Lower Wall Shear Rate of the Common Carotid Artery in Treated Type 2 Diabetes Mellitus with Metabolic Syndrome

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Summary

Arterial sites with low wall shear stress (WSS) are more prone to the development of atherosclerotic plaques, as was observed in carotid arteries in subjects with atherosclerosis risk factors. Type 2 diabetes mellitus (DM), hypertension, hyperlipidemia and other components of the metabolic syndrome, are associated with high risk for symptomatic cerebrovascular disease. It was shown by others that untreated type 2 DM is associated with lower WSS in common carotid arteries. However, the cardiovascular risk of type 2 DM could be modified by therapy. The aim of our study was to test the hypothesis that treated type 2 DM subjects with metabolic syndrome still have lower WSS in common carotid arteries than healthy controls. We enrolled 26 compensated DM subjects with metabolic syndrome, treated by metformin, statins and ACEI for more than 6 months, and 22 aged-comparable healthy controls. Wall shear rate (WSR) was used as a measure of WSS. A linear 3-11 MHz probe was used to measure blood velocity and internal diameter in the common carotid arteries. We compared observed values of WSR adjusted for age by ANCOVA. Wall shear rate was significantly lower in DM group than in control subjects: peak (systolic) values of wall shear rate were 410 ± 130 s^{-1} vs. 487 ± 111 s^{-1} (p<0.005). DM subjects had significantly lower WSR, because of both thinner lumen and slower blood flow velocities. Lower WSR was accompanied by higher IMT (0.73 ± 0.12 mm vs. 0.64 ± 0.11 mm, p<0.001). Treated subjects with compensated type 2 DM with metabolic syndrome still have atherogenic hemodynamic profile. These findings might help to understand faster progression of atherosclerosis in diabetic subjects with metabolic syndrome despite up-to-date medication.

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Introduction

Atherosclerosis, the leading cause of death in the developed countries, is a consequence of both, the genetic predisposition and the traditional risk factors such as diabetes mellitus (DM) (Mayer-Davis *et al.* 1998), hypertension (Berenson *et al.* 1998), hyperlipidemia (Waters *et al.* 1995), cigarette smoking (Howard *et al.* 1998). Patients with type 2 DM are at high risk for cardiovascular disease (CVD); their risk is 2- to 6-fold higher than in subjects without diabetes (Kannel and McGee 1979, Stamler *et al.* 1993). Accelerated atherosclerosis in diabetic subjects is partially explained by the atherogenic risk factors associated with the metabolic syndrome (Alberti *et al.* 2005).

Atherosclerosis is a systemic, multifactorial disease. Nonetheless, atherosclerosis remains a geometrically focal disease, preferentially affecting the outer edges of vessel bifurcations (Fox *et al.* 1982). Local hemodynamic factors participate in the physiopathology of atherogenesis, accounting for the focal nature of the process (Asakura and Karino 1990). The main local hemodynamic factor is wall shear stress (WSS), the frictional force acting tangentially to the endothelial surface as a result of blood flow (Malek *et al.* 1999). Wall shear stress is directly related to whole-blood viscosity and to wall shear rate (WSR) (Girerd *et al.* 1996). WSR is defined as the difference between

adjacent velocities in the vascular lumen. The ratio between the maximum velocity at the center of the artery and vessel radius is a common approximation of WSS (Hoeks *et al.* 1995). Wall shear rate can be used as an approximation of WSS (Hoeks *et al.* 1995, Malek *et al.* 1999). In the atherosclerosis-prone sites, blood flow is slow and changes its direction during the cardiac cycle, resulting in a low net hemodynamic shear stress. In contrast, vessel regions that are exposed to steady blood flow and a higher magnitude of shear stress remain comparatively plaque-free e.g. flow-dividers in bifurcation (Zarins *et al.* 1983, Gnasso *et al.* 1997). Lower WSS induces rather larger lesions with vulnerable plaque phenotype, whereas vortices with oscillatory WSS induce stable lesions (Cheng *et al.* 2006). Wall shear stress is not implicated only in vascular pathobiology, but also in vascular remodeling (Gibbons and Dzau 1994), and is a critical determinant of vessel caliber (Langille and O'Donnell 1986). The mechanisms, by which low WSS can cause arterial damage are known for a long time: the resultant stagnation of blood permits increased uptake of atherogenic blood particles as a result of increased residence time (Zarins *et al.* 1983). Wall shear stress can change the morphology and orientation of endothelial cell layer (Malek *et al.* 1999). Moreover, exposure of the arterial wall to a relatively low WSS may increase the vulnerability of these regions of the vessel to atherosclerosis (Malek *et al.* 1999). Low WSS modulates the transcription of genes for nitric oxide, and increased local production of mitogenic substances (Traub and Berk 1998). This complex endothelial cell response to shear stress may also provide a mechanism by which known risk factors act to promote atherosclerosis.

Carotid atherosclerosis is also locally influenced by WSS. Irace *et al.* (1999) demonstrated that subjects suffering from carotid atherosclerosis, but with low calculated cardiovascular risk have lower WSS of the common carotid artery (CCA) than controls. Investigation on patients affected by unilateral carotid atherosclerosis demonstrated that shear stress is lower in the carotid arteries with plaques than in contralateral plaque-free arteries (Gnasso *et al.* 1997). According to the study carried out by Irace *et al.* (1999) DM subjects have lower WSS of compared with age-matched healthy control subjects.

The high risk of CVD that accompanies metabolic syndrome and DM mandates comprehensive and complex preventive care. Drug therapy indicated for cardiovascular risk reduction includes insulin sensitisers (metformin), angiotensin-converting-enzyme inhibitors (ACEI) (Yusuf *et al.* 2000, Chitravas *et al.* 2007) and lipid lowering therapy (statins) (Furberg *et al.* 1994, Crouse *et al.* 1995, Amarenco and Tonkin 2004). However, despite this medication the diabetics have high risk of cardiovascular events. The aim of our study was to test the hypothesis that DM subjects with metabolic syndrome compensated by metformin, with established statin and ACEI therapy, still have lower WSR in the common carotid artery than healthy controls.

Methods

Diabetics with metabolic syndrome according NCEP ATP III criteria (Alberti *et al.* 2005), treated with metformin, statin and ACEI for more than 6 months were included into this study. All diabetics were overweight with central obesity (waist circumference above 102 cm in men and 88 cm in women). Diabetics were enrolled from the outpatient department, Third Department of Internal Medicine General University Hospital. We also examined healthy, age- and sex comparable subjects.

Blood pressure (BP) was measured on the right arm, after 5 min rest, in a sitting position. Serum total cholesterol, triglycerides and HDL-cholesterol levels were measured using automated analyzer methods. LDL-cholesterol concentrations were calculated using the Friedewald's formula. No participants had values of triglycerides above 4 mmol/l. Ultrasound examination was performed by the use of a linear-array 3-11 MHz probe of SONOS 5500 (Philips, Andover, Massachusetts, USA). The examinations were performed in fasting state (at least 12 h) during morning hours. The subjects were kept in supine position with their heads slightly extended. We scanned common carotid artery (CCA), carotid bifurcations and the origins of the internal carotid arteries in longitudinal and transverse planes. Subjects with stenosis of the carotid tree or atherosclerotic plaque were excluded. Plaque was defined as a focal structure encroaching into the arterial lumen of at least 0.5 mm or 50 % of the surrounding intima-media thickness (IMT) value, or demonstrates a thickness >1.5 mm as measured from the media-adventitia interface to the intima-lumen interface (Meijer *et al.* 2006). Ultrasound measurements were performed in CCA 1-2 cm proximal to the bifurcation. The distal segments of CCA were recorded digitally for further analysis. Blood flow velocity was

detected with the sample volume placed in the center of CCA. Peak systolic (V_{peak}), end-diastolic (V_{min}), and mean velocities (V_{mean}) were recorded.

Both, the internal diameter (ID) and the common carotid intima-media thickness (CIMT) were analyzed off-line by specialized software Image Pro-Plus version 4.0 (Media Cybernetics, Silver Spring, MD, USA). The reader was the same throughout the study and was blinded with regard to the subject investigated. CIMT, defined as the distance between two parallel lines: the lumen-intima and media-adventitia boundaries, was measured on the far wall of CCA and the average of three measurements was calculated. ID, defined as the distance between the leading edge of the echo produced by the intima-lumen interface of the near arterial wall and to the leading edge of the echo produced by the lumen-intima interface of the far wall, was measured on the top of the R-wave of QRS complex.

Wall shear rate was calculated using the Poiseuillian parabolic model of velocity distribution across the arterial lumen based on the assumption of laminar blood flow, according to the following formula (Gnasso *et al.* 1996, Jiang *et al.* 2000):

$$WSR_{peak} = 4 \times V_{peak} / ID$$
$$WSR_{mean} = 4 \times V_{mean} / ID,$$

where WSR is wall shear rate (s^{-1}), V is the velocity ($cm \cdot s^{-1}$) and ID is the arterial diameter. WSR was calculated separately for peak and mean blood flow velocity.

Statistical analysis

Because the right and left sides were analyzed separately, the analyses concerning carotid parameters were based on 52 samples in the diabetic group and 44 in the control subjects.

Statistical analysis was performed by Statistica for Windows ver.6.0 statistical software (StatSoft, In., Tulsa, OK, USA). Clinical differences between diabetics and controls were analyzed using an unpaired *t*-test. Data are expressed as mean ± S.D.. The analysis of covariance (ANCOVA) was used to compare hemodynamic differences between age-adjusted DM subjects and controls. Univariate correlation analysis was performed to test a possible influence of age, ID, systolic blood pressure (SBP) and body mass index (BMI) on WSR and CIMT. Data are expressed as mean ± S.D. and p<0.05 was considered statistically significant.

Table 1. Clinical characteristics of diabetic and control subjects.

	Diabetes mellitus	Controls	p - value
Age (years)	63 ± 9	60 ± 8	0.08
Body mass index (kg/m²)	29 ± 3	26 ± 3	< 0.001
Diabetes mellitus duration (years)	7 (1 - 18)	–	
HbA1c (%)	5.5 ± 0.6	3.8 ± 0.4	< 0.001
Systolic blood pressure (mmHg)	137 ± 17	125 ± 20	0.005
Diastolic blood pressure (mmHg)	80 ± 8	79 ± 11	0.68
Total cholesterol (mmol/l)	4.4 ± 0.9	5.1 ± 0.8	< 0.001
HDL- cholesterol (mmol/l)	1.2 ± 0.2	1.6 ± 0.4	< 0.001
LDL- cholesterol (mmol/l)	2.4 ± 0.6	2.9 ± 0.7	0.001
Triglycerides (mmol/l)	1.8 ± 1.4	1.4 ± 0.5	0.01

Data are expressed as mean ± S.D. Clinical differences between diabetics and controls were analyzed using an unpaired *t*-test.

Results

Table 1 shows clinical and biochemical characteristics of diabetics and that of controls. Diabetics had higher systolic blood pressure than healthy controls (137±17 mmHg vs. 125±20 mmHg, p<0.08) despite being treated with ACEI. Subjects in both groups were overweight, but body mass index was significantly higher in diabetics (29±3 vs. 26±3, p<0.001). The total cholesterol (4.4±0.9 mmol/l vs. 5.1±0.8 mmol/l, p<0.001) and LDL cholesterol (2.4±0.6 mmol/l vs. 2.9±0.7 mmol/l, p<0.001) were lower in diabetics, as a result of statin treatment. HDL cholesterol was lower in DM subjects (1.2±0.2 mmol/l vs. 1.6±0.4 mmol/l, p<0,001) and the triglycerides were higher (1.8±1.4 mmol/l vs. 1.4±0.5 mmol/l, p<0.01).

Hemodynamic parameters are listed in Table 2. Peak WSR was significantly lower in diabetics than in healthy controls (410±130 s^{-1} vs. 487±111 s^{-1}, p=0.003), because of both thinner lumen (6.7±0.92 mm vs. 5.9± 0.64 mm, p<0.001) and slower blood flow velocity (67.0 ±16.7 $cm \cdot s^{-1}$ vs. 71.4±12.2 $cm \cdot s^{-1}$, p=0.16). Common carotid intima-media thickness was significantly lower in

Table 2. Hemodynamic parameters.

	Diabetes mellitus	Controls	p - value
Number	52	44	
Internal diameter (mm)	6.7 ± 0.92	5.9 ± 0.64	< 0.001
Intima-media thickness (mm)	0.73 ± 0.12	0.64 ± 0.11	< 0.001
Pulse pressure (mmHg)	53.1 ± 10.7	45.7 ± 2.0	0.02
Velocity $_{peak}$ (cm. s^{-1})	67.0 ± 16.7	71.4 ± 12.2	0.16
Velocity $_{mean}$ (cm. s^{-1})	33.0 ± 8.1	39.4 ± 6.7	< 0.001
WSR $_{peak}$ (s^{-1})	410 ± 130	487 ± 111	0.003
WSR $_{mean}$ (s^{-1})	204 ± 71	269 ± 62	< 0.001

ANCOVA - Analysis of covariance, values shown as mean ± S.D.

healthy controls (0.73±0.12 mm vs. 0.64±0.11 mm, p<0.001). These results remained significant even when adjusted for systolic BP.

In diabetic group, we found significant correlations between peak wall shear rate and age (r = –0.48, p<0.0001) as well as between mean wall shear rate and age (r = –0.39, p=0.004).

Discussion

Our data demonstrate that treated diabetics with metabolic syndrome still have lower wall shear rate of the common carotid artery compared to healthy controls. These findings suggest that DM subjects with metabolic syndrome have a local carotid hemodynamic profile more prone to plaque development.

In our study, the decrease of WSR was the consequence of both lower blood flow velocity and enlargement of CCA. It was shown that the diameter of CCA increases with age (Eigenbrodt *et al.* 2006). Our diabetics were slightly older than healthy controls, but we believe that this difference was not so high to explain different CCA diameters. Moreover, we added the age as a confounding factor to the analysis of covariance.

Eigenbrodt *et al.* (2006) reported that persons with pre-existing atherosclerotic disease or atherosclerotic risk factors have larger carotid diameters than persons without such attributes. This is thought to be an adaptive phenomenon (Safar *et al.* 1998). Hypertension *per se* is a known variable associated with large internal and interadventitial diameter of CCA (Boutouyrie *et al.* 1999). In the present study, we found a significant correlation between ID and SBP. Our results are in agreement with previous observations of such a relationship between ID and blood pressure in treated hypertension (Chironi *et al.* 2003). The hypertension exerts a fatiguing effect on the elements of the arterial wall (i.e. elastin and collagen), resulting in degenerative changes and interadventitial enlargement. The role of DM in enlargement of CCA is not completely clarified. Henry *et al.* (2004) reported that DM is associated with a pattern of compensatory remodeling, i.e. preservation ID at increased CIMT. Diabetics in our study had dilated CCA, i.e. outward arterial remodeling (characterized by an increase of ID caused by a greater change in interadvencial diameter than in CIMT). This pattern of arterial remodeling of CCA appears maladaptive, and is associated with ischemic stroke independently of carotid atherosclerosis and cardiovascular risk factors (Bai *et al.* 2007).

Decreased blood flow velocity in the CCA was reported in hypertensive subjects with left ventricular hypertrophy (Jiang *et al.* 1998, Kohara *et al.* 1999). Widening of pulse pressure in stiffer arteries was proposed as the mechanism of the alteration of blood flow velocity (Kohara *et al.* 1999). Another possibility for explaining decreased blood flow velocity is an abnormality of cardiac function in DM subjects. Several studies demonstrated left ventricular diastolic dysfunction as a typical abnormality at early stage of DM, independent of the confounding role of myocardial ischemia, body weight, and blood pressure (Celentano *et al.* 1995, Di Bonito *et al.* 1996).

As expected, CIMT was higher in our diabetic group than in healthy controls. One of the premises of CIMT is that it reflects atherosclerosis and cardiovascular risk. Atherosclerosis is restricted primarily to the intimal layer of the vessel wall. Ultrasound imaging cannot discriminate between the intimal layer and the medial layer of the vessel wall. An increased CIMT may reflect either intimal thickening, thickening of the medial layer, or a combination of both. In the present study, we found a strong inverse correlation between WSR and CIMT in agreement with previous observations (Gnasso *et al.* 1996, Zarins *et al.* 1983). We also found a significant

influence of SBP and glycated hemoglobin on CIMT. Our results support the hypothesis of interactions between systemic and local risk factors able to influence atherogenesis. Association between glucose tolerance and CIMT was explained by hyperglycemia (fasting glucose or HbA1c) and/or insulinemia and insulin resistance (Wagenknecht *et al.* 2003). Insulin in physiological concentrations stimulates the proliferation of smooth muscle cells and increases lipid activity and synthesis. High levels of glucose can damage or alter the endothelial barrier, thus allowing insulin to interact with the underlying smooth muscle cells. Systolic blood pressure is known to be associated with intima-media thickening (Vaudo *et al.* 2000) and this finding was confirmed in our study. Linhart *et al.* (1996) demonstrated that carotid artery structure may be representative of LV mass even when it is within normal limits.

Although WSR is a common approximation of WSS (Gnasso *et al.* 1997, Irace *et al.* 1999, Carallo *et al.* 2006, Tuka *et al.* 2006), the use of WSR might potentially lead to some inaccuracy. First limitation is that endothelial cells sense and respond to WSS, not WSR. Gnasso *et al.* (1996) measured blood viscosity in vitro by use of a cone/plane viscosimeter. It was shown that even actual measurements of viscosity must not lead to real values of shear stress (Setty *et al.* 2002). Some authors (Zarins *et al.* 1983, Paszkowiak and Dardik 2003) use an arbitrary value for blood viscosity to estimate shear stress, but the use of an arbitrary value of blood viscosity would not change the statistical significance of the results. For these reasons, we used WSR measurements only.

It can be concluded from this study that treated subjects with compensated DM still have atherogenic hemodynamic profile. These findings might help to understand fast progression of atherosclerosis in diabetic subjects despite up-to-date medication.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

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