106,['HSCRP : ',num2str(HSCRP)],'FontSize',12);
text(-140, 106,['Primary Diagnosis : ',num2str(Primary_Diagnosis)],'FontSize',12);
text(-40, 106,['SBP/DBP/HR : ',num2str(SDHR)],'FontSize',12);
text(110, 135,['Mean Fitness Averaged Model in BL =
',num2str(round(Mean_Fitness_avr_BL)), ' %'],'FontSize',12);
text(110, 120,['Mean Fitness Averaged Model in RLS =
',num2str(round(Mean_Fitness_avr_RLS)), ' %'],'FontSize',12);
text(110, 106,['Mean Fitness Averaged Model in window =
',num2str(round(Mean_Fitness_avr_RLS_window1)), ' %'],'FontSize',12);
text(230, 135,['Endothelial Reaction = ',num2str(EP)],'FontSize',12);
```

% Export Data into the Excel File

```
xlswrite('Endothelial Performance by Code.xls', EP1_mean, 1, ['A'
num2str(Subject)]);
xlswrite('Endothelial Performance by Code.xls', EP1_slope,1, ['B' num2str(Subject)]);
xlswrite('Endothelial Performance by Code.xls', EP2_mean,1, ['C' num2str(Subject)]);
xlswrite('Endothelial Performance by Code.xls', EP2_slope,1, ['D' num2str(Subject)]);
xlswrite('Endothelial Performance by Code.xls', EP2_stability,1, ['E'
num2str(Subject)]);
xlswrite('Endothelial Performance by Code.xls', EP3_mean,1, ['F' num2str(Subject)]);
xlswrite('Endothelial Performance by Code.xls', EP3_slope,1, ['G' num2str(Subject)]);
xlswrite('Endothelial Performance by Code.xls', EP3_stability,1, ['H'
num2str(Subject)]);
xlswrite('Endothelial Performance by Code.xls', EP4_mean,1, ['I' num2str(Subject)]);
xlswrite('Endothelial Performance by Code.xls', EP4_slope,1, ['J' num2str(Subject)]);
```

```
xlswrite('Endothelial Performance by Code.xls', EP4_stability,1, ['K'
num2str(Subject)]);
xlswrite('Endothelial Performance by Code.xls', EP5_mean,1, ['L' num2str(Subject)]);
xlswrite('Endothelial Performance by Code.xls', EP5_slope,1, ['M' num2str(Subject)]);
xlswrite('Endothelial Performance by Code.xls', EP5_stability,1, ['N'
num2str(Subject)]);
xlswrite('Endothelial Performance by Code.xls', EP,1, ['P' num2str(Subject)]);
```

APPENDIX E

LIST OF PUBLICATIONS

International Conferences

1. **Vahid Sohani**, Edmond Zahedi, KalaivaniChellappan and MohdAlauddinMohd Ali. “A Review of Commercially Available Non-Invasive Vascular Screening Technologies for Clinical Applications”, *IEEE conference*, 2012, Langkawi, Malaysia.

2. **Vahid Sohani**, Edmond Zahedi, M. A. Mohd. Ali, GanKokBeng, KalaivaniChellappan, "A Dynamic Model between Central Aortic Pressure and Radial Photoplethysmogram: Experimental Proof of Concept", *IEEE conference*, 2014, KLCC, Malaysia.

Journals

3. Edmond Zahedi, **Vahid Sohani**, M. A. Mohd. Ali, KalaivaniChellappan, GanKokBeng: “Experimental Feasibility Study of Estimation of the Normalized Central Blood Pressure Waveform from Radial Photoplethysmogram”, *Journal of Healthcare Engineering*, Vol. 6, No. 1, 2015, Pages 121-144.

4. Shahab Rooznow, **Vahid Sohani**, "Integrate Band-pass Filter Design for 13.56MHz RFID Reader", *Journal of Applied Sciences Research*,Vol. 8(2), 2012, Pages. 1018-1025.

Symposium

5. **Vahid Sohani**, Edmond Zahedi, M. A. Mohd. Ali, Axel Hunger, GanKokBeng, KalaivaniChellappan: "Estimation of the central blood pressure waveform from the radial photoplethysmogram", 1st YRA MedTech Symposium, April 2015, Duisburg, Germany.

APPENDIX F

DEFINITION of BASIC STATISTICAL PARAMETERS

a) Objective of Statistics

The main objective of doing statistical analysis in clinical research is to investigate the suitability of a proposed technique to be clinically used. To this end, a proposed technique should be evaluated by enlarging the sample size while including different groups of subjects (e.g. male and females, old and young, etc.). Therefore, outcome of the statistical analysis should provide some information about suitability of a proposed approach among specific groups.

b) Basic Statistical Parameters

Population (*N*): Population refers to all (available) subjects in a clinical research. In other words, population equals to total number of participants including male, female, healthy, non-healthy, young, old and etc.

Sample (*n*): A sample can be considered as a subset of population which contains specific subjects of whole population. Criteria of such selection depend on research objectives. For instance, if a clinical research aims to find out the possibility of a technique among non-healthy people then male patients may consider as a specific group of study.

Median: Median is a statistical parameter used for indicating lower and higher half of a data. The median is normally obtained by sorting all observations (values of data) from smallest to largest and then selecting a middle value. So that the so-called low half of data can be distinguished from high half of data.

Mean ($\bar{x}$): In statistics, mean is used for representing the central tendency of a set of data. Using the following equation, a mean can be obtained by averaging among all values:

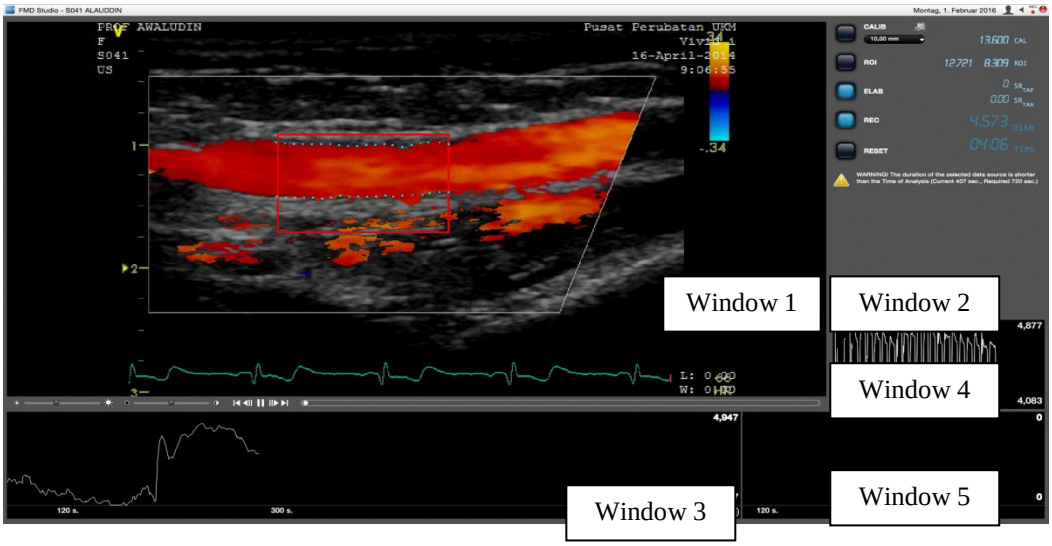
$$\bar{x} = \frac{1}{n}\sum_{i=1}^{n} x_i \qquad (3.4)$$

where n is a number of data and x_i is a set of data.

Variance: Variance can be considered as a statistical index to show how close (far) a set of numbers are from each other. In fact, it is a measure of the spread of the values in a distribution.

APPENDIX G

DETAILS OF THE CARDIOVASCULAR SUITE PROGRAM



Window 1: This window shows the brachial artery and its surrounding tissue. The red color shows the artery at the time of systole in which blood is rushed in to the brachial artery. In addition, the ECG signal can be seen at the bottom of this window (the green signal). Some basic information about subject (e.g. name, ID) and place of data collection can be also found at the top of this window.

Window 2: This window can be known as a control panel. In fact, the basic parameters of measurement (e.g. calibration, region of interest) should be initially determined here. Figure 3.15 shows this window.

Figure 3.15 The control panel of the cardiovascular suite program

The "CALIB" refers to the calibration. In fact, it provides the program some basic information about the size of the image obtained by ultrasound system. This parameter should be determined before starting a new examination. In order to setup

the calibration, the CALIB button should be pressed and then, a known distance be determined across the ultrasound image (i.e. in front of the scaling part of the ultrasound image (left side of the window 1)). It can be done by clicking on one end and drag the mouse to the other extreme. The scaling can be saved by selecting the appropriate value from the drop-down menu which is in front of the CALIB button.

The "ROI" is the abbreviation of "region of interest" which indicates that part of the brachial artery that needs to be measured by the program. Such a measurement is done pixel by pixel. In order to run this application, the button of ROI should be activated so that the region of interest can be determined by dragging the mouse across the brachial artery. This region plays an important role on accuracy of the measurement. Hence, special attention should be paid to determine this region. There is a numerical range in front of the ROI button which indicates the size (width and height) of the ROI in millimeter. Such a range is measured as 8.695 - 5.072 in Figure 3.15. Figure 3.16 shows a close view of the ROI box across the brachial artery. As can be seen, the length of window is well located alongside the brachial artery.

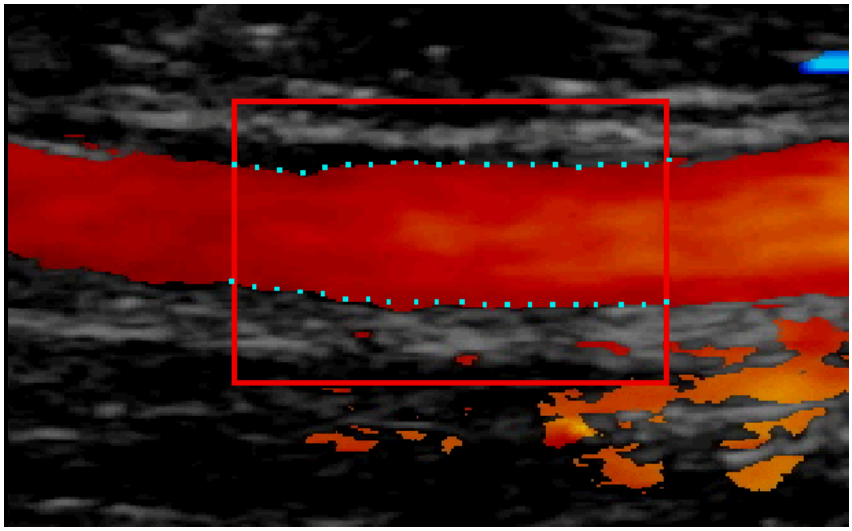


Figure 3.16 Graphical determination of ROI across the brachial artery

The "ELAB" refers to the word enable. The measurement can be done once this button is pressed. There are two parameters in front of this button; SR_{TAP} and SR_{TAN} which are related to the Doppler analysis. Since this analysis was not used in this research, these parameters are considered zero.

In order to be able to record the measurement and its parameters, the button of REC must be pressed. The "REC" refers to the record which activates the process of recording. There is a value in front of this button which represents the current diameter of the brachial artery. As can be seen in Figure 4.20 this value is 3.023.

The last button in control panel is the "RESET" which is an action for deleting the values of brachial diameter which is already measured. This option is used in case of wrong determination of region of interest. A number in front of this option indicates the time (e.g. 00:27 in Figure 4.20).

Window 3: This window plots values of brachial diameter over the time. In other words, this curve is a graphical representation of diameter changes from the beginning till present. The dimension of the brachial diameter and time are millimeter and second respectively. The curve in window 3 of the Figure 4.20 shows vascular dilatation caused by the FMD stimulus.

Window 4: The cardiovascular suite program has an option to trace (measure) the brachial diameter while data is obtaining from an ultrasound machine. In other words, it provides a way of a real-time measurement during the test. Window 4 shows the instantaneous diameter chart. This chart can feedback sonographer to ensure that the setup (e.g. subject's arm, ultrasound probe, etc.) remains fix during data collection.

Window 5: This window shows a curve which is used for the Doppler analysis. In this research, the Doppler analysis was not used so that this window is deactivated. Once the measurement is done, result box is appeared.