Temporal pattern of pulse wave velocity during brachial hyperemia reactivity

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Abstract. Endothelial function can be assessed non-invasively with ultrasound, analyzing the change of brachial diameter in response to transient forearm ischemia. We propose a new technique based in the same principle, but analyzing a continuous recording of carotid-radial pulse wave velocity (PWV) instead of diameter. PWV was measured on 10 healthy subjects of 22±2 years before and after 5 minutes forearm occlusion. After 59 ± 31 seconds of cuff release PWV decreased 21 ± 9% compared to baseline, reestablishing the same after 533 ± 65 seconds. There were no significant changes observed in blood pressure. When repeating the study one hour later in 5 subjects, we obtained a coefficient of repeatability of 4.8%. In conclusion, through analysis of beat to beat carotid-radial PWV it was possible to characterize the temporal profiles and analyze the acute changes in response to a reactive hyperemia. The results show that the technique has a high sensitivity and repeatability.

1. Introduction
The vascular endothelium is a large paracrine organ that secretes numerous factors regulating vascular tone, cell growth, leukocyte interactions and thrombogenicity. The endothelium senses and responds to a myriad of internal and external stimuli through complex cell membrane receptors and signal transduction mechanisms, leading to synthesis and release of various vasoactive, thromboregulatory and growth factor substances. Endothelial function is altered by many pathophysiological insults, such as tobacco, arterial hypertension and diabetes. It is known that the endothelial dysfunction play a seminal role in atherogenesis [1], [2] and in reducing circulatory efficiency [3], [4].

Endothelial function can be evaluated non-invasively in brachial artery, with a technique known as flow mediated dilation (FMD) [5]-[8]. Basically, ultrasound images of the brachial artery are analyzed, before (baseline) and during an induced hyperemia by occlusion and release of a pneumatic cuff placed on the forearm. Increased post ischemia flow induces the release of nitric oxide (NO) in that artery, resulting in local vasodilation. The FMD is estimated as the percentage of change between maximum diameter achieved during hyperemia with respect to baseline diameter. Despite its widespread use, it is an expensive method and critically dependent on operator skills, limiting its application in everyday clinical practice [8], [9].

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Pulse wave velocity (PWV), which is inversely related to distensibility, has attracted much interest in recent years as a measure of conduit artery stiffness. Distensibility, however, is influenced by dynamic changes in vascular tone as well as by chronic structural changes in the artery wall and may thus be used to measure acute changes in vascular tone. PWV has indeed been shown to be acutely influenced by constitutively released NO. Therefore, the PWV may be used to analyze the acute changes in vascular smooth muscle tone.

In this work we propose to analyze the use of carotid-radial PWV, as a tool to assess endothelial dynamics using the basic concept of the flow mediated dilation. This approach would evaluate endothelial dysfunction, in an independent way from the operator using low cost and widely available devices. The aims are: 1) characterize the beat to beat carotid-radial PWV temporal profile in response to transient ischemia in the forearm and 2) analyze the repeatability of the proposed technique.

2. Material and methods

2.1. Subjects
Ten healthy students (6 female, 22±2 years, 64±19 Kg, 1.7±0.1 m) from the Medicine School (Universidad de la República, Uruguay) were invited and agreed to participate in the non-invasive study. Measurements were performed in the morning, in a quite room, with temperature controlled at 21-23 ºC. Caffeine, alcohol and vitamin C ingestion, as well as strenuous exercise were avoided prior to the examination. The study protocol was approved by the ethics committee and all the participants gave informed consent.

2.2. Study protocol and recordings
All the procedures agreed with international consensus to evaluate the endothelial function through FMD [12]. The subjects remained rested in supine position for 15 minutes before starting the study. Right brachial blood pressure was measured with a sphygmomanometer every two minutes throughout the study, recording the systolic pressure (SP) and the diastolic pressure (DP). A pneumatic cuff was placed in the left forearm to induce a transient ischemia of 5 minutes (see figure 1).

The carotid-radial PWV was measured using mechano-transducers placed simultaneously on the skin over the left carotid and radial arteries (see figure 1), using a similar device previously described [13]. Upon completion of each study, signals were analyzed using a software previously developed in our laboratory that allows beat to beat PWV calculation [13]. The PWV was obtained from the ratio of the distance between the sensors (ΔL) and the temporal delay (Δt) between carotid and radial.

Figure 1. Instrumental employed to realize transient ischemia (pneumatic cuff) and the measurement of carotid-radial pulse wave velocity (mechano-transducers).
waveforms. The obtained PWV recordings were: before cuff inflation (baseline) and during 10 minutes after cuff release (post-occlusion status).

In order to analyze the repeatability of the technique, in a subgroup of 5 randomly selected subjects the study was repeated one hour later under the same conditions. All the measurements were done by the same trained operator.

2.3. Data analysis
To analyze the endothelial dynamics, the relative carotid-radial PWV change was quantified in reactive hyperemia (PWV\textsubscript{RH}), defined in equation (1):

\[
PWV_{RH} = \frac{\text{PWV}_{\text{Basal}} - \text{PWV}_{\text{Min}}}{\text{PWV}_{\text{Basal}}} \times 100
\]

where PWV\textsubscript{Basal} represents the average PWV of at least 10 beats of baseline recording prior to the occlusion and PWV\textsubscript{Min} is the minimum value of PWV reached during hyperemia. To analyze the temporal evolution of carotid-radial PWV, after transient ischemia, the response of the artery of each subject was normalized, with respect to baseline.

For the repeatability study the coefficient of repeatability (CR) was calculated from the absolute difference (D\textsubscript{i}) between the n=5 values of PWV\textsubscript{HR} obtained in studies 1 and 2, as defined in equation (2):

\[
CR = 2 \times \sqrt{\frac{\sum D_i^2}{n}}
\]

We also evaluate the coefficient of variation (CV) as the ratio between the standard deviation of the two measures and the average.

Data are expressed as mean ± standard deviation. Comparison between variables were analyzed by paired Student t test or ANOVA as appropriate. A value of p<0.05 was considered statistically significant.

3. Results
Baseline SP and DP values were 118±11 y 74±9 mmHg respectively. There were no significant changes observed in SP and DP, between the records at baseline, occlusion and post-occlusion. Figure 2 shows the carotid-radial PWV temporal profile normalized with respect to baseline of each student, for the entire group. The initial time of the profile represent the moment when the cuff is deflated and the first radial beat is displayed. The minimum time (t\textsubscript{min}), calculated as the time at which the PWV is equal to PWV\textsubscript{Min} after reactive hyperemia, was 59±31 seconds. The recovery time (t\textsubscript{rec}), calculated as the time at which the PWV reaches a maximum, for at least 5% of baseline, was 533±65 seconds. PWV\textsubscript{Min} value was significatively lower than PWV\textsubscript{Basal} (664±137 vs. 830±107 cm/s; p<0.05). The percentage change in PWV during the hyperemia compared to baseline was PWV\textsubscript{RH}=20.7±9.2 %. The individual values obtained before and after transient ischemia, reflected in PWV measurements, are showed in table 1.
Figure 2. Average carotid-radial PWV temporal profile, normalized with respect to baseline value, calculated during 10 minutes after transient ischemia, for the 10 subjects. Solid line: average. Dotted line: standard error of the mean.

There were no significant changes in SP and DP, or heart frequency between the considered studies for the repeatability test, ensuring that endothelial dynamics was evaluated in stable hemodynamic conditions. There were no significant differences between PWVRH in study 1 (PWV1RH=15.1±2.7%) and study 2 (PWV2RH=14.1±2.3%).

Table 1. Derived parameters from carotid-radial PWV before and after transient ischemia.

| Subject | PwvBasal (cm/sec) | PwvMin (cm/sec) | PWVHR (%) | tmin (sec) | trec (sec) |
|---------|------------------|-----------------|-----------|------------|------------|
| 1       | 860±41           | 715             | 16.9      | 47         | 609        |
| 2       | 850±37           | 734             | 13.6      | 42         | 612        |
| 3       | 784±34           | 679             | 13.4      | 46         | 508        |
| 4       | 620±10           | 399             | 35.6      | 98         | 540        |
| 5       | 897±18           | 613             | 31.7      | 37         | 512        |
| 6       | 979±45           | 854             | 12.8      | 129        | 468        |
| 7       | 949±25           | 769             | 19.0      | 55         | 526        |
| 8       | 795±20           | 654             | 17.7      | 27         | 404        |
| 9       | 716±33           | 476             | 33.5      | 57         | 597        |
| 10      | 858±29           | 748             | 12.8      | 51         | 552        |

We obtained a CR of 4.8% and a CV of 9.7%. Individual values for the subgroup of 5 subjects considered for the repeatability study are shown in table 2.
Table 2. Results of the repeatability study.

| Subject | Pwv1$_{HR}$ (%) | Pwv2$_{HR}$ (%) |
|---------|-----------------|-----------------|
| 1       | 16.9            | 16.2            |
| 2       | 13.6            | 12.8            |
| 3       | 13.4            | 16.1            |
| 6       | 12.8            | 10.9            |
| 7       | 19.0            | 14.9            |

Figure 3 shows the carotid-radial temporal profiles, normalized with respect to baseline, for the first and the second measurement performed one hour later. It is noted that the two temporal evolutions of PWV show the same trend, i.e. both reach a minimum value around the minute, with a very similar relative decrease, and then begin to increase to recover its initial value.

4. Discussion

This paper presents a new methodology in order to evaluate no invasively endothelial dynamics. This technique is based on the beat to beat carotid-radial PWV measurement, before and during reactive hyperemia induced by the occlusion and release of a cuff placed on the forearm.

From the analysis of carotid-radial PWV temporal profiles, there is a clear decrease of PWV during hyperemia compared to baseline and subsequent recovery after several minutes. From the Moens Korteweg relation, it is possible to relate the square of pulse wave velocity, directly to the product of the elastic modulus and wall thickness, and inversely with the product of the arterial diameter and the density of blood [13]. Therefore, the sharp decline in PWV observed during hyperemia may be related mainly to an increase of diameter and/or a decrease of elastic modulus. In this sense, it was shown that local activation of vascular smooth muscle by nitroglycerin, causes isobaric increases of diameter and isobaric decreases of elastic modulus [14]. We found that the percentage of change between PWV$_{min}$
achieved during hyperemia compared to baseline was near 21%. These findings are consistent with an
other work that applying a similar methodology, compares the technique of FMD with the humerus-
radial PWV measurement [15]. In that work, the relative reduction of PWV respect to baseline values
evaluated in 17 healthy subjects of 33±11 years, was 14±5 %. Due to the fact that in healthy
individuals, diameter changes assess by the ultrasound FMD technique yields values on the order of 7-
9 % [7], [10], one could infer that the present methodology is more sensitive. Similarly, an interesting
point to emphasize is that although the time to reach the minimum value for PWV is similar to the
time to reach the maximum dilation in FMD technique (both within the minute of cuff release),
recovery times are significantly different. The recovery time here found was about 9 minutes, while in
the FMD technique was around 3 minutes [12], [16]. In a recent study involving FMD, using an
automatic method that allows the continuous diameter detection, a better sensitivity was achieved
analyzing the ascendand speed of the profile instead of extreme values [17]. Perhaps our methodology
will require in a future, to incorporate a descendent profile rate evaluation, in order to analyze in more
detail the recovery phase. Therefore, in future studies of FMD in addition to the temporal signal of
diameter, it would be very interesting to obtain simultaneously the evolution of the elastic modulus.
An alternative could be the simultaneous measurement of diameter and PWV, using the here proposed
methodology.
Although PWV measurement is a simple and widely used technique to assess arterial stiffness, the
approximate determination of the separation distance between sensors required for its calculation,
result in an under or overestimation of the true value. In this work analyzing the relative changes in
PWV, each individual was self-control. Moreover, PWV is strongly related to blood pressure. An
increase in blood pressure level, will increase PWV. Therefore one might think that the decrease of
PWV during hyperemia was actually due to a passive phenomenon, as a response of a decrease in
blood pressure. If this were the case, FMD studies should not show a vasodilator response. On the
contrary, if during hyperemia pressure level increases, a decrease of PWV is not expected. Therefore,
the changes found during hyperemia can not be entirely attributed to a pressure phenomenon.
It is known that endothelial function studies based on analysis of ultrasound images are extremely
variable, with coefficients of variations up to 40% [9]. The use of automated methods have noticeably
reduce the variability, presenting CV values of 16% for one hour repeated studies, and 18% for one
month repeated studies [16]. In the present study using the beat to beat PWV technique, the resulting
repeatability coefficient for PWV$_{BB}$ was less than 5% and the CV less than 10%. It is to note, that
future studies involving inter-operator variability are required.
Finally, the results were obtained in a small sample for a young and healthy population. It is
therefore necessary to perform other studies, including a higher number of patients of different ages
and with different pathologies that produce endothelial dysfunction, to confirm the results here found.
These issues, as well as the possibility to compare the results with simultaneous recordings of other
techniques such as FMD, are currently being planned by our working group for the near future.

5. Conclusion
Through analysis of beat to beat carotid-radial PWV it was possible to characterize in healthy subjects
the temporal profiles and to analyze the acute changes in response to a transient ischemia. The results
show that the technique has a high sensitivity and repeatability.

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008-020) from Uruguay.
References

[1] Ross R 1993 The pathogenesis of atherosclerosis: a perspective for the 1990s *Nature* **362** 801-9
[2] Alexander R W and Dzau V J 2000 Vascular biology: the past 50 years 2000 *Circulation* **102** 112-6
[3] Griffith T M, Edwards D H, Davies R L, Harrison D J and Evans K T 2000 NO coordinates the behavior of vascular resistance vessels *Nature* **329** 442-5
[4] Henderson A H 1997 Endothelial dysfunction: a reversible clinical measure of atherogenic susceptibility and cardiovascular inefficiency *Int. J. Cardiol.* **62** 543-8
[5] Laurent S, Lacolley P, Brunel P, Laloux B, Pannier B and Safar M 1990 Flow-dependent vasodilation of brachial artery in essential hypertension *Am. J. Physiol.* **258** 1004-11
[6] Anderson E A and Mark A L 1989 Flow-mediated and reflex changes in large peripheral artery tone in humans *Circulation* **79** 93-100
[7] Celemajer D S, Sorensen K E, Gooch V M, Spiegelhalter D J, Miller O I, Sullivan I D, Lloyd J K and Deanfield J E 1992 Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis *Lancet* **340** 1111-5
[8] Sorensen K E, Celemajer D S, Spiegelhalter D J, Georgakopoulos D, Robinson J, Thomas O and Deanfield J E 1995 Non-invasive measurement of human endothelium dependent arterial responses: accuracy and reproducibility *Brit. Heart J.* **74** 247-53
[9] Malik J, Wichterle D, Haas T, Melenovsky V, Simek J and Stulc T 2004 Repeatability of noninvasive surrogates of endothelial function *Am. J. Cardiol.* **94(5)** 693-6
[10] Ramsey M W, Goodfellow J, Jones C J H, Luddington L A, Lewis M J and Henderson A H 1995 1995 Endothelial control of arterial distensibility is impaired in chronic heart failure *Circulation* **92** 3212-19
[11] Kinlay S, Creager M A, Kukumoto M, Hikita H, Frang J C, Selwyn A P and Ganz P 2001 Endothelium-derived nitric oxide regulates arterial elasticity in human arteries in vivo *Hypertension* **38** 1049-53
[12] Correti M C et al 2002 Guidelines for ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force *J. Am. Coll. Cardiol.* **39** 257-65
[13] Graf S 2008 Detección no invasiva de las alteraciones arteriales inducidas por la ateroesclerosis y la hipertensión arterial. PhD thesis. Facultad de Medicina. Universidad de Buenos Aires. Exp. No 506.644/01, Res: No 1727
[14] Bank A J, Kaiser D R, Rajala S, Cheng A 1999 In vivo human brachial artery elastic mechanics: effects of smooth muscle relaxation *Circulation* **100** 41-7
[15] Naka K K, Tweddel A C, Doshi S N, Goodfellow J and Henderson A H 2006 Flow-mediated changes in pulse wave velocity: a new clinical measure of endothelial function *Eur. Heart J.* **27** 302-9
[16] Craiem D, Chironi G, Gariepy J, Miranda-Lacet J, Levenson J and Simon A 2007 New monitoring software for larger clinical application of brachial artery flow-mediated vasodilatation measurements *J. Hypertens.* **25(1)** 133-40
[17] Chironi G, Craiem D, Miranda-Lacet J, Levenson J, Simon A 2008 Impact of shear stimulus, risk factor burden and early atherosclerosis on the time-course of brachial artery flow-mediated vasodilation *J. Hypertens.* **26(3)** 508-15