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Carotid-radial pulse wave velocity as a discriminator of intrinsic wall alterations during evaluation of endothelial function by flow-mediated dilatation

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Abstract—**Flow-mediated dilatation (FMD) is the most accepted technique for the evaluation of endothelial function. However, it has been show a great inter-subject variability limiting its clinical use. Carotid-radial pulse wave velocity (PWVcr) was proposed as an alternative tool for the evaluation of endothelial function. At the present, there is no doubt that PWVcr reduces its values in response to reactive hyperemia test (RHT) in healthy subjects. *Aims:* a) to determine simultaneously the temporal profile of FMD, PWVcr and shear rate in response to RHT and b) to describe and analyze how subjects "FMD responders" or "non-responders" behave regards to PWVcr changes. *Methods:* 34 healthy young subjects were included. The PWVcr (strain gauge mechano-transducers), brachial diameter (B-Mode ultrasound and blood flow velocity (Doppler ultrasound) were measured before (baseline) the cuff was inflated and after its deflation (5 minutes). 10th percentiles FMD and PWVcr changes in the population were used for the definition of the subjects ("responders and non-responders"). *Results:* Changes in PWVcr, brachial arterial diameter and shear rate were evidenced after the cuff release ($p<0.05$). There were differences in the PWV and FMD temporal profiles. Within "FMD responders" there were "PWV responders and non-responders". *Conclusion:* Assessing RHT-related changes in PWVcr in the context of a FMD evaluation, could be useful as a discriminator of intrinsic wall alterations giving additional information of vascular dynamics.**

I. Introduction

ENDOTHELIAL dysfunction (ED) represents an early event in atherosclerotic plaque formation and it is recognized as a loss in endothelial cells capability to produce nitric oxide (NO), a powerful anti-atherogenic molecule [1]. Flow-mediated dilatation (FMD) of the brachial artery assessed by ultrasound devices is the widespread adopted technique for the evaluation of endothelial health. Although it does not give a direct measurement of endothelium local NO production, fundamental to the significance of this technique is the close relationship demonstrated between endothelial NO participation and FMD [2]. Despite of the growing use that has been acquiring FMD, it has not a well defined place in the clinical practice. An explanation that has been given, is the great inter-subjects variability reported. This variability has been analyzed and authors found biological, technical and interpretative discrepancies [3-6]. In this context, normal and reference values of FMD are not available at the moment, limiting its clinical use.

In the past five years, among other approaches, pulse wave velocity (PWV) has been proposed as a potential tool to evaluate endothelial function [6, 7]. PWV is recognized as the "gold standard" parameter of regional arterial stiffness [10]. At the present, there is no doubt that PWV reduces its values in response to reactive hyperemia test (RHT) in healthy subjects [6-9]. However, although PWV changes are determined by geometrical factors, i.e. arterial diameter, there are other determinants included in Moens and Korteweg equation that could also explain RHT-related changes in PWV [7]. Taking into account this, we hypothesized that carotid-radial PWV (PWVcr) changes owing RHT could not necessarily follow FMD response and PWVcr evaluation could offer additional information of endothelial function.

We measured simultaneously in a sample of 34 healthy subjects, FMD and PWV before and after provoked the RHT. After that, we identified those subjects who showed an adequate FMD response ("FMD responders") and those who do not ("FMD non-responders"). Once the groups were determined, the same distinction regard to PWV changes was carried out and an intra-subject study was carefully performed.

Therefore, the objectives of the present work were: a) to determine the temporal profile of FMD, carotid-radial PWV and shear rate in brachial diameter in response to transient forearm ischemia in 34 healthy participants and b) to describe and analyze how subjects "FMD responders" or "non-responders" behave regards to PWVcr changes.

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II. METHODS

A. Subjects

A group of healthy and untrained medicine students randomly selected (n=34) were invited and agreed to participate in the study. Following the guidelines for the ultrasonic assessment of endothelial dependent FMD of the brachial artery [4], subjects were asked to abstain from physical activity, tobacco products, and vitamin supplementation for at least 6 hours prior the examination. The study protocol was approved by the ethics committee and all the participants gave informed consent.

The subjects' main characteristics are detailed in Table I.

Table 1: Demographic characteristics of the subjects.

Variable	Value
n	34
Sex, M/W	9/25
Age (years)	21.1 ± 0.1
Systolic pressure (mmHg)	124.2 ± 2.1
Diastolic pressure (mmHg)	70.1 ± 0.8
Heart rate (b.p.m)	76.9 ± 2.1
Body mass index (kg/m2)	21.4 ± 0.4
Height (cm)	168.6 ± 1.9
Weight (kg)	60.8 ± 2.2
Total cholesterol (mg/dL)	183.9 ± 6.4
High density lipoprotein cholesterol (mg/dL)	65.0 ± 2.6
Low density lipoprotein cholesterol (mg/dL)	101.2 ± 4.6
Triglycerides (mg/dL)	88.0 ± 5.3
Fasting glucose (mg/dL)	82.2 ± 0.9
Creatinine (mg/dL)	0.7 ± 0.0

Values expressed as MV ± SEM.

B. Study protocol, blood samples and recordings

The first part of the study included anthropometric measurements like height and weight (body mass index was calculated) and a fasting venous blood draw to obtain total cholesterol, low density lipoprotein cholesterol, high density lipoprotein cholesterol, triglycerides and creatinine. After that, subjects were instructed to lie in supine position for 15 minutes to establish a hemodynamic steady state in a temperature-controlled (21º-23ºC) room. Heart rate (HR) was determined and right brachial blood pressure (BP) was measured by using sphygmomanometer every two minutes during the whole study.

To provoke the endothelial stimulus, i.e. the increase of shear stress evoked by a transient increase of blood flow, five minutes of ischemia were induced by occluding the brachial artery. The occlusion was performed inflating a pneumatic cuff placed on the left forearm just below the elbow to at least 50 mm Hg above systolic pressure.

Before (baseline) and after the arterial occlusion and cuff release were carried out, carotid and radial pressure waveforms were simultaneously obtained using strain gauge mechano-transducers (Motorola MPX 2050, Motorola Inc., Corporate 1303 E. Algonquin Road, Schaumburg, Illinois 60196, USA) by placing them on the skin over the carotid and radial arteries. The signals were recorded and analyzed off-line using a software that allows PWVcr calculation, taking into account the given distance (Δx) between these arterial sites and the time delay (Δt) between the carotid and radial waveforms onset. The algorithm used for the detection of the foot waves was explained in previous work [9].

At the same time, left brachial artery was visualized longitudinally above the antecubital crease using high resolution B-Mode ultrasound (SonoSite, MicroMaxx, SonoSite Inc., 21919 30th Drive SE, Bothell, WA 98021, USA; Sampling rate: 12.5 Hz; 5 to 10 MHz probe, Model: L38e). Video-sequences were recorded and analyzed off-line using an automated step- by- step algorithm applied to each digitalized image that allows the brachial diameter obtainment. In addition, Doppler signals were performed to acquire blood flow velocity in baseline and in specific moments during the reactive hyperaemia period. Doppler signals were used for the characterization of endothelial stimulus. All measurements were done by the same trained operator.

C. Data analysis

As was mentioned, PWVcr was quantified using specific software developed by our group. PWVcr levels corresponding to baseline and to the first 5 minutes after cuff deflation were determined by averaging four beats in each specific time. After that, the percent of PWVcr change (with respect to basal levels) was quantified:

$$PWVcr[\%] = PWVcr_{\text{after cuff-deflation}} - PWVcr_{\text{baseline}} / PWVcr_{\text{baseline}} .100$$

(Equation 1)

To analyze the endothelial function taking into account the "gold standard" accepted methodology, the FMD was quantified as the percentage change in the arterial diastolic diameter (DD), considering the basal levels and those measured after the cuff deflation (first 5 minutes):

$$FMD[\%] = DD_{\text{after cuff deflation}} - DD_{\text{baseline}} / DD_{\text{baseline}} .100$$

(Equation 2)

The mean blood flow velocity (*Vm* [cm/s]) and brachial mean diameter (*Dm*) were used for the shear rate (SR) calculation, an estimate of shear stress without accounting for blood viscosity [5] following the equation:

$$SR = Vm/Dm$$

(Equation 3)

SR was obtained for the characterization of the endothelial stimulus.

D. Statistics

Changes in BP, HR, PWV, arterial diameter and shear rate, were evaluated using ANOVA + Bonferroni test. Differences in the variables (PWV, arterial diameter and shear rate) percentual changes were evaluated using two tailed paired Student t-test. All data are presented as mean value (MV) ± standard error of the mean (SEM). A $p<0.05$ indicated significant statistical differences.

III. Results

HR and brachial BP showed no significant changes during the experimental sessions, ensuring stable hemodynamic conditions during the whole measurements.

Fig. 1 shows carotid and radial pressure, B-Mode and Doppler signals recordings before and after the cuff-occlusion and deflation in a typical subject. Immediately after the cuff-deflation, there is an acute and transient increase of brachial blood flow.

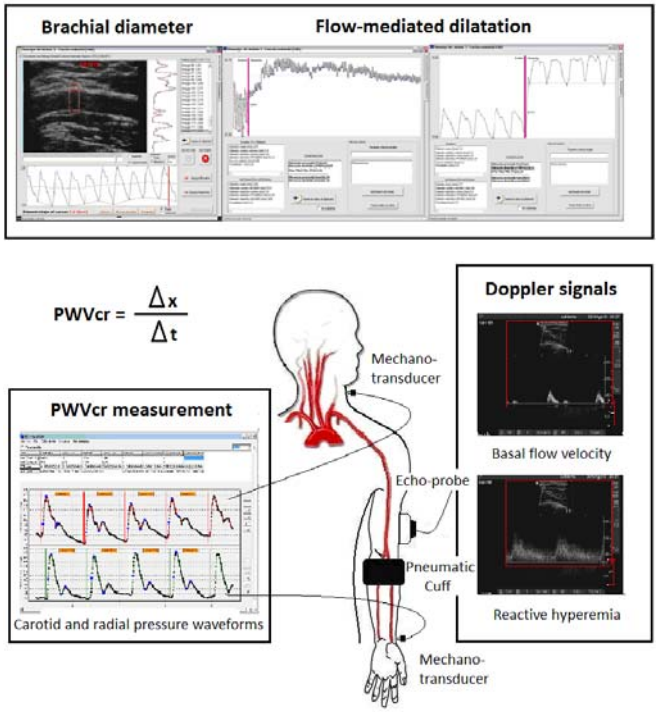

Fig. 1. Schema of the instrumental approach employed to measure the PWVcr (mechano-transducers), brachial artery diameter (B-Mode echography) and blood flow velocity (Doppler signals).

In Fig. 2 is represented the mean values of PWVcr (top), brachial diastolic diameter (middle) and shear rate stimulus (bottom) from baseline and during the cuff deflation. Basal diastolic diameter changed (3.05 ± 0.07 vs. 3.36 ± 0.08 mm; $p<0.05$) 10.6% 60 seconds post-ischemia. A reduction 10.3% were evidenced at the same time in PWVcr values (8.1 ± 0.2 vs. 7.3 ± 0.2 m/s; $p<0.05$). Shear rate values were higher immediately when the cuff was released ($p<0.05$). In function of FMD and PWVcr percentage changes at the first minute of cuff release, the subjects were classified into 4 different groups (G1-G4):

- G1 (n=23): subjects, who had a DD increase with a simultaneously PWVcr reduction;
- G2 (n=6): subjects, who had a DD increase with no PWVcr decrease or even an increase (paradoxical response);
- G3 (n=4): subjects, who had no DD increase but with PWVcr reduction;
- G4 (n=1): subjects, who had no changes in both parameters or showed a paradoxical response.

The cut points or thresholds to define those groups were a 5% or more of FMD percentage change increase ("FMD responders") and a reduction of 2.1% or less of PWVcr values ("PWVcr responders"). That points represent the 10th percentiles of the subject population studied and were arbitrary selected. The different groups are represented in Table 2.

Table 2: Groups of "FMD and PWVcr responders and non-responders".

		FMD[%]		
		≥5%	**<5%**	
PWVcr[%]	**≤-2.1%**	23	4	27
	>-2.1%	6	1	7
		29	5	*n=34*

This work main results were:

a) Changes in PWVcr, brachial arterial diameter and shear rate were evidenced after the cuff release (fig. 2).
b) There were differences in the PWV and diastolic diameter temporal profiles in response to the transient ischemia (fig. 2).
c) Among "FMD responders" (85%, 29/34), there were "PWVcr responders and non-responders" (Table 2).
d) Almost 30% (10/34) of the subjects showed a discrepancy in PWVcr and FMD response (Table 2).

IV. Discussion

The exact mechanisms that could explain PWV reduction associated with RHT remain still unknown.

PWV is determined by arterial diameter, elastic modulus, blood density and mean arterial wall thickness as can be observed in Moens and Korteweg equation. If hyperemic changes in PWV were determined only by diameter changes, PWVcr temporal profile would show similar behavior to diameter response. However, there is a dissociation between both parameters in response to RHT, indicating that FMD response could not only explain by it self PWVcr changes. Thus, modifications in intrinsic wall properties, i.e. elastic modulus, could play a key role in determining PWVcr reduction. About this, Kinlay et al. [11] demonstrated that

PWV decreased and augmented in response to nitroglycerin (NO-donor) and L-NMMA (NO synthase inhibitor) administration respectively. Therefore, there is a well known link between NO production and regional arterial stiffness

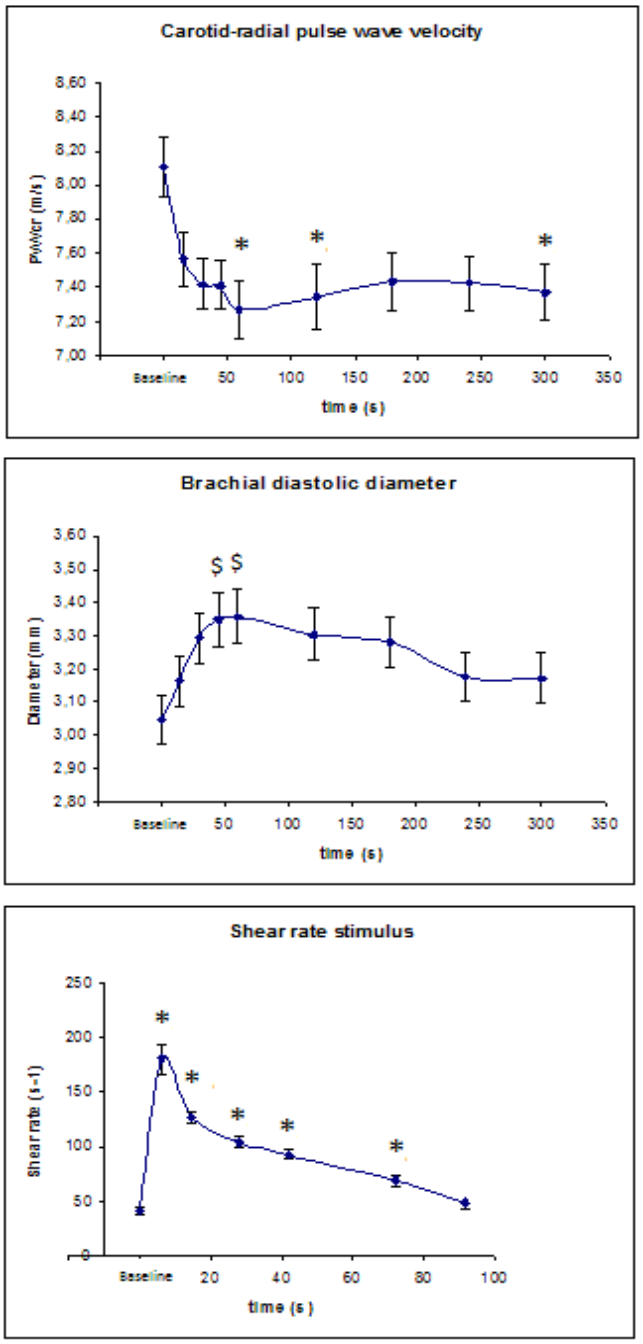

Fig 2. PWV (Top), diastolic diameter (Middle) and shear rate (Bottom) measured values at rest (baseline) and after cuff deflation. * and $: $p<0.05$ comparing baseline with post occlusion times with ANOVA and Student t-test respectively.

determination. On the other hand, FMD is abolished by the administration of a NO blockade [12]. Few studies analyzed PWV changes in response to RHT in hypertensive patients [9] and with chronic heart failure [6]. They evidenced a low, blunted or an altered PWVcr response in comparing with healthy subjects, indicating probably a potential clinical usefulness of PWV. In our work, we found in a sample of young healthy subjects without known cardiovascular risk factors, within the "FMD responders", a group who diminished PWVcr values (G1) and another who did not (G2). Assessing RHT-related changes in PWVcr in the context of a FMD assessment could discriminate subjects who really show an alteration in intrinsic arterial wall properties. Therefore, PWVcr changes could be employed not only for the evaluation of subjects with well known CV disease and/or risk factors, but also for a screening assessment in apparently healthy subjects.

Finally, inter-subject variability of FMD has been a problem in its clinical application. Future works are needed to analyze if discriminating changes in intrinsic wall properties by means of PWVcr during RHT could contribute to reduce FMD inter-subject variability.

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