

Reference Values of Whole Blood Viscosity and Its Correlation with Hematology and Serum Chemistry in Beagle Dogs

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(Received: September 20, 2017 / Accepted: December 20, 2017)

Abstract : The aim of this study was to measure whole blood viscosity (WBV) and then to establish its reference values and identify correlation between WBV and hematology or serum chemistry in beagle. The experiment was made up of 82 healthy beagle dogs. Jugular vein blood samples (10 ml) were collected. WBV (cP) measured within 4 hours after collection by U-shaped scanning capillary-tube viscometer (BVD-PRO1[®]) which is capable of measuring yield stress and viscosity of whole blood continuously over a wide range of shear rates from 1 s^{-1} to 1000 s^{-1} is new type of capillary tube viscometer and calculates viscosity using Casson fluid model. Measured values of WBV, Complete blood cell count, serum chemistry were analyzed by RM-ANOVA test. Mean diastolic and systolic WBV (cP) were 29.032 ± 6.137 and 4.528 ± 0.865 . Bodyweight (BW), Red blood cell count (RBC), Hemoglobin (HGB), Hematocrit (HCT), Alkaline phosphatase (ALP), Cholestetol (CHOL), Total protein (TP), Globulin, Chloride (Cl), Fibrinogen, were statistically correlated with WBV over whole range of shear rates ($p < 0.05$). This study newly evaluated reference values of WBV by U-shaped viscometer in beagle. Correlation between WBV and BW, RBC, HGB, HCT, ALP, CHOL, TP, Globulin, Cl, Fibrinogen was presented.

Key words : Whole blood viscosity, Hematology, Serum chemistry, Reference values, beagle.

Introduction

Blood test including hematological and serum chemical test give the information to understand the current condition and disease of patient (1). Diseases related with vascular disorders are limited to be early diagnosed by common blood examination such as hematology and serum chemistry. And these common blood tests are difficult to propose any therapeutic guide. Hemorheology analyzes blood flow changed by RBC aggregation and deformation and their interaction with blood vessel wall. Therefore, vascular disorders have a strong association with hemorheology (2). Main parameter of hemorheology is whole blood viscosity (WBV) which depends on the shear stresses and pressure gradients and determined by hematocrit, plasma viscosity, plasma proteins concentration, erythrocyte aggregation, deformability, and mechanical properties of red blood cells (RBC) (3). WBV is a function of the quality and quantity of suspended materials, which are RBC, and the diverse plasma proteins and of the interaction between these elements (4). So a study on the relationship between WBV and RBC, plasma proteins is essential for more understanding of hemorheology. In human studies, many researches on blood viscosity have been already studied. For example, relationship between coronary disease, cerebrovascular disease, blood pressure, aging, etc. and blood viscosity are revealed (5-7). But, even though blood viscosity has been researched,

there is lack of studies about blood viscosity in dogs. So even more understanding of normal reference values of blood viscosity is necessary for the early diagnosis of conditions such as circulatory problem, cardiac insufficiency, retinal hemorrhage, renal disease and hypertension caused by hyperviscosity in dogs (8-11). Viscometers, which have been used conventionally in these days, are divided into three groups: capillary tube viscometers, falling-body viscometers, rotational viscometers. But most of them have some limitations such as measuring viscosity only specified, constant shear rate and often making experimental errors owing to their mechanical devices. For this reasons, measured values of blood viscosity by these viscometers has margin of errors. New type of capillary tube viscometer, U-shaped scanning capillary-tube viscometer (U-SCTV) (BVD-PRO1[®]) used this study is capable of measuring yield stress and viscosity of whole blood continuously over a wide range of shear rates from 1 s^{-1} to 1000 s^{-1} (12). The aim of this study is to measure WBV using by new viscometer and then to establish its reference values and identify correlation between WBV and hematology or serum chemistry in beagle dogs. Consequently, establishment of normal reference values of WBV by this study give an early diagnosis of vascular disorders in dogs.

Material and Methods

Experimental Animals

The experiment was made up of 82 healthy beagle dogs. Whole dogs are intact male. Mean body weight of dogs was

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9.918 ± 1.537 kg. Beagles were raised under controlled environment with consistent feeding management. C-reactive protein (CRP) concentration, a systemic inflammation marker were measured all dogs, then result of CRP concentration of all dogs was below 35 mg/L.

Blood Sample Collection

Jugular vein blood samples (10 ml) were collected. Each withdrawn blood sample was divided into three standard tubes which are EDTA-coated tube for complete blood cell count (CBC) and WBV, plain tube for serum chemistry and sodium citrate-coated tube for plasma fibrinogen concentration. CBC, serum chemistry and viscosity measurements were made on whole blood samples within 4 hours of blood collection. The blood samples collected for serum chemistry in plain tubes were centrifuged at 3500 rpm, 4°C for 10min. Serum, supernatant liquid was separated and also measured within 4 hours. The blood samples for plasma fibrinogen concentration in sodium citrate tubes were also centrifuged at 3500 rpm, 4°C for 10min. Plasma for measuring fibrinogen concentration was separated and stored in -70°C until analysis.

Whole Blood Viscosity Analysis

WBV (cP) was measured by U-shaped scanning capillary-tube viscometer (BVD-PRO1[®], Bio-visco, Inc., Republic of Korea) which is capable of measuring yield stress and viscosity of whole blood continuously over a whole range of shear rates from 1 s⁻¹ to 1000 s⁻¹. It is new type of capillary tube viscometer and calculates viscosity using Casson fluid model.

Hematological Analysis

CBC was measured using the automatic blood cell counter (Vet ABC[®], ABX Diagnostics, Montpellier, France). red blood cell count (RBC, 10⁶/mm³), hemoglobin concentration (HGB, g/dl), hematocrit (HCT, %), white blood cell count (WBC, 10³/mm³), platelet (PLT, 10³/mm³), mean corpuscular volume (MCV, μm³), mean corpuscular hemoglobin (MCH, pg), mean cell hemoglobin concentration (MCHC, g/dl), red cell distribution (RDW, %), mean platelet volume (MPV, μm³) were measured.

Serum Chemical Analysis

Serum chemistry was measured using the automatic chemistry analyzer (Hitachi Auto Analyzer 7020[®], Hitachi Co., Tokyo, Japan). The concentration of albumin (ALB, g/dl), alkaline phosphatase (ALP, U/L), alanine transaminase (ALT, U/L), amylase (AMY, U/L), total bilirubin (TBIL, mg/dl) blood urea nitrogen (BUN, mg/dl), Calcium (Ca, mg/dl), cholesterol (CHOL, mg/dl), creatinine (CRE, mg/dl), glucose (GLU, mg/dl), phosphorus (P, mg/dl), total protein (TP, g/dl), globulin (GLOB, g/dl), sodium (Na⁺, mmol/L), potassium (K⁺, mmol/L), chloride (Cl⁻, mmol/L) were measured.

CRP concentration was measured using portable magnetic permeability-based analyzer (LifeAssays[®] Veterinary Reader, LifeAssays AB, Lund, Sweden).

Fibrinogen concentration (mg/dl) was measured by automated coagulation instrument (STA R evolution[®], Diagnostica Stago, Asnières sur Seine, France) using the Clauss clotting method.

Table 1. Reference values of WBV for beagles: males (n = 82)

Total group	Shear rates	Mean	SD	Reference range	
				25th	75th
Whole Blood Viscosity (cP)	SR 1 s ⁻¹	29.032	6.137	25.735	31.833
	SR 5 s ⁻¹	12.099	2.339	10.838	13.133
	SR 10 s ⁻¹	9.157	1.702	8.240	9.853
	SR 50 s ⁻¹	5.870	1.013	5.378	6.240
	SR 100 s ⁻¹	5.204	0.923	4.763	5.520
	SR 150 s ⁻¹	4.922	0.887	4.505	5.220
	SR 300 s ⁻¹	4.528	0.865	4.128	4.823
	SR 1000 s ⁻¹	4.050	0.862	3.615	4.350

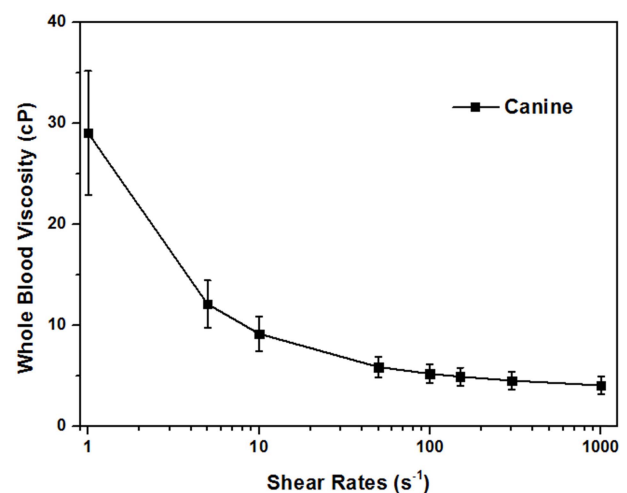


Fig 1. Mean WBV (cP) at shear rates from 1 s⁻¹ to 1000 s⁻¹ in beagles. The graph illustrates the mean values with S.D. (error bars).

Statistical Analyses

In present study, to analyze data of WBV, Mean, standard deviation (SD) and 25th and 75th percentile values were calculated. For finding correlation between WBV and hematology and serum chemistry, Pearson's correlation analysis was used. SPSS 18.0.0 (PASW Statistics; IBM Co., Armonk, NY, USA) and GraphPad Prism 5.0 (GraphPad Software, Inc., San Diego, CA, USA) were used for statistical analysis. P values under 0.05 or less were considered statistically significant.

Results

Reference values of WBV

Mean, SD and 25th and 75th percentile values were acquired by statistical analysis. Owing to the number of dogs (82 male beagle dogs), values within 25th and 75th percentile distance were used as the reference range.

Correlation analysis

Coefficient of correlation, *r* values resulted from correlation analysis are described in Table 2-4. *r* values are presented where there is significant correlation (*p* < 0.05 or less). Scatter diagram with *r* values of correlation were illustrated

Table 2. *r* values between WBV and hematology are presented

Shear rates	WBC	RBC	HGB	HCT
SR 1 s⁻¹	-0.3048**	0.7638**	0.8017**	0.7929**
SR 5 s ⁻¹	-0.2954**	0.7623**	0.8057**	0.7964**
SR 10 s ⁻¹	-0.2905**	0.7595**	0.8054**	0.7959**
SR 50 s ⁻¹	-0.2787*	0.7472**	0.7991**	0.7889**
SR 100 s ⁻¹	-0.2689*	0.7415**	0.7952**	0.7847**
SR 150 s ⁻¹	-0.2653*	0.7392**	0.7938**	0.7833**
SR 300 s⁻¹	-0.2633*	0.7342**	0.7928**	0.7824**
SR 1000 s ⁻¹	-0.2679*	0.7045**	0.7726**	0.7641**

p* < 0.05, *p* < 0.01, ****p* < 0.001

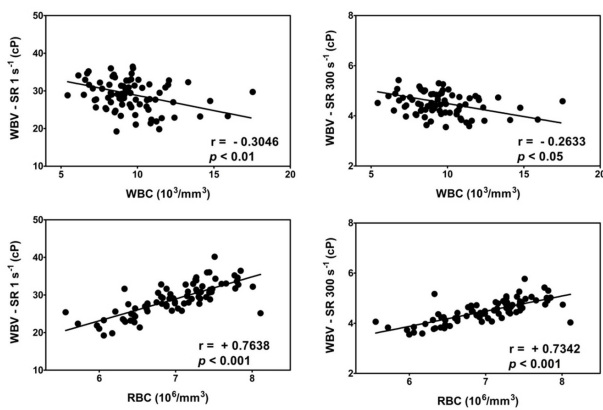


Fig 2. Scatter diagram with *r* values between diastolic (Shear rate 1 s⁻¹) and systolic (Shear rate 300 s⁻¹) blood viscosity and WBC and RBC.

in Figs 2~7.

Correlation between WBV and hematology

WBC, RBC, HGB, HCT were statistically correlated with WBV over whole range of shear rates (Table 2). Correlation between WBC, RBC, HGB, HCT and WBV were illustrated by Scatter diagram with *r* values (Figs 2~3).

Correlation between WBV and serum chemistry

TP, GLOB, Fibrinogen, CHOL, Cl concentration statistically correlated with WBV over whole range of shear rates while AMY concentration correlated with low shear rates

Table 3. *r* values between WBV and serum chemistry are presented

Shear rates	TP	GLOB	Fibrinogen	CHOL	CL	AMY
SR 1 s⁻¹	0.4893**	0.5041**	0.4216**	0.7929**	-0.2990**	0.2209*
SR 5 s ⁻¹	0.4449**	0.4369**	0.4421**	0.7964**	-0.3014**	0.2221*
SR 10 s ⁻¹	0.4370**	0.4400**	0.4510**	0.7959**	-0.2946**	0.2181*
SR 50 s ⁻¹	0.4177**	0.4453**	0.4654**	0.7889**	-0.2781*	-
SR 100 s ⁻¹	0.4045**	0.4534**	0.4770**	0.7847**	-0.2650*	-
SR 150 s ⁻¹	0.3982**	0.4569**	0.4805**	0.7833**	-0.2585*	-
SR 300 s⁻¹	0.3840**	0.4436**	0.4795**	0.7824**	-0.2533*	-
SR 1000 s ⁻¹	0.3535**	0.4138**	0.4690**	0.7641**	-0.2455*	-

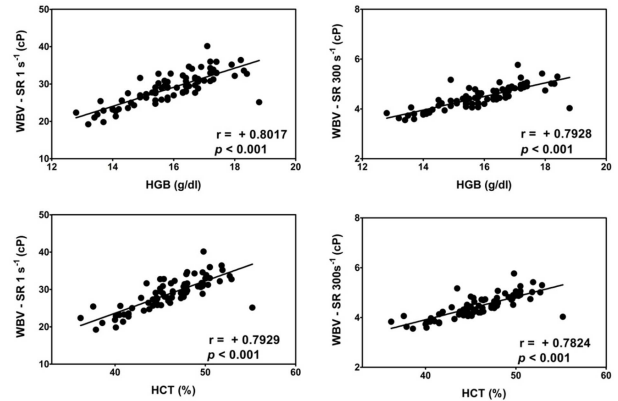


Fig 3. Scatter diagram with *r* values between diastolic (Shear rate 1 s⁻¹) and systolic (Shear rate 300 s⁻¹) blood viscosity and HGB and HCT.

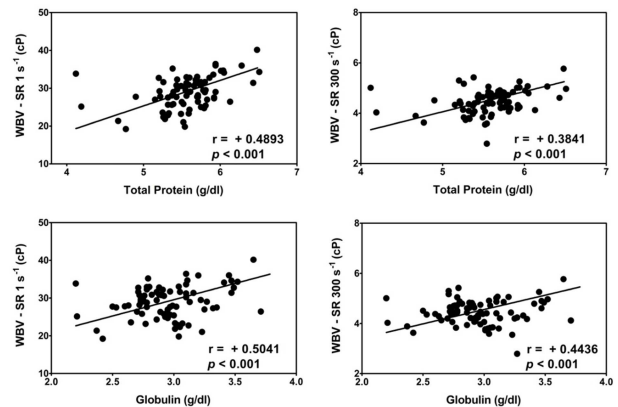


Fig 4. Scatter diagram with *r* values between diastolic (Shear rate 1 s⁻¹) and systolic (Shear rate 300 s⁻¹) blood viscosity and TP and GLOB.

(Table 3). Correlation between TP, GLOB, Fibrinogen, CHOL, Cl, AMY and WBV were illustrated by Scatter diagram with *r* values (Figs 4~6).

Correlation between WBV and body weight

Body weight (BW) was statistically correlated with WBV over whole range of shear rates (Table 4). Correlation between bodyweight and WBV was illustrated by Scatter diagram with *r* values (Figs 7~8).

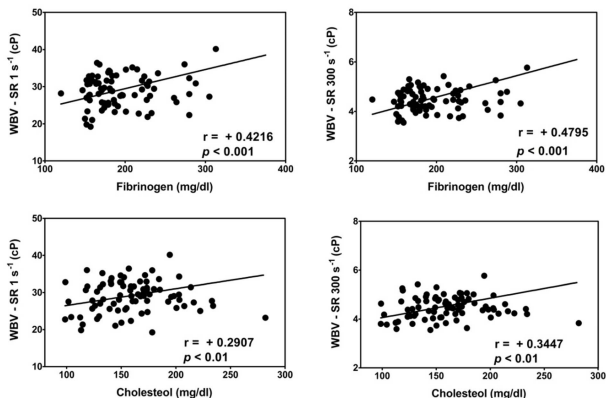


Fig 5. Scatter diagram with *r* values between diastolic (Shear rate 1 s⁻¹) and systolic (Shear rate 300 s⁻¹) blood viscosity and fibrinogen and CHOL.

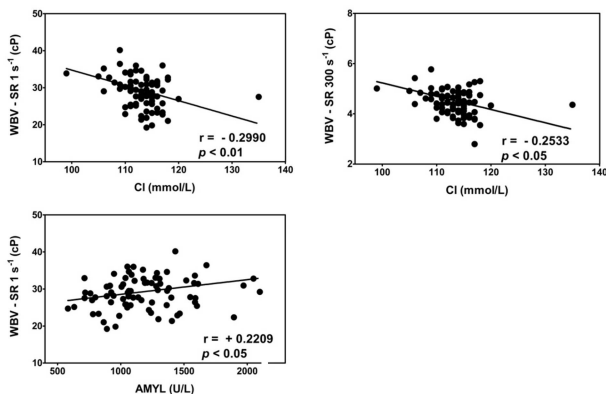


Fig 6. Scatter diagram with *r* values between diastolic (Shear rate 1 s⁻¹) and systolic (Shear rate 300 s⁻¹) blood viscosity and Cl and AMY.

Discussion

To our knowledge, this is the first study of whole blood viscosity values measured concurrently from a population of healthy, non-inflammatory dogs which were raised under controlled environment with consistent feeding management. Secondary influences on viscosity values: inflammatory disorders, sex hormones were minimized on this group (13,14). Acute phase reactants resulted from inflammatory response is a common cause of increased RBC aggregation (3). On this experiment, CRP of whole dogs was under 35 mg/L. Therefore inflammatory factors were controlled in this study (15). Concerning hemorheological gender differences in several data about difference species such as human, dog, and rat can be found in the literature. The male blood has high viscosity, hematocrit and fibrinogen concentration. In female, hemorheological parameters are influenced by physiological changes such as menstrual cycle. It was reported that the gender-differences on WBV can be even statistically significant and thus influencing the evaluation and comparability of data collected in different experimental models of unisex or mixed-gender groups (14,16,17). In the present study, blood samples were collected from only intact male beagles. Influence of sex hormones on viscosity values were reduced and thus

Table 4. *r* values between WBV and BW are presented

Whole Blood Viscosity (cP)	Body weight	
	SR	<i>r</i> value
SR 1 s ⁻¹	0.4015**	
SR 5 s ⁻¹	0.3842**	
SR 10 s ⁻¹	0.3744**	
SR 50 s ⁻¹	0.3500**	
SR 100 s ⁻¹	0.3381**	
SR 150 s ⁻¹	0.3316**	
SR 300 s ⁻¹	0.3292**	
SR 1000 s ⁻¹	0.3186**	

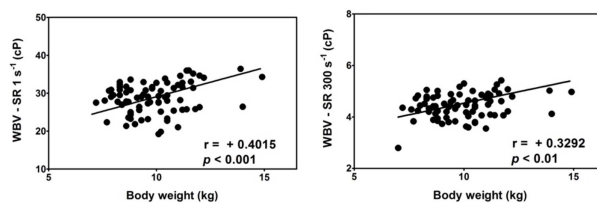


Fig 7. Scatter diagram with *r* values between diastolic (Shear rate 1 s⁻¹) and systolic (Shear rate 300 s⁻¹) blood viscosity and BW.

more reliable data could be obtained. Influence factors such as environment, CRP and gender, affecting the viscosity were minimized, therefore we achieved more stable standard values of WBV in dogs. In the result from previous study, WBVs at 0.7 s⁻¹, 2.4 s⁻¹, and 94 s⁻¹ were 22.885, 14.584 and 5.591 cP with 40% HCT, respectively in various breeds of dog (24 of 40 were beagles) (18). The other study presented that WBVs at 0.227 s⁻¹, 128.5 s⁻¹ were 49.0 and 4.77 cP with native HCT, respectively in beagle dogs (15 beagles). After calculation to standard hematocrit (45%), the former was 36.1 and the latter was 4.31 (10). Because of HCT concentration difference of each study, absolute comparison between previous studies and present' is impossible. And above-mentioned datas were measured only at specific shear rates and those datas cannot fully reflect the shear-thinning hemorheological characteristics of whole blood (12). However, values of WBV obtained in this study were measured over a range of shear rates continuously from high to low shear rates (as low as 1 s⁻¹). Therefore, the mean and reference values of present study were suggested over whole range of shear rates from 1 s⁻¹ to 1000 s⁻¹ and worthwhile to be used in futher study. In this study, there is some limitation because entire experimental dogs are male. In future studies, investigation the gender differences of WBV in beagles may be valuable to use as a practical data. Depending on increasing shear forces, the viscosity of blood decreases. For this reason, blood would be described as a non-Newtonian fluid (19). There are two distinct feature of RBC to contribute to blood viscosity at high and low shear rates. RBC deformability is a significant factor to determine WBV at high shear rates, while WBV at low shear rates is mainly influenced by RBC aggregation. At low shear conditions, non-Newtonian feature of blood is more deepened by rouleaux formation of RBC which mean that RBC aggregate into stack of coins. These coin-shaped aggregate

gative conditions tend to grow the frictional resistance. Therefore increase blood viscosity under low shear rates (3). At high shear conditions, RBC, flexible cells, can change more easily to ellipsoidal structures. Such transitions promote their orientation to flow stream lines in vessels, and thus lead to decreased blood viscosity (3,20). For all those reasons, blood viscosity is primarily influenced by RBC. Correlation between WBV and RBC, HGB, HCT was presented in many previous reports (3,4,10,21,22). Similar with those reports in this study, RBC, HGB, HCT were statistically correlated with WBV over whole range of shear rates ($p < 0.01$). The more shear rates were decreased, the more r values were increased. Correlation between TP, GLOB, fibrinogen, CHOL concentration and WBV can be found in the results from previous studies. RBC aggregation correlated with total plasma protein concentration. And Plasma viscosity correlated with total serum protein (9,18). Correlation between TP and WBV was not found in the dogs in Windberger et al. 2003. In contrast, Correlation between TP and WBV was observed in our study ($p < 0.01$). At diastolic (Shear rate 1 s^{-1}) and systolic (Shear rate 300 s^{-1}) blood viscosity, each r values between TP and WBV were 0.4893 and 0.3840. The more shear rates were decreased, the more r values were increased. Immunoglobulins have an influence on WBV by inducing red cell aggregation (23). The grade of hyperviscosity correlated with size of the paraprotein and severity of hyperglobulinemia. Large molecules have a greater effect to increase serum viscosity. Therefore hyperviscosity syndrome is shown most commonly in macroglobulinemia such as IgM gammopathies. And IgA aggregates its molecule to make polymers which have a huge effect on serum viscosity (8,11,24). In the present study, GLOB was statistically correlated with WBV over whole range of shear rates ($p < 0.01$). At diastolic (Shear rate 1 s^{-1}) and systolic (Shear rate 300 s^{-1}) blood viscosity, each r values between GLOB and WBV were 0.5041 and 0.4436. The more shear rates were decreased, the more r values were increased. Fibrinogen also induces RBC aggregation. And raised levels of macromolecules such as fibrinogen contribute to increase plasma viscosity. Therefore, fibrinogen is a significant determinant of WBV (18,25). In this study, correlation between fibrinogen and WBV was seen statistical significance ($p < 0.01$). At diastolic (Shear rate 1 s^{-1}) and systolic (Shear rate 300 s^{-1}) blood viscosity, each r values between fibrinogen and WBV were 0.4216 and 0.4795. The more shear rates were decreased, the more r values were increased. In human study, It is presented that high-density lipoprotein cholesterol (HDL) has specific viscoelastic properties (26). HDL decreases RBC aggregation and may permit RBC to maintain deformability (27). In present study, correlation between CHOL and WBV was seen statistical significance ($p < 0.01$). At diastolic (Shear rate 1 s^{-1}) and systolic (Shear rate 300 s^{-1}) blood viscosity, each r values between CHOL and WBV were 0.2907 and 0.3447. The more shear rates were increased, the more r values were increased. The role of AMY is to hydrolysis complex carbohydrates. The pancreatic acinar cells produce a calcium-dependent enzyme, AMY which passes directly from the pancreas into the circulation (28). Whether there is any causal relationship between AMY and WBV, however, in this study, AMY was correlated with

WBV at low shear rates only ($p < 0.01$). Correlation between AMY and WBV wasn't presented in previous study. We need further studies about this correlation. Cl is the major anion and plays a leading part in acid-base equilibrium and maintenance of osmolality. Changes in water balance lead to changes in chloride ion and sodium ion concentrations (29). When blood is dehydration, blood viscosity is increased by relative concentration. Watery Change such as dehydration in blood has an effect on Cl, sodium concentration and blood viscosity. So we think that there is some causal relationship. In this study, Cl and WBV were statistically inversely correlated ($p < 0.05$). But existence of a relationship between Cl and WBV does not necessarily indicate that the relationship is causal. Thus, further studies would be needed. In the present study, body weight was also statistically correlated with WBV in both shear rates ($p < 0.01$). But there is no investigation about relationship between body weight and WBV in previous studies. Therefore, further study would be needed to investigate correlation between those factors. In conclusion, The results suggested average and reference values of WBV. And it was presented that correlation between WBV and regular blood tests such as hematology and serum chemistry in beagle dogs. These results can be used as a reference data for further investigation.

Acknowledgement

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (Grant No. NRF 2014R1A2A2A01007969).

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