Whole Blood Passage Time: What Does It Mean? – Reply –

We thank Dr Okamoto and colleagues for their comments on our work.¹ Exercise training has become an accepted therapeutic modality in patients with heart diseases, so the negative short-term effects of exercise on whole blood rheology, as well as the beneficial long-term effects such as cardiovascular protection and anti-atherogenesis, need to be understood to ensure appropriate exercise prescriptions are tailored for individual patients. The microchannel array flow analyzer (MCFAN) is generally accepted as a reliable tool for assessing whole blood rheology; it is superior to other methods in terms of the accuracy of the uniform microchannel dimensions and the high reproducibility of the time required for whole blood to pass through the microchannels.^{2,3} We validated this rapid and simple method of measuring whole blood passage time (WBPT) using the MCFAN in a pilot study ($n=45$; intraobserver and interobserver variability of 7.3% and 9.1%, respectively). Thus, we recently used this ex vivo capillary model to evaluate whole blood rheology and leukocyte properties such as adhesiveness and deformability.^{1,4–6} Okamoto et al proposed an alternative method using nickel mesh filtration to assess more specific parameters such as erythrocyte deformability.⁷ They used that method to measure the flow rate of erythrocyte suspensions with a hematocrit of 2% under various levels of filtration pressure.⁷ However, we disagree with this for the following reasons. (1) Erythrocytes and endothelial cells are coated by plasma proteins comprising mostly albumin, which function as an extracellular matrix. The flow rate of erythrocyte suspensions without plasma proteins might overestimate erythrocyte deformability. (2) A hematocrit of 2% is too low to estimate erythrocyte deformability in vivo. (3) Erythrocyte suspensions without filtering are supposed to include erythrocyte membrane fragments, which would lead to artifacts that critically affect flow rates by plugging the pores of the nickel mesh. We suggest that erythrocyte membrane fragments be excluded by filtering and that washed erythrocytes are suspended in a solution containing albumin or in plasma before analysis.

Our statistical analysis¹ by analysis of variance with a post-hoc test was accurate and our judgments were correct, even though the standard deviations among blood cell counts and

hemorheological data were relatively large because of the limited number of participants ($n=10$).

The density ratio of erythrocytes and leukocytes is 1,000:1 in blood sampled from the vein or aorta. However, where the hematocrit falls in capillaries to half of that in the large vessels,⁸ leukocyte rheology contributes to changes in vascular resistance, as well as in the hematocrit and erythrocyte deformability, even in spontaneously hypertensive rats.⁴

WBPT was always corrected using the passage time for saline, which was determined immediately before each blood sample according to the manufacturer's instructions.

References

1. Ikeda N, Yasu T, Tsuboi K, Sugawara Y, Kubo N, Umemoto T, et al. Effects of submaximal exercise on blood rheology and sympathetic nerve activity. *Circ J* 2010; **74**: 730–734.
2. Kikuchi Y, Sato K, Mizuguchi Y. Modified cell-flow microchannels in a single-crystal silicon substrate and flow behavior of blood cells. *Microvasc Res* 1994; **47**: 126–139.
3. Kikuchi Y. Effect of leukocytes and platelets on blood flow through a parallel array of microchannels: Micro- and macroflow relation and rheological measures of leukocyte and platelet activities. *Microvasc Res* 1995; **50**: 288–300.
4. Fukuda S, Yasu T, Kobayashi, Ikeda N, Schmid-Shönbein GW. Contribution of fluid shear response in leukocytes to hemodynamic resistance in the spontaneously hypertensive rat. *Circ Res* 2004; **95**: 100–108.
5. Arai K, Yasu T, Umemoto T, Jinbo S, Ikeda N, Ueda S, et al. Effects of pitavastatin on postprandial endothelial function, oxidative stress, and hemorheology in patients with stable angina pectoris. *Circ J* 2009; **73**: 1523–1530.
6. Azekoski Y, Yasu T, Watanabe S, Tagawa T, Abe S, Yamakawa K et al. FFA causes leukocyte activation and resultant endothelial dysfunction through enhanced angiotensin II production in mononuclear and polymorphonuclear cells. *Hypertension* 2010 (in press).
7. Ariyoshi K, Maruyama T, Odashiro K, Akashi K, Fujino T, Uyesaka N. Impaired erythrocyte filterability of spontaneously hypertensive rats: Investigation by nickel mesh filtration technique. *Circ J* 2010; **74**: 129–136.
8. House SD, Lipowsky HH. Microvascular hematocrit and red cell flux in rat cremaster muscle. *Am J Physiol* 1987; **252**: H211–H222.

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