Ultrasound assessment of maternal endothelial function: a tool for epidemiology

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ABSTRACT

The endothelium, the inner layer of vessels, represents a huge organ, an important regulator and mediator of vasoactive factors. A number of common diseases including preeclampsia, hypertension, coronary heart disease and diabetes have been linked to poor endothelial function. Tests have been developed to measure endothelial function, but have not been suitable for population-based studies until they became non-invasive. Ultrasound imaging of the vessel diameter and the blood velocity has opened that door. The techniques are used to assess vascular distension and increased flow velocity as a response to reactive hyperemia after arterial occusion or administration of different drugs. Here we discuss the background, describe the techniques currently in use and their potential application in epidemiological studies.

INTRODUCTION

Endothelium is a single layer of cells lining the inner wall of blood vessels. This endothelial lining provides an interface with the blood stream and plays an important role in sensing and responding to stimuli activating various vasoactive systems that function as mediators. These endothelium-derived substances include vasodilators (e.g. nitric oxide (NO) and prostaglandins) and vasoconstrictors (e.g. endothelin-1). Substances derived from the endothelium stabilize platelets, control the migration of white blood cells and lipoproteins into the intima, and discourage the influx of inflammatory cells. Furthermore, they control the dimension of the blood vessels by their action on the vascular smooth muscle cells of the media. An impaired balance between vasoconstrictors and vasodilators, increased vascular permeability and enhanced expression of cell adhesion molecules, which support a procoagulatory proinflammatory state, characterizes endothelial dysfunction. Endothelial dysfunction has been associated with the pathogenesis of a variety of disorders, such as ischemic heart disease (1,2), essential hypertension (3), dyslipidemia (4), diabetes mellitus (5,6) and preeclamptic toxemia of pregnancy (7,8) that have a significant impact on the community health. Understanding the role of endothelium in the pathogenesis has led to better strategies in the management of conditions such as ischemic coronary heart disease, pulmonary hypertension, erectile dysfunction etc. in recent years. A number of epidemiological studies have shown a link between endothelial dysfunction with later development of cardiovascular events (9-12).

Certain diseases that are pregnancy specific, such as gestational diabetes and preeclampsia, not only adversely affect the fetal and maternal wellbeing during pregnancy but also have a long-term effect on the health of the woman and her offspring. For some conditions, such as gestational trophoblastic disease and gestational diabetes, there exists a well-defined follow up policy to subsequently identify and manage their consequences even though the incidence of adverse events may be relatively low. For example, following a diagnosis of gestational trophoblastic disease (even after benign hydatidiform mole) checking urinary or serum beta-hCG at about 6 weeks after any subsequent pregnancy event is a routine in most developed countries. Women who had gestational diabetes are usually offered a glucose tolerance test (or at least a fasting blood glucose measurement) 3 months postpartum and yearly thereafter. The women who had preeclampsia are at increased risk of cardiovascular events, such as hypertension, ischemic heart disease and stroke, later in life (13-16). However, no general policy exists to counsel, prevent, diagnose or screen for adverse health consequences following preeclampsia and provide early intervention. One of the difficulties is, not knowing who among these women are likely to suffer the adverse effects and who may benefit from interventions. There is some indication that these women have had an underlying endothelial dysfunction, but more observational as well as larger epidemiological studies are required to verify these assumptions. Even

the physiological changes in maternal endothelial function during pregnancy are not well defined yet. One of the major problems has been that the invasive methods of studying endothelial function are not appropriate in this regard and noninvasive methods have not been validated well until recently.

METHODS FOR ASSESSING ENDOTHELIAL FUNCTION

A variety of methods can be used *in vitro* and *in vivo* to assess endothelial function. In the experimental settings, investigators have used nitroglycerin to assess the effect of maximum vascular smooth muscle relaxation, N^G-monomethyl-L-arginine (L-NMMA) to inhibit NO production by endothelium, L-arginine to stimulate NO production, antioxidants to slow down and oxidants to hasten the breakdown of NO, phosphodiesterase 5 (PDE5) inhibitors to potentiate and prolong the effect of NO, and acetylcholine or reactive hyperemia to stimulate NO production by endothelium. However, vascular reactivity tests are mostly used to assess endothelial function in the clinical settings. Today two invasive (brachial artery catheterization with venous occlusive strain-gauge plethysmography and intracoronary agonist infusion with Doppler flow wire to measure changes in coronary artery blood flow) and two non-invasive methods (ultrasonographic assessment of changes in brachial artery diameter ± blood flow in response to postocclusive reactive hyperemia and non-invasive assessment of coronary flow reserve using Doppler echocardiography) represent the commonly employed methods in clinical practice. Interestingly, there appears to be a close relationship between endothelial function in the coronary and peripheral circulations (17). However, whether endothelial dysfunction in one vascular bed correlates with endothelial function in other vascular beds and reflects global endothelial function remains controversial.

Invasive methods are obviously not appropriate for studying asymptomatic subjects and are therefore not suitable for population-based epidemiological studies. Two other methods have been proposed for the evaluation of endothelial function: laser Doppler iontophoresis to assess response of forearm skin microvessels to acetylcholine (18-21) and pulse-wave analysis using vascular tonometry (22-24). Although these methods show correlation with flow-mediated dilatation (FMD) they are still not generally accepted methods of measuring endothelial function. Their results may be affected by several factors, such as skin conductivity, current induced vasodilatation, hemodynamic status, vascular structure and vessel wall properties etc. Therefore, FMD of brachial artery is perhaps the most appropriate method for large-scale epidemiological studies and for serial evaluation of endothelial function, whereas investigation of coronary flow reserve using Doppler echocardiography may be contemplated in high-risk population such as in women with a previous history of severe preeclampsia (in spite of pitfalls and being time-consuming).

FLOW-MEDIATED VASODILATATION (FMD)

A sudden increase in the endothelial shear stress acting on the arterial endothelium induces vasodilatation of the peripheral conduit arteries in vivo, which is mainly mediated by an increased endothelial NO release (25). This capacity of endothelium to generate bioactive NO in conduit arteries can be evaluated by measuring the flow-mediated dilatation of the brachial artery (percentage increase in diameter and/or volume blood flow above the baseline values) evoked by post-occlusive reactive hyperemia. High-resolution ultrasonography with a 10 MHz linear array transducer is commonly employed for this purpose and authoritative guidelines (26), technical details (27,28) and extensive reviews (29-31) have been published on this subject.

In brief, the examination is preferably performed in the morning in a fasting state as food intake can alter the arterial response (32). Smoking, tea, coffee or any other stimulants should be avoided before examination. It is advisable to perform the examination in a temperature controlled (23±1 degree centigrade) room with the subjects lying in supine position (pregnant women should be examined in a semi recumbent or left lateral position to avoid hypotension due to the pressure of the gravid uterus on the vena cava inferior). In premenopausal women, it is important to investigate the FMD consistently during a particular phase in the menstrual cycle (33). After a 10 min rest, blood pressure is measured in the left arm using an automatic equipment. An electrocardiogram is continuously recorded and displayed on the screen of the ultrasound machine. A conventional mercury sphygnomanometer cuff is loosely fitted around the upper arm (some investigators prefer to fit it around the forearm, but the proximal limb occlusion causes larger increase in brachial artery diameter leading to more reproducible measurements). Brachial artery of the right arm is located approximately 5 cm proximal to the antecubital fossa using a high-resolution linear ultrasound probe (7.5-12MHz). Colour Doppler is used to help the identification of the artery. A longitudinal image of the artery is obtained keeping the ultrasound beam close to 90 degrees to the vessel walls and the inner diameter is measured in a frozen two-dimensional B-mode image (figure 1a). Blood flow velocity waveforms (figure 1c) are obtained from the brachial artery using pulsed-wave Doppler keeping the angle of insonation <60 degrees. After the baseline measurements are performed the brachial artery is occluded by quickly inflating the upper arm cuff to a pressure 50 mmHg above the systolic blood pressure. After 5 minutes the cuff is released (at least 4.5 minutes of occlusion is required to obtain maximum response) (27). The blood flow velocity waveforms are recorded within 15 seconds of cuff deflation (figure 1d) and the diameter after 60 se-

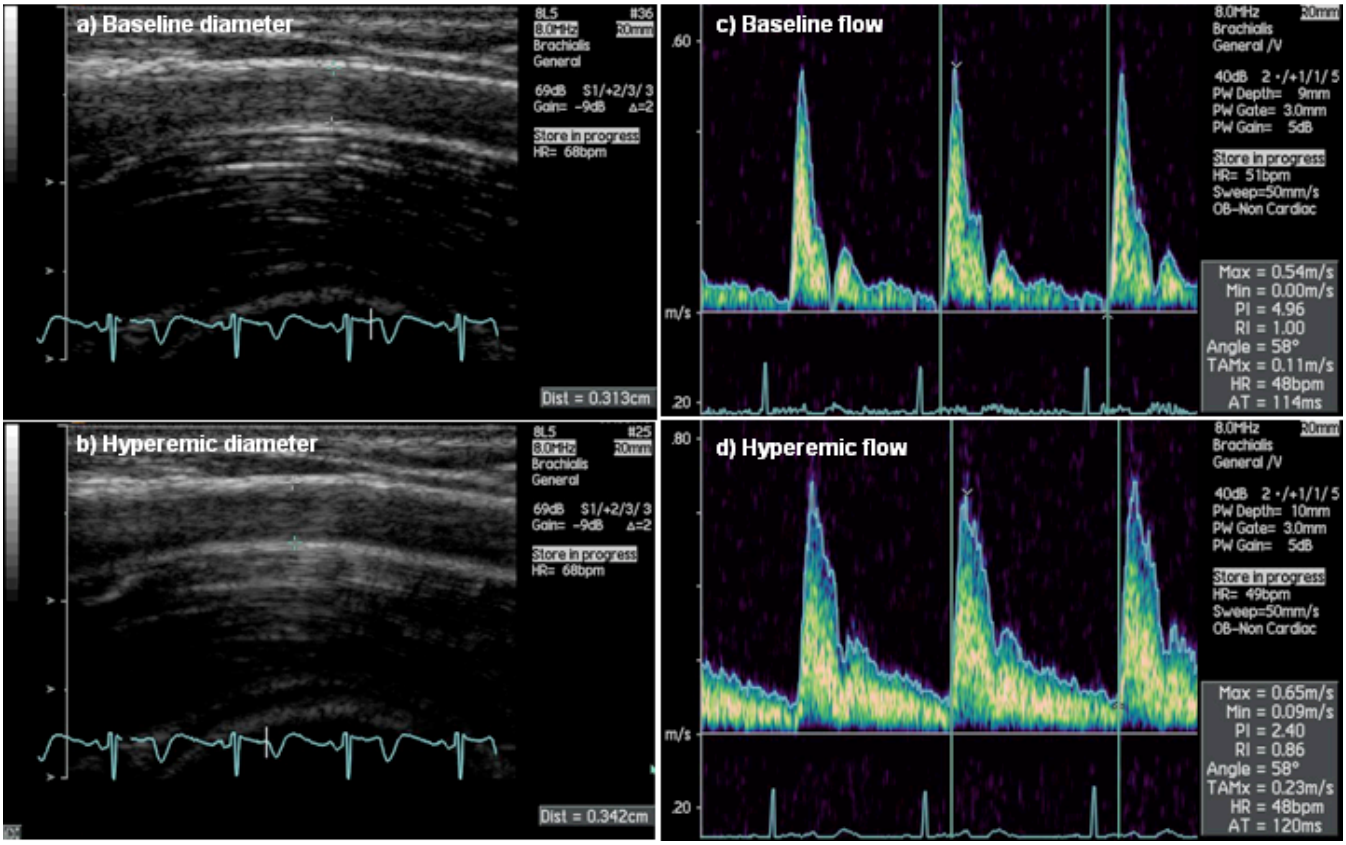

Figure 1. Inner diameter (a) and blood flow velocity waveform (c) of the brachial artery in a healthy woman at baseline, and during reactive hyperemia (b and d).

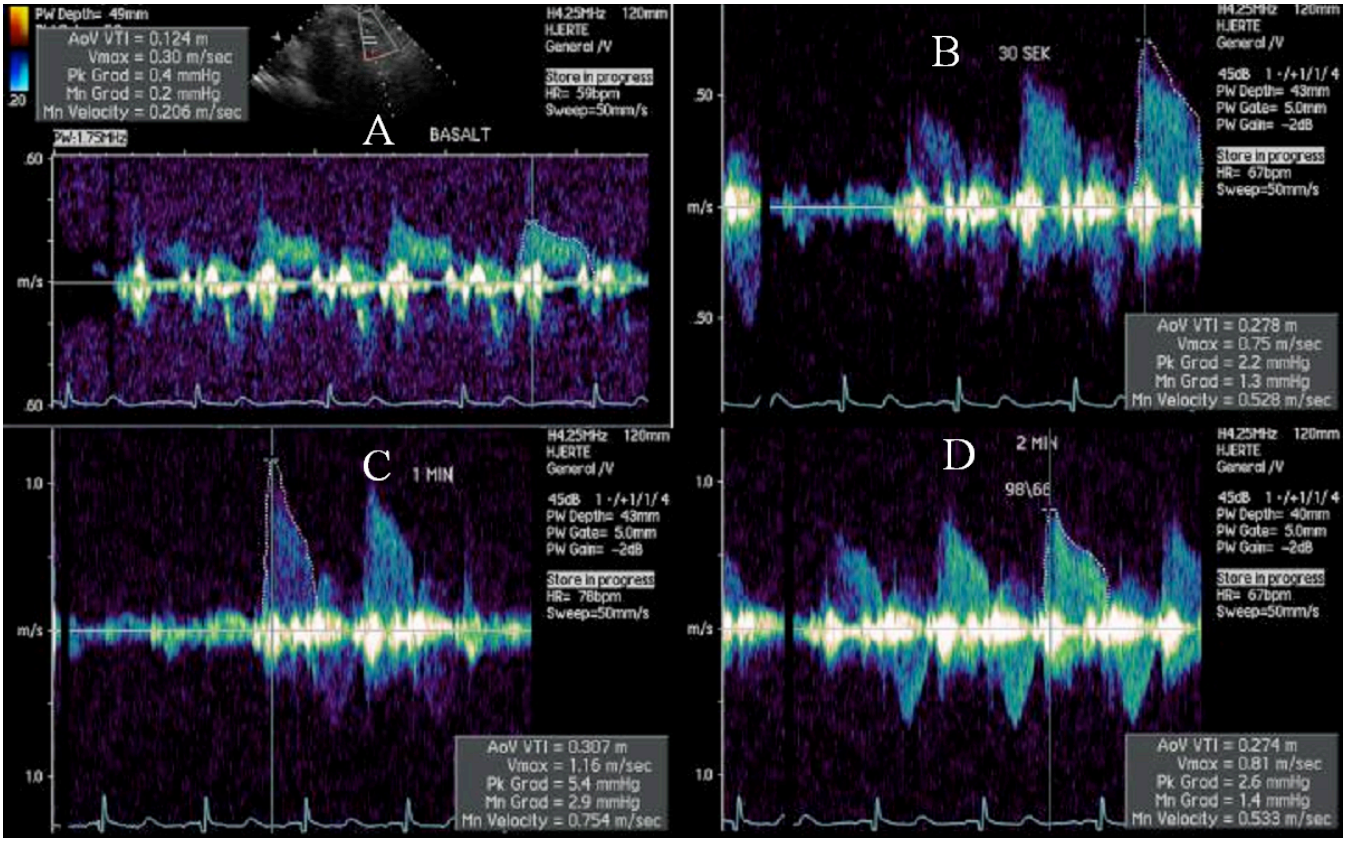

Figure 2. Left descending coronary artery blood flow velocity waveform at baseline (A), 30 seconds (B), 1 minute (C) and 2 minutes (D) after the start of intravenous adenosine infusion in a healthy woman. The mean (time-averaged maximum) diastolic coronary flow velocity increased from 20.6 cm/s at baseline to 75.4 cm/s at 1 minute indicating normal coronary flow reserve.

conds (figure 1b) as the peak diameter increase occurs between 60 to 70 seconds after the cuff release (34). The diameter is measured consistently at the same period of cardiac cycle, which is identified with the help of EKG and the cine-loop facility of the ultrasound machine. An average of at least 3 measurements should be used.

The FMD is calculated as the percentage change in vessel diameter compared with baseline, i.e. FMD % = 100*(diameter after cuff deflation – baseline diameter)/ baseline blood flow. Blood flow is calculated as: Time-averaged maximum velocity * π (brachial artery diameter/2)^2. Increase in blood flow % = 100*(blood flow after cuff deflation – baseline blood flow)/baseline blood flow. Other parameters such as pulsatility index and resistance index can also be calculated from the flow velocity waveforms.

Peak FMD is approximately 8.5 (7-10)% of baseline diameter and may be even higher during pregnancy depending on the gestational age. A FMD of ≤ 4.5% from the baseline has been suggested as the cut-off value for identifying endothelial dysfunction (11) but such a level has not been established for the pregnant population.

CORONARY FLOW RESERVE (CFR)

Coronary flow reserve is the ratio of maximum (stimulated) to baseline (resting) coronary blood flow that has been used as a measure of coronary endothelial function. Invasive studies have shown a link between impaired coronary microvascular dilatation (endothelial dysfunction) and adverse cardiovascular events (35,36). Until recently, methods of assessing CFR had been invasive (coronary sinus thermodilution, coronary sinus sampling of diffusible tracers, intracoronary Doppler guidewire), semi-invasive (transesophageal echocardiography) or expensive and cumbersome (nuclear magnetic resonance, positron emission tomography, single positron emission computed tomography, myocardial scintigraphy). Because of the easier accessibility, brachial artery FMD has been used to reflect the reactivity of other vascular beds including coronary, although there are clear differences among them in regards to vascular architecture, vascular resistance blood flow pattern, and metabolism. With advances in ultrasound technology it became possible to visualize and measure coronary artery blood flow using transthoracic Doppler echocardiography (37-42) that allowed direct investigation of the coronary vasculature noninvasively. Although coronary blood flow at baseline and during maximal arteriolar dilatation following agonist (e.g. adenosine or dipyridamole) infusion is difficult to quantify accurately using echo Doppler, the ratio between hyperemic and baseline coronary blood flow velocity provides a good measure of endothelial function. CFR evaluated by transthoracic Doppler echocardiography corresponds to invasively measured CFR using intracoronary Doppler guidewire technique (43,44).

The CFR is measured usually in the left descending coronary artery (LAD) using conventional echo Doppler equipment with a 3.5 to 6 MHz phased-array ultrasound transducer. The LAD is visualized using colour Doppler (pulse repetition frequency adjusted at a relatively low velocity range of 12-24 cm/s and sample volume 3-5 mm) as a circular vessel in the interventricular groove with predominant diastolic flow signal. The transducer is then rotated 80-90 degrees to obtain longitudinal view of the LAD. The coronary blood velocity waveforms (figure 2) are obtained using pulsed-wave Doppler keeping the angle of insonation as low as possible. Following baseline measurements, intravenous infusion of adenosine in the cubital vein (140µg/kg/min) is started and coronary blood flow velocity is recorded at 30 seconds, 1 minute and 2 minutes after the start of infusion. Adenosine acts instantaneously achieving maximum coronary vasodilatation in 40-60s. The CFR is calculated as the ratio of hyperaemic to resting (baseline) mean (time-averaged maximum) diastolic coronary flow velocity. A CFR value of ≤ 2 is generally considered as a cut-off value to identify significant coronary artery endothelial dysfunction. However, the method requires considerable expertise and patience.

ENDOTHELIAL FUNCTION IN NORMAL PREGNANCY

Few observational studies have indicated that FMD is enhanced in normal pregnancy (45-48) and the NO-dependent vasodilatation appears to improve with advancing gestational age regardless of concurrently appearing lipid changes (49) although some studies suggest that the FMD may be lower in the late third trimester (45,50).

ENDOTHELIAL DYSFUNCTION, PREECLAMPSIA AND MATERNAL HEALTH

Endothelial dysfunction is commonly observed in preeclampsia (8,51). Even though the link between shallow trophoblast invasion, inadequate spiral artery remodeling, abnormal placentation and generalized maternal endothelial dysfunction remains unclear, impaired endothelial function is considered to be an important factor in the pathogenesis of preeclampsia. Impaired vasodilatory response to endothelium-dependent agonists like acetylcholine and bradykinin in isolated myometrial (52,53), omental (54) and cutaneous (55) resistance arteries have provided direct evidence of vascular endothelial dysfunction in preeclampsia. Investigators have also used other indirect markers (e.g. fibronectin, Von Willebrand factor, endothelin 1, asymmetric dimethyl arginine etc.) to show that endothelial function is impaired in peeclampsia (56). Reduced FMD in second trimester has also been reported to be associated with increased risk of preeclampsia (57). However, endothelial dysfunction may not be generalized and may be confined to or be more

pronounced in the utero-placental vasculature, as it is shown that endothelin 1 concentrations are higher in the uterine vein compared to the brachial vein in women with preeclampsia (58). Nevertheless, failure of shear stress-mediated dilation in myometrial arteries might contribute to impaired uteroplacental blood flow in preeclampsia (59), and abnormal uterine artery Doppler waveform (Figure 3) in the second trimester, which is an indicator of increased utero-placental resistance and a risk factor for the development of preeclampsia, is commonly associated with reduced FMD in normotensive pregnant women (60).

Women with cardiovascular risk factors are predisposed to preeclampsia (61) and vice versa (13-16). Therefore, endothelial dysfunction may be a cause or a consequence of preeclampsia. It is not clear whether endothelial function is already impaired even before pregnancy in women who later develop preeclampsia, and whether it further deteriorates during pregnancy. Similarly, it is not known whether women who had preeclampsia continue to have endothelial dysfunction following delivery, how long the dysfunction lasts, and whether it continues to deteriorate. Impaired FMD has been shown to be present 1-3 years after preeclamptic pregnancy (62,63) and microvascular dysfunction after 15-25 years (64). Are these women who continue to have endothelial dysfunction more at risk of developing cardiovascular events than those who do not? Longitudinal studies are urgently needed to answer these questions. Assessment of endothelial function may help in risk stratification during pregnancy as well as later in life.

CONCLUSION

Ultrasonographic assessment of maternal endothelial function can be used as an epidemiological tool. It may help in predicting the development and severity of preeclampsia, especially in combination with other tests. Additionally, it may identify a subgroup of women who are at increased risk of developing cardiovascular disease later in life following adverse pregnancy events, such as preeclampsia and intrauterine fetal growth restriction. These women may benefit from counselling, appropriate follow up, advice on lifestyle modification, diet, and in some cases primary pharmacological prophylaxis. Incorporation of maternal endothelial function testing using appropriate methods into ongoing population-based epidemiological studies in Norway could be a step in the right direction in this regard.

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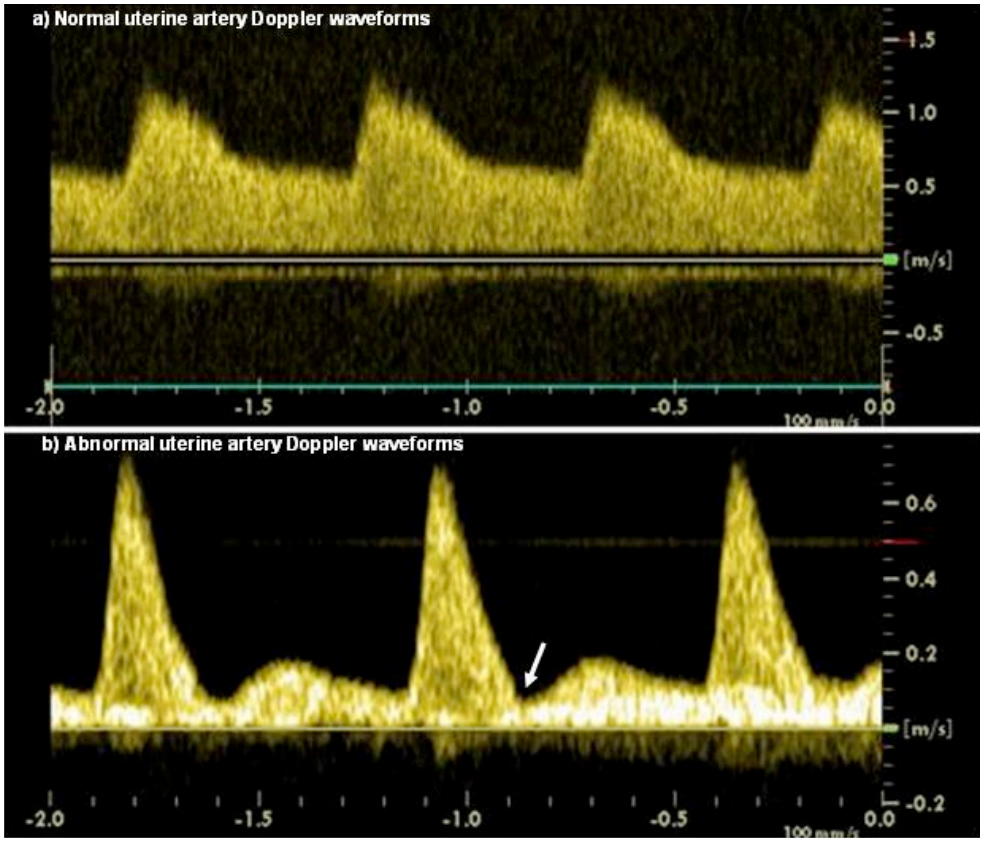

Figure 3. Normal uterine artery blood flow velocity waveforms at 23 weeks of gestation is characterized by low pulsatile velocity (a). An augmented pulsatile waveform signifies increased utero-placental vascular impedance (b). Arrow points to the protodiastolic notch, another sign of increased impedance.

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