Arterial Wall Structure and Dynamics in Type 2 Diabetes Mellitus Methodological Aspects and Pathophysiological Findings

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Abstract: Type 2 Diabetes Mellitus (DM), or adult-onset diabetes, is being considered as a new pandemic. Cardiovascular disease is the major cause of morbidity and mortality in type 2 DM, due to arterial structure and functional changes. Assessment of arterial structure and biomechanics, by non-invasive methods and parameters, can be used to detect early alterations related to DM. Three markers of vascular disease may help to a better evaluation of vascular dysfunction in type 2 DM patients: carotid intima–media thickness (IMTc), arterial stiffness, assessed by pulse wave velocity (PWV), and endothelial function, evaluated through the brachial artery flow-mediated dilation (FMD). Among these parameters, IMTc is considered a marker of structural vessel wall properties, and arterial stiffness reflects functional wall properties. Endothelial function represents the arterial way to actively regulate its diameter (smooth muscle-dependent actions) and its visco-elastic properties (wall elasticity and viscosity).

IMTc is increased in patients with type 2 DM and other independent risk factors, such as: age, hyperlipidemia and duration of DM. Subjects with DM have shown increased arterial stiffness. Type 2 DM is associated with reductions in FMD (endothelial dysfunction), which has already been reported to be inversely and strongly related to the extent of hyperglycemia. The underlying patho-physiological mechanisms are complex and remain to be fully elucidated.

A complete understanding of the association between arterial alterations and early detection, and type 2 DM, may be critical for the primary prevention of DM-related macro-vascular disease.

Keywords: Type 2 diabetes mellitus, Cardiovascular diseases, Arterial wall structure, Vascular disease, Endothelial function.

INTRODUCTION

The World Health Organization (WHO) estimates that more than 180 million people worldwide have diabetes. Patients with type 2 Diabetes Mellitus (DM), formerly called adult-onset diabetes, currently comprise over 90% of people with diabetes around the world. Moreover, the number of patients with type 2 DM is estimated to double by 2030, and almost half of diabetes deaths occur in people under the age of 70; 55% of diabetes deaths are women [1]. Besides, 80% of people with diabetes live in low- and middle-income countries, and they are middle-aged (45-64 years), not elderly (over 65 years). Diabetes deaths are likely to increase by more than 50% in the next 10 years without urgent action [2]. In addition, the prevalence of type 2 DM is causing growing expenses for the health care system in addition to life-years lost [3].

Cardiovascular disease is the major cause of mortality in type 2 DM [4]. In particular, alterations in normal vascular structure and functions are the primary cause of mortality and morbidity in patients with type 2 DM. Changes in the normal arterial structure and function associated with DM may, in part, explain the increased cardiovascular disease risk and mortality observed in this disease. The assessment of arterial structure and biomechanics alterations (*i.e.* increased wall thickness and/or wall stiffness), a common feature of aging, exacerbated by type 2 DM, has become an attractive and promissory way for identifying arterial structural and functional abnormalities in the preclinical early stages of the atherosclerotic vascular disease. Although many techniques and indices are currently available, their large clinical application is limited by a lack of standardization; so, researchers find serious difficulties when trying to measure, quantify, and compare these parameters. In our opinion, lack of awareness about the available tools to detect early vascular alterations is frequently observed in low- and middle-income countries.

In this review, we deal with main arterial wall structure and dynamics alterations related to DM and analyze the most important non-invasive methods and parameters available to detect arterial changes in type 2 DM patients. Our goal is to provide an insight into the knowledge of the structural and functional arterial changes associated with DM, and a short

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and updated analysis of the methods used, by our group and others, for their early detection.

A complete understanding of the association between arterial alterations and early detection, and type 2 DM, may be critical for the primary prevention of DM-related macrovascular disease [5].

CARDIOVASCULAR DISEASES IN PATIENTS WITH TYPE 2 DM: A GENERAL OVERVIEW

The injurious effects of hyperglycemia in DM are classified into micro-vascular and macro-vascular diseases, which include retinopathy, neuropathy and nephropathy (microvascular), and cerebro-vascular disease, ischemic heart disease and peripheral vascular disease (macro-vascular) [6]. A constellation of potentially modifiable risk factors for vascular disease are present in type 2 DM, including those that constitute the various definitions of the metabolic syndrome, in a very complex interaction: hyperglycemia, insulin resistance, dyslipidemia, hypertension, abdominal obesity, oxidative stress, accelerated ageing, alterations in coagulation and fibrinolysis, and smoking [7-9]. This may be due to the fact that both cardiovascular disease and DM are multi-factorial polygenic diseases, a consequence of a complex interaction of multiple genetic defects and several environmental factors exposure [10].

The traditional concept consists in atherosclerosis as a complication of diabetes. However, an alternative theory has proposed that both diseases have identical origins: genetic and environmental, the "common pathway" hypothesis. Thus, the cluster of risk factors, components of the insulin resistance syndrome, influenced by adverse fetal and early life nutritional experiences, result in the same pathway that leads to both diabetes and cardiovascular disease [11].

It is well known that DM confers a two- to four-fold risk of cardiovascular disease [12]. WHO reports that 50% of people with DM die of cardiovascular disease (primarily heart disease and stroke), the greatest cause of death in diabetic patients [1,12]. The overall risk of death among people with DM is at least double than the risk of control individuals without DM [1]. Furthermore, type 2 DM increases 5 times the risk for first acute myocardial infarction (AMI) and twice the risk for recurrent AMI compared with non-diabetic subjects' [13]. Type 2 DM is also a strong independent predictor of cerebro-vascular disease and stroke, with an elevated risk up to 4 times, compared with non-diabetic patients. Moreover, the risk of stroke recurrence, stroke-related dementia and mortality is also increased in these patients [14, 15].

In patients with type 2 DM the risk of diabetic vascular complications and mortality is strongly associated with previous hyperglycemia over time [16]. Besides, treatment to glycosilated hemoglobin (Hb A1C) targets below or around 7% in the years soon after the diagnosis of diabetes is associated with long-term reduction in risk of microvascular and macrovascular diseases [17]. In addition, DM-associated vascular impairment includes anatomic, structural and functional alterations.

ARTERIAL ALTERATIONS IN DM: CAROTID INTIMA-MEDIA THICKNESS, ARTERIAL BIOMECHANICAL PROPERTIES AND ENDOTHELIAL FUNCTION

Arterial system alterations in type 2 DM include anatomic, structural and functional changes resulting in multi-organ dysfunction [17]. Chronic hyperglycemia, the hallmark of DM, is the major metabolic alteration in the beginning of diabetic vascular impairment, by means of several pathways: increase of reactive oxygen species concentration, production of advanced glycation end products (AGEs), impairment of vasodilatory response due to Nitric Oxide (NO) inhibition, accumulation of endothelial growth factors, stimulation of hemodynamic regulation systems (Renin Angiotensin-Aldosterone System) and of signaling cascades (C Reactive Protein), and, finally, vascular smooth muscle cell dysfunction. Also, alterations in coagulation are reported: chronic inflammation impaired fibrinolytic mechanism and enhanced platelet aggregation [17].

In particular, studies performed in patients have suggested that arterial alterations (*i.e.* increased stiffness) could be yet another feature of insulin resistance [18]. Insulin, at physiological range, has acute vascular smooth muscle relaxant effects that lead to reduce arterial stiffness. However, these important and beneficial actions at physiological levels, that allow keeping reduced the left ventricle afterload, are blunted in insulin-resistant states, such as type 2 DM, and are closely related to whole-body glucose uptake. Besides, the chronic effects of insulin resistance on arterial function (wall stiffness) have also been evaluated, and a negative association between insulin-mediated glucose uptake and arterial stiffness was observed in healthy subjects [19]. It has been shown in a large population-based study that insulin concentrations (a more indirect measure of insulin sensitivity) were associated with carotid artery stiffness, and this association was stronger in women than in men [20]. As arterial stiffness is highly dependent on blood pressure levels, hypertension may affect the stimulation of glucose uptake by insulin. In this regard, recent reports have shown additive adverse effects of insulin resistance on arterial stiffness in the context of hypertension [21, 22]. Yet, in the only longitudinal study that has addressed the individual and combined effects of raised blood pressure and raised glucose levels on the progression of arterial stiffness, the estimated rate of increase in arterial stiffness was higher in individuals with both abnormalities than in those with either abnormality alone [23]. Moreover, persistence of both abnormalities may synergistically accelerate the rate of increase in arterial stiffness. It was found three times higher than in those who persisted with raised levels of blood pressure or glucose alone. Consequently, insulin resistance contributes to increased arterial stiffness independently of arterial pressure in type 2 DM patients, but also it was observed in apparently healthy subjects. Our group has reported results obtained from hypertensive patients with and without type 2 DM, which agree with the aforementioned conclusion [24].

Metabolic abnormalities showed in type 2 DM may lead to all the processes that contribute to the vascular event: thrombosis, inflammation, vasoconstriction, vascular wall lesion formation and remodelling, and plaque rupture [25].

Concerning this, three markers of vascular disease may help to a better evaluation of vascular dysfunction in type 2 DM patients: carotid intima–media thickness (IMTc), arterial stiffness and endothelial function (evaluated through the brachial artery flow-mediated dilation). Among these parameters, IMTc is considered a marker of structural vessel wall properties, and arterial stiffness (a general and non-specific term) reflects functional wall properties. Endothelial function represents the arterial way to actively regulate its diameter (smooth muscle-dependent actions) and its visco-elastic properties (wall elasticity and viscosity).

A) CAROTID INTIMA-MEDIA THICKNESS IN TYPE 2 DM

Methodological Aspects

Non-invasive B-mode ultrasound imaging allows the quantitative measurement of large artery wall thickness, specifically the intima–media thickness (IMT). Defined as, the distance separating blood/intima and media/adventitia echogenic interfaces, the IMT is mainly measured in extracranial carotid arteries where both interfaces of interest are best visible. IMT is measured on the far wall, because IMT values from the near wall depend in part on gain settings, and are less reliable. Since, its introduction in the early 1990s, the IMT, especially measured in the carotid artery, has increasingly been used as a surrogate marker of atherosclerotic disease. As previously demonstrated, computerized measurement of common IMTc is more reliable, in terms of precision and reproducibility, with approximately 3% difference between two successive measurements [26]. As a result, the measurement of the IMT on the common carotid artery has become a valid tool for large-scale multicentre studies. However, this segment is less likely to show intrusive plaques than its bifurcation or internal carotid arteries. So, in order to quantify IMT and analyze the potential existence of atherosclerotic plaques, the internal and external carotid arteries should be scanned. Recently, the recommended carotid ultrasound scanning protocol stated that IMTc measurements should be limited to the far wall of the common carotid artery and should be supplemented by a thorough scan of the extracranial carotid arteries for the presence of plaques, to increase sensitivity for identifying subclinical vascular disease [27].

Current ultrasound technology is not sensitive enough in order to measure the thickness of the intima *per se*. Therefore, IMT may reflect not only early atherosclerosis, but also non-atherosclerotic compensatory enlargement with medial growth, as a result of smooth muscle cell hyperplasia and fibrocellular hypertrophy [28]. Moreover, the carotid diameter change may passively alter wall thickness by stretching the arterial wall, as a consequence of the non-compressibility of the arterial wall mass. One possibility to minimize a potential influence of diameter change on IMT alteration is to calculate the area of the circular section of IMT [29].

The IMTc evaluation includes, a high-resolution B-mode system operating with preferentially linear ultrasound transducers at frequencies >7 MHz and an automated computerized operator-independent software [30]. Scanning of the carotid artery is performed in the antero-posterior projection, with the patient lying on his/her back with the head in axis. During the scanning, the sound beam is adjusted perpendicularly to the arterial surface of the far wall of the common carotid artery to achieve two parallel echogenic lines corresponding to the lumen-intima and media-adventitia interfaces. Once the two parallel echogenic lines of the far wall are clearly visible, along 1 cm of the segment to measure, at least, a fixed digital image (end-diastolic electrocardiogram triggering) is stored. Then, IMT is automatically computerized by specially designed software as the mean distance between those two lines. Intraluminal diameter is also computerized [31] (Fig. (**1**)).

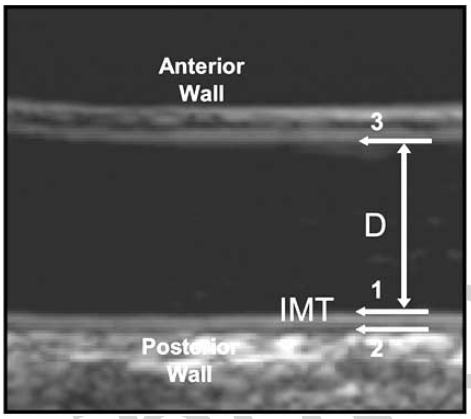

Fig. (1). B-mode image of the internal carotid artery in a diabetic patient. Internal diameter (D) and automatically computerized determination of intima media thickness (IMT) are shown. Two parallel echogenic lines, corresponding to the lumen-intima (1) and media-adventitia (2) interfaces of the posterior arterial wall, as well as the echogenic line representing the anterior arterial wall (3), are represented with arrows.

Clinical and Patho-Physiological Aspects

The IMTc is increased in patients with type 2 DM and other independent risk factors, such as: age, hyperlipidemia and duration of DM [32]. In these patients, a personal history of obesity and a family history of hypertension were independently related to higher values of IMTc [33]. Different authors have shown that the mean IMTc in middle-aged population ranged from 0.71–0.98 mm in type 2 DM patients, and 0.66–0.85 mm in healthy individuals (controls) [34-36].

Additionally, in several prospective studies, IMTc has proved to be a consistent and independent adequate predictor for stroke and coronary accidents in the general population [37-39]. Moreover, it has been used to re-stratify cardiovascular risk in dyslipemic population [40]. In Type 2 DM subjects without a history of myocardial infarction, the IMTc was similar to that in non-DM patients with a history of myocardial infarction [36], suggesting that DM is a marker of IMTc alteration. In this sense, in the study of Wagenknecht *et al.* the progression of maximal IMTc in subjects with DM was twice as high as in control individuals [41]. However, this result is not conclusive, since other studies report lower rates [42]. Overall, in type 2 DM, prevalent cardiovascular disease is associated with higher IMTc [42].

Vascular wall thickening seems to be one of the main compensatory mechanisms to preserve circumferential wall stress in hypertension [25, 43, 44]. Moreover, arterial wall

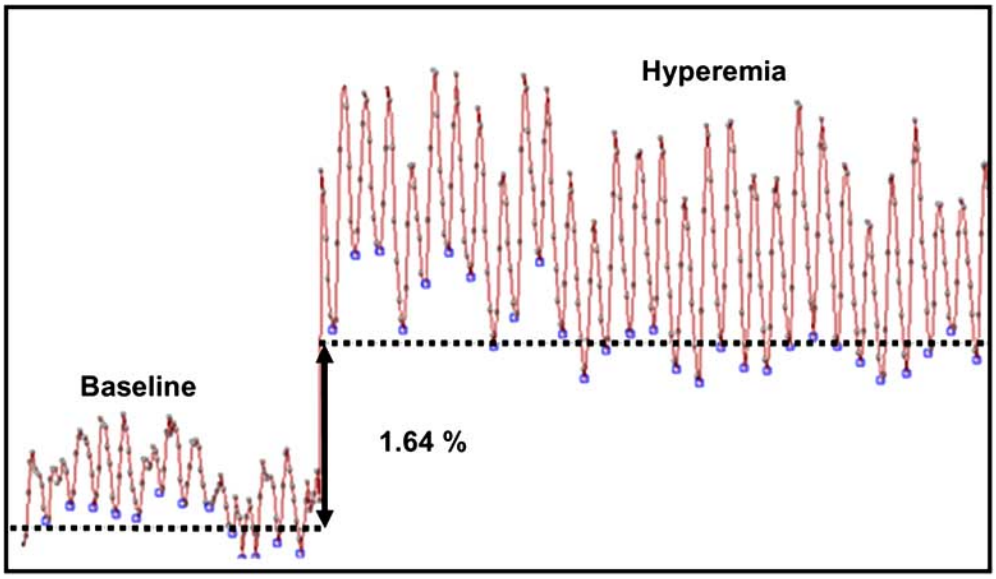

Fig. (2). Graphic of the measurements of baseline and maximum post-ischemia diameters in the brachial artery of a diabetic patient, by the non-invasive ultrasound flow-mediated dilatation method. The number obtained is the percentage increase in arterial diastolic diameter from baseline conditions to maximum arterial diameter during hyperemia.

viscosity (see below) is associated with higher IMTc, suggesting that intima-media thickening might be related to the vascular smooth muscle alterations found in hypertension [45, 46]. As previously demonstrated, the increased wall viscosity, co-existing with the enlarged IMTc, could contribute to raise the arterial wall "damping capability". In other words, the apparent disadvantage of an increased IMTc could positively play a significant role in maintaining the cushioning effect exerted by the arterial wall in transferring pressure to stretching. Consequently, vibrations from high-frequency harmonic components may develop structure injuries. The mechanism of damping consists in reducing accelerating oscillations. In this way, our group suggested that wall remodelling (*i.e.* IMTc increase) described in hypertensive patients, with and without type 2 DM, exerts a protective effect against high-frequency stretching, adjusting energy dissipation [47].

B) ARTERIAL STIFFNESS IN TYPE 2 DM

Arterial Conduit and Buffering Function: Arterial Wall as a Visco-Elastic Material

Large and medium arteries have two different but interrelated activities: conduit and buffer functions. The conduit function of the main systemic arteries allows blood transfer from the heart to the peripheral vessels, while maintaining an elevated intraluminal pressure to overcome the vascular peripheral resistance. Large and medium arteries have a large cross-sectional area (lumen) with a distensible wall adaptable to ventricular ejection. So, low arterial impedance must be presented to the pulsatile blood flow ejected by the heart to maintain an adequately high level of mean pressure and to minimize ventricular work. The buffer function is a major arterial wall property that determines the pressure and flow waves cushioning. It is accepted that the arterial wall buffering function is determined not only by arterial elastic properties, but also by the viscous properties of the wall [47]. Hence, a proper mechanical characterization of the arterial buffer function must consider both wall elasticity and viscosity. One of the most relevant functions of the elastic properties of the arterial wall is to store part of the mechanical energy generated by the heart during systole and to restore it in diastole, optimizing the heart-vessel coupling and ensuring a continuous flow towards the tissues [48]. Another amount of this energy is dissipated by means of the viscous properties of the arterial wall [49].

Changes in arterial stiffness (or more specifically in the elastic and/or viscous modulus) basically determine impairment in the arterial conduit and buffering function. Both effects lead to an increase in systolic blood pressure as the heart ejects into a stiffer arterial system, which must generate higher maximal and end-systolic pressures for the same net stroke volume. Consequently, it reduces the arterial volume at the onset of diastole and decreases the diastolic blood pressure as well, particularly in the elderly. Greater arterial stiffness also affects blood pressure regulation through pulse wave velocity and arterial wave reflection. The direct clinical consequences of increased arterial stiffness are: (a) increase of the risk of stroke as a result of higher maximal systolic pressure; (b) development of left ventricular hypertrophy due to increased left ventricle after-load; and (c) decreased coronary perfusion due to the decrease in diastolic blood pressure [50].

Methodological Aspects

Arterial stiffness can be non-invasively assessed by several methodologies, including the evaluation of "regional" and "local" arterial stiffness levels [50]. Arterial stiffness can be estimated either "locally", at specific arterial sites (i.e. carotid, femoral, brachial, radial), or "regionally", over a given arterial segment length (i.e. the aorta or the upper or lower limb) [50]. However, the local and regional stiffness estimates are closely related. If arterial diameter pulsation falls while distending pulse pressure is kept constant (increased stiffness), the pressure wave travels at a higher speed.

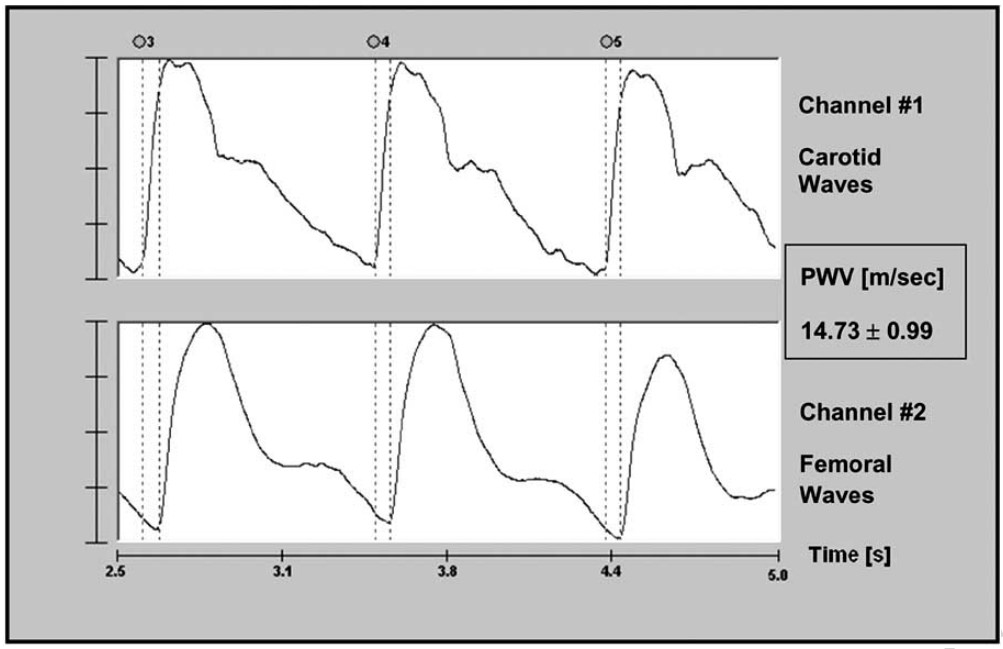

Fig. (3). Pulse wave velocity (PWV) measured in a diabetic patient with the foot-to-foot methodology. The first systolic upstroke corresponds to the carotid arterial wave (Channel #1), and the second systolic upstroke corresponds to the femoral arterial wave (Channel #2). The difference between them is the time that takes the arterial waveform to pass between the two points of measurement at a known distance (m/sec). The software processes the latter and PWV value is obtained.

The "regional" arterial stiffness is assessed by quantifying the arterial pulse wave velocity (PWV). PWV can be defined as the speed at which the forward wave (pressure, distension or flow) is transmitted from the aorta through the vascular tree. PWV is calculated by measuring the time (t) the arterial waveform takes to pass between two known points at a measured distance (D); thus, PWV=D/t, where D is measured in metres, and t in seconds. A crucial issue is the reference point considered on the waveforms. So, the foot-to-foot methodology is usually used, as it avoids the confounding influence of wave reflection. The foot of the pulse wave is the point of its systolic upstroke, and the "second derivative method", as well as the "intersecting tangent method", is the most reproducible computer algorithm available for its determination [51] (Fig. (**3**)).

The pulse waves in each artery can be non-invasively recorded using applanation tonometers, mechanographic transducers or continuous Doppler probes, applied onto the skin [50]. Automated computation of PWV involves taking readings from two sites simultaneously, or gating separate successive recordings of both arterial pulses to a fixed point in the cardiac cycle, usually the R wave of the electrocardiogram. The latter approach imposes to check accurately that the heart rates are quite similar during each recording.

To simplify the method, the distance between the two recording sites is assimilated to the distance measured transcutaneously, using a tape meter. The measurement of carotid to femoral pulse wave velocity, as arterial pulse moves in opposite ways, leads to a systematic overestimation by an amount close to 30%. This could be corrected by subtracting the distance between the carotid site and the sternal notch from the overall distance. Although, this method provides values closer to invasive measurements, it becomes complex and a source of error [50].

The "local" arterial stiffness is clinically evaluated using several complementary parameters, based on the local non-invasive pressure and diameter determinations. Local mechanical properties of particular arterial segments may be of special interest, because atherosclerosis is more frequent in some arteries, such as carotid arteries. Most of pathophysiological and pharmacological studies have used pressure (tonometry) and diameter (echo-tracking or B-mode echography) recordings. A major advantage is that local arterial stiffness is directly determined from the change in pressure, pulse pressure, driving the change in internal diameter, without using any model of the circulation. However, as it requires a high degree of technical expertise and it takes longer than measuring PWV, local measurement of arterial stiffness is now a days indicated only for mechanistic analyses in pathophysiology, pharmacology and therapeutics, rather than for epidemiological studies.

Diameter waveform (*i.e.* carotid) is commonly assessed with high-resolution ultrasound system operating with linear ultrasound transducers at frequencies >7 MHz. Detection of the displacement of arterial near and far walls can be obtained using an echo-tracking system (radiofrequency tracking) or analyzing the sequence of B-mode images with automatic image processing software. In both the cases, the internal diameter waveform, calculated as the difference between the far and near wall movements, is given as an output [26].

Applanation tonometry has been proposed to assess non-invasively arterial pressure waveforms and magnitudes in both central and peripheral arteries. This technique provides pressure waves, being almost identical to those obtained intra-arterially [52]. Calibration of the pressure waveform is based on the observation that mean arterial blood pressure (MAP) is constant throughout the large artery tree, and that

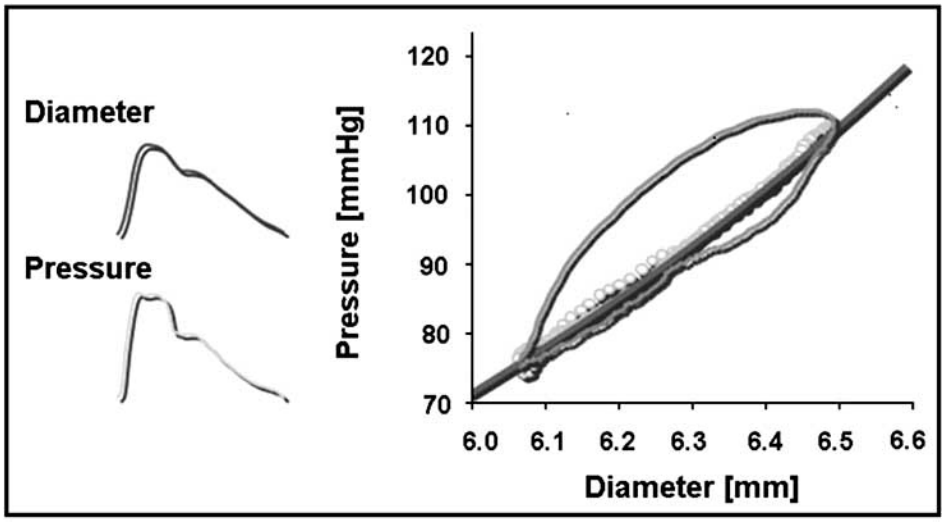

Fig. (4). Measurements obtained from a diabetic patient´s common carotid artery. Left pannel: Averaged pressure and diameter waveforms; Right pannel: Corresponding plot of pressure-diameter loop. The latter shows the measured pressure-diameter viscoelastic loop (continuous light grey line) and the purely elastic relationship (line of circles) obtained after the elimination of the viscous components. Continuous dark grey line: exponential fit to the purely elastic relationship.

diastolic pressure does not change substantially in the supine position [53]. Basically, mean and diastolic pressures are obtained from the brachial artery. By assigning the same mean and diastolic pressures to the target artery under study, the pressure wave at this artery is calibrated throughout the cardiac cycle.

Parameters commonly used to characterize the viscoelastic behavior of arteries can be obtained from static or dynamic approaches. Thus, arterial compliance and distensibility are the expression of the relationship between the absolute and relative changes in the arterial volume and the distending pressure, respectively. This relationship can be expressed as a function of diameter rather than volume, assuming the artery lumen as circular in cross-section, and the length as constant, so that the change in volume during the cardiac cycle is caused by a change in lumen cross-sectional area alone [54].

Static analysis considers only extreme values (systolic and diastolic pressures and systolic and diastolic diameters), assuming a linear relationship between pressure and diameter. Consequently, using this approach, compliance and distensibility are considered constant during the cardiac cycle. However, it is well known that due to non-linear relationship between diameter and pressure, arterial properties change during the cardiac cycle. As a result, arteries become stiffer at higher distending pressures, decreasing compliance and distensibility according to increasing blood pressure. Therefore, proper intersubject comparison of arterial wall properties should be done at the same distending pressure (isobaric condition). Some researchers have tried to calculate the arterial compliance independent of the blood pressure effect, using the beta stiffness index, applying a logarithmic conversion of systolic and diastolic blood pressure ratio [50].

In the dynamic analysis procedure, arterial pressure waveform is recorded at the same place where the diameter waveform is obtained, after the echographic recording. A surface electrocardiogram is also acquired and stored together with the diameter and pressure signals. An automated computerized procedure allows the construction of the pressure-diameter hysteresis loop. The area within the pressure-diameter loop is a measure of energy dissipated within the wall during a cardiac cycle, and is related to the arterial wall viscosity [45, 49] (Fig. (**4**)).

After subtracting the viscous component, the procedure determines also the purely elastic properties (the purely elastic pressure-diameter relationship runs along the same curve both for increasing and decreasing diameters). For example, the inverse slope of the pressure-diameter loop at any time represents a local measure of compliance. Using this approach, compliance and distensibility, as well as other local arterial parameters, such as pulse wave velocity and incremental elastic modulus, can be derived as a function of the distending pressure during the cardiac cycle, providing the possibility of isobaric comparison of arterial wall properties among subjects.

Clinical and Pathophysiological Aspects

In cross-sectional studies, arterial stiffness was strongly associated with age and classical risk factors for cardiovascular disease [55-57]. Also, it has been reported as related to coronary atherosclerosis [58]. Besides, arterial stiffness (aortic PWV) was considered as a predictor of a composite of cardiovascular events outcome above and beyond traditional risk factors [59].

In particular, subjects with DM have increased arterial stiffness [58, 60]. ***"Local"*** (carotid) and ***"regional"*** (PWV) arterial stiffness have been reported as altered even before the onset of diabetes in patients with impaired glucose tolerance. In addition, PWV seems to have a reasonable predictive value for mortality in patients with impaired glucose tolerance and type 2 DM [60]. Furthermore, PWV has been significantly correlated with amplified wave reflections and greater values in the central arteries of patients with type 2 DM [61, 62]. Similar findings were reported with carotid-femoral PWV, associated with age and duration of type 2 DM as independent risk factors [32]. Thus, aortic PWV has

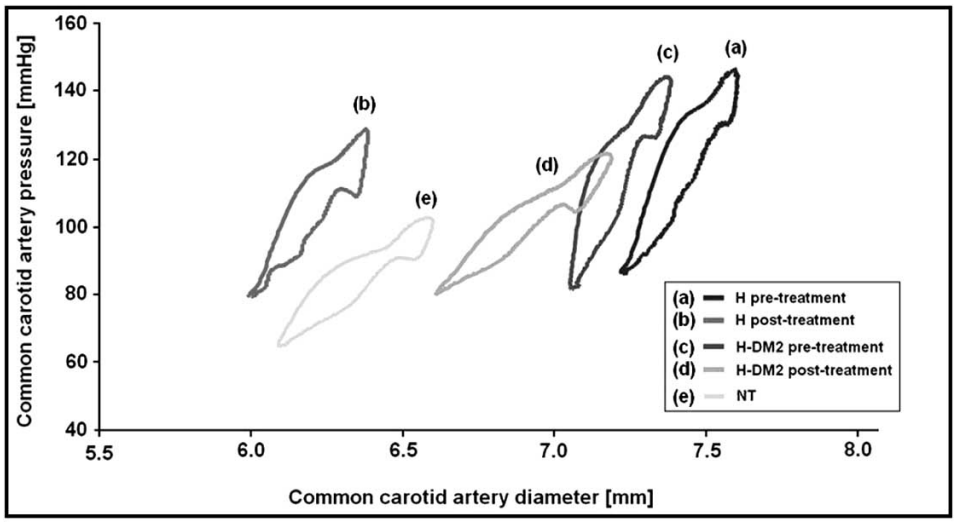

Fig. (5). Pressure and diameter plot showing instantaneous pressure-diameter loops obtained from: (a) hypertensive patients without DM before (H pre-treatment) administration of ramipril; (b) hypertensive patients without DM after (H post-treatment) administration of ramipril; (c) hypertensive patients with type 2 DM before ramipril administration (H-DM2 pre-treatment); (d) hypertensive patients with type 2 DM after (H-DM2 post-treatment) ramipril administration; (e) normotensive subjects (NT).

proved to be a useful integrated index of vascular status and a powerful independent predictor of mortality [60].

Interestingly, the age-related increase in arterial stiffness is steeper in subjects with type 2 DM than in their non-diabetic counterparts [32, 61, 63, 64]. This is consistent with observations of steeper increases in pulse pressure with aging in patients with type 2 DM [65]. More importantly, the increased pulse pressure observed in type 2 DM patients has been found to be predictive of future cardiovascular mortality [65, 66].

The association between diabetes and arterial wall properties may differ between central and peripheral arteries. In a recent study, we measured PWV in the upper limb (carotid-humeral PWV) and in the aortic region (carotid-femoral PWV) in patients with and without diabetes; we found that diabetic patients have an increased arterial stiffness mainly localized in the aortic region. In addition, we found that, with respect to non-diabetic patients, diabetic patients have a greater regional difference between the upper limb and aortic stiffness [67].

Our results partially confirm previous results. Regional stiffness estimates have been compared in different arterial segments and have shown that in individuals with DM the central rather than the peripheral part of the arterial tree was affected preferentially [61, 68, 69]. Besides, in studies where stiffness measurements were performed locally at different arterial sites, the detrimental effects of DM were higher at the more muscular (femoral) rather than the more elastic (carotid) arteries [19, 70, 71]. Therefore, preferential stiffness of peripheral arterial segments suggests that the buffering function of the central circulatory system would be preserved unlikely the peripheral conduit function, facilitating cardiac stroke volume expulsion into a circulatory system that stiffens increasingly with age.

Recently, our group quantified the arterial wall elastic and viscous modulus, the energy storage and dissipation in the arterial wall and the wall buffering function, by means of a non-invasive recording of pressure (tonometry) and diameter (high-resolution mode-B echography). Type 2 DM patients and non-diabetic individuals, matched by sex, age and hemodynamic characteristics, were evaluated, and a robust mathematical approach that used the pressure-diameter relationship was applied (Fig. (**5**)). The results showed that large arteries viscoelasticity and functions were impaired in hypertensive patients, but the alterations in elasticity differed between hypertensive without and with type 2 DM. As shown in (Fig. **5**), in hypertensive patients, independently of type 2 DM, the slope of the pressure-diameter relationship (associated with wall elasticity) is steeper than in control subjects, and the loop area (related to wall viscosity) is greater than those found in normotensive individuals. As it was stated, an increase in the wall elasticity (stiffness) and viscosity in hypertensive patients could be related to the wall remodeling due to the load imposed on the arterial wall. However, it is noteworthy that for the same hypertension level, type 2 DM patients show a higher modulus of elasticity. Hence, other factors than hemodynamics or hypertension would contribute to the increased arterial stiffness found in type 2 DM hypertensive patients.

Finally, effects of angiotensin converting enzyme (ACE) inhibition with ramipril could be different between hypertensive patients with and without type 2 DM. This pharmacological intervention showed pressure-independent effects on the common carotid artery wall dynamics that differed between groups of hypertensive patients. Despite of a similar reduction in pressure values, the viscoelastic beneficial changes were higher in type 2 DM individuals. Therefore, treatment with ACE inhibition seemed to induce favorable effects on large arteries dysfunction, which may prevent outcomes in this specific population affected by hypertension and type 2 DM.

C) ENDOTHELIAL DYSFUNCTION IN TYPE 2 DM

Endothelial dysfunction accomplishes a number of functional alterations in vascular endothelium, such as: impaired

vasodilatation, angiogenesis and barrier function, inflammatory activation, and increased plasma levels of endothelial products, all of which are generally associated with cardiovascular disease [7]. The relationship between endothelial dysfunction and type 2 DM is complex. In type 2 DM, a common cause may underlie both the endothelial dysfunction and the development of hyperglycemia, whereas other factors, such as dyslipidemia, may additionally contribute to clinical situations. Endothelial dysfunction may, thus, play a primary role in the development of the vascular complications of type 2 DM, complications that are aggravated by hyperglycemia, but that are not primarily dependent on the development of hyperglycemia [11].

Methodological Aspects

Non-invasive ultrasound flow-mediated dilatation (FMD) of the brachial artery is the most widely used method for measuring the endothelial function. Briefly, brachial arteries are scanned, with high-resolution ultrasound imaging, under baseline conditions (at rest) and during hyperemia. This is induced by inflation and deflation of a cuff mostly around the forearm distal to the site scanned with ultrasound. The shear stress caused by the increased blood flow following ischemia induces NO release, which, in turn, causes local arterial vasodilatation. FMD is estimated as the percentage increase in arterial diastolic or mean diameter from baseline conditions to maximum vessel diameter during hyperemia [72, 73].

Due to the lack of a standardized method to measure brachial artery reactivity, mean FMD values differ widely among studies of the same populations. The location of the occlusion (upper arm/lower arm) and the duration of the occlusion (shear stimulus) may explain some of the differences, whereas the measurement location (antecubital fossa/upper arm), and the occlusion pressure (above or below 275 mmHg) do not [48]. FMD fluctuates during the day and is influenced by temperature, stress, diet, glucose levels and menstrual cycle, among other factors [50]. Within-subject variability of FMD is high, with coefficient of variations ranging from 14–50% [50]. Despite this biological variation, under appropriate operator training, there is good intra- and inter-observer reproducibility for measurements of baseline and maximum post-ischemia diameters in the brachial artery (diameter variations of approximately 4%) [74].

Although the optimal methodology for the assessment of brachial FMD is still under debate, recent studies suggest that the equipment should include a high-resolution ultrasound system operating with linear array probe (5-13 MHz) fixed to a stereotactic clamp, a computer image acquisition and automated analysis software. The brachial artery should be imaged in longitudinal section, 5-10 cm proximal to placement of a blood pressure cuff, just below the antecubital fossa. The baseline image should be recorded for 30 s to 1 min, and the cuff should be inflated to supra-systolic pressure (300 mmHg for adult or 50 mmHg above the patient's systolic pressure) for 5 minutes. After cuff release, the brachial artery should be scanned continuously for 2 to 5 min. Each image should be measured at end diastole using an automated computer-based edge detection algorithm [75, 76] (Fig. (**2**)).

Clinical and Patho-Physiological Aspects

The brachial FMD, estimated as the percentage change in the diameter of the brachial artery after reactive hyperemia induced by hypoxia, ranges from about 5-10% in young healthy subjects to 0% in patients with established coronary heart disease. The FMD has proven to be predictive for the presence of coronary heart disease and for future coronary events in high-risk populations, and has also shown to be closely related to coronary vaso-reactivity [77-79].

Type 2 DM is associated with reductions in FMD (endothelial dysfunction), which has already been reported to be inversely and strongly related to the extent of hyperglycemia [80]. Besides, oscillating glucose can have more deleterious effects than constant high glucose on endothelial function and oxidative stress [81]. These alterations have also been described after high-AGEs meals or even a single loading of test meal in type 2 DM patients [82, 83].

The underlying mechanisms related to FMD reduction are suspected to be associated with hyperglycemia and insulin resistance, which results in mitochondria superoxide overproduction, and thus decreased NO availability. Clustering of risk factors, such as dyslipidemia, hypertension and obesity in the metabolic syndrome play an additional role. Insulin-mediated vasodilatation is at least in part NO dependent, thus explaining how insulin resistance may cause endothelial dysfunction. The predictive value of endothelial dysfunction in epicardial coronary arteries of diabetic patients has been established for long-term coronary event [84].

It has been suggested that FMD contributes to atherogenesis, as atherosclerosis is frequently present when type 2 DM is first diagnosed. Thus, glycemic burden can elicit endothelial dysfunction, which may take part in the beginning of atherosclerosis before development of overt diabetes, and it occurs simultaneously with insulin resistance. In turn, endothelial dysfunction induces alterations in vessels that worsen vasculopathy and induces progression of atherosclerotic disease [7, 85].

CONCLUSIONS

There is clear evidence that type 2 DM is associated with higher intima-media thickness and arterial viscoelasticity (stiffness), and with endothelial dysfunction. The underlying patho-physiological mechanisms are complex and remain to be fully elucidated.

In this review, the main arterial wall pathophysiological findings in type 2 DM patients are described, and the main methodological aspects that should be considered in order to understand the relevance of its clinical application to assess the early macro-vascular disease detection is discussed.

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ABBREVIATIONS

ACE	=	Angiotensin converting enzyme
AGEs	=	Advanced glycation end products
AMI	=	Acute myocardial infarction
D	=	Distance
Di	=	Internal diameter
DM	=	Diabetes mellitus
FMD	=	Flow-mediated dilatation
Hb A1C	=	Glycosilated hemoglobin
IMT	=	Intima-Media Thickness
IMTc	=	Carotid intima-media thickness
MAP	=	Mean arterial blood pressure
NO	=	Nitric oxide
PWV	=	Pulse wave velocity
t	=	Time
WHO	=	World health organization

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