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Exercise-induced shear stress is associated with changes in plasma von Willebrand factor in older humans

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Abstract

Shear stress is the frictional force of blood against the endothelium, a stimulus for endothelial activation and the release of von Willebrand factor (vWF). This study tested the hypothesis that the increase in shear stress associated with exercise correlates with plasma vWF. Young ($n = 14$, 25.7 ± 5.4 y) and older ($n = 13$, 65.6 ± 10.7 y) individuals participated in 30 min of dynamic handgrip exercise at a moderate intensity. Brachial artery diameter and blood flow were measured using ultrasound Doppler and blood samples were collected before, immediately after, and following 30 min of recovery from exercise with plasma levels of vWF. Plasma levels of vWF increased ($P < 0.05$) by $6 \pm 2\%$ in young individuals and $4 \pm 1\%$ in older individuals immediately after exercise. The change in plasma vWF was linearly correlated with the increase in shear stress during exercise in older individuals (post-exercise: $r = 0.78$, 30 min recovery: $r = 0.77$, $P < 0.01$), but no association was found in the young individuals. These changes in plasma levels of vWF in humans suggest that aging influences endothelial activation and hemostasis.

Keywords

shear stress; handgrip; endothelial function; aging

Introduction

Von Willebrand factor (vWF) is a large multimeric glycoprotein that upon endothelial activation or injury is released from Weibel-Palade bodies with subsequent modulation of factor VIII activity in plasma and binding of platelets to the subendothelium. A model for release of vWF is strenuous exercise that increases vWF in plasma (Claus et al. 2006; Van den Burg et al. 1995) due to β -adrenergic and V1 receptor activation by epinephrine and vasopressin, respectively (Small et al. 1984). The increase in shear stress accompanying exercise may also contribute to endothelial activation as studies using cultured endothelial cells have shown increased release of vWF with the time and magnitude of shear stress (Galbusera et al. 1997; Sun et al. 2000). Aging may be of importance for the release of vWF since older individuals may have an inability of the endothelium to maintain a balanced hemostatic potential due to decreased endothelial nitric oxide bioavailability (Taddei et al. 2001) and elevated basal levels of coagulation activation products (Mari et al. 2008). The consequence is the progression towards a more prothrombotic state which may contribute to atherogenesis.

Accordingly, we hypothesized that exercise-induced shear stress would be positively related to changes in plasma levels of vWF and that this relationship would be greater in older as compared to young individuals. First, we determined whether the increase in hemodynamic shear stress accompanying exercise is associated with the increase in plasma levels of vWF. Second, we evaluated the influence of age on the relationship between changes in shear stress and plasma levels of vWF during exercise.

Methods

Fourteen young (18-34 y, 8 women) and thirteen older individuals (44-86 y, 8 women) participated in this study. Individuals reported to be either sedentary or recreationally active, but none were currently involved in regular exercise training. Young individuals were void of metabolic and cardiovascular disease as assessed through a medical history questionnaire. Young women were tested during the early follicular phase of their menstrual cycle. In the older subject group, three individuals reported having no metabolic or cardiovascular diseases while ten individuals had a previous history of hypercholesterolemia and/or hypertension that was controlled by medication at the time of the study. The study was approved by the Human Individuals Research Committee at The University of Toledo and is in accordance with guidelines set forth by the Declaration of Helsinki. All individuals provided written informed consent after being explained all experimental procedures, the exercise protocol, and possible risks associated with participation in the study.

Experimental Protocol

Individuals participated in two study visits separated by no less than 48 hrs. During the first visit, anthropometric measurements were obtained including height, weight, percent body fat as estimated using bioelectrical impedance (Tanita, Tokyo, Japan), and forearm volume using water displacement. Maximal forearm strength was measured using a handgrip dynamometer (Takei, Tokyo, Japan). The average of three maximal voluntary isometric contractions (MVC) was used as the value of forearm muscle strength. No less than 2 min of recovery was allowed between each MVC. Following a brief rest, individuals were asked to perform handgrip exercise whereby the workload progressively increased in a ramp fashion ($0.5 \text{ kg} \cdot \text{min}^{-1}$) until the required contraction rate (30 cpm) could no longer be maintained. The weight achieved at task failure was used to calculate the workload used during constant load exercise in the subsequent visits. Lastly, individuals practiced the contraction rate that would be performed during the subsequent visit until they were familiar with the duty cycle.

During the second visit, individuals arrived in a fasted state and rested for 15 min before baseline measurements were obtained including blood pressure and blood sample collection. The exercise protocol consisted of dynamic ($0.2 \text{ m} \cdot \text{s}^{-1}$, 0.55 duty cycle) handgrip exercise performed with the right hand for 30 min. To prevent muscle fatigue during exercise, individuals were given 1-2 min breaks between every 5 min of exercise. Contraction frequency (30 cpm), workload (65% of end ramp workload), and the distance the resistance traveled (0.08 m) were kept constant. Blood samples were collected before exercise, immediately after exercise, and following 30 min of recovery.

Brachial artery blood velocity was measured at rest and during exercise using a Doppler ultrasound velocimetry system (model 500-M, Multigon Industries, Yonkers, NY) operating in pulsed mode. The transducer, with an operating frequency of 4 MHz and fixed transducer crystal and sound beam angle of 45° relative to the skin, was placed flat on the right arm 6-10 cm above the medial humeral condyle. Blood velocity was measured by one investigator exhibiting a test-retest and between-day coefficient of variation of 6.4% and 10.4% for exercise blood velocities, respectively. Electrocardiography (ECG) recordings were obtained using a modified 3-lead placement. Blood velocity was averaged over each cardiac cycle (R-R wave)

using custom software (Hoelting et al. 2001). The continuous cardiovascular (blood velocity, ECG) data were digitized at 100 Hz (PowerLab 16SP, ADInstruments, Grand Junction, CO) and stored for offline analysis.

Images of the brachial artery were obtained at rest and during steady-state exercise using an ultrasound Doppler imaging system (Logiq 400, GE Medical Systems, Milwaukee, WI) operating in B-mode. The transducer, with an operating frequency of 7 MHz, was placed flat on the skin of the upper arm over the same region where blood velocities were measured. The average diameter measurements of 10-15 longitudinal images were used to calculate the cross-sectional area ($CSA = \pi r^2$) of the artery, which in turn was multiplied by the corresponding blood velocity to obtain forearm blood flow ($FBF = \text{blood velocity} * CSA * 60$). A 20 s average of forearm blood flow was obtained at rest and during the steady-state phase of each of the five bouts of dynamic exercise. For each subject, an average of the five steady-state blood flow values was determined and used for statistical analysis.

Blood pressure was measured from the radial artery at the left wrist at rest and during exercise using an automated blood pressure device (Vasotrac, Medwave Inc., St. Paul, MN). Forearm vascular conductance ($\text{ml} \cdot \text{min}^{-1} \cdot \text{mmHg}^{-1}$) was calculated by dividing mean blood flow ($\text{ml} \cdot \text{min}^{-1}$) by mean arterial pressure. Shear rate was calculated using Poiseuille's equation: $\text{shear rate (s}^{-1}) = (4 * \text{mean FBF}) / \pi r^3$ (Silber et al. 2005). Shear stress (dyn/cm^2) was calculated by multiplying shear rate by blood viscosity. A whole blood viscosity value of $0.05 \text{ dyn} \cdot \text{s} \cdot \text{cm}^{-2}$ ($5 \text{ mPa} \cdot \text{s}$) was used to calculate shear stress as this value is similar between young and older individuals (Dammers et al. 2002). Whole blood viscosity was assumed not to change during exercise due to the small active muscle mass and submaximal level of intensity. The term shear stress is used to represent the hemodynamic forces acting on the vascular walls since shear rate is directly proportional to shear stress.

Blood Sample Collection

Blood was collected anaerobically by venipuncture from an antecubital vein into tubes (BD Vacutainer, Franklin Lakes, NJ) containing 0.109 M sodium citrate (3.2% final concentration). Immediately upon obtaining the blood sample, tubes were centrifuged at 1500 g for 20 min and the plasma stored at -80°C until analysis.

Quantification of Plasma vWF

Considered a marker of endothelial cell activation, vWF levels in plasma were quantified using gel electrophoresis (Laurell 1966). Each sample was measured in duplicate. Plasma concentration of vWF was expressed relative to normal pooled plasma (%NP) which was collected from 14 healthy young men and women with no medical history of metabolic or cardiovascular disease.

Statistical Analysis

Linear regression was used to identify correlations between variables. Independent-sample t-tests were used to test for differences between age groups in anthropometric and cardiovascular data. Paired t-tests were used to test for differences in cardiovascular data between rest and exercise data within each age group. A one-way analysis of variance with repeated measures (RMANOVA) was used to test for differences in vWF across time. Two-way RMANOVA was used to test for differences in plasma levels of vWF between and within age groups (age x time). Newman-Keuls post-hoc test was used to test for differences if a significant main effect or interaction was found. Statistical significance was set at $P \leq 0.05$. Values are expressed as mean $\pm$ SD unless stated otherwise.

Results

Basal plasma levels of vWF were higher ($P < 0.05$) in older individuals as compared to young individuals (Table 1). No significant correlations were found between the anthropometric variables and basal plasma levels of vWF. No correlations were found between resting hemodynamics (heart rate, MAP, mean blood flow, shear stress) and plasma levels of vWF.

Older individuals were on average shorter, had lower maximal forearm strength, higher body mass index, and higher percent body fat than young individuals (Table 1). Older individuals exhibited a higher resting MAP, mean blood velocity, and shear stress than young individuals ($P < 0.05$) (Table 2). During exercise, the pressor response was greater ($P < 0.05$) in older as compared to young individuals, but mean blood flow and forearm vascular conductance was lower ($P < 0.05$) in older than young individuals due to differences in the absolute exercise workload. The amount of exercise-induced shear stress was similar between young and older individuals.

Plasma levels of vWF increased by $6 \pm 2\%$ in young individuals immediately following exercise ($P < 0.05$) and returned to resting levels following 30 min of recovery (Fig. 1). No correlation was found between shear stress and the increase in plasma vWF. In older individuals, plasma levels of vWF were increased by $4 \pm 1\%$ immediately after exercise ($P < 0.05$) and remained elevated after 30 min of recovery. The increase in plasma levels of vWF following exercise was correlated to shear stress in older individuals (immediately after exercise: $r = 0.78$, $y = 0.13x + 2.77$; 30 min recovery: $r = 0.77$, $y = 0.15x + 2.42$; $P < 0.01$) such that increased shear stress was associated with increased plasma levels of vWF (Figure 2). No significant difference was found in the exercise-induced increase in plasma vWF between age groups.

Discussion

We used ultrasound Doppler to measure brachial artery blood flow to active forearm muscle and the associated hemodynamic response to determine if exercise-induced shear stress is associated with changes in plasma levels of vWF, a biomarker of endothelial activation. The results demonstrate that plasma levels of vWF change similarly in young and older individuals following submaximal dynamic handgrip exercise, in agreement with studies examining the effect of whole-body exercise on the increase in vWF in plasma (Van den Burg et al. 1995; Van den Burg et al. 2000). Exercise-induced shear stress was positively correlated with plasma levels of vWF in older, but not young individuals.

One other study has investigated the effect of exercise hemodynamics on the increase of plasma vWF in humans. Through the analysis of brachial pulse pressure contour obtained from arterial catheterization, Smith et al. (1993) found maximal treadmill exercise to strengthen the negative correlation found between plasma levels of vWF and the compliance of the large and small arteries. Although this is indirect evidence, these findings suggest that vascular regions with increased arterial stiffness and therefore higher shear stress are related to increased plasma levels of vWF during exercise. The present study extends these findings by calculating changes in wall shear stress of a conduit artery during exercise and showing that exercise-induced shear stress is related to the increase in plasma levels of vWF in older individuals. Whether our findings are related to structural alterations of the vessel wall with aging such as changes in arterial compliance (Tanaka et al. 1998) or were a consequence of altered endothelial cell function requires further investigation.

The age-specific difference found in the relationship between exercise-induced shear stress and plasma levels of vWF was not specific to different levels of shear stimuli since shear stress during exercise was similar between age groups. Rather, the difference between groups may be associated with age-related changes in adrenergic receptor activation. The increase in plasma

levels of vWF during exercise is mediated by adrenergic stimulation since this response can be inhibited by the β -adrenoreceptor antagonist propranolol (Small et al. 1984). Furthermore, Bühler et al. (1980) found that older individuals have a decreased forearm vasodilator response to the β -adrenoceptor agonist isoproterenol as compared to young individuals. This is believed to be the consequence of β -adrenoceptor desensitization caused by increased levels of endogenous catecholamines associated with aging. The increase in plasma levels of vWF in young individuals following exercise may be due to adrenergic receptor-mediated stimulation, while in older individuals, shear forces induced by the increase in exercise blood flow was the primarily mediator of endothelial activation since β -adrenoceptor sensitivity was likely reduced in this age group.

The average mean shear stress values ($5\text{--}6\text{ dyn/cm}^2$) calculated at rest is similar to mean shear stress levels ($4\text{--}7\text{ dyn/cm}^2$) reported in the brachial artery of humans (Cheng et al. 2007). During exercise, the average mean shear stress ($\sim 27\text{ dyn/cm}^2$) were within the range of expected physiologic arterial shear stress values ($10\text{--}70\text{ dyn/cm}^2$) and below shear stress levels associated with thrombosis ($>70\text{ dyn/cm}^2$) (Malek et al. 1999). The ability of physiological levels of shear stress to induce endothelial activation has been demonstrated. Galbusera et al. (1997) has shown that 6 h of laminar shear stress ranging from $8\text{--}12\text{ dyn/cm}^2$ can stimulate cultured endothelial cells to release vWF. Sun et al. (2000) has demonstrated that 2 h of low shear stress ($2\text{--}10\text{ dyn/cm}^2$) can also induce vWF release from cultured endothelial cells. The amount of shear stress measured during exercise in the present study was well within the level of shear stress capable of stimulating vWF release from endothelial cells.

Several limitations are associated with this study. First, the mode of exercise utilizes a small muscle group (forearm muscles), and therefore, we were unable to observe large changes in plasma vWF as compared to studies using whole-body exercise (Claus et al. 2006; Paton et al. 2004; Van den Burg et al. 1995; Van den Burg et al. 2000). Second, the majority of older individuals in this study had a medical history of cardiovascular disease that may have influenced the relationship between shear stress and endothelial function. We find this unlikely since we found no difference in shear stress or plasma levels of vWF between older individuals with or without a medical history. Lastly, this study failed to measure vWF protease (ADAMTS-13) activity. Intense treadmill exercise is demonstrated to decrease ADAMTS-13 activity (Claus et al. 2006) but not antigen levels (Stakiw et al. 2008) in plasma of healthy individuals. However, the reduction in ADAMTS-13 activity is found to be significant hours after exercise ends while the increase in plasma vWF reaches its highest level immediately post-exercise (Claus et al. 2006).

In conclusion, physiological levels of shear stress induced during dynamic handgrip exercise are associated with plasma levels of vWF in older individuals, but not in young individuals. This age-specific difference may be due to the attenuation of β -adrenergic receptor activation with aging.

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Figure 1.

Exercise-induced increase in plasma levels of vWF in young and older individuals. Values are mean $\pm$ SE. *, significant difference from Pre-exercise ($P < 0.05$).

Figure 2.

Relationship between exercise-induced increase in plasma vWF and shear stress in young (A) and older (B) individuals. The increase in plasma vWF in older individuals after exercise was linearly correlated (post: $r^2 = 0.61$, recovery: $r^2 = 0.59$) with shear stress during exercise. Closed and open circles represent post-exercise and 30 min recovery, respectively. Solid and dotted regression lines are for post-exercise and 30 min recovery, respectively.

Table 1

Baseline characteristics for young and older subjects

	Young (n=14)	Older (n=13)
Age (y)	25.7 ± 5.4	65.6 ± 10.7 [†]
Weight (kg)	69.4 ± 12.8	76.5 ± 19.4
Height (cm)	171.9 ± 10.3	163.9 ± 7.6 [†]
Body Mass Index (kg/m ²)	23.1 ± 2.4	27.8 ± 6.1 [†]
Percent Body Fat (%)	20.0 ± 10.1	28.6 ± 12.9 [†]
Forearm Volume (ml)	954.7 ± 227.0	975.7 ± 262.5
Isometric MVC (kg)	42.0 ± 11.1	33.7 ± 10.8 [†]
Exercise Workload (kg)	3.4 ± 0.9	2.3 ± 0.7 [†]
vWF (%NP)	93.7 ± 19.0	107.6 ± 16.6 [†]

Values are mean ± SD.

[†] significant difference between age groups ($P \leq 0.05$)

Table 2

Hemodynamic variables measured at rest and during dynamic forearm exercise

	Young (n=14)		Older (n=13)	
	Rest	Exercise	Rest	Exercise
Heart Rate (bpm)	62.2 ± 13.8	73.0 ± 12.0	63.0 ± 10.8	74.8 ± 14.6
Mean Arterial Pressure (mmHg)	82.0 ± 5.8	103.0 ± 17.6	97.6 ± 12.0 [†]	123.3 ± 20.5 [†]
Mean Blood Velocity (cm/s)	3.9 ± 1.0	32.3 ± 7.0	5.5 ± 2.2 [†]	28.4 ± 5.4
Mean Blood Flow (ml/min)	30.1 ± 10.9	358.7 ± 111.2	37.1 ± 15.2	255.2 ± 106.5 [†]
FVC (ml/min/mmHg)	0.4 ± 0.1	3.5 ± 1.2	0.4 ± 0.2	2.2 ± 1.1 [†]
Shear Rate (s ⁻¹)	81.8 ± 28.3	558.8 ± 168.5	118.8 ± 53.1 [†]	544.9 ± 116.5
Shear Stress (dyn/cm ²)	4.1 ± 1.4	27.9 ± 8.4	5.9 ± 2.7 [†]	27.2 ± 5.8

Values are mean ± SD. FVC - forearm vascular conductance.

[†] significant difference between age groups ($P \leq 0.05$)