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Reactive hyperemia-related changes in carotid-radial pulse wave velocity as a potential tool to characterize the endothelial dynamics

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Abstract—**Current methods used to evaluate the endothelial function have limitations. The analysis of the pulse wave velocity (PWV) response to transient ischaemia could be an alternative to evaluate the endothelial dynamics. *Aims:* To analyze (a) the carotid-radial PWV temporal profile during flow mediated dilatation test, and (b) the PWV changes considering its main vascular geometrical (diameter) and intrinsic (elastic modulus) determinants. *Methods:* Sixteen healthy young adults were included. The carotid-radial PWV (strain gauge mechano-transducers), wall thickness and brachial diameter (B-Mode ultrasound) were measured before (basal state), during a forearm cuff inflation (5 minutes) and after its deflation (10 minutes). The PWV, brachial diameter and elastic modulus changes and temporal profile were analyzed (basal state, 15, 30, 45, and 60 seconds after cuff deflation). *Results:* Transient ischaemia was associated with arterial stiffness changes, evidenced by carotid-radial PWV variations. The PWV and diastolic diameter changes, and temporal profiles differed. The arterial stiffness changes could not be explained only by geometrical (diameter) changes. *Conclusion:* The carotid-radial PWV analysis, evaluated using robust and simple available techniques, could be used in the clinical practice to study the vascular response to transient ischaemia and the endothelial function.**

I. Introduction

ENDOTHELIAL dysfunction represents an early event in the development of atherosclerosis, a main cause of cardiovascular (CV) events (i.e. stroke and myocardial infarction) [1], [2]. The impairment in the endothelium-dependent flow-mediated vasodilatation (FMD) has been assessed using ultrasound devices, providing a non-invasive measure of potentially cumulative but therapeutically amenable, composite CV risk [3]. However, several limitations, including the critical dependence on operator skills, have been ascribed to the method, limiting its clinical use. In this context, considering the clinical requirements, meaning and potential applicability of the endothelial function evaluation, there is great interest in generating new tools to evaluate it using simple and robust available techniques.

The pulse wave velocity (PWV) is generally accepted as a simple, non-invasive, economic, robust and reproducible method to evaluate the arterial stiffness [4]. The main vascular geometrical and intrinsic properties that determine the PWV are, respectively, the arterial diameter and the arterial wall elastic modulus. Hence, the transient ischaemia stimulus with the following reactive hyperemia could be associated with changes in the PWV as a result of the FMD and of potential changes in the intrinsic arterial properties. Then, the changes in PWV in response to transient ischaemia could be used to evaluate the endothelial dynamics. About this, Naka *et al.* [5] have proposed a new method to evaluate the endothelial function, measuring, simultaneously, by oscillometry, the PWV in upper and lower limbs before and after a transient ischaemic stimulus. However, the methodology and equipment employed by Naka *et al.* differed from those widely available and used to determine PWV in the clinical practice.

In this work, we propose analyzing the use of the *carotid-radial PWV,* measured with strain-gauge mechano-transducers, as a tool to evaluate the endothelial dynamics, using the conceptual basis of the FMD, that is to say the reactive hyperemia determined in the upper arm by transitory distal ischaemia [6]. This would be an advantageous approach to the endothelial function evaluation since it could be assessed in a simple, operator-independent way, using widespread devices.

This work aims were: a) to characterize the temporal profile of the carotid-radial PWV and the FMD in response to transient forearm ischaemia and b) to analyze the PWV changes considering its main vascular geometrical (diameter) and intrinsic (elastic modulus) determinants.

II. Methods

A. Subjects

Sixteen healthy medicine students were invited and agreed to participate in the study. Recordings were done in the morning, in a quiet room, with temperature controlled at 21–23ºC. 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

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and all the participants gave informed consent. The subjects' main characteristics are detailed in Table I.

TABLE I
GROUP CHARACTERISTICS

Age [years]	22 ± 2
Body weigth [Kg,]	72 ± 18
Body heigth [m]	1.7 ± 0.1
BMI [Kg/m^2]	23.3 ± 2.0
Heart rate [b.p.m]	71 ± 9
Systolic pressure [mmHg]	115 ± 13
Diastolic pressure [mmHg]	80 ± 6

Values expressed as mean ± standard deviation

B. Study protocol, recordings and data analysis

All procedures agreed with international consensus to evaluate the endothelial function through FMD [7]. The subjects (n=16) were rested in supine position for 15 minutes before starting the study. Right brachial blood pressure (BP) was measured (sphygmomanometer) every 2 minutes. A pneumatic cuff was placed in the left forearm to induce transient ischaemia [Figure 1]. Heart rate (HR) was registered.

The carotid-radial PWV was measured using strain gauge mechano-transducers (Motorola MPX 2050, Motorola Inc., Corporate 1303 E. Algonquin Road, Schaumburg, Illinois 60196, USA) placed simultaneously on the skin over the carotid and radial arteries [Figure 1] [8]. The signals were recorded and analyzed off-line, using a software that allows the PWV calculation, given the sensors distance (ΔL) and considering the time delay (Δt) between the carotid and radial waveforms onset [4]. All measurements were done by the same trained operator. The PWV variation coefficient was less than 5%. To evaluate the wall thickness and diameter the brachial artery was visualized longitudinally above the antecubital crease using high resolution B-Mode ultrasound (Sampling rate: 30 Hz; 7.5-MHz probe; Portable Ultrasound System, Model: Aloka SSD210, ALOKA CO., LTD. Tokyo, Japan) [8] [Figure 1]. Video sequences were recorded and analyzed off-line using an automated step-by-step algorithm applied to each digitalized image [Figure 1] [9].

Data were obtained before (basal state), during cuff inflation (5 minutes) and after its deflation (10 minutes).

C. Data analysis

The PWV and the brachial artery diameter corresponding to the basal state and the first 15, 30, 45, 60 seconds after the cuff deflation were analyzed.

The PWV main determinants are the arterial diameter and elastic modulus, as described by Moens-Korteweg [10]: $PWV = \sqrt{\frac{E \cdot h}{DD \cdot \rho}}$, where ρ is the blood density (assumed equal to 1.055 g/ml), DD the diameter at end-diastole, h the mean wall thickness and E the elastic modulus. Then, using the measured PWV, h and DD the E was calculated.

Finally, to analyze the endothelial dynamic (function) taking into account the "gold standard" accepted methodology [7], the FMD was quantified as the percentual change in the arterial diastolic diameter, considering the basal levels and those after the cuff deflation (15, 30, 45, 60 seconds):

$FMD_{[\%]} = \frac{DD_{after\ cuff-deflation} - DD_{basal}}{DD_{basal}} \cdot 100$., where DD is the arterial diastolic diameter at end-diastole. In addition, the same procedure was used to quantify the endothelial dynamics using the measured carotid-radial PWV and the calculated arterial wall elastic modulus (E):

$PWV_{[\%]} = \frac{PWV_{after\ cuff-deflation} - PWV_{basal}}{PWV_{basal}} \cdot 100$,

$E_{[\%]} = \frac{E_{after\ cuff-deflation} - E_{basal}}{E_{basal}} \cdot 100$.

D. Statistics

Changes in BP, HR, PWV, arterial diameter and E, were evaluated using ANOVA + Bonferroni test. Differences in the variables (PWV, arterial diameter and E) percentual changes were evaluated using two tailed paired Student t-test. A $P<0.05$ indicated significant statistical differences.

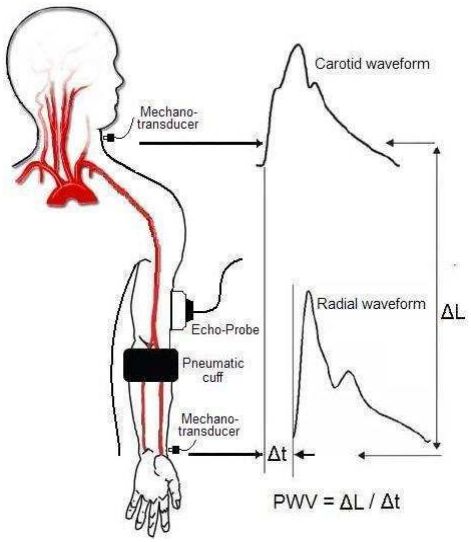

Fig 1. Schema of the instrumental approach employed to measure the carotid-radial pulse wave velocity (mechano-transducers) and brachial artery diameter (B-Mode echography).

III. RESULTS

There were not significant changes in HR or BP during the experimental sessions, ensuring that the endothelial dynamics was evaluated in stable hemodynamic conditions.

Figure 2 shows typical carotid and radial pressure recordings and diameter waveforms obtained after the cuff-deflation, in

a typical subject. Immediately after the cuff-deflation, the radial pressure waveform is visualized and an increase in the diastolic diameter (flow-mediated dilatation) is evidenced.

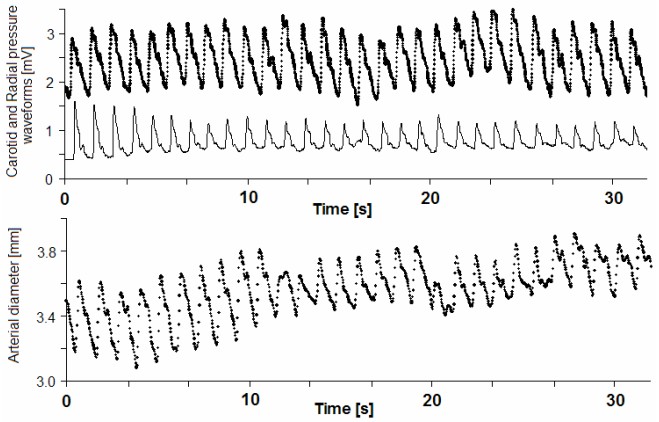

Fig 2. Top: Carotid (dotted line) and Radial (solid line) pressure waveforms obtained during 30 seconds after cuff-deflation (time = 0 s), in a typical subject. Note the instantaneous visualization of the radial recording after cuff deflation. Bottom: arterial diameter waveform obtained during 30 seconds after cuff-deflation (time = 0 s), in a typical subject. Note that shortly after cuff deflation the flow-mediated dilatation is observed.

Figure 3 shows the carotid-radial PWV (top) and brachial diastolic diameter (bottom) temporal profiles from basal state until the first minute after the cuff deflation.

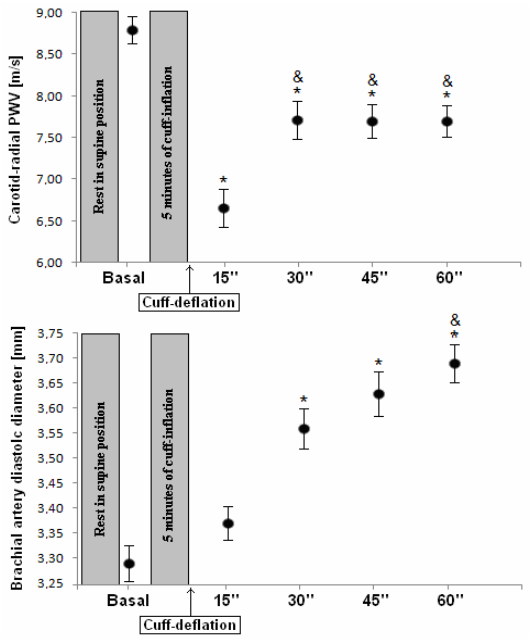

Fig 3. PWV (Top) and diastolic diameter (Bottom) measured values at rest (basal state) and 15, 30, 45 and 60 seconds after cuff deflation. * and &: p<0.05 with respect to Basal and 15'' state, respectively.

Figure 4 shows the arterial elastic modulus temporal profile from basal state until the first minute after cuff deflation.

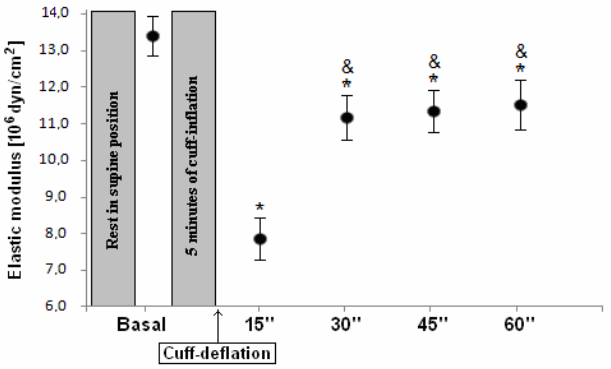

Fig 4. Elastic modulus computed values at rest (Basal state) and 15, 30, 45 and 60 seconds after cuff deflation. * and &: p<0.05 with respect to Basal and 15'' state, respectively.

Figure 5 shows PWV, diastolic diameter and elastic modulus percentual changes respect to the basal state for the 15, 30, 45 and 60 seconds after the cuff deflation.

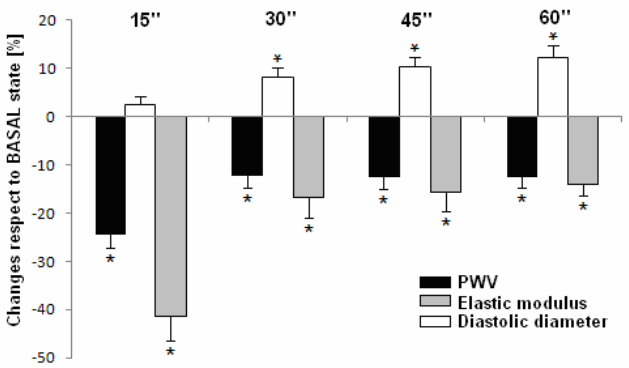

Fig 5. PWV, diastolic diameter and elastic modulus percentual changes after (15, 30, 45 and 60 seconds) cuff deflation. *: p<0.05 with respect to inexistent change.

IV. DISCUSSION

This work main results were:

a) Changes in the regional arterial stiffness, associated with transient ischaemia and FMD were evidenced using the carotid-radial PWV measured applying standard methods (mechano-transducers).

b) There were differences in the PWV and diastolic diameter percentual changes in response to the transient ischaemia.

c) The PWV, brachial diastolic diameter and elastic modulus temporal profiles in the first 60 seconds after cuff deflation differed. Then, the arterial stiffness acute changes could not be explained only by geometrical (arterial diameter) changes.

The PWV is an index widely used to evaluate the arterial stiffness and is considered the 'gold standard' to characterize the regional arterial stiffness using non-invasive studies; the higher the stiffness, the higher the PWV [4]. The arterial geometry (diameter) and arterial wall intrinsic

properties (elastic modulus) are main determinants of the PWV. So, the PWV can be modified by both, geometrical and intrinsic arterial wall changes. On the other hand, the endothelium modulates both the arterial geometry and the biomechanical properties. Then, we proposed evaluating the arterial stiffness by means the PWV measure as a new approach to assess the endothelial function.

In normal endothelial function conditions, it is expected a brachial diameter increase (FMD) during the reactive hyperemia associated with transient ischaemia. This could result in changes, actually in a decrease, in the PWV, as stated in Moens and Korteweg equation [10]. In this work, after transient forearm ischaemia we found a reduction in carotid-radial PWV, measured with standard methodologies. This results agrees with that reported by Naka *et al*.[5].

The relative changes in the carotid-radial PWV (~40%) were higher than those obtained in the diastolic diameter. So, at least in theory the PWV could be a more "sensitive" parameter to analyze the endothelial function after transient ischaemia.

It is noteworthy that in the first 15 seconds after cuff deflation we found changes in the PWV despite the arterial diameter variations were not significant [Figure 3]. Then, in this early stage, pressure changes and/or humoral factors could contribute to explain the PWV variations. Following this early stage, there was a diameter increase, which reached a maximum by the first minute after the cuff deflation, a finding that agrees with previous results [10]. On the contrary, after the first stage (15 seconds after cuff deflation) the PWV increased but its levels kept below the basal values during all the period analyzed [Figure 3]. Then both, the DD and PWV changes and their temporal profiles were different.

During reactive hyperemia the arterial wall elastic modulus (E) decreased [Figure 4]. This compliant effect during a diameter expansion would be somehow controversial. How during a diameter dilatation, the artery would become more compliant? We propose two explanations. First, this event might be explained by a smooth muscle relaxation. If there were a right shift in the brachial pressure-volume loop during hyperemia but with a slope decrease due to smooth muscle relaxation, the global response might suit our results. Second, the carotid-radial PWV averaged a larger effect than the particular brachial mechanical response. These hypotheses should be verified in future protocols with concomitant pressure and diameter measurements.

Finally, from a physiological point of view, it is of great interest analyzing the PWV temporal profile after cuff deflation. At least in theory, two stages could be identified in the PWV profile. The first (15 seconds after cuff deflation), in which there was a great reduction in the PWV, could be mainly related with the action vaso-relaxing factors (i.e. adenosine generated during the ischaemia) stored during the cuff-occlusion. The second stage, in which the PWV increased and stabilized at levels lesser than those found in basal conditions, could be related with the blood-flow dependent washout of the vaso-relaxing factors. In other words, in the first stage both, the stored factors and the blood flow-related increase in shear stress could result in a PWV reduction, while in the second stage mainly blood-flow related mechanisms could be responsible of the PWV levels.

V. CONCLUSION

In this work, we demonstrated that in normal young adults the response to transient ischaemia can be evaluated measuring the carotid-radial PWV. Then, at least in theory, the carotid-radial PWV analysis could be used in the clinical practice to evaluate the endothelial dynamics. This could represent an advantageous approach over those nowadays employed in the endothelial function evaluation given its simplicity, operator-independence, requirement of widespread available devices and relative low costs.

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