

Wall Shear Stress – an Important Determinant of Endothelial Cell Function and Structure – in the Arterial System in vivo

Discrepancies with Theory

Robert S. Reneman^a Theo Arts^b Arnold P.G. Hoeks^b

Departments of ^aPhysiology and ^bBiophysics, Cardiovascular Research Institute Maastricht, University of Maastricht, Maastricht, The Netherlands

Key Words

Endothelial cell function · MRI · Non-invasive vascular ultrasound · Poiseuille's law · Ultrasound · Velocity profiles · Velocity tracers · Viscosity · Wall shear rate · Wall shear stress

Abstract

It has been well established that wall shear stress is an important determinant of endothelial cell function and gene expression as well as of its structure. There is increasing evidence that low wall shear stress, as present in artery bifurcations opposite to the flow divider where atherosclerotic lesions preferentially originate, expresses an atherogenic endothelial gene profile. Besides, wall shear stress regulates arterial diameter by modifying the release of vasoactive mediators by endothelial cells. Most of the studies on the influence of wall shear stress on endothelial cell function and structure have been performed in vitro, generally exposing endothelial cells from different vascular regions to an average wall shear stress level calculated according to Poiseuille's law, which does not hold for the in vivo sit-

uation, assuming wall shear stress to be constant along the arterial tree. Also in vivo wall shear stress has been determined based upon theory, assuming the velocity profile in arteries to be parabolic, which is generally not the case. Wall shear stress has been calculated, because of the lack of techniques to assess wall shear stress in vivo. In recent years, techniques have been developed to accurately assess velocity profiles in arterioles, using fluorescently labeled particles as flow tracers, and non-invasively in large arteries by means of ultrasound or magnetic resonance imaging. Wall shear rate is derived from the in vivo recorded velocity profiles and wall shear stress is estimated as the product of wall shear rate and plasma viscosity in arterioles and whole blood viscosity in large arteries. In this review, we will discuss wall shear stress in vivo, paying attention to its assessment and especially to the results obtained in both arterioles and large arteries. The limitations of the methods currently in use are discussed as well. The data obtained in the arterial system in vivo are compared with the theoretically predicted ones, and the consequences of values deviating from theory for in vitro studies are considered. Applications of wall shear stress

as in flow-mediated arterial dilation, clinically in use to assess endothelial cell (dys)function, are also addressed. This review starts with some background considerations and some theoretical aspects.

Copyright © 2006 S. Karger AG, Basel

Background Considerations

During left ventricular ejection, the forces generated by the heart expel blood into the arterial system, resulting in the exertion of hemodynamic forces on the artery wall. The endothelial cells, lining the artery wall on the luminal side, sense a pressure pulse, i.e. the difference between diastolic and systolic blood pressure, and a tangential stress exerted by the flowing blood. The pressure pulse induces distension of the artery wall, resulting in mainly radial and circumferential wall strain, i.e. the systolic increase in diameter and cross-sectional area relative to the end-diastolic level, respectively. The tangential stress is known as wall shear stress. It can be estimated as the product of wall shear rate and blood viscosity, wall shear rate being defined as the radial derivative of blood flow velocity at the wall. It has been well established that, in addition to biochemical mediators, these biomechanical forces are important determinants of endothelial cell function. For example, wall shear stress regulates arterial diameter by modifying the production of vasoactive mediators by endothelial cells [1, 2], while both wall shear stress and circumferential strain are determinants of endothelial gene expression [3]. As assessed *in vitro*, endothelial genes upregulated by shear stress include transcription factors, growth factors, adhesion molecules and enzymes; they can be transiently or more permanently upregulated [4]. It is of interest to note that enhanced shear stress upregulates intercellular adhesion molecule-1 (ICAM-1) [5, 6], but that the effect on vascular cell adhesion molecule-1 (VCAM-1) is dependent on the type of shear stress applied. Upregulation of VCAM-1 is observed when the cells are exposed to steady shear stress [7], but downregulation when they are exposed to oscillatory shear stress [6]. Also the redox state of endothelial cells was found to be dependent on the type of shear stress applied [8]. Walpole et al. [9] showed that *in vivo* the effect of shear stress on the expression of ICAM-1 and VCAM-1 is shear level dependent. In their experiments, low shear stress enhanced VCAM-1 expression substantially and suppressed ICAM-1 expression, while high shear stress enhanced ICAM-1 expression substantially and VCAM-1 expression only mildly. Shear

stress downregulates endothelin-1, a vasoactive molecule [10], and thrombomodulin [11]; the latter downregulation being completely reversible [11]. These findings indicate that endothelial cells can discriminate between subtle variations in loading conditions which may include differences in temporal and spatial gradients of shear stress [12].

Atherosclerotic lesions preferentially originate in areas of disturbed flow associated with low shear stress [13–15]. Recently, it has been shown that genes are differently expressed in areas of undisturbed and in areas of disturbed flow [16]. *In vitro*, shear stress levels of 1.0 [17] to 1.5 Pa [18] induce atheroprotective endothelial gene expression profiles, while a shear stress level of 0.4 Pa stimulates the expression of an atherogenic phenotype [18]. Despite the increasing evidence that fluid dynamical forces play a role in the expression of endothelial genes that are likely involved in atherogenesis, further investigations are needed to rate these findings at their true value. More insight into the level and the type of shear stress responsible for the expression of a specific gene pattern is particularly required.

Biomechanical forces not only affect endothelial cell function, but also its structure. Endothelial cells tend to align with wall shear stress: the higher the wall shear stress, the more elongated the cells [19–21]. These changes are associated with redistribution and rearrangement of intracellular stress fibers [20] and with their number [22]. One has to realize, however, that the shape change may depend on the type of shear stress applied. Under oscillatory shear stress, bovine aortic endothelial cells maintain their polygonal shape as in static culture, while the cells do not exhibit actin stress fibers [19].

The interaction between biomechanical forces and endothelial cell function, called mechanotransduction, is an intriguing mechanism and has been the subject of investigation in the past decade [23–28]. In mechanotransduction, the mechanical forces acting on the luminal side of the endothelial cells, thereby deforming these cells, are transmitted through the cytoskeleton to other sites in the cell [29]. These forces are especially sensed at the basal adhesion points, where the endothelial cell is attached to the extracellular matrix, cell junctions and the nuclear membrane, leading to redistribution of forces throughout endothelial cells [24] and to acute and more delayed processes in these cells [23, 24]. In the cellular membrane, that may respond to the induced deformation directly, shear stress activates stretch-sensitive ion channels, phospholipids and integrins, for example [23, 24]. The biomechanical forces transferred to the nuclei can be sensed by

the stretch-sensitive sequences in the promoter of a variety of genes, which include the A and B chain of platelet-derived growth factor, tissue plasminogen activator, transforming growth factor- β , endothelin-1, an endothelial isoform of nitric oxide synthase (NOS3) and ICAM-1 [3, 25]. It has been proposed that the glycocalyx, a network of proteoglycans and glycoproteins of a few hundred nanometers in thickness covering the endothelial cells [30, 31], may sense the fluid dynamic forces [27] and transfer these forces into tensile stress [31]. If this is indeed so, the glycocalyx acts as the first step in the process of mechanotransduction. This hypothesis also implies that endothelial cells are not seeing wall shear stress. This stress, however, remains a driving force for endothelial cell deformation and, hence, its function and structure.

Especially *in vivo*, it is difficult to distinguish between pressure- and shear stress-induced changes in endothelial cell function, because changes in radial and circumferential strain and changes in wall shear stress are inextricably connected and even may interact [2]. Therefore, except for the wall shear-stress-induced changes in arterial diameter, most of the studies on the effect of cyclic strain or shear stress on endothelial cell function and gene expression as well as on their structure have been performed *in vitro*, generally exposing cultured endothelial cells to acute increases in either of these biomechanical forces. Although these studies have provided important information, the experimental conditions in these studies are substantially different from those *in vivo*, where the endothelial cells are continuously exposed to varying levels of circumferential strain and wall shear stress. Besides, it can be argued whether endothelial cells behave similarly *in vitro* and *in vivo*. Moreover, in these experiments the shear stresses applied are generally based upon calculations according to Poiseuille's law, which holds for stiff and straight pipes, Newtonian fluid, steady laminar flow and a fully developed parabolic velocity profile, conditions which are not met *in vivo* (see Theoretical Aspects). Besides, in *in vitro* experiments, endothelial cells derived from different areas are exposed to average shear stress values, assuming wall shear stress to be constant along the arterial tree as predicted by theory. In this light it is of utmost importance to be informed of wall shear stress, being such an important determinant of endothelial cell function and structure *in vivo*, regarding both the level of wall shear stress and the variations in this level along the arterial tree.

The first assessments of wall shear stress *in vivo* were performed in arterioles, either directly by means of pressure, length and diameter measurements [32] or indirect-

ly by deriving wall shear rate [33] from velocity profiles, i.e. the velocity distribution over the cross-sectional area of the vessel, using fluorescently labeled platelets as velocity tracers [34] and estimating wall shear stress from the product of shear rate and plasma viscosity [35]. More recently, fluorescently labeled nanometer particles, providing a better spatial resolution than blood platelets, are in use to assess velocity profiles in vessels accessible to microscopic imaging [36, 37]. It was not until the 90s of the past century that ultrasound [38, 39] and magnetic resonance imaging (MRI) [40, 41] techniques became available to non-invasively assess time-dependent velocity profiles in human peripheral arteries directly, enabling the determination of wall shear rate in these vessels. In large arteries, whole blood viscosity is used to calculate wall shear stress. In human coronary arteries, estimations of wall shear stress have been made by combining flow velocity measurements and angiography [42]. These developments have improved our insights into the level of wall shear stress in arteries and its distribution along the arterial tree. Besides, they allow comparison between the values derived from *in vivo* measurements and those determined on the basis of theory. Especially in large arteries, however, the assessment of wall shear stress remains an approximation due to the limited spatial resolution of the systems in use to determine time-dependent velocity profiles. Moreover, at the present state of the art, *in vivo* studies on the relation between wall shear stress and changes in the artery wall are limited to changes observed in excised arteries or changes which can be observed non-invasively, as those in intima-media thickness (IMT).

In the present review, we will discuss wall shear stress in the arterial system *in vivo*, paying attention to its assessment and especially to the results obtained in both arterioles and arteries. The limitations of the methods in use are discussed as well. Comparisons are made between the wall shear stress values derived from *in vivo* measurements and those derived theoretically. The consequences of wall shear stress values deviating from theory for *in vitro* experiments are considered. Biological consequences of changes in wall shear stress and of its application *in vivo*, as in flow-mediated arterial dilation to assess endothelial (dys)function in the clinic, are addressed as well. Recent findings on the relation between wall shear stress and IMT, as obtained in humans, are presented.

Theoretical Aspects

In all theoretical considerations and nearly all experiments on the interaction between shear stress (τ) and endothelial cell function, either *in vitro* or *in vivo*, this tangential force is calculated on the basis of Poiseuille's law. In *in vitro* and in animal experiments, shear stress is often derived from the measured flow q , the lumen radius r and the medium viscosity η according to the equation:

$$\tau = \frac{4\eta q}{\pi r^3} \quad (1)$$

In clinical studies, shear stress is often calculated from whole blood viscosity and shear rate (γ) as estimated from the measured blood flow velocity and the internal diameter of the artery, assuming Poiseuille flow according to the equation [43]:

$$\gamma = \frac{8v_m}{d}, \quad (2)$$

where v_m is the mean flow velocity of the blood and d the end-diastolic internal arterial diameter. Alternatively, only center line flow velocity is determined. When peak flow velocity v_{max} is used to calculate γ and assuming a parabolic velocity profile, the numerator in this equation becomes $4v_{max}$ (see also equation 5). Equation 2 has also been applied in animal experiments.

The shear stress values calculated in this way might hold for *in vitro*, provided that the conditions meet Poiseuille's law. The latter cannot be achieved in arteries *in vivo*, where we are dealing with non-Newtonian fluid, distensible vessels, unsteady flow and, due to the branching arterial tree, the effect of too short entrance lengths.

The shear stress value for arteries obtained by applying Poiseuille's law is estimated to be 1.5 Pa (15 dyn·cm⁻²) \pm 50% [44]. Based upon the principle of minimal work according to Murray's law [45, 46], which states that the cube of the radius of a parent vessel is equal to the sum of the cubes of the radii of the daughter vessels, it is assumed that mean wall shear stress is constant along the arterial tree [47–49]. As can be learned from equation 1, shear stress critically depends on the radius of the artery. Therefore, in the concept of mean wall shear stress being constant, adaptation of the arterial diameter to this tangential force, i.e. an increase and a decrease in wall shear stress is associated with an increase and a decrease in arterial diameter, respectively, has been considered from early on [50–52], a consideration supported by the experimental findings of Kamiya and Togawa [53]. To date, it has been well established that wall shear stress is an

important determinant of arterial diameter, both acutely and chronically, and that this adaptation process is of great importance in maintaining mean wall shear stress within limits with changing flow requirements [1, 2, 54] or changes in blood viscosity [53, 55, 56].

The unsteady blood flow in a branching arterial system deserves special attention. In arteries, the velocity profile will not develop to a full parabola as a consequence of unsteadiness and short entrance lengths. In arterioles (diameter \sim 100 μ m or smaller), where flow is practically steady, the velocity profile does not develop to a full parabola either, due to branching and the dominating viscous forces in the center of these vessels. In both arteries and arterioles, the velocity profiles are flattened parabolas (see Assessment of Wall Shear Rate *in vivo*). Therefore, shear rate, i.e. the velocity gradient relative to the arterial radius (dv/dr), is low in the center of the vessel and high towards the artery wall. The viscosity is higher in the center of the vessel, because red blood cells tend to stream in the center of the vessel, thereby reducing the shear stress gradients they are exposed to. Less red blood cells travel along the artery wall, where, in addition to a thin layer of plasma, blood platelets are traveling. They are likely dispersed from the center of the vessel due to collision with the larger red blood cells [57, 58]. The velocity profiles are not only flattened, but often also skewed due to curvature effects (fig. 1c). As a consequence, wall shear rate often differs over the cross-sectional area of the vessel (fig. 1d). The skewness of the velocity profile introduces an additional error in calculating wall shear rate from the center line velocity (equation 2).

Blood flow velocity, and, hence, wall shear stress, is high in systole and relatively low in diastole. Because diastole comprises approximately two thirds of the cardiac cycle, the level of wall shear stress during this phase of the cardiac cycle contributes substantially to the calculated mean wall shear stress. During systole, not only the increase in pulse pressure but also the increase in wall shear stress is limited due to the increase in arterial diameter, especially in elastic arteries [59]. It has been shown that distensibility of the artery wall, i.e. the relative increase in the arterial cross-sectional area for a given increase in pulse pressure, reduces wall shear rate by about 30% as compared with rigid arteries [60, 61].

At branch points, the laminar flow field is disturbed. This has been well established in model studies [62, 63], in numerical analyses [64, 65], and in *in vitro* [66] and *in vivo* [67] investigations. When arteries divide, especially where diameter increases in the daughter vessel, as in the carotid artery bulb, regions with predominantly axial and

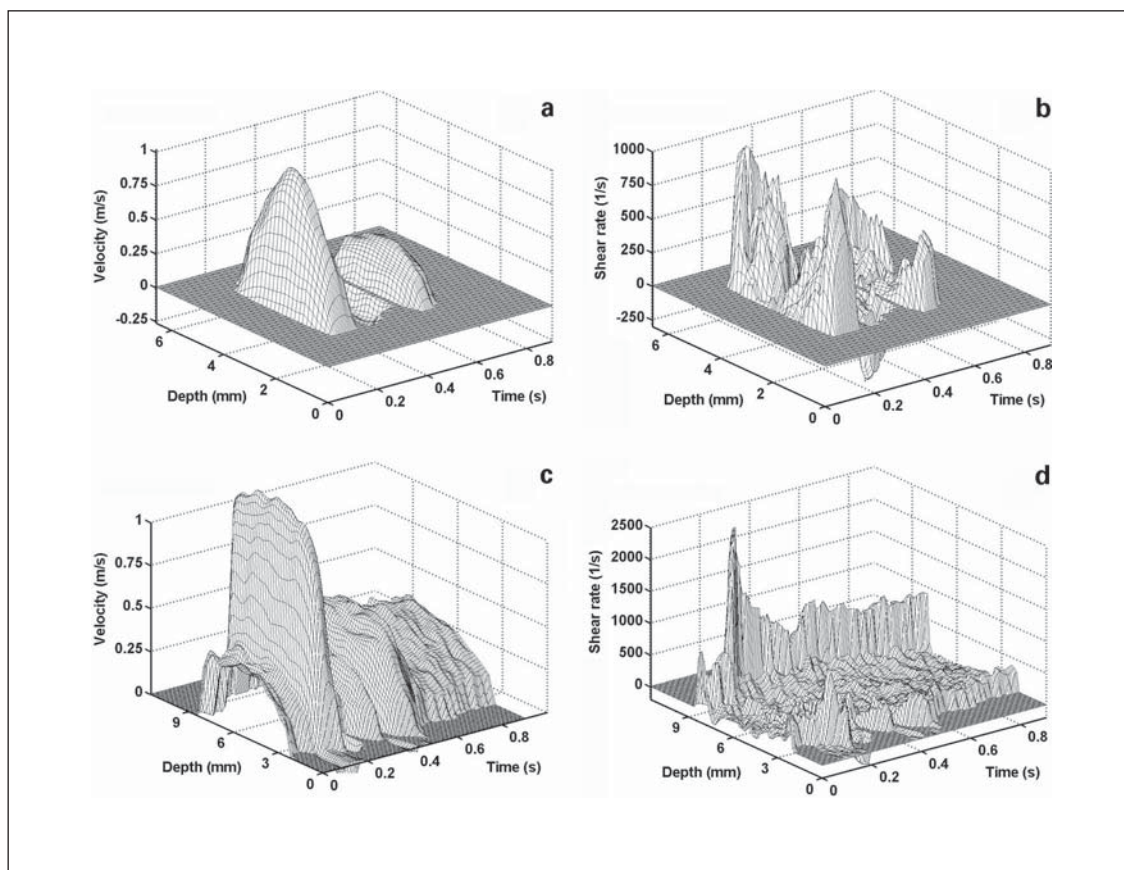


Fig. 1. The velocity profiles (**a, c**) and the shear distributions (**b, d**) as recorded in the brachial (**a, b**) and the common carotid (**c, d**) artery of a presumed healthy volunteer.

unidirectional flow are observed on the side of the flow divider, while on the side opposite to this divider flow separation occurs, and areas of recirculation and flow reversal develop, with flow remaining laminar. Wall shear stress is high near the flow divider and low, and even negative, opposite to this divider [68, 69], which is the preferred site of atherogenesis. Both at and opposite to the flow divider, the flow pattern is oscillatory in nature.

When deriving wall shear rate from the actual velocity profile *in vivo*, blood flow velocities have to be determined accurately close to the wall. This can be achieved in arterioles, using nanometer particles as fluorescent velocity tracers. Therefore, in these vessels, plasma viscosity can be used to calculate wall shear stress, especially since the plasma layer is relatively large in this part of the arterial system. In large arteries, however, wall shear rate is assessed at a distance from the wall due to the limited resolution of the ultrasound and MRI systems employed

to record the velocity profiles *in vivo*. Therefore, in these vessels, the values obtained have to be considered as least estimates, because shear rate increases towards the wall. Because the sample volumes of the ultrasound and MRI systems are mainly seeing whole blood viscosity, this viscosity is used in the calculation of wall shear stress in large arteries. Despite the underestimation of wall shear rate, the wall shear stress values estimated at a distance from the artery wall will not be too different from those at the wall, because viscosity decreases towards the wall; shear stress can be considered as a continuum from the center of the vessel to the wall. Although assessed in venules, extrapolation of the data on shear stress and shear rate as a function of vessel radius, as presented by Long et al. [36], indicates that shear stress determined 250–300 μm from the wall (as in ultrasound systems), which converts to a relative radial position of 0.9 for an artery of 6 mm in diameter, will underestimate shear stress at the wall by about 10%.

One should bear in mind that at the present state of the art the relation between wall shear stress and endothelial cell function and structure can be studied in vivo only at a global level. Investigation of subcellular effects remains restricted to in vitro studies, making, for example, use of atomic force microscopy [20] combined with computational fluid mechanics [70]. In the latter study, it could be demonstrated that the shear-stress-induced reorganization of endothelial cells results in flattening of these cells, thereby reducing the shear stress gradient across endothelial cells. Interestingly, through this mechanism, the shear stress gradient across these cells decreases with increasing wall shear stress.

Direct Assessment of Wall Shear Stress in vivo

In this review we have included wall shear stress in arterioles in vivo to be able to make comparisons with theory along the whole arterial tree and to provide an indication of the shear stress levels endothelial cells are exposed to in these vessels.

Lipowsky et al. [32] were one of the first to assess wall shear stress in vivo directly. In cat mesenteric arterioles, wall shear stress was determined by means of micropressure measurements upstream and downstream, and length and diameter measurements. The measurement of micropressure, however, needs a lot of skill and can only be realized in a limited number of experienced centers.

In more recent years, techniques became available to determine velocity profiles in arterioles and, non-invasively, in arteries in vivo, enabling the assessment of wall shear rate from these profiles and, hence, the calculation of wall shear stress.

Assessment of Wall Shear Rate in vivo

In Arterioles

In the assessment of velocity profiles in arterioles, fluorescent particles are used as velocity tracers. To assess the profiles, pairs of flashes are given, and a short preset time interval between the two flashes provides in one video field two images of the same tracer displaced over a certain distance for the given time interval. The time interval between the two flashes (range 1–5 ms) is selected so that the concomitant images of the tracer show no or only little overlap. By triggering the sequence of light flashes by the R wave of the ECG, within a measuring

period all flash pairs can be recorded at a selected moment in the cardiac cycle. A preset delay is used to determine velocity profiles in systole and diastole. Video recordings are analyzed frame by frame. To determine the velocity profiles, the centroids of the images of the tracers are identified and the following parameters are measured: (1) the displacement of the tracer in the preset time interval, yielding its velocity, and (2) its relative radial position in the vessel. Originally, fluorescently labeled blood platelets were used as velocity tracers [34]. Because in their study the centroid of the blood platelet image was used for the measurements, no data points could be obtained closer to the wall than 0.5 μm because of the physical size [71] and the orientation [72] of blood platelets. In more recent years, nanometer particles are in use to assess the flow velocity distribution, basically using similar processing techniques, [36, 37]. Because of their smaller size (0.4–0.5 μm in diameter), flow velocities can be determined closer to the wall than with the fluorescently labeled blood platelets. Originally, the velocity of the tracers and their position were determined by hand, a time-consuming procedure, but recently a computerized two-dimensional correlation technique to assess displacement and position of the tracers has been developed [37].

The experimentally determined velocity profiles can be adequately described with the following equation, modified after Røevros [73]:

$$v(r) = v_{max} \left(1 - \left| a \frac{r}{R} + b \right|^K \right), \quad a > 0, \quad (3)$$

where $v(r)$ is the flow velocity at the radial position r , the vertical lines denote absolute values, v_{max} is the maximal flow velocity in the vessel, R is the radius of the vessel, a is a scale factor allowing a non-zero intercept of the fit with the vessel wall, b is a parameter correcting for a shift of the top of the profile away from the vessel center and K describes the degree of flattening of the profile. $K = 2$ for a fully developed parabolic velocity profile; the flatter the velocity profile is, the higher K will be.

The velocity profiles in arterioles assessed with labeled blood platelets as markers and described in this way are flattened parabolas in both systole and diastole [34, 35] with K factors varying between 2.3 and 4. The ratio of the maximum and the mean velocity of the profile, being 2 in case of a parabolic profile, was found to range from 1.39 to 1.54, which also indicates flattening of the profile. Because of asymmetry of the velocity profiles, differences in wall shear rate at opposite walls may have to be appreciated [33]. Therefore, when interested in global wall

shear rate in arterioles, the average of the values at opposite walls is taken as the representative reading. In arterioles the velocity profile of fluorescently labeled red blood cells is similar to that of blood platelets, but most of the red blood cells are traveling at streamlines with higher velocities (see Theoretical Aspects), that is more towards the center of the vessel [34, 35].

By describing the velocity profiles recorded by the best fit through the measuring points with the use of equation 3, and a linear extrapolation from the point closest to the wall where velocity can be measured (transition point) to zero flow at the wall, a least estimate of wall shear rate (WSR) can be determined by means of the following equation [33]:

$$WSR = \frac{2v(x)}{D(1-|x|)}, \quad (4)$$

where $v(x)$ is the velocity at x , which is the relative radial position of the transition point, and D is the vessel diameter.

The wall shear rate values expected in the case of a parabolic velocity distribution (WSR_p), but with the same volume flow in the arteriole can be derived with the following equation:

$$WSR_p = \frac{4v_{max}}{D} = \frac{8v_{mean}}{D} \quad (5)$$

The extent to which the wall shear rate values derived from the actual velocity profiles in vivo are higher than those calculated on the basis of a parabolic velocity distribution for the same volume flow is given by the relation:

$$\frac{WSR}{WSR_p} = \frac{0.25 v(x)}{(1-|x|) \cdot v_{mean}} \quad (6)$$

In Large Arteries

Initially, the velocity distribution in large arteries was assessed by means of multi-gate pulsed Doppler systems [67, 74]. Although this technique has provided interesting information about velocity patterns and artery wall dynamics in, for example, the carotid artery bifurcation [67], it has several important limitations [74]. In more recent years, ultrasound [38, 39] and MRI [40, 75] techniques became available to more accurately determine velocity profiles in large arteries.

Ultrasound

Accurate measurement of low blood flow velocities close to the vessel wall can only be achieved when the high-amplitude low-frequency signals reflected by the artery wall are adequately suppressed without losing the low blood flow velocity information near the wall. This can be accomplished by considering the time-dependent aspects of the reflections and using a band stop filter which adapts its rejection range to the mean frequency of the reflections from the artery wall [76]. In this adaptive filtering technique, these reflections are suppressed by shifting the temporal frequency distribution towards zero frequency, the shift being given by the estimated mean frequency of the reflected signal. Subsequently, the reflections, then centered around zero frequency, are selectively suppressed by a high-pass filter with a low cut-off frequency.

For the assessment of wall shear rate, a conventional two-dimensional imager (Mark 9 HDI; Advanced Technology Laboratories, Bothell, Wash., USA) with a C9-5 curved array is combined with dedicated signal processing to measure the blood flow velocity distribution along a selected line of observation across the center of the artery. After localization of the region of interest, the system is switched to a single line of observation (Motion mode; M-mode) with short emission bursts (2 periods) to retain spatial resolution and a high pulse repetition frequency to facilitate blood flow velocity detection. The radio frequency (RF) signals are captured at a sample rate of 20 MHz and stored on a computer for off-line analysis. After automatic identification of the wall-lumen interfaces, cursors, representing sample volumes, are positioned on the reflections from the anterior and posterior walls [77]. The time-dependent blood flow velocity distribution is obtained with the use of a modeled cross-correlation function applied to the RF data between the cursors after elimination of the wall signals with the adaptive high-pass filter. Calculation of mean blood flow velocity for all RF segments provides a time-dependent velocity profile (fig. 1a, c). The length of the RF segments is selected according to the actual bandwidth of the RF signals (2.5 MHz) and corresponds to 300 μm in depth; the segments are spaced at 150 μm intervals (50% overlap). The shear rate distribution is derived from the radial derivative of the velocity profile at each site and each time instant (fig. 1b, d). Because of the limited resolution of the system, due to the finite size of the sample volume, blood flow velocities cannot be determined at the wall. Therefore, the maximum value of the radial derivative of the

velocity profile is considered as the estimate of instantaneous wall shear rate. In general, the maximum shear rate is assessed 250–300 μm from the blood-intima boundary. From the shear distribution, mean wall shear rate, the time-averaged shear rate over one cardiac cycle, peak wall shear rate, the value at peak systole, and the maximal cyclic change in wall shear rate within a cardiac cycle can be determined. When interested in wall shear rate values in a particular artery in general, the values recorded near the anterior and the posterior wall are averaged to minimize the influence of skewness of the velocity profile and of secondary flows. In studies on the relation between wall shear rate and artery wall structure, the shear rate values assessed locally are used. The assessment of wall shear rate, arterial diameter, the change in arterial diameter during the cardiac cycle (distension) and IMT has been integrated in one system [78], facilitating studies on the relation between these parameters under normal and pathological circumstances. For further details regarding this ultrasound technique, the reader is referred to previous publications of our group [38, 39].

In the common carotid artery, the intra-subject inter-session variability on different days varies between 13 and 15% for peak wall shear rate and between 10 and 12% for mean wall shear rate (coefficient of variation), while the inter-subject variability varies between 16 and 19% for peak wall shear rate and between 11 and 17% for mean wall shear rate [79]. In the femoral artery [80] and the brachial artery [81], these values are somewhat higher. Therefore, measurements over about 16 cardiac cycles are considered to obtain reliable values of peak and mean wall shear rate, i.e. a methodological variation about four times lower than the biological one.

Using similar approaches as in arterioles, it could be demonstrated that also in such elastic arteries as the common carotid artery, the velocity profile is substantially flattened (fig. 1c) with a K factor of 4 in systole [82]. In the femoral artery, the velocity profile is also flattened in systole [75]. In the brachial artery, however, in systole, the velocity profile was found to be close to parabolic [75] (fig. 1a) with a K factor of 2.1 [82]. This difference can likely be explained by the greater relative distension of the common carotid artery [60, 82] and a relatively longer entrance length in the brachial artery. It cannot be excluded, however, that the more parabolic profile in the brachial artery partially results from the smaller diameter of this vessel in relation to the resolution of the ultrasound system.

An important limitation of the ultrasound technique presently in use is that wall shear rate can only be deter-

mined reliably in relatively straight arteries. Therefore, reliable information about wall shear stress in artery bifurcations, the site of preference of atherosclerosis, cannot be obtained. Methods are under development that may allow the assessment of wall shear rate at these sites of interest.

Magnetic Resonance Imaging (MRI)

In MRI, flow velocity distribution in a cross-section of an artery can be visualized as a function of time by placing the artery of interest in a strong static magnetic field ($\sim 1\text{--}2$ T) and modulating the static field with high-frequency magnetic pulses. In this way, a small fraction of the hydrogen atoms (protons) is brought into resonance (~ 150 MHz). After adding a gradient field to the static magnetic field, the phase shift of the resonating atoms becomes position dependent. If there is no flow-related motion, a linear gradient in phase shift is generated. However, if an atom travels along the direction of the magnetic gradient, the phase shift of the position where the atom was excited is brought to a new position in the image. Thus, an additional phase shift is generated proportionally with blood flow velocity. The related deviation in phase shift can be detected by means of the velocity-encoded phase-contrast method. In this method two images are acquired, a reference scan and a velocity-encoded sensitized scan. The phase shift between both images is denoted as the phase contrast image. A velocity map is obtained by multiplication of the observed phase shift with a calibration factor, defined as the aliasing velocity (AVL) divided by 180° .

In MRI flow velocity mapping, the settings have to be optimized for a given experimental condition, because the sensitivity to blood flow velocity increases with decreasing AVL. If blood flow velocity exceeds AVL, the phase shift exceeds the interval of $\pm 180^\circ$ and it will be falsely measured as a phase shift within this interval. This artifact, known as aliasing, requires that AVL should not be lower than the maximum blood flow velocity to be measured. In finding the optimum value for AVL, one should realize that AVL cannot be elevated too much to avoid reduction of the signal-to-noise ratio in the phase assessment, resulting in enhancement of noise in the velocity signal.

The spatial resolution of MRI in the assessment of blood flow velocity is determined by the pixel size in the imaging plane and by slice thickness in the direction perpendicular to the imaging plane. A convenient pixel size

Table 1. Units of the parameters described in this review

Description	Units
Velocity	
Microcirculation	mm·s ⁻¹
Large arteries	cm·s ⁻¹
Wall shear rate	s ⁻¹
Wall shear stress	Pa ^a
Viscosity	mPa·s ^b
Distension (Δd)	mm
Wall strain ($\Delta d/d$)	dimensionless
Distensibility	MPa ⁻¹
Compliance	mm ² ·MPa ⁻¹
IMT	mm

^a 1 Pa = 10 dyn·cm⁻².

^b 1 mPa·s = 0.01 dyn·s·cm⁻².

is 0.5–1.0 mm. For reliable blood flow velocity measurements, the information of 4 pixels has to be collected, limiting the true spatial resolution of velocity assessment to about 1–2 mm. Slice thickness is generally around 4–8 mm. Diminution of slice thickness would enhance the resolution in depth, but at the cost of signal power. Misalignment of the artery with the perpendicular direction of the image further deteriorates spatial resolution. Fortunately, the latter effect is of limited importance in most applications.

The temporal resolution of blood flow velocity mapping is determined by three factors, being the number of cardiac beats for averaging, the measurement interval and the acquisition duration within the cardiac cycle. Averaging over several heart beats requires good periodicity of the flow velocity signal, which may be improved by breath holding during the MRI measurement. By averaging over 2–16 heart beats, flow velocity profiles during the cardiac cycle can be obtained with a repetition time of 25 ms [41]. In complicated flow fields as in turbulence, e.g. near valves or stenoses, the periodicity of the flow velocity signal becomes a problem, hampering reliable multiple-beat averaging. The latter is also difficult during such interventions as reactive hyperemia, when the flow velocity signal becomes noisy due to unsteadiness. For further details regarding flow velocity imaging in arteries by means of MRI, the reader is referred to a recent review by Gatehouse et al. [83].

Following the approach as described above, in the common carotid artery flow velocity maps over the cross-

section of an artery can be obtained with a spatial resolution of 1–2 mm and a temporal resolution of 25 ms, while averaging over 10 heart beats [41, 84]. Since the flow velocity map provides simultaneously flow velocities at various distances from the artery wall, the velocity profile, and, hence, shear rate can be accurately determined in the field of observation. As a consequence of the limited spatial resolution, however, the wall shear rate values are underestimated and will generally be lower than those estimated by means of ultrasound, because with the latter blood flow velocities are measured closer to the wall than with MRI (250–300 and 1,000–1,200 μm , respectively). As with ultrasound, accurate velocity profile recordings can only be obtained in relatively straight arteries.

Calculation of Wall Shear Stress

In arterioles, plasma viscosity can be used to calculate wall shear stress the endothelial cells are exposed to (see Theoretical Aspects). Plasma viscosity can be accurately determined in vitro by means of commercially available glass capillary viscometry systems.

In large arteries, whole blood viscosity is used to calculate wall shear stress (see Theoretical Aspects). In this calculation, the influence of plasma viscosity can be ignored, because in arteries the plasma layer is only 3–7 μm thick [85], which is negligibly small relative to the size of the sample volumes of the ultrasound and MRI systems (see above). Whole blood viscosity (WBV) can be determined using the approximation proposed by Weaver et al. [86]:

$$\log(WBV) = \log(\eta_0) + (0.03 + 0.0076 \log(\gamma)) Ht, \quad (7)$$

where η_0 is plasma viscosity, γ is wall shear rate and Ht is hematocrit.

Under the shear rate conditions derived from the in vivo measurements, i.e. $100 \text{ s}^{-1} < \gamma < 1,000 \text{ s}^{-1}$ in large arteries, the effect of changes in plasma viscosity on whole blood viscosity, calculated by means of equation 7, is negligible [82], leaving shear rate and hematocrit as the relevant parameters. Alternatively, whole blood viscosity can be determined in vitro by means of a cone/plate viscometer. A limitation of this approach is that viscosity cannot be determined at appropriate and sufficiently high shear rates in all subjects studied [87], resulting in too high estimates of whole blood viscosity.

The units of the parameters, as described in the present review, are presented in table 1.

Table 2. The peak (PWSR) and mean wall shear rate (MWSR), and the peak (PWSS) and mean wall shear stress (MWSS) values determined in the common carotid artery (CCA) of presumed healthy volunteers and published in literature

Characteristics	Hoeks et al. [39] ^a		Gnasso et al. [87] ^b	Samijo et al. [79] ^a		Samijo et al. [92] ^a			Kornet et al. [91] ^a	Dammers et al. [82] ^a	
	CCA	CCA	CCA	CCA	CCA	CCA	CCA	CCA	CCA	CCA	
Artery	CCA	CCA	CCA	CCA	CCA	CCA	CCA	CCA	CCA	CCA	
Subjects	9 (M)	7 (M)	21	11 (M)	11 (F)	12 (M)	7 (M)	12 (F)	8 (F)	53	10 (7 M)
Age, years	20–30	60–70	35.9 (21–64)	24 (20–36)	25 (19–32)	20–29	50–59	20–29	50–59	18–67	20–30
D, mm	6.5 ± 0.4	7.7 ± 0.7	5.5 ± 0.7 (4.3–8.2)	6.3 ± 0.4	6.1 ± 0.2	6.5 ± 0.6	6.4 ± 0.5	6.0 ± 0.3	6.3 ± 0.3		6.7 ± 0.5
PWSR, s ⁻¹	1,100 ± 231	765 ± 138	640	1,338 ± 376	1,074 ± 244	1,050 ± 264	767 ± 114	965 ± 182	793 ± 136	900	1,047 ± 345
MWSR, s ⁻¹	342 ± 48	310 ± 79	260	414 ± 82	379 ± 53	408 ± 83	357 ± 81	375 ± 52	341 ± 99	310	360 ± 111
PWSS, Pa			2.9 ± 0.8 (1.4–5.3)	4.3 ± 1.3	3.3 ± 0.7	3.6 ± 0.8	2.6 ± 0.3	2.9 ± 0.5	2.5 ± 0.4		3.4 ± 0.8
MWSS, Pa			1.2 ± 0.3 (0.9–3.0)	1.3 ± 0.27	1.2 ± 0.2	1.4 ± 0.2	1.2 ± 0.2	1.1 ± 0.2	1.1 ± 0.2		1.2 ± 0.2
V _{peak} , cm · s ⁻¹	95 ± 11	57 ± 13	97 ± 23 (58–156)	105 ± 21	82 ± 13	90 ± 11	67 ± 10	76 ± 14	62 ± 9		87 ± 26
V _{mean} , cm · s ⁻¹	30 ± 4	20 ± 6	30 ± 6 (20–42)	32 ± 4	27 ± 6	32 ± 5	30 ± 7	27 ± 4	28 ± 5		30 ± 8
Blood viscosity mPa · s			4.6 ± 0.3 (4.0–5.2) ^c	3.2 ± 0.3 ^d	3.1 ± 0.3 ^d	3.0 ^d	3.2 ^d	2.9 ^d	3.2 ^d		

The diameter (D), the peak (V_{peak}) and mean (V_{mean}) blood flow velocity, and the blood viscosity values, when assessed, are presented as well. Means ± SD and occasionally ranges (within parentheses) are presented. M = Males; F = females.

^a Wall shear rate determined from the in vivo recorded velocity profile by means of ultrasound.

^b Wall shear rate determined by means of ultrasound, assuming a parabolic velocity profile.

^c Determined in vitro at a shear rate of 225 s⁻¹.

^d Determined with the Weaver equation (see text, equation 7).

Wall Shear Rate/Stress Values in vivo

In Arterioles

Wall shear rate in rabbit mesenteric arterioles, derived from actual velocity profiles, was found to be on the average 1,700 s⁻¹, ranging from 472 to 4,712 s⁻¹ [33]. The average wall shear stress, calculated from these shear rate values and a plasma viscosity of 1.07 mPa · s, a value commonly found in rabbits, is 1.82 Pa with a range of 0.51–5.0 Pa [35]. The substantial spreads in wall shear rate and wall shear stress in these arterioles can be explained by the variations in flow velocity, peak flow velocity varying between 1.3 and 14.4 mm · s⁻¹, and the absence of any regulatory property of these arterioles: their diameter does not change in response to changes in blood flow velocity [88]. The wall shear stress values found in arterioles of the rabbit mesentery are significantly lower than those found by Lipowsky et al. [32] by means of pressure gradient and diameter measurements in arterioles of the cat mesentery. In the latter vessels, wall shear stress was found to be 4.71 ± 2.34 Pa (mean ± SD). In their study, however, the reduced velocity, defined as mean flow velocity divided by vessel diameter, was significantly higher than ours in the rabbit mesentery (on the average 208 vs. 87 s⁻¹, respectively). Considering the non-regulatory properties of mesenteric arteries, differences in reduced velocity may explain the differences in wall shear stress values found by Lipowsky et al. [32] and our group [33].

Comparison of the wall shear rate values derived from the in vivo determined platelet velocity profiles with those calculated on the basis of a parabolic velocity profile, using equation 6, reveals on the average a 2.1 times lower wall shear rate (range: 1.5–3.9 times), when assuming a parabolic velocity profile. This underestimation of wall shear rate, and, hence, of wall shear stress, is even more pronounced when nanometer particles, allowing flow velocity assessments closer to the wall, rather than platelets are used as velocity tracers [36].

In Large Arteries

An important observation made in human arteries is that mean wall shear stress, calculated from wall shear rate, derived from in vivo recorded velocity profiles, and whole blood viscosity is far from constant along the arterial tree as predicted by theory. In the common carotid artery of presumed healthy volunteers, mean wall shear stress was found to be within the limits of the theoretically predicted value of 1.5 Pa ± 50%, varying on the average between 1.1 and 1.4 Pa in the different study populations (table 2). In muscular conduit arteries of presumed healthy volunteers, however, mean wall shear stress is substantially lower, reaching average values in the common femoral artery, the superficial femoral artery and the brachial artery varying between 0.3 and 0.4 Pa, around 0.5 Pa and between 0.4 and 0.5 Pa, respectively, in the different populations studied (table 3). The wall

Table 3. The peak (PWSR), mean (MWSR) and cyclic wall shear rate (CWSR), and the peak (PWSS), mean (MWSS) and cyclic wall shear stress (CWSS) values assessed in the common (cFA) and superficial femoral arteries (sFA), and the brachial artery (BA) of presumed healthy volunteers and published in literature

Characteristics	Kornet et al. [89] ^a		Dammers et al. [81] ^a				[82] ^a		Silber et al. [75] ^b		
Artery	cFA	sFA	cFA	sFA	BA	BA	BA	BA	BA	BA (MRI)	FA (MRI)
Subjects	11	10	6	12	7 (M)	7 (F)	8 (M)	8 (F)	10 (7M)	24 (9 M/15 F)	24 (9 M/15 F)
Age, years	20–29	20–29	60–74	60–74	36±4.7	37±5.6	59±4.6	58±4.5	23.7±3.4	27±6	27±6
D, mm	7	6.3	7	6.3	4.4±0.6	3.9±0.6	4.2±0.5	3.1±0.4	3.7±0.7		
PWSR, s ⁻¹									770±170		
MWSR, s ⁻¹									95±24		
CWSR, s ⁻¹									971±234		
PWSS, Pa	4.0±1.3	3.4±0.6	3.8±1.2	4.0±0.1	3.3±0.7	2.7±0.6	3.3±0.5	2.9±1.2	3.9±0.8	1.2±4	1.3±0.3
MWSS, Pa	0.4±0.3	0.5±0.1	0.3±0.1	0.5±0.2	0.5±0.1	0.4±0.2	0.5±0.3	0.5±0.2	0.5±0.2		
CWSS, Pa	5.1±1.6	5.1±1.1	4.8±1.2	5.1±1.9					4.9±1.1		
V _{peak} , cm·s ⁻¹					59±15	45±15	49±12	46±13	68±16		
V _{mean} , cm·s ⁻¹					7.5±2.0	8.1±4.3	8.3±6.4	8.1±4.3	7.7±2.7		
Blood viscosity mPa·s					5.0±0.2 ^c	4.8±1.3 ^c	5.0±0.2 ^c	4.9±1.3 ^c			

The diameter (D), the peak (V_{peak}) and mean blood flow velocity (V_{mean}), and the blood viscosity values, when assessed, are presented as well. Means ± SD and occasionally ranges are presented. M = Males; F = females.

^a Wall shear rate determined from the in vivo recorded velocity profile by means of ultrasound.

^b Wall shear rate determined by means of MRI, assuming a parabolic velocity profile.

^c Determined with the Weaver equation (see text, equation 7).

shear stress values presented in these tables are based upon the average of the shear rate values recorded near the anterior and posterior walls (see Assessment of Wall Shear Rate in vivo and Ultrasound).

The lower mean wall shear stress values in these conduit arteries at rest can be explained by the high peripheral resistance in these vessels, reducing mean volume flow and inducing reflections. In the femoral artery, during vasodilatation, induced by reactive hyperemia, adaptation of the peripheral resistance results in mean wall shear stress values not significantly different from those in the common carotid artery [89]. This observation indicates that mean wall shear stress is regulated locally and strongly depends on the characteristics of the peripheral circulation. In case of matching of the characteristic and input impedances, as in the brain circulation, reflections are practically absent, while they are dominant in the arm and leg circulation where these impedances are not well matched at rest. The idea that mean wall shear stress is regulated locally is supported by the observation that no correlation could be found between wall shear rate and wall shear stress in the common carotid and brachial arteries assessed in the same study population [82]. The low mean wall shear stress in the femoral and brachial arteries at rest allows for an increase in mean wall shear stress

during exercise, without reaching levels that could be damaging to the endothelial cells.

In normal coronary arteries, mean shear stress, derived from intra-coronary velocity measurements and coronary angiography, was found to be on the average 0.68 Pa (range 0.33–1.24 Pa) [42]. One should realize, however, that these values are likely to be underestimations, because they were calculated by means of a modification of equation 1, assuming the velocity profile to be parabolic (see below).

Although large arteries adapt their diameter to changes in blood flow velocity, maintaining mean wall shear stress within limits, inter-individual variations in this parameter have to be appreciated (tables 2, 3). Despite this variability, even at the same site in a bifurcation, in the carotid artery bulb specific areas of low wall shear stress can be identified [90].

Peak wall shear stress is not significantly different between elastic and muscular arteries and varies between 2.5 and 4.3 Pa in the common carotid artery, between 3.4 and 4.0 Pa in the femoral arteries and between 2.7 and 3.9 Pa in the brachial artery in the different populations studied (tables 2, 3).

Mean wall shear stress does not only vary along the arterial tree, but also within artery bifurcations. In the

femoral artery bifurcation, mean wall shear stress was found to be significantly lower in the common than in the superficial femoral artery; the former artery seeing reflections from both the superficial and the deep femoral artery, resulting in a longer-lasting negative flow during diastole in the common femoral artery [89]. Peak and maximal cyclic stress are not significantly different between the common and superficial femoral arteries [89]. Similarly, in the common carotid artery, mean wall shear rate, and, hence mean wall shear stress, was found to be lower near the bifurcation than about 3 cm more proximally, probably because at the latter site the influence of reflections from the external carotid artery has greatly disappeared [91].

No significant differences in mean wall shear stress could be detected between men and women, either in the common carotid artery [92] or in the brachial artery [81]. Only peak wall shear stress in the common carotid artery was found to be higher in males than in females [92].

Mean and peak wall shear rate are higher in elastic than in muscular conduit arteries. In the common carotid artery, mean and peak wall shear rate varies in the different study populations between 310 and 414 and between 900 and 1,338 s^{-1} , respectively (table 2). In the brachial artery, mean and peak wall shear rate was found to be on the average 95 and 770 s^{-1} , respectively (table 3). The lower wall shear rate in the brachial than in the common carotid artery implies that whole blood viscosity is higher (equation 7) in the former artery. Indeed, in these studies, the whole blood viscosity values range from 4.8 to 5.0 $mPa \cdot s$ in the brachial artery and from 2.9 to 3.2 $mPa \cdot s$ in the common carotid artery (tables 2, 3). Whole blood viscosity in the common carotid artery increases with age in both men and women, reaching the level of significance only in the latter.

As in arterioles, in large arteries, the wall shear rate values derived from the in vivo recorded velocity profiles, and, hence, the wall shear stress values, are higher than those calculated on the basis of a parabolic velocity profile. In the common carotid artery, mean wall shear rate is underestimated by a factor of 2–3 [39, 82] and mean wall shear stress by a factor of 2 [82], when assuming a parabolic velocity profile. In the brachial artery, the underestimation of mean wall shear rate and mean wall shear stress is less pronounced [82], likely due to the more parabolic shape of the velocity profile in this artery (fig. 1a). The underestimation, when assuming a parabolic velocity profile, is also illustrated by the relatively low mean and peak shear rate values (260 and 640 s^{-1} , respectively) obtained by Gnasso et al. [87] in the common ca-

rotid artery (table 2) and the low peak shear stress values found by Silber et al. [75] in the brachial and femoral arteries (on the average 1.2 and 1.3 Pa, respectively; table 3). Erroneously, correct wall shear stress values can be obtained, when assuming a parabolic velocity profile (table 2) [87]. When underestimating wall shear rate and using these too low values to assess whole blood viscosity, too high viscosity values will be found (see equation 7), resulting in calculated wall shear stress values close to the values derived from the in vivo recorded velocity profiles (table 2).

Comparison of Theory and in vivo Measurements

Based upon theory, mean wall shear stress was calculated to be 1.5 Pa \pm 50%, and according to the theory of minimal energy expenditure it should be constant along the arterial tree (see Theoretical Aspects). As shown above, in vivo mean wall shear stress varies substantially along the arterial tree due to local peripheral influences. Mean wall shear stress is on the average comparable to the theoretically predicted value only in rabbit arterioles and in the human common carotid artery (table 2) and is substantially lower in human conduct arteries, like the femoral and brachial arteries (table 3).

Theory also predicts that mean wall shear stress should be regulated via diameter adaptation. In several in vivo studies it has indeed been shown that if volume flow is forced to change from its physiological state, and thus mean wall shear stress, the arterial diameter adapts and mean wall shear stress is restored towards its baseline value. This adaptation does not only occur in large arteries [53, 93], but also in arterioles, where mean wall shear stress is kept relatively constant over a wide range of flow values [54]. Diameter adaptation to maintain mean wall shear stress within limits also holds for changes in blood viscosity [55, 56]. Also, in end-stage renal failure patients, mean and peak wall shear stress are kept constant when whole blood viscosity changes substantially following hemodialysis, mainly due to a reciprocal change in wall shear rate [94]. It is of interest to note that in embryogenesis, mean shear rate, and, hence, shear stress, is rather constant along the arterial tree in the developing arterial system down to a diameter of about 40 μm [95]. This indicates that during development arterial segments adapt their lumen size to the flow to be carried. Interestingly, at the stage of development studied, a vessel wall media, differentiated vascular smooth muscle cells and active

regulation of vascular tone are still lacking. Thus, even in the absence of vascular smooth muscle, arteries adapt their lumen to shear rate and, hence, shear stress, a process probably controlled by endothelial cells [95].

In vivo measurements have shown that in the human common carotid artery mean wall shear stress decreases with increasing age in both males and females, only in the latter reaching the level of significance [92]. The values reached at the age of 60 years amount to 1.1–1.2 Pa, which are still within the range of optimal values predicted by theory. The decrease in mean wall shear stress in this elastic artery with increasing age may be explained by the increase in arterial diameter to reduce the loss of arterial compliance, i.e. the absolute change in arterial cross-sectional area for a given increase in pulse pressure, at older age [59, 96], a good example of interaction between two parameters to be regulated. Peak wall shear stress in the common carotid artery significantly decreases with age in both males and females [92]. In the brachial artery, neither mean nor peak wall shear stress changes significantly as a function of age in both men and women [81]. Also, in the common and superficial femoral arteries, peak, mean and cyclic wall shear stress do not change significantly with increasing age [89]. These data demonstrate that, within limits, mean wall shear stress is not only maintained at its basic level when hemodynamics change acutely, but also when hemodynamic changes occur more chronically as in aging.

Mean Wall Shear Stress and Structural and Functional Aspects of the Artery Wall in vivo

By relating stationary flow behavior in scale models of human carotid artery bifurcations to intimal thickening in a corresponding series of autopsy specimens, it could be shown that maximal intimal thickening occurs in regions of flow separation and relatively low wall shear stress [13, 14]. In in vitro experiments, intimal thickening was found to correlate negatively with the amplitude of cyclic changes in wall shear stress in a human coronary artery branch [97] and in a human aortic bifurcation [98]. In vivo IMT, measured at one site in the human carotid artery, was found to correlate negatively with estimated averaged wall shear stress [87]. Also in normal coronary arteries, a negative correlation between wall thickness and shear stress has been observed [99]. It is of interest to note that local differences in mean wall shear stress, as in artery bifurcations (see Wall Shear Stress/Rate Values in vivo), do have structural consequences. The lower

mean wall shear stress at the posterior wall in the common than in the superficial femoral artery was found to be associated with a greater IMT at this wall in the former artery [80]. A similar observation has been made in the common carotid artery. Also in this artery at the posterior wall, IMT is greater at the site of lower wall shear rate near the bifurcation than 3 cm more upstream, where wall shear rate is higher [91]. These findings may explain the substantial variations in IMT observed within a relatively short common carotid artery segment [100]. In the brachial artery, no clear correlation could be found between IMT and wall shear stress [81].

Although the compliance of the common femoral artery was found to be greater in athletes, carrying high flow levels during exercise, than in sedentary volunteers, neither peak nor mean wall shear stress is different between these two populations [101]. Also in the brachial artery, no correlation between mean wall shear stress and any of the mechanical artery wall parameters determined could be detected [81].

As has been shown in in vivo and in in vitro experiments, gene expression is not only dependent on the level, but also on the type of shear stress applied (see Background Considerations). Although at the present state of the art no firm conclusions can be drawn regarding the type of wall shear stress that induces changes in the artery wall in vivo, our data suggest that mean wall shear stress has to be considered as a candidate, at least as far as the induction of structural changes is concerned. In the femoral artery bifurcation, after all, the differences in IMT between the common and the superficial femoral artery are associated with differences in mean wall shear stress, but not in peak or cyclic wall shear stress; the values of the latter two parameters are not significantly different in both arteries.

Flow and Arterial Diameter

It has been well established that arterial diameter adapts to acute changes in volume flow [102], and, hence, in wall shear stress. Adaptation of the diameter occurs by modifying the production of autacoids by endothelial cells [2, 54, 103]. In both arterioles and large arteries, nitric oxide and prostaglandins are the most important autacoids involved in this process [2, 54]. Diameter regulation will take place as long as these autacoids are produced by the endothelial cells [54]. The release of nitric oxide and prostaglandins by enhanced shear forces also prevents blood platelets from additional aggregation dur-

ing thrombo-embolic processes; without the production of these autacoids platelet aggregation is enhanced with increasing shear stress in these processes [104, 105].

The arterial diameter does not only adapt to acute changes in wall shear stress, but also to chronic changes in this parameter. Radial artery diameter increases in response to chronically enhanced shear stress [106], while carotid artery diameter decreases following a chronic reduction in shear stress [107]. Similar observations have been made in endurance-trained athletes and paraplegic patients; femoral artery diameter is substantially larger in the former than in the latter, which complies with the higher flow to be carried by this artery in the athletes than in the patients [101]. Femoral artery diameter is also significantly smaller in paraplegic patients than in sedentary volunteers. As a consequence of the smaller diameter mean and peak shear rate [101] as well as mean and peak shear stress [108] are substantially higher in paraplegic patients than in healthy volunteers. The flow-mediated dilation in the femoral artery of paraplegic patients, however, is preserved and was found to be even higher in these patients than in control subjects [109]. Another example of arterial diameter adaptation to chronically applied shear stress is the development of collaterals in ischemic disease: this development is enhanced by increased shear stress [110]. One should bear in mind that the increase in arterial diameter in response to acutely and chronically enhanced wall shear stress has its limits, the maximum increase in diameter of the brachial artery being about 15 [111] and 35% [112], respectively.

Flow-Mediated Arterial Dilation

It is basically the arterial dilation in response to an acute increase in shear stress that is made use of clinically to test the integrity of the endothelium [113–115]. Flow, and, hence, shear stress, is increased by the administration of vasodilators or by inducing reactive hyperemia, and the induced increase in arterial diameter is recorded by ultrasound or MRI. The assessments are made in the brachial artery. In patients with such diseases as hypertension and atherosclerosis, increased volume flow is followed by no or only reduced arterial dilation which is interpreted as disturbed endothelial cell function. In community-based studies, flow-mediated arterial dilation was found to be inversely related to risk factors as smoking, systolic blood pressure and elevated mass index, positively related to prior exercise [116], and inversely associated with carotid artery IMT [117]. Al-

though straightforward at first sight, the test presently in use is subject to criticism.

Although the ultrasound systems used to non-invasively track the artery wall, enabling the assessment of changes in arterial diameter, can resolve displacements of a few micrometers within a cardiac cycle, they are unsuited to track changes during arterial dilation. Repeated assessment of the end-diastolic diameter results in a measurement error on the order of the anticipated flow-induced diameter changes, i.e. 0.2–0.4 mm [111]. Processing of two-dimensional echo video images, a rather time-consuming procedure, improves the resolution, but not the repeatability of the measurement [118]. Moreover, in conduit arteries, as the brachial artery, the degree of flow-mediated dilation may depend on the original diameter of the artery: the smaller the diameter of the artery is, the greater the dilation will be [75, 119], which does not necessarily reflect better endothelial cell function [75]. The degree of flow-mediated dilation also depends on the resting wall shear stress level, the response being greater at higher resting levels [119]. Moreover, the response to a flow stimulus is tested under extreme flow conditions, driving the associated increase in diameter to extreme values over a relatively short period of time. This should be kept in mind, especially since the mechanism of flow-mediated dilation is highly sensitive to the nature of the shear stress stimulus [120]. It has been argued as to whether the reduced flow-mediated dilation, as seen in relation to cardiovascular risk factors (see above), represents disturbed endothelial cell function or results from a reduced stimulus for dilation under these circumstances [121]. All these uncertainties, which cannot adequately be explained at the present state of the art, make the outcome of the test questionable. Another complicating factor is that endothelial cell (dys)function is not determined in the arterial system at a site of preference of atherogenesis, but in the brachial artery which is relatively devoid of atherosclerosis. Therefore, it would be more appropriate to assess the relation between changes in flow, or preferably in wall shear rate, and changes in diameter in arteries prone to the development of atherosclerosis. This could be achieved by following a similar approach as in the newly developed technique to determine stretch-induced baroreceptor sensitivity [59, 122] and to continuously record spontaneous variations in wall shear rate and arterial diameter, and to assess the transfer function from shear changes to diameter changes. The continuous recording of arterial diameter changes is operational in our institute, but the continuous recording of wall shear rate is still problematic because of the high processing power

required. The procedure can be simplified considerably, if in large arteries the shape of the velocity profile will reproduce over consecutive cardiac cycles as in arterioles [34]. Then, instead of wall shear rate center-stream flow velocity or cross-sectional mean flow velocity can be used as input parameter. This approach is under investigation in our institute.

Discussion and Conclusions

Wall shear stress has been shown to be an important determinant of endothelial cell function and the gene expression by these cells as well as of their structure. There is increasing evidence that genes are differently expressed, depending on the level and the type of shear stress they are exposed to. Most of the information about the effect of shear stress on endothelial cell function and structure has been obtained in *in vitro* studies, generally exposing endothelial cells to shear stress levels derived from theory.

It was not until recent years that methods became available to assess wall shear rate, and, hence, wall shear stress, *in vivo*. The approach most commonly used nowadays is to derive wall shear rate from the actual velocity profiles recorded in the microcirculation with the use of fluorescent nanometer particles as velocity tracers and in large arteries by means of ultrasound or MRI. Shear rate distributions across the arterial diameter are derived by determining the radial derivative of the velocity profile at each site and each time instant. From these distributions, peak, mean and cyclic shear rate can be obtained. In arterioles, wall shear stress is estimated as the product of wall shear rate and plasma viscosity, because in these vessels blood flow velocity can be measured close to the wall. In large arteries, whole blood viscosity is used in this calculation, because in these vessels the layer of plasma skimming is negligibly small (3–7 μm) relative to the size of the sample volume of the ultrasound and MRI systems. Blood flow velocities cannot be measured closer to the wall than 250–300 μm with ultrasound systems and than 1,000–1,200 μm with MRI. Therefore, especially in large arteries and more specifically with MRI, the wall shear rate values presented in this review have to be considered as least estimates, because shear rate increases towards the wall. However, in large arteries, the wall shear stress values estimated at a distance from the wall will not be too different from those at the wall, because viscosity is lower near the artery wall than more centrally in the vessel; the underestimation of shear stress at the wall being

approximately 10%. Despite the limitations, MRI and especially ultrasound, being both non-invasive techniques, have provided valuable information about the level of wall shear stress along the arterial tree in humans. One should bear in mind that in MRI not only the spatial resolution, but also the temporal resolution is limited, the latter requiring special precautions to be able to study dynamic processes. An advantage of MRI over ultrasound techniques is that the artery walls can be identified more reliably with MRI.

An important lesson learned from the human studies is that mean wall shear stress is far from constant along the arterial tree as predicted on the basis of the theory of minimal energy expenditure. Only in the human elastic common carotid artery and in rabbit arterioles mean wall shear stress is on the average within the limits of the value predicted by theory, i.e. 1.5 Pa \pm 50% (table 2). In the human muscular femoral and brachial arteries, mean wall shear stress is substantially lower, varying on the average between 0.3 and 0.5 and between 0.4 and 0.5 Pa, respectively, in the different populations studied (table 3). The lower mean wall shear stress in the brachial and femoral arteries than in the common carotid artery at rest can be explained by the high peripheral resistance in the former two arteries, causing reflections thereby reducing mean wall shear stress. Because adaptation of peripheral resistance by vasodilatation results in mean wall shear stress values not significantly different from those in the common carotid artery, it may be concluded that mean wall shear stress is regulated locally. The low mean wall shear stress in the femoral and brachial arteries at rest is logic from a physiological point of view: it allows for an increase in mean wall shear stress during exercise without reaching high levels that could be damaging to endothelial cells.

The arteriolar wall shear stress values presented in this review are determined in non-regulating mesenteric arterioles and are not necessarily representative of the values in regulating arterioles as in skeletal muscle. Insight into the latter values is important, among others, to be informed of the level of wall shear stress at which endothelial cells control autoregulation. To the best of our knowledge, no values of wall shear stress in skeletal muscle arterioles, based upon *in vivo* measurements, have been published.

Within artery bifurcations, differences in mean wall shear stress have to be appreciated, too. It is of interest to note that the differences in mean wall shear stress observed near the posterior walls in the carotid and femoral artery bifurcations of presumed healthy volunteers do

have structural consequences: in these walls, IMT is greatest in the areas of lowest mean wall shear stress. Also, in normal coronary arteries, wall shear stress and IMT correlate negatively. In the limited studies available, no correlation could be found between wall shear stress and artery wall dynamics.

The prediction of theory that mean wall shear stress is a regulated parameter is confirmed by *in vivo* observations. In case of acute changes in flow or in viscosity, mean wall shear stress barely changes due to diameter adaptation and in the latter also due to a reciprocal change in wall shear rate. Also, over longer periods of time, mean wall shear stress seems to be regulated well. In the common carotid artery, mean wall shear stress decreases only slightly, in both men and women, between the age of 20 and 60 years, the values at older age remaining within the limits predicted by theory. In the brachial and femoral arteries, mean wall shear stress does not change with increasing age at all. The slight, but significant decrease in mean wall shear stress in the elastic common carotid artery can be explained by the increase in diameter of this artery at older age to limit the loss of arterial compliance as a consequence of the stiffening of this artery with increasing age. This is an example of competition between two regulatory mechanisms. Although mean wall shear stress seems to be regulated well, in humans inter-individual differences have to be appreciated, while in non-regulatory vascular areas, like the mesenteric artery, wall shear stress varies substantially with blood flow velocity.

The wall shear rate values derived from the *in vivo* recorded velocity profiles, and, hence, the wall shear stress values, are substantially higher than those calculated on the basis of a parabolic velocity profile. In both arterioles and large arteries, mean wall shear rate is on the average underestimated by a factor of about 2, when assuming a parabolic velocity profile. This underestimation does not only affect calculated wall shear stress, but also calculated or determined whole blood viscosity, which strongly depends on shear rate. Therefore, calculation or determination of whole blood viscosity at too low wall shear rate, leading to overestimation of whole blood viscosity, may erroneously result in correct wall shear stress values.

The differences in mean wall shear stress along the arterial tree observed *in vivo* do have consequences for *in vitro* studies regarding the effect of shear stress on endothelial cell function and structure. This effect should be studied at the wall shear stress value the endothelial cells are exposed to in real life; no general value can be taken for endothelial cells derived from different vascular areas.

What is normal for one type of endothelial cell can be too high or too low for another. Because in most of the *in vitro* studies the shear stress levels applied to the endothelial cells are theoretically assumed or calculated based upon Poiseuille's law, the underestimation of mean wall shear stress, when assuming a parabolic velocity profile, has to be considered as well. Application of the right level of shear stress to specific endothelial cells is of utmost importance, because the pattern of gene expression has been shown to be dependent on the level of shear stress applied. For example, shear stress levels of 1.0–1.5 Pa induce atheroprotective endothelial gene expression profiles, while shear stress around 0.4 Pa induces the expression of an atherogenic phenotype. The latter complies with the observation that in artery bifurcations the development of atherosclerosis preferentially occurs in areas of disturbed laminar flow, associated with low wall shear stress. Also, the type of shear stress applied affects gene expression. It is still incompletely understood whether negative shear stress, present at sites of preference of atherogenesis, expresses an atherogenic endothelial gene profile.

Although the ultrasound and MRI techniques presently available to non-invasively assess wall shear rate, and, hence, wall shear stress, *in vivo* have provided important information, they do have their limitations. Not only is the spatial resolution of the systems limited (see above), but at the present state of the art, wall shear rate can reliably be determined only in relatively straight arteries. This is a serious limitation, because information about the level of wall shear stress and its direction in artery bifurcations, especially opposite to the flow divider, is of utmost importance when studying the role of wall shear stress in atherogenesis. In our institute, we are working on a different approach to the assessment of wall shear rate, using multiple lines of observation (multiple M-line technique), that may allow the assessment of the shear rate distribution in the carotid artery bulb. This approach may also be used to record wall shear rate over longer periods of time, making it possible to relate temporal changes in wall shear rate to temporal changes in diameter to provide a better method to assess endothelial cell (dys)function in the clinic. If successful, this approach can be used in arteries prone to atherosclerosis, provided that they are accessible by ultrasound. This can be considered as a major advantage, because at the present state of the art endothelial (dys)function is assessed in the brachial artery that is practically devoid of atherosclerosis.

References

- 1 Koller A, Huang A: Development of nitric oxide and prostaglandin mediation of shear stress-induced arteriolar dilation with aging and hypertension. *Hypertension* 1999;34:1073–1079.
- 2 Busse R, Fleming I: Pulsatile stretch and shear stress: physical stimuli determining the production of endothelium-derived relaxing factors. *J Vasc Res* 1998;35:73–84.
- 3 Gimbrone MA, Topper JN: Biology of the vessel wall; in Chien KR (ed): *Molecular Basis of Cardiovascular Disease*. Philadelphia, Saunders, 1999, pp 331–348.
- 4 Topper JN, Gimbrone MA: Blood flow and vascular gene expression: fluid shear stress as a modulator of endothelial phenotype. *Mol Med Today* 1999;5:40–46.
- 5 Nagel T, Resnick N, Atkinson WJ, Dewey CF Jr, Gimbrone MA Jr: Shear stress selectively upregulates intercellular adhesion molecule-1 expression in cultured human vascular endothelial cells. *J Clin Invest* 1994;94:885–891.
- 6 Chappell DC, Varner SE, Nerem RM, Medford RM, Alexander RW: Oscillatory shear stress stimulates adhesion molecule expression in cultured human endothelium. *Circ Res* 1998;82:532–539.
- 7 Ando J, Tsuboi H, Korenaga R, Takada Y, Toyama-Sorimachi N, Miyasaka M, Kamiya A: Shear stress inhibits adhesion of cultured mouse endothelial cells to lymphocytes by downregulating VCAM-1 expression. *Am J Physiol* 1994;267:C679–C687.
- 8 De Keulenaer GW, Chappell DC, Ishizaka N, Nerem RM, Alexander RW, Griendling KK: Oscillatory and steady laminar shear stress differentially affect human endothelial redox state: role of a superoxide-producing NADH oxidase. *Circ Res* 1998;82:1094–1101.
- 9 Walpolo PL, Gotlieb AI, Cybulsky MI, Langille BL: Expression of ICAM-1 and VCAM-1 and monocyte adherence in arteries exposed to altered shear stress. *Arterioscler Thromb Vasc Biol* 1995;15:2–10.
- 10 Malek AM, Greene AL, Izumo S: Regulation of endothelin 1 gene by fluid shear stress is transcriptionally mediated and independent of protein kinase C and cAMP. *Proc Natl Acad Sci USA* 1993;90:5999–6003.
- 11 Malek AM, Izumo S: Molecular aspects of signal transduction of shear stress in the endothelial cell. *J Hypertens* 1994;12:989–999.
- 12 Barbee KA: Role of subcellular shear-stress distributions in endothelial cell mechanotransduction. *Ann Biomed Eng* 2002;30:472–482.
- 13 Zarins CK, Giddens DP, Bharadvaj BK, Sottiura VS, Mabon RF, Glagov S: Carotid bifurcation atherosclerosis. Quantitative correlation of plaque localization with flow velocity profiles and wall shear stress. *Circ Res* 1983;53:502–514.
- 14 Ku DN, Giddens DP, Phillips DJ, Strandness DE: Hemodynamics in the normal human carotid bifurcation: in vitro and in vivo studies. *Ultrasound Med Biol* 1985;11:13.
- 15 Friedman MH, Deters OJ, Barger CB, Hutchins GM, Mark FF: Shear-dependent thickening of the human arterial intima. *Atherosclerosis* 1986;60:161–171.
- 16 Davies PF, Shi C, Depaola N, Helmke BP, Polacek DC: Hemodynamics and the focal origin of atherosclerosis: a spatial approach to endothelial structure, gene expression, and function. *Ann NY Acad Sci* 2001;947:7–16; discussion 16–17.
- 17 Topper JN, Cai J, Falb D, Gimbrone MA Jr: Identification of vascular endothelial genes differentially responsive to fluid mechanical stimuli: cyclooxygenase-2, manganese superoxide dismutase, and endothelial cell nitric oxide synthase are selectively up-regulated by steady laminar shear stress. *Proc Natl Acad Sci USA* 1996;93:10417–10422.
- 18 Malek AM, Alper SL, Izumo S: Hemodynamic shear stress and its role in atherosclerosis. *JAMA* 1999;282:2035–2042.
- 19 Helmlinger G, Geiger RV, Schreck S, Nerem RM: Effects of pulsatile flow on cultured vascular endothelial cell morphology. *J Biomech Eng* 1991;113:123–131.
- 20 Barbee KA, Davies PF, Lal R: Shear stress-induced reorganization of the surface topography of living endothelial cells imaged by atomic force microscopy. *Circ Res* 1994;74:163–171.
- 21 Sato M, Ohshima N: Flow-induced changes in shape and cytoskeletal structure of vascular endothelial cells. *Biorheology* 1994;31:143–153.
- 22 Walpolo PL, Gotlieb AI, Langille BL: Monocyte adhesion and changes in endothelial cell number, morphology, and F-actin distribution elicited by low shear stress in vivo. *Am J Pathol* 1993;142:1392–1400.
- 23 Davies PF, Tripathi SC: Mechanical stress mechanisms and the cell. An endothelial paradigm. *Circ Res* 1993;72:239–245.
- 24 Davies PF: Flow-mediated endothelial mechanotransduction. *Physiol Rev* 1995;75:519–560.
- 25 Resnick N, Gimbrone MA Jr: Hemodynamic forces are complex regulators of endothelial gene expression. *FASEB J* 1995;9:874–882.
- 26 Chien S: Molecular and mechanical bases of focal lipid accumulation in arterial wall. *Prog Biophys Mol Biol* 2003;83:131–151.
- 27 Moon JJ, Matsumoto M, Patel S, Lee L, Guan JL, Li S: Role of cell surface heparan sulfate proteoglycans in endothelial cell migration and mechanotransduction. *J Cell Physiol* 2005;203:166–176.
- 28 Li YS, Haga JH, Chien S: Molecular basis of the effects of shear stress on vascular endothelial cells. *J Biomech* 2005;38:1949–1971.
- 29 Helmke BP, Davies PF: The cytoskeleton under external fluid mechanical forces: hemodynamic forces acting on the endothelium. *Ann Biomed Eng* 2002;30:284–296.
- 30 Vink H, Duling BR: Identification of distinct luminal domains for macromolecules, erythrocytes, and leukocytes within mammalian capillaries. *Circ Res* 1996;79:581–589.
- 31 Smith ML, Long DS, Damiano ER, Ley K: Near-wall micro-PIV reveals a hydrodynamically relevant endothelial surface layer in venules in vivo. *Biophys J* 2003;85:637–645.
- 32 Lipowsky HH, Kovalchek S, Zweifach BW: The distribution of blood rheological parameters in the microvasculature of cat mesentery. *Circ Res* 1978;43:738–749.
- 33 Tangelder GJ, Slaaf DW, Arts T, Reneman RS: Wall shear rate in arterioles in vivo: least estimates from platelet velocity profiles. *Am J Physiol* 1988;254:H1059–H1064.
- 34 Tangelder GJ, Slaaf DW, Muijtjens AM, Arts T, oude Egbrink MG, Reneman RS: Velocity profiles of blood platelets and red blood cells flowing in arterioles of the rabbit mesentery. *Circ Res* 1986;59:505–514.
- 35 Reneman RS, Woldhuis B, oude Egbrink MGA, Slaaf DW, Tangelder GJ: Concentration and velocity profiles of blood cells in the microcirculation; in Hwang NHC, Turitto VT, Yen MRT (eds): *Advances in Cardiovascular Engineering*. New York, Plenum, 1992, pp 25–40.
- 36 Long DS, Smith ML, Pries AR, Ley K, Damiano ER: Microviscometry reveals reduced blood viscosity and altered shear rate and shear stress profiles in microvessels after hemodilution. *Proc Natl Acad Sci USA* 2004;101:10060–10065.
- 37 Vennemann P, Kiger KT, Lindken R, Groenendijk BC, Stekelenburg-de Vos S, Ten Hagen TL, Ursem NT, Poelmann RE, Westerweel J, Hierck BP: In vivo microparticle image velocimetry measurements of blood-plasma in the embryonic avian heart. *J Biomech*, in press, available electronically.
- 38 Brands PJ, Hoeks APG, Hofstra L, Reneman RS: A noninvasive method to estimate wall shear rate using ultrasound. *Ultrasound Med Biol* 1995;21:171–185.
- 39 Hoeks APG, Samijo SK, Brands PJ, Reneman RS: Assessment of wall shear rate in humans: an ultrasound study. *J Vasc Invest* 1995;1:108–117.
- 40 Oyre S, Pedersen EM, Ringgaard S, Boesiger P, Paaske WP: In vivo wall shear stress measured by magnetic resonance velocity mapping in the normal human abdominal aorta. *Eur J Vasc Endovasc Surg* 1997;13:263–271.
- 41 Oyre S, Ringgaard S, Kozerke S, Paaske WP, Erlandsen M, Boesiger P, Pedersen EM: Accurate noninvasive quantitation of blood flow, cross-sectional lumen vessel area and wall shear stress by three-dimensional paraboloid modeling of magnetic resonance imaging velocity data. *J Am Coll Cardiol* 1998;32:128–134.

- 42 Doriot PA, Dorsaz PA, Dorsaz L, De Benedetti E, Chatelain P, Delafontaine P: In-vivo measurements of wall shear stress in human coronary arteries. *Coron Artery Dis* 2000;11:495–502.
- 43 Hoeks APG, Reneman RS: Flow patterns and arterial wall dynamics; in: Hennerici MG, Meairs SP (eds): *Cerebrovascular ultrasound*. Cambridge: University Press; 2001, pp 77–87.
- 44 Kamiya A, Bukhari R, Togawa T: Adaptive regulation of wall shear stress optimizing vascular tree function. *Bulletin Math Biol* 1984;46:127–137.
- 45 Murray CD: The physiological principle of minimum work. I: The vascular system and the cost of blood volume. *Proc Nat Acad Sci USA* 1926;12:207–214.
- 46 Murray CJ: The physical principle of minimum work applied to the angle of branching of arteries. *J Gen Physiol* 1926;9:835–841.
- 47 Kassab GS, Fung YC: The pattern of coronary arteriolar bifurcations and the uniform shear hypothesis. *Ann Biomed Eng* 1995;23:13–20.
- 48 LaBarbera M: Principles of design of fluid transport systems in zoology. *Science* 1990;249:992–1000.
- 49 Pries AR, Secomb TW, Gaethgens P: Design principles of vascular beds. *Circ Res* 1995;77:1017–1023.
- 50 Rodbard S: Vascular Caliber. *Cardiology* 1975;60:4–49.
- 51 Zamir M: Shear forces and blood vessel radii in the cardiovascular system. *J Gen Physiol* 1977;69:449–461.
- 52 Sherman TF: On connecting large vessels to small. The meaning of Murray's law. *J Gen Physiol* 1981;78:431–453.
- 53 Kamiya A, Togawa T: Adaptive regulation of wall shear stress to flow change in the canine carotid artery. *Am J Physiol* 1980;239:H14–H21.
- 54 Koller A, Kaley G: Shear stress dependent regulation of vascular resistance in health and disease: role of endothelium. *Endothelium* 1996;4:247–272.
- 55 Melkumyants AM, Balashov SA: Effect of blood viscosity on arterial flow induced dilator response. *Cardiovasc Res* 1990;24:165–168.
- 56 Melkumyants AM, Balashov SA, Khayutin VM: Control of arterial lumen by shear stress on endothelium. *NIPS* 1995;10:204–210.
- 57 Tangelder GJ, Teirlinck HC, Slaaf DW, Reneman RS: Distribution of blood platelets flowing in arterioles. *Am J Physiol* 1985;248:H318–H323.
- 58 Eckstein EC: Rheophoresis—a broader concept of platelet dispersivity. *Biorheology* 1982;19:717–724.
- 59 Reneman RS, Meinders JM, Hoeks AP: Non-invasive ultrasound in arterial wall dynamics in humans: what have we learned and what remains to be solved. *Eur Heart J* 2005;26:960–966.
- 60 Perktold K, Thurner E, Kenner T: Flow and stress characteristics in rigid walled and compliant carotid artery bifurcation models. *Med & Biol Eng & Comput* 1994;32:19–26.
- 61 Duncan DD, Barger CB, Borchardt SE, Deters OJ, Gearhart SA, Mark FF, Friedman MH: The effect of compliance on wall shear in casts of a human aortic bifurcation. *J Biom Eng* 1990;112:183–188.
- 62 Bharadvaj BK, Mabon RF, Giddens DP: Steady flow in a model of the human carotid bifurcation. Part I – flow visualization. *J Biomech* 1982;15:349–362.
- 63 Bharadvaj BK, Mabon RF, Giddens DP: Steady flow in a model of the human carotid bifurcation. Part II – laser-Doppler anemometer measurements. *J Biomech* 1982;15:363–378.
- 64 Rindt CCM, Van Steenhoven AA, Janssen JD, Reneman RS, Segal A: A numerical analysis of steady flow in a three-dimensional model of the carotid artery bifurcation. *J Biomechanics* 1990;23:461–473.
- 65 Rindt CC, Steenhoven AA: Unsteady flow in a rigid 3-D model of the carotid artery bifurcation. *J Biomech Eng* 1996;118:90–96.
- 66 Motomiya M, Karino T: Flow patterns in the human carotid artery bifurcation. *Stroke* 1984;15:50.
- 67 Reneman RS, Van Merode T, Hick P, Hoeks APG: Flow velocity patterns in and distensibility of the carotid artery bulb in subjects of various ages. *Circulation* 1985;71:500–509.
- 68 Van de Vosse FN, Van Steenhoven AA, Janssen JD, Reneman RS: A two-dimensional numerical analysis of unsteady flow in the carotid artery bifurcation. A comparison with three-dimensional in-vitro measurements and the influence of minor stenoses. *Biorheology* 1990;27:163–189.
- 69 Reneman RS, Hoeks APG, Van de Vosse F, Ku D: Three-dimensional blood flow in bifurcations: computational and experimental analyses and clinical applications. *Cerebrovasc Disc* 1993;3:185–192.
- 70 Barbee KA, Mundel T, Lal R, Davies PF: Sub-cellular distribution of shear stress at the surface of flow-aligned and nonaligned endothelial monolayers. *Am J Physiol* 1995;268:H1765–H1772.
- 71 Frojmovic MM, Panjwani R: Geometry of normal mammalian platelets by quantitative microscopic studies. *Biophys J* 1976;16:1071–1089.
- 72 Teirlinck HC, Tangelder GJ, Slaaf DW, Muijtjens AM, Arts T, Reneman RS: Orientation and diameter distribution of rabbit blood platelets flowing in small arterioles. *Biorheology* 1984;21:317–331.
- 73 Roelvros JM: Analogue processing of C.W.-Doppler flowmeter signals to determine average frequency shift momentarily without the use of a wave analyzer; in Reneman RS (ed): *Cardiovascular applications of ultrasound*. Amsterdam-London, North-Holland Publ. Co., 1974, pp 43–54.
- 74 Reneman RS, Van Merode T, Hick P, Hoeks APG: Cardiovascular applications of multi-gate pulsed Doppler systems. *Ultrasound Med Biol* 1986;12:357–370.
- 75 Silber HA, Ouyang P, Bluemke DA, Gupta SN, Foo TK, Lima JA: Why is flow-mediated dilation dependent on arterial size? Assessment of the shear stimulus using phase-contrast magnetic resonance imaging. *Am J Physiol Heart Circ Physiol* 2005;288:H822–H828.
- 76 Brands PJ, Hoeks APG, Reneman RS: The effect of echo suppression on the mean velocity estimation range of the rf cross-correlation model estimator. *Ultrasound Med Biol* 1995;21:945–959.
- 77 Meinders JM, Brands PJ, Willigers JM, Kornet L, Hoeks AP: Assessment of the spatial homogeneity of artery dimension parameters with high frame rate 2-D B-mode. *Ultrasound Med Biol* 2001;27:785–794.
- 78 Brands PJ, Hoeks APG, Willigers J, Willekes C, Reneman RS: An integrated system for the non-invasive assessment of vessel wall and hemodynamic properties of large arteries by means of ultrasound. *Eur J Ultrasound* 1999;9:257–266.
- 79 Samijo SK, Willigers JM, Brands PJ, Barkhuyzen R, Reneman RS, Kitslaar PJEHM, Hoeks APG: Reproducibility of shear rate and shear stress assessment by means of ultrasound in the common carotid artery of young human males and females. *Ultrasound Med Biol* 1997;23:583–590.
- 80 Kornet L, Hoeks APG, Lambregts J, Reneman RS: In the femoral artery bifurcation differences in mean wall shear stress within subjects are associated with different intima-media thicknesses. *Arterioscler Thromb Vasc Biol* 1999;19:2933–2939.
- 81 Dammers R, Tordoir JHM, Hameleers JMM, Kitslaar PJEHM, Hoeks APG: Brachial artery shear stress is independent of gender or age and does not modify vessel wall mechanical properties. *Ultrasound Med Biology* 2002;28:1015–1022.
- 82 Dammers R, Stiff F, Tordoir JH, Hameleers JM, Hoeks AP, Kitslaar PJ: Shear stress depends on vascular territory: comparison between common carotid and brachial artery. *J Appl Physiol* 2003;94:485–489.
- 83 Gatehouse PD, Keegan J, Crowe LA, Masood S, Mohiaddin RH, Kreitner KF, Firmin DN: Applications of phase-contrast flow and velocity imaging in cardiovascular MRI. *Eur Radiol* 2005;15:2172–2184.
- 84 Nayak KS, Hargreaves BA, Hu BS, Nishimura DG, Pauly JM, Meyer CH: Spiral balanced steady-state free precession cardiac imaging. *Magn Reson Med* 2005;53:1468–1473.
- 85 Wazer JR: Viscosity and flow measurements 1) A laboratory handbook of rheology. New York, Interscience Publishers, 1963.
- 86 Weaver JPA, Evans A, Walder DN: The effect of increased fibrinogen content on the viscosity of blood. *Clin Sci* 1969;36:1–10.
- 87 Gnasso A, Carallo C, Irace C, Spagnuolo V, De Novara G, Mattioli PL, Pujia A: Association between intima-media thickness and wall shear stress in common carotid artery in healthy male subjects. *Circulation* 1996;94:3257–3262.

- 88 Broeders MA, Tangelder GJ, Slaaf DW, Reneman RS, oude Egbrink MG: Endogenous nitric oxide protects against thromboembolism in venules but not in arterioles. *Arterioscler Thromb Vasc Biol* 1998;18:139–145.
- 89 Kornet L, Hoeks AP, Lambregts J, Reneman RS: Mean wall shear stress in the femoral arterial bifurcation is low and independent of age at rest. *J Vasc Res* 2000;37:112–122.
- 90 Zhao SZ, Ariff B, Long Q, Hughes AD, Thom SA, Stanton AV, Xu XY: Inter-individual variations in wall shear stress and mechanical stress distributions at the carotid artery bifurcation of healthy humans. *J Biomech* 2002;35:1367–1377.
- 91 Kornet L, Lambregts JAC, Hoeks APG, Reneman RS: Differences in near-wall shear rate in the carotid artery within subjects are associated with different intima-media thicknesses. *Arterioscler Thromb Vasc Biol* 1998;18:1877–1884.
- 92 Samijo SK, Willigers JM, Barkhuysen R, Kitslaar PJEHM, Reneman RS, Hoeks APG: Wall shear stress in the common carotid artery as function of age and gender. *Cardiovasc Res* 1998;39:515–522.
- 93 Zarins CK, Zatina MA, Giddens DP, Ku DN, Glagov S: Shear stress regulation of artery lumen diameter in experimental atherogenesis. *J Vasc Surg* 1987;5:413–420.
- 94 Samijo SK, Barkhuysen R, Willigers JM, Leunissen KM, Ledoux LA, Kitslaar PJ, Hoeks AP: Wall shear stress assessment in the common carotid artery of end-stage renal failure patients. *Nephron* 2002;92:557–563.
- 95 le Noble F, Fleury V, Pries A, Corvol P, Eichmann A, Reneman RS: Control of arterial branching morphogenesis in embryogenesis: go with the flow. *Cardiovasc Res* 2005;65:619–628.
- 96 Reneman RS, Van Merode T, Hick P, Muyltjens AMM, Hoeks APG: Age-related changes in carotid artery wall properties in man. *Ultrasound Med Biol* 1986;12:465–471.
- 97 Friedman MH, Barger CB, Deters OJ, Hutchins GM, Mark FF: Correlation between wall shear and intimal thickness at a coronary artery branch. *Atherosclerosis* 1987;68:27–33.
- 98 Friedman MH, Hutchins GM, Barger CB, Deters OJ, Mark FF: Correlation between intimal thickness and fluid shear in human arteries. *Atherosclerosis* 1981;39:425–436.
- 99 Wentzel JJ, Janssen E, Vos J, Schuurbiers JC, Krams R, Serruys PW, de Feyter PJ, Slager CJ: Extension of increased atherosclerotic wall thickness into high shear stress regions is associated with loss of compensatory remodeling. *Circulation* 2003;108:17–23.
- 100 Willekes C, Brands PJ, Willigers JM, Hoeks APG, Reneman RS: Assessment of local differences in intima-media thickness in the human carotid artery. *J Vasc Res* 1999;36:222–228.
- 101 Schmidt-Trucksass A, Schmid A, Brunner C, Scherer N, Zach G, Keul J, Huonker M: Arterial properties of the carotid and femoral artery in endurance-trained and paraplegic subjects. *J Appl Physiol* 2000;89:1956–1963.
- 102 Furchgott RF: Role of endothelium in responses of vascular smooth muscle. *Circ Res* 1983;53:557–573.
- 103 Pohl U, Holtz J, Busse R, Bassenge E: Crucial role of endothelium in the vasodilator response to increased flow in vivo. *Hypertension* 1986;8:37–44.
- 104 Broeders MA, Tangelder GJ, Slaaf DW, Reneman RS, Egbrink MG: Endogenous nitric oxide and prostaglandins synergistically counteract thromboembolism in arterioles but not in venules. *Arterioscler Thromb Vasc Biol* 2001;21:163–169.
- 105 oude Egbrink MG, Van Gestel MA, Broeders MA, Tangelder GJ, Heemskerk JM, Reneman RS, Slaaf DW: Regulation of microvascular thromboembolism in vivo. *Microcirculation* 2005;12:287–300.
- 106 Girerd X, London G, Boutouyrie P, Mourad JJ, Safar M, Laurent S: Remodeling of the radial artery in response to a chronic increase in shear stress. *Hypertension* 1996;27:799–803.
- 107 Langille BL, O'Donnell F: Reductions in arterial diameter produced by chronic decreases in blood flow are endothelium-dependent. *Science* 1986;231:405–407.
- 108 Boot CR, Groothuis JT, Van Langen H, Hopman MT: Shear stress levels in paralyzed legs of spinal cord-injured individuals with and without nerve degeneration. *J Appl Physiol* 2002;92:2335–2340.
- 109 De Groot PC, Poelkens F, Kooijman M, Hopman MT: Preserved flow-mediated dilation in the inactive legs of spinal cord-injured individuals. *Am J Physiol Heart Circ Physiol* 2004;287:H374–H380.
- 110 Schaper W, Pipp F, Scholz D, Boehm S, Deindl E, Barancik M, Eitenmuller I, Ziegelhoeffer T, LKluge A, Schmitz-Rixen T: Physical forces and their translation into molecular mechanisms; in Schaper W, Schaper J (eds): *Arteriogenesis*. Dordrecht, Kluwer Academic Publ; 2004, pp 73–113.
- 111 Hijmering ML, Stroes ES, Pasterkamp G, Siervogel M, Banga JD, Rabelink TJ: Variability of flow mediated dilation: consequences for clinical application. *Atherosclerosis* 2001;157:369–373.
- 112 Dammers R, Tordoir JH, Kooman JP, Welten RJ, Hameleers JM, Kitslaar PJ, Hoeks AP: The effect of flow changes on the arterial system proximal to an arteriovenous fistula for hemodialysis. *Ultrasound Med Biol* 2005;31:1327–1333.
- 113 Celermajer DS, Sorensen KE, Gooch VM, Spiegelhalter DJ, Miller OI, Sullivan ID, Lloyd JK, Deanfield JE: Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. *The Lancet* 1992;340:1111–1115.
- 114 Van Guldener C, Lambert J, Janssen MJFM, Donker AJM, Stehouwer CDA: Endothelium-dependent vasodilatation and distensibility of large arteries in chronic haemodialysis patients. *Nephrol, Dial Transplant* 1997;12(S2):14–18.
- 115 Cardillo C, Kilcoyne CM, Quyyumi AA, Cannon RO, Panza JA: Selective defect in nitric oxide synthesis may explain the impaired endothelium-dependent vasodilatation in patients with essential hypertension. *Circulation* 1998;97:851–856.
- 116 Benjamin EJ, Larson MG, Keyes MJ, Mitchell GF, Vasani RS, Keaney JF Jr, Lehman BT, Fan S, Osypuk E, Vita JA: Clinical correlates and heritability of flow-mediated dilation in the community: the Framingham Heart Study. *Circulation* 2004;109:613–619.
- 117 Juonala M, Viikari JS, Laitinen T, Marniemi J, Helenius H, Ronnema T, Raitakari OT: Interrelations between brachial endothelial function and carotid intima-media thickness in young adults: the cardiovascular risk in young Finns study. *Circulation* 2004;110:2918–2923.
- 118 De Roos NM, Bots ML, Schouten EG, Katan MB: Within-subject variability of flow-mediated vasodilation of the brachial artery in healthy men and women: implications for experimental studies. *Ultrasound Med Biol* 2003;29:401–406.
- 119 Gnasso A, Carallo C, Irace C, De Franceschi MS, Mattioli PL, Motti C, Cortese C: Association between wall shear stress and flow-mediated vasodilation in healthy men. *Atherosclerosis* 2001;156:171–176.
- 120 Pyke KE, Tschakovsky ME: The relationship between shear stress and flow-mediated dilation: implications for the assessment of endothelial function. *J Physiol* 2005;568:357–369.
- 121 Mitchell GF, Parise H, Vita JA, Larson MG, Warner E, Keaney JF Jr, Keyes MJ, Levy D, Vasani RS, Benjamin EJ: Local shear stress and brachial artery flow-mediated dilation: the Framingham Heart Study. *Hypertension* 2004;44:134–139.
- 122 Kornet L, Hoeks AP, Janssen BJ, Willigers JM, Reneman RS: Carotid diameter variations as a non-invasive tool to examine cardiac baroreceptor sensitivity. *J Hypertens* 2002;20:1165–1173.