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Gender, Race, and Diet Affect Platelet Function Tests in Normal Subjects Contributing to a High Rate of Abnormal Results

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Summary

To assess sources of variability in platelet function tests in normal subjects, 64 healthy young adults were tested on 2–6 occasions at 2 week intervals using 4 methods: platelet aggregation (AGG) in platelet-rich plasma (PRP) in the Bio/Data PAP-4 Aggregometer (BD) and Chrono-Log Lumi-Aggregometer (CL); and AGG in whole blood (WB) in the CL and Multiplate Platelet Function Analyzer (MP), with ATP release (REL) in CL-PRP and CL-WB. Food and medication exposures were recorded prospectively for 2 weeks prior to each blood draw. At least one AGG abnormality was seen in 21% of 81 drug-free specimens with CL-PRP, 15% with CL-WB, 13% with BD-PRP, and 6% with MP-WB, increasing with inclusion of REL to 28% for CL-PRP and 30% for CL-WB. Epinephrine AGG and REL were significantly reduced in males ($P<0.0001$). Ristocetin AGG and collagen and thrombin REL were significantly reduced in Blacks ($P<0.0001$). One-third of specimens drawn following flavonoid-rich food exposures had aberrant results, compared to 8.5% of specimens without such exposures ($P=0.0035$). PRP tests had less intra-individual variation than WB tests. Gender, race, diet, and test system affected results of platelet function testing in healthy subjects, suggesting caution when interpreting the results of platelet function testing in patients.

Keywords

Platelets; platelet function tests; platelet aggregation; ristocetin; flavonoids

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Author contribution

C. H. Miller and S. Stein designed the study, directed the research, analyzed data, and wrote the paper. A. S. Rice collected data, performed testing, and analyzed data. K Garrett recruited participants, collected data, and coordinated study activities. All authors edited and approved the final paper.

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

Competing interests

The authors state that they have no competing interests to declare.

Introduction

Measurement of platelet function *in vitro* is the primary clinical method used for the diagnosis of an inherited or acquired platelet function defect in a patient with excessive bleeding, although specific receptor, granule, or DNA studies are often used for confirmation. Light transmission aggregometry (LTA) in platelet-rich plasma (PRP), which has been the gold standard for platelet function testing for fifty years, measures the change in optical density produced when platelets form aggregates in fluid phase. Abbreviations used are defined in Table I. More recently, the simultaneous measurement of adenosine triphosphate (ATP) release as an indicator of platelet activation and granule secretion has been added. Impedance aggregometry allows measurement of aggregation and ATP release in whole blood (WB); it requires that platelets first adhere to a pair of electrodes and then form stationary aggregates which increase the resistance across the electrode gap. There have been few direct comparisons of the various measurement systems available for diagnosis of platelet function defects (Ingerman-Wojenski *et al*, 1983; Ingerman-Wojenski and Silver, 1986; Riess *et al*, 1986; Podczasy *et al*, 1997; Seyfert *et al*, 2007).

In clinical practice, it is common to test a group of “normal” subjects to set reference ranges for response to specific agonists and to test an individual normal subject with each patient tested to verify appropriate function of the test system (Christie *et al*, 2008; Hayward *et al*, 2010; Cattaneo *et al*, 2013). Numerous drugs have been documented to affect aggregation and ATP release (Rao, 2007). In addition, certain foods, alcohol, caffeine, and smoking have been reported to have effects. Foods rich in flavonoids, such as chocolate, red wine, and tea have been particularly implicated (Pearson *et al*, 2005; Holt *et al*, 2005). Certain flavonoids have been shown to inhibit platelet signaling pathways through binding to the thromboxane A_2 receptor (Guerrero *et al*, 2004). In clinical platelet function testing, use of medications is usually considered (Christie *et al*, 2008; Hayward *et al*, 2010; Cattaneo *et al*, 2013); the impact of other exposures has not been evaluated.

In a recent study of women with menorrhagia, using a standardized instrument and protocol for aggregation and ATP release testing at 6 sites, we found 20% of 169 control subjects collected for reference range determination to be abnormal with arachidonic acid alone (Miller *et al*, 2011). This led us to attempt to define the degree and sources of variation in an unselected group of normal subjects studied over time using four different test systems with prospective collection of exposure data.

Methods

Subjects

Subjects were recruited from among students and employees at Emory University in Atlanta. Each completed a questionnaire on history of bleeding symptoms and chronic disease. Those with no history of excessive bleeding or diagnosed chronic disease were tested for coagulation parameters as previously described (Miller *et al*, 2011). All subjects had normal coagulation function except one subject with decreased factor XII, who was not excluded. The 64 enrolled subjects ranged in age from 18–35 and included 30 males and 34 females. They self-identified their ethnicities as 34 (53%) White non-Hispanic, 22 (34%) Black non-

Hispanic, 3 (5%) Asian, 2 (3%) White Hispanic, and 3 (5%) other. The study was conducted with the approval of the Institutional Review Boards of Emory University and the CDC. Written informed consent was obtained from all participants.

Food and Medication Data Collection

Data were collected prospectively on daily drug, food, and alcohol intake and illness through self-recorded standardized diaries which were collected prior to each blood draw and reviewed after testing was concluded. Each specimen was scored as positive or negative for intake of drugs other than oral contraceptives and multi-vitamins within the 2 weeks prior to specimen collection. No subject began or discontinued oral contraceptives during the study. Alcohol and flavonoid intake was scored as positive or negative for two time periods: morning exposure within 6 hours of blood draw and evening exposure within 12–18 hours of blood draw. The list of flavonoid-rich foods was compiled from the literature (Pearson *et al*, 2005; Holt *et al*, 2005) and included cocoa, chocolate, tea, grapes and grape products, fish, onions, garlic, broccoli, apples, citrus fruits, nuts, peanuts, soy, and red, blue, and purple berries.

Platelet Function Tests

Blood was collected into evacuated siliconized glass tubes (Becton Dickinson, Franklin Lakes, NJ) containing 3.2% sodium citrate in a ratio of 1:9 with blood and maintained at room temperature. For PRP, blood was centrifuged at 22°–25°C for 8 minutes at 200 × g. After transfer of two-thirds of PRP with a plastic pipette to a polypropylene tube, the remaining PRP was centrifuged at 22°–25°C for 20 minutes at 1,600 × g to produce platelet-poor plasma (PPP), which was transferred with a plastic pipette to another polypropylene tube. PRP was standardized to a platelet count of 250×10^9 platelets L^{-1} by addition of PPP. For WB testing, whole blood was diluted with an equal volume of 0.9%NaCl.

LTA was measured in PRP using a BioData Platelet Aggregation Profiler, Model PAP-4 (BD) (BioData Corp.) and a Chrono-Log platelet lumi-aggregometer Model 560-CA (CL) (Chrono-Log Corp, Haverton, PA, USA) by change in optical density and expressed as % maximal aggregation. WBA was measured by change in impedance and expressed as ohms in the CL and in aggregation units (AU) in the Multiplate analyzer (MP) (Dynabyte GmbH, Munich, Germany). In CL-PRP and CL-WB, REL was measured by luminescence using luciferin-luciferase reagent (Chrono-Log Corp.) added at a ratio of 50 microliters (μL) to 450 μL PRP or 100 μL to 900 μL diluted WB. REL was calculated by comparison of peak luminescence recorded from the subject sample with that of a 2 μM ATP standard (Chrono-Log Corp) and expressed in μmoles (μM). Reactions were initiated by the addition of agonists to produce the final concentrations recommended by the manufacturers, as shown in Table II.

Statistical Methods

Distributions were checked for normality by the D'Agostino and Pearson omnibus normality check. Multivariate analysis was performed for each method and agonist using gender and race. A *P*-value was calculated for multiple comparisons by the Bonferroni correction. For 56 comparisons at a cut-off of 0.025, a *P*-value of <0.0004 was required to achieve

significance. Coefficient of variation (CV) was calculated for each subject with four or more specimens for each agonist and method. Reference ranges were calculated with a lower limit of 2.5 percentile and upper limit of 97.5 percentile using results from the first blood draw on each subject with no drugs other than oral contraceptives and multi-vitamins reported during the previous two weeks. Chi-square and Fisher's exact test were used for comparisons. Statistical analysis was carried out using SAS Version 9.2 (SAS Institute, Cary, NC, USA) and GraphPad Prism 5 (GraphPad Software, Inc., LaJolla, CA, USA).

Results

Platelet aggregation was performed on the same blood specimen by each of four methods: LTA using platelet-rich plasma (PRP) in two instruments, one with simultaneous measurement of ATP release (REL), and WBA in two instruments, one with simultaneous measurement of REL. Key findings are summarized in Table III.

Specimen characteristics

Sixty-four subjects were studied at two-week intervals over a three month period. A total of 349 specimens were tested, an average of 5.4 specimens per subject. Subjects were not asked to abstain from drug use but were asked to record drugs and foods ingested during the two-week period prior to each blood draw. Exposure to drugs was reported for 217 of 349 specimens (62.2%), 59 (16.9%) with drugs known to affect platelet function. All subjects reported drug use prior to at least one specimen. Specimens with a single drug exposure included 35 with ibuprofen, 10 with acetaminophen, 7 with acetylsalicylic acid, and 5 with loratadine. A total of 162 specimens had multiple drug exposures. Results on specimens with drug exposures will be reported in a separate publication.

A total of 129 specimens from 41 subjects with no drug exposure reported in the previous two weeks other than oral contraceptives or multi-vitamins were classified as drug-free. Characteristics of these subjects were similar to those of the entire study group. Seven specimens from 4 subjects with platelet counts below $150 \times 10^9\ L^{-1}$ were excluded from analysis, leaving 122 drug-free specimens. Distributions of test results for these specimens are shown in Figure 1.

Evaluation of test profiles

Reference ranges for each test were calculated using the first drug-free specimen from each individual (Table IV). The remaining 81 drug-free specimens were used as a test group. The profile of tests from a single method, excluding ristocetin, was evaluated for each specimen (Table V). For REL in CL-PRP, ADP and EPI results were excluded, because their reference ranges included zero. The numbers of profiles with one or more abnormal AGG measurements were 21% for CL-PRP, 15% for CL-WB, 13% for BD-PRP, and 6% for MP-WB. When REL was included, 28% of CL-PRP and 30% of CL-WB profiles had at least one abnormality. If an abnormality with 2 or more agonists was required to consider a profile as abnormal, as suggested by Hayward *et al* (2009), AGG profiles were abnormal in 8.6% of CL-PRP, 3.7% of CL-WB, 1.7% of BD-PRP, and no MP-WB profiles, and profiles combining AGG and REL were abnormal in 9.9% (CL-PRP) and 13.6% (CL-WB).

Effects of race and gender

To investigate sources of the variability observed, a multivariate analysis was performed on the 122 drug-free specimens using the variables race and gender and a *P*-value adjusted for multiple comparisons. EPI aggregation in CL-PRP and BD-PRP and REL in CL-PRP were significantly reduced in males ($P<0.0001$) (Figure 2). Blacks had significantly more aggregation than Whites using ADP in CL-WB ($P=0.0001$) and significantly less REL using collagen and thrombin in PRP and WB and AA in WB ($P<0.0001$). Ristocetin agglutination in Blacks was significantly lower than in Whites in CL-PRP ($P<0.0001$) (Figure 3) but not in BD-PRP or either WB system. No significant gender or race differences were seen using MP-WB.

Ristocetin response

Platelet response to ristocetin varied by instrument (Figure 1A–D, Figure 3), with BD-PRP showing similar medians at all concentrations. Because of these results, a further study was performed using additional concentrations on a new panel of 7 healthy control subjects (Figure 4). In CL-PRP, median agglutination dropped to near zero at 1.00 mg mL^{-1} but in BD-PRP was strong at 0.75 mg mL^{-1}. At 0.50 mg mL^{-1}, a concentration often used to detect von Willebrand disease Type 2B (VWD2B), CL-PRP showed little response (range 0–2%), while BD-PRP continued to show a median agglutination of 10% (range 0–90%).

Intra-individual variation

47gures 5–7 show variation in individual test results over time by subject. To assess intra-individual variation, a coefficient of variation (CV) for each subject with four or more specimens was calculated by agonist and method (Table VI). For PRP aggregation, median CVs were below 15% with collagen, AA, ADP, and EPI using both instruments. For WB aggregation, median CVs were between 15 and 30%. REL showed the greatest intra-individual variation. Eighteen subjects had large CVs due to extremely low results on individual specimens. When these diverged from results on the subject's other specimens, as illustrated by open circles in Figures 5–7, they were termed "aberrant" specimens.

Effect of food exposures

The histories of all subjects were examined for reported illness or intake of flavonoid-rich foods or alcohol prior to each specimen. Of 75 specimens drawn after flavonoid-rich food exposures, 24 (32.0%) had aberrant results, compared to 4 of 47 specimens (8.5%) without such exposures ($P=0.0035$). The distribution of exposures was significantly different between the aberrant and non-aberrant specimens ($P<0.0001$) (Table VII). Of 28 specimens with aberrant results, 24 (85.7%) were drawn after flavonoid-rich food intake within 18 hours, compared to 51/94 (54.3%) with consistent results ($P=0.0035$). Exposure within 1–6 hours with or without earlier exposure characterized 19/94 specimens (20.2%) with non-aberrant results and 15/28 (53.6%) with aberrant results ($P=0.0004$). No other alcohol intake or illnesses were reported in the aberrant group. The tests affected and the specific foods consumed are shown in Table VIII.

Discussion

Guidelines for platelet function testing (Christie *et al*, 2008; Hayward *et al*, 2010; Cattaneo *et al*, 2013) stress the importance of insuring that the patient and normal controls have not taken drugs influencing platelet function. Clinical and Laboratory Standards Institute guidelines also recommend that the patient be fasting (Christie *et al*, 2008). The characteristics of the normal subjects for reference range definition and daily controls are not otherwise defined. Most clinical laboratories rely on a group of proven donors who have demonstrated normal platelet function on previous occasions. Repeated study of 5 such subjects with LTA showed good reproducibility over a 2 year period (Hevelow *et al*, 2007). Repeated WBA in 7 subjects over 5 years, however, showed abnormal results on one-third of 86 studies (Refaii *et al*, 2010). We tested 64 unselected healthy subjects, finding striking differences that must be considered in interpretation of patient results.

Abnormal results in a control subject are often ascribed to the subject having forgotten that they took medication. To improve on simple recall, we prospectively collected exposure data. All subjects had drug exposure on at least one specimen; more than 60% of specimens followed some drug exposure. Although only 17% of exposures were to drugs known to affect platelet function, we excluded all in order to focus on other sources of variability. To mimic the clinical situation, we used the first drug-free specimen from each subject to calculate reference ranges by non-parametric methods, including 95% of the normal population, as recommended (Hayward *et al*, 2008), and used the 81 remaining drug-free specimens as a test group. Diagnosis of platelet function disorders involves a panel of tests, ranging from at least 5 tests when measuring aggregation alone to a total of 10 when both aggregation and release are measured. The chance that a single test result will fall below a 2.5 percentile cut-off is 12.5% for an aggregation profile using 5 agonists and approaches 25% for a profile including both aggregation and release. Our findings that 6–21% of aggregation profiles and 28–30% of profiles measuring both aggregation and release were abnormal are close to that expectation. The strategy suggested to reduce the number of false positive profiles by requiring abnormality with >1 agonist to classify a profile as abnormal (Hayward *et al*, 2009) is effective; however, it would exclude patients with a true bleeding disorder affecting response to a single agonist. In spite of the statistical expectation of a large number of false positive tests, the frequency of specimens with minimal aggregation and absent release among healthy subjects is concerning.

Females have been observed to have greater aggregation than males in some studies (Johnson *et al*, 1975; Meade *et al*, 1985; Silver *et al*, 1993; Becker *et al*, 2006) but not in others (Beyan *et al*, 2006; Seyfert *et al*, 2007; Rubak *et al*, 2012). Here, only epinephrine showed a gender difference. The number of abnormalities seen in healthy subjects using a relatively high concentration of epinephrine (10 μM) in two different instruments makes one question its utility for diagnosis. Although it is included on recommended agonist panels (Hayward *et al*, 2009; Dawood *et al*, 2012; Cattaneo *et al*, 2013), we believe that its use in the initial screening panel may lead to misdiagnosis, particularly in males.

Ristocetin response showed a significant race difference. A single nucleotide polymorphism in von Willebrand factor, D1472H, which is common in Blacks, interferes with ristocetin

binding to von Willebrand factor and falsely lowers ristocetin cofactor (Flood et al, 2010). DNA was not available on our subjects to investigate this possibility. The race difference was evident only in CL-PRP. BD-PRP and the WB systems did not show this difference. Black subjects also showed decreased release with collagen and thrombin in PRP and WB and with AA in WB. These differences might also be due to common genetic variants.

Subjects who consumed flavonoid-rich foods prior to testing were significantly more likely to show results differing from their usual pattern than those without such consumption. Studies on the effects of foods on platelet function have focused primarily on their potential long-term use in the prevention of cardiovascular disease and have been conducted after exposure for 1–4 weeks (Pearson *et al*, 2005; Holt *et al*, 2005). Flavonoids, components of such common foods as chocolate, tea, red wine, and beer, have been most extensively studied. Black tea (Wolfram *et al*, 2002) and chocolate (Innes *et al*, 2003) have been demonstrated to inhibit LTA. Chocolate (Murphy *et al*, 2003) and grape products, including red wine and grape juice (Keevil *et al*, 2000; Pignatelli *et al*, 2002), have shown an effect on WBA. Few studies have assessed how rapidly these effects occur and their persistence. Chocolate prolonged the Platelet Function Analyzer-100 closure time within 4–6 hours (Murphy *et al*, 2003). Red wine retained an effect at 12 hours post consumption (Pignatelli *et al*, 2002). In this study, more flavonoid-rich foods were consumed in the evening, but those with aberrant results most often had both morning and evening exposures. These data suggest that a morning fast prior to blood draw is **not** sufficient to eliminate the effect of inhibitory foods; an abstention from specific foods of 24 hours may be required to eliminate their effect. Other studies have shown no effect of eating breakfast (Stegnar *et al*, 2010) or lunch (Silver *et al*, 1993) prior to LTA, but neither considered the intake of specific foods. Our study was limited by the large number of specimens excluded due to drug exposure and the complexity of the dietary information collected. Flavonoid-rich foods are common and often consumed together. Longitudinal studies will be required to determine the timing and magnitude of food effects on clinical platelet function testing.

The test systems used vary in basic mechanism, type of specimen, and the specific proprietary reagents provided as agonists. The BD-PRP system showed the least intra-individual variation over time. Both WB systems showed more variability than PRP, perhaps due to standardization of the platelet count in PRP. The MP-WB system produced the fewest abnormal results in these healthy subjects. CL-PRP and CL-WB results, using the same instrument and reagents, were similar; however, in the CL-PRP system, no ATP release was detected on 6.6% of AA, 8.2% of ADP, and 13.1% of EPI tests, preventing calculation of a lower limit of the reference range. Exclusion of such low results from reference range calculations and yet considering them to be diagnostic in patients is not an acceptable practice. Overall, addition of ATP release measurements to aggregation profiles increased the number of abnormal findings in healthy subjects.

The panels of agonists and concentrations used (Table II) were those recommended by the manufacturers at the time of the study. They are similar but not identical to the recommendations published more recently (Dawood *et al*, 2012; Cattaneo *et al*, 2013). Reagent formulations vary by manufacturer, and this may contribute to the differences observed among the systems tested. BD-PRP showed less variability than CL-PRP with

different ristocetin concentrations. From this study, it could not be determined if the BD system can detect clinically important differences in ristocetin response, such as that seen in VWD2B. In VWD2B, aggregation with low ristocetin concentrations is expected to be increased over the minimal response usually seen in normal individuals; however, normal subjects had up to 90% aggregation with 0.50 mg/mL ristocetin in the BD system, suggesting that it might be difficult to identify VWD2B with this system.

We conclude that gender, race, diet, and test system affect results of platelet function testing in healthy subjects and that these differences should be considered when interpreting results in patients referred for evaluation of bleeding. Epinephrine and ristocetin are particularly problematic; abnormal results seen with either of these agonists alone may reflect population variation and should be interpreted cautiously. Exclusion of flavonoid-rich foods from the diet for 24 hours prior to testing may decrease false positive results. While it might be presumed that the systems with the least intra-individual variability and the fewest false-positive results would be the best choice for patient testing, increased specificity often results in decreased sensitivity. A similar method comparison using patients with known platelet function defects would be required to determine whether the methods showing the least variability retain sufficient sensitivity for detection of mild platelet defects and whether release measurements, which decrease specificity, add appreciably to diagnostic efficacy. Because of the high rate of abnormal results observed in normal individuals, confirmation of all abnormal results by demonstrating the reproducibility of the defects in a different specimen, along with specific receptor, granule, or DNA studies are needed to ensure accurate diagnosis and avoid incorrectly labeling patients as having an abnormality of platelet function.

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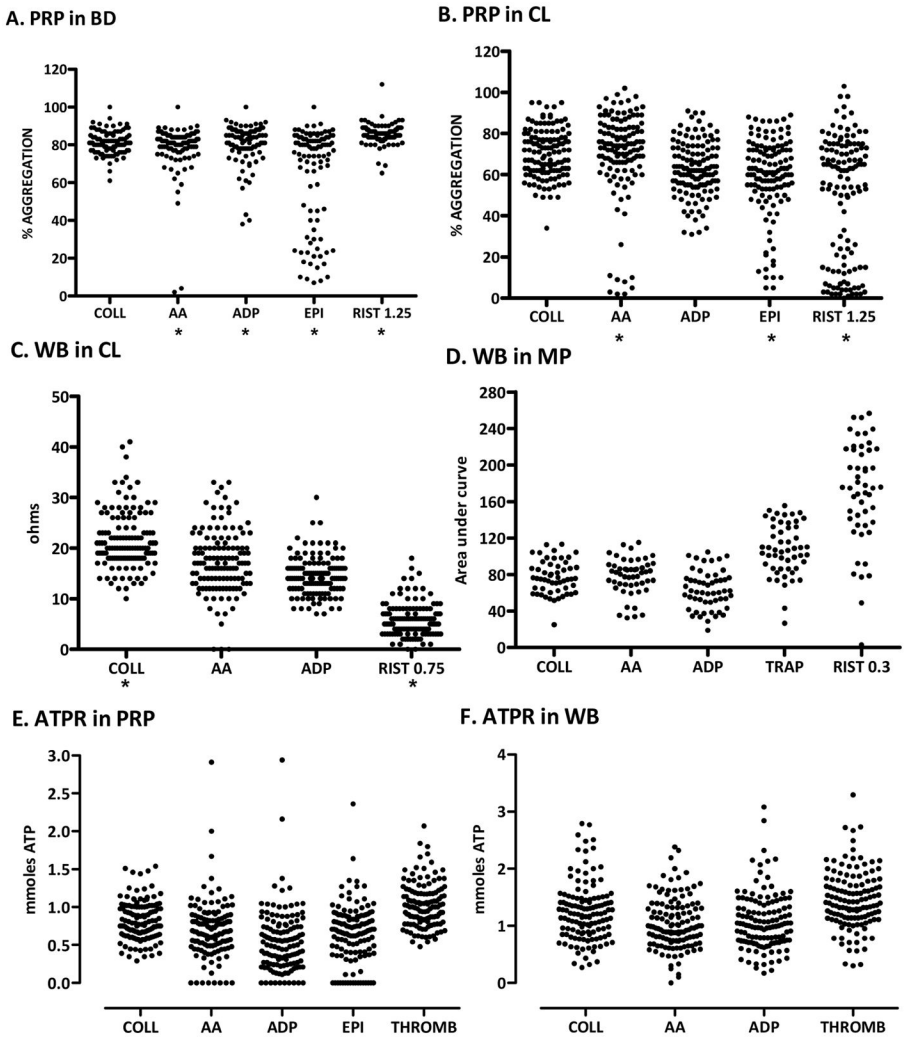

Figure 1.

Platelet aggregation and ATP release (REL) in drug-free subjects using collagen (COLL), arachidonic acid (AA), adenosine diphosphate (ADP), epinephrine (EPI), ristocetin (RIST), and thrombin (THROMB) in platelet-rich plasma (PRP) (A, B, E) or whole blood (WB) (C, D, F) in BioData system (BD), ChronoLog system (CL), and Multiplate system. * not normally distributed

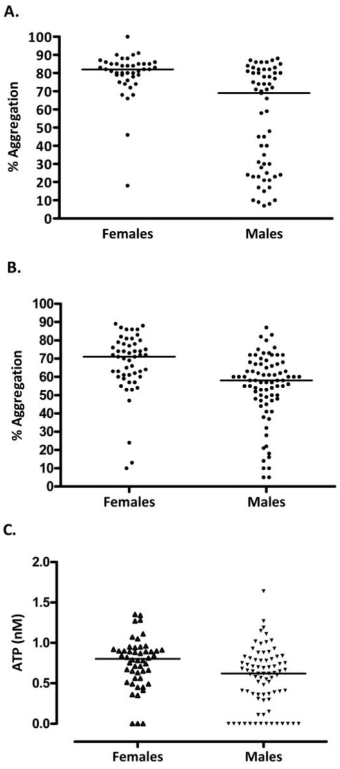

Figure 2.

Gender differences in response to epinephrine in platelet-rich plasma. A. BioData system. B. Chrono-Log system aggregation. C. ATP release. The bar is placed at the median.

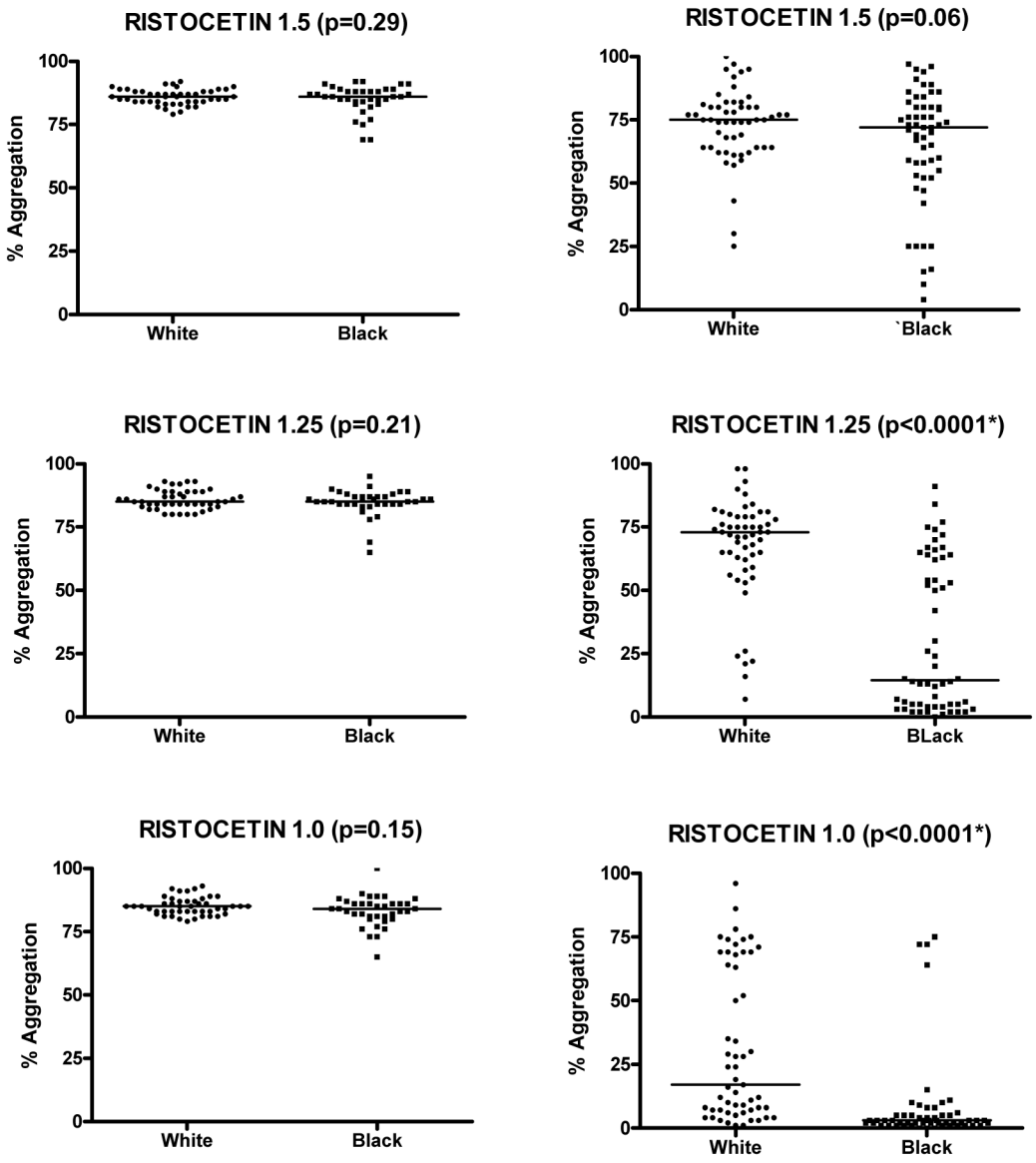

Figure 3.

Race differences in aggregation with ristocetin at different concentrations (mg mL−1) in BioData (left) and Chrono-Log (right) systems. The bar is placed at the median.

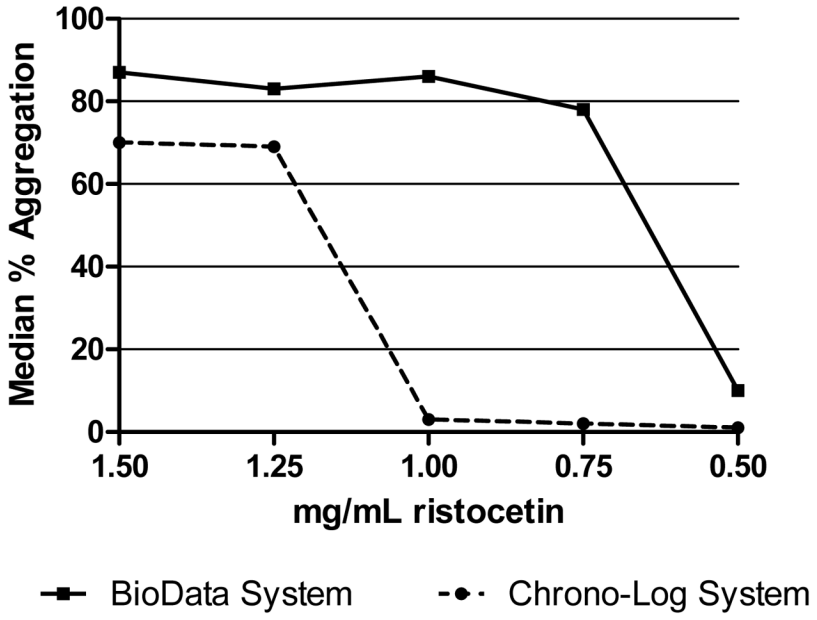

Figure 4.
Comparison of median platelet aggregation to various concentrations of ristocetin in the BioData and Chrono-Log systems among 7 healthy subjects.

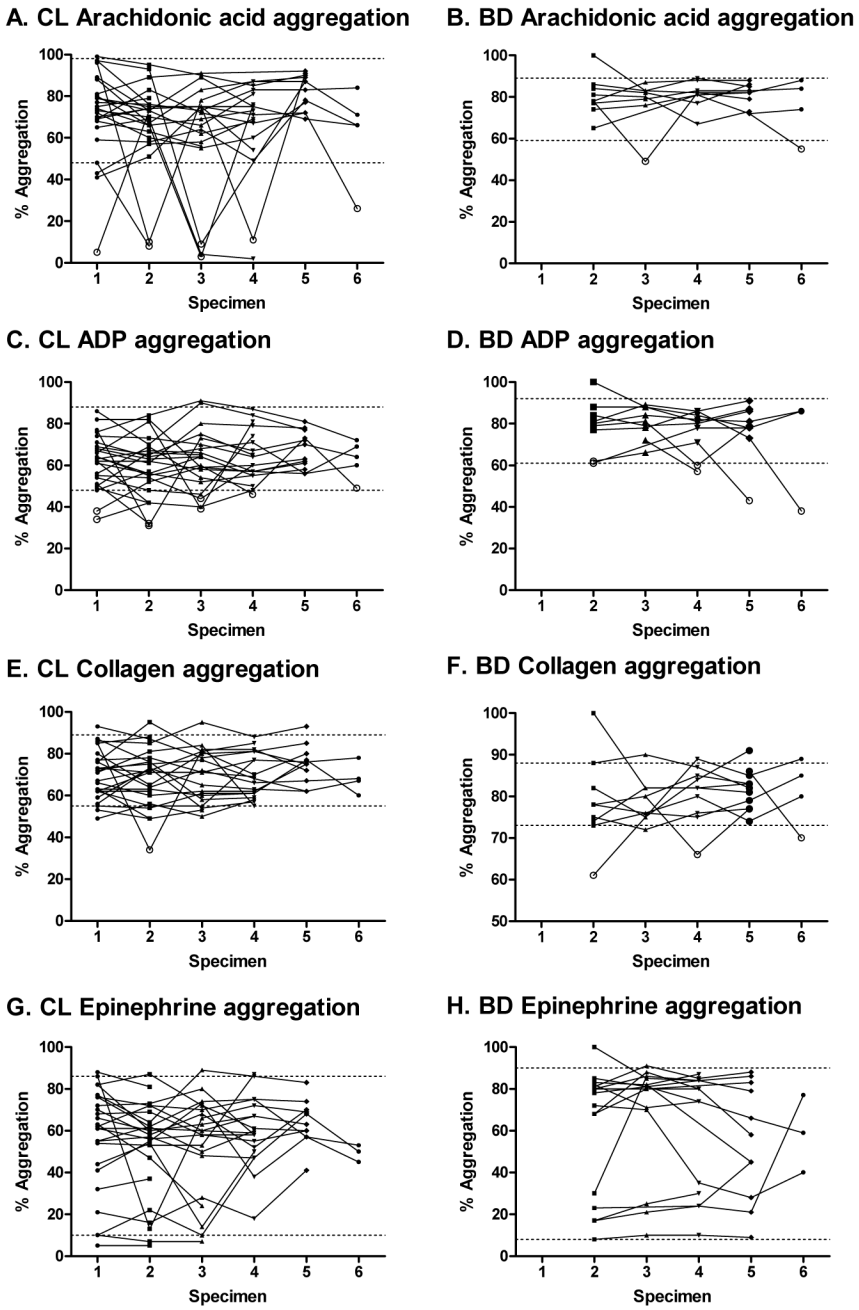

Figure 5.

Platelet aggregation in platelet-rich plasma (PRP) from drug-free subjects in the Chrono-Log (CL) and BioData (BD) systems. Dashed lines represent reference ranges (2.5–97.5 percentiles) from Table IV. Aberrant specimens are shown as open circles.

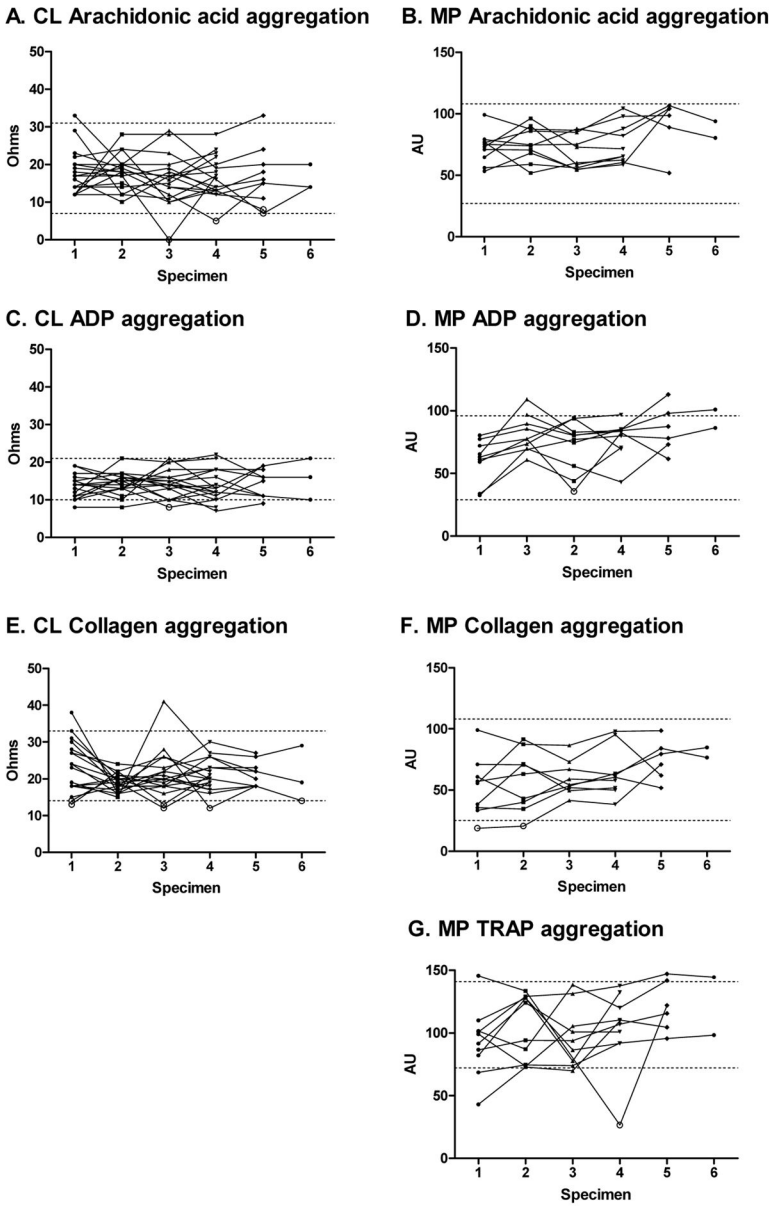

Figure 6.

Platelet aggregation in whole blood (WB) from drug-free subjects in the Chrono-Log (CL) and Multiplate (MP) systems. Dashed lines represent reference ranges (2.5–97.5 percentiles) from Table IV. Aberrant specimens are shown as open circles.

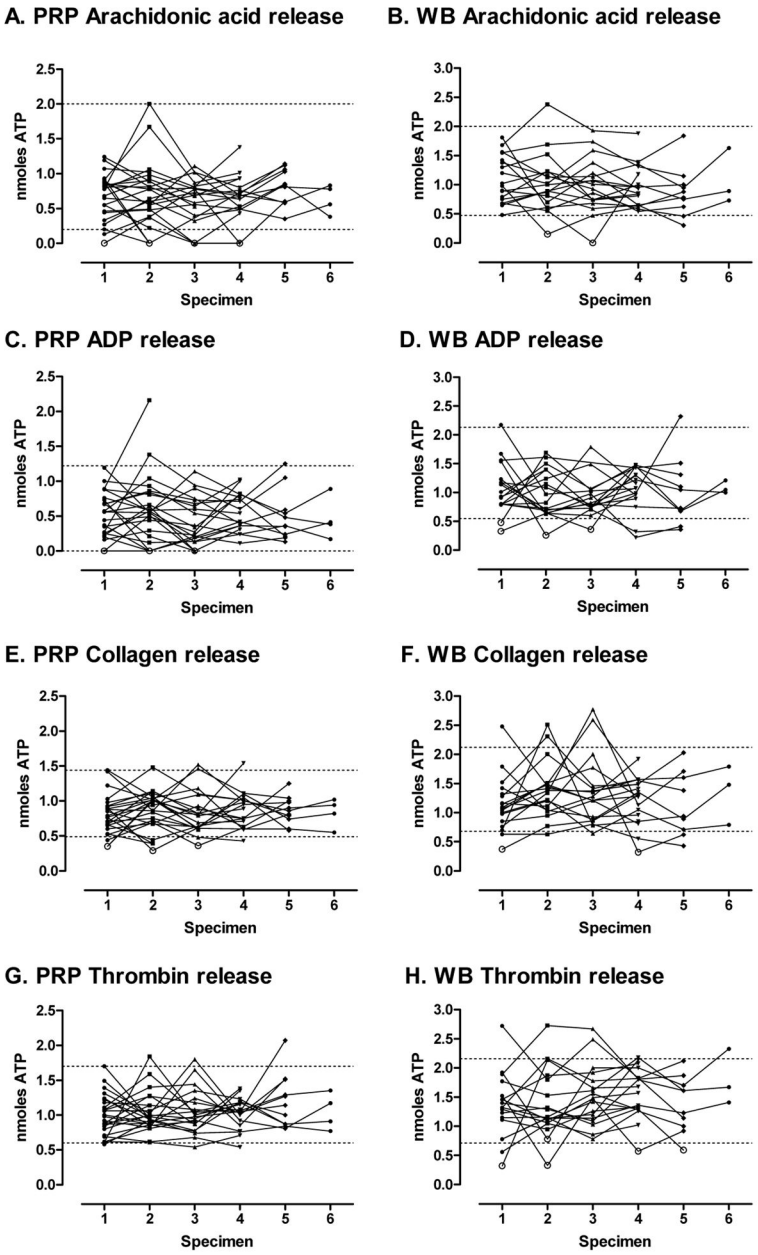

Figure 7.

Platelet ATP release in platelet-rich plasma (PRP) and whole blood (WB) from drug-free subjects in the Chrono-Log system. Dashed lines represent reference ranges (2.5–97.5 percentiles) from Table IV. Aberrant specimens are shown as open circles.

Table I

Abbreviations used

AGG	Platelet aggregation
ATP	Adenosine triphosphate
AA	Arachidonic acid
ADP	Adenosine diphosphate
BD	Bio/Data PAP-4 Aggregometer
BD-PRP	PRP aggregation in the Bio/Data PAP-4 Aggregometer
CL	Chrono-Log Lumi-Aggregometer
CL-PRP	PRP aggregation in the Chrono-Log Lumi-Aggregometer
CL-PRP REL	ATP release in PRP in the Chrono-Log Lumi-Aggregometer
CL-WB	WB aggregation in the Chrono-Log Lumi-Aggregometer
CL-WB REL	ATP release in WB in the Chrono-Log Lumi-Aggregometer
CV	Coefficient of variation
COLL	Collagen
EPI	Epinephrine
LTA	Light transmission aggregometry
MP	Multiplate Platelet Function Analyzer
MP-AGG	PRP aggregation in the Multiplate Platelet Function Analyzer
PRP	Platelet-rich plasma
REL	ATP release
RIST	Ristocetin
THROMB	Thrombin
TRAP	Thrombin receptor activating peptide
VWD2B	von Willebrand disease type 2B
WB	Whole blood
WBA	Whole blood aggregometry

Table II

Final concentration of agonists by instrument and method (PRP=platelet-rich plasma, WB=whole blood)

Reagent	Method			
	BioData PRP	**Chrono-Log PRP**	**Chrono-Log WB**	**Multiplate WB**
ADP	20 μM	10 μM	20 μM	6.5 μM
Arachidonic Acid	0.5 mM	0.5 mM	0.5 mM	0.5 mM
Collagen	1.9 μg mL^{-1}	2 μg mL^{-1}	2 μg mL^{-1}	3.2 μg mL^{-1}
Epinephrine	10 μM	10 μM	--	--
Thrombin	--	1 U mL^{-1}	1 U mL^{-1}	--
TRAP*	--	--	--	32 μM
Ristocetin	1.50 mg mL^{-1} 1.25 mg mL^{-1} 1.0 mg mL^{-1}	1.50 mg mL^{-1} 1.25 mg mL^{-1} 1.00 mg mL^{-1} 0.50 mg mL^{-1}	0.75 mg mL^{-1} 0.25 mg mL^{-1}	0.3 mg mL^{-1}

*Thrombin receptor activating peptide

Table III

Summary of key findings in drug-free specimens. AA=arachidonic acid, ADP=adenosine diphosphate, COLL=collagen, EPI=epinephrine, RIST=ristocetin, THROMB=thrombin, TRAP=thrombin receptor activating peptide

	Platelet-rich plasma			Whole blood		
	Chrono-Log			Chrono-Log		
	Aggregation	ATP Release	BioData Aggregation	Aggregation	ATP Release	Multiplate Aggregation
Males versus females	EPI↓	EPI↓	EPI↓			
Blacks versus Whites	RIST↓	COLL↓ THROMB↓		ADP↑	COLL↓ THROMB↓ AA↓	
Flavonoid-rich food exposure	AA↓ ADP↓ COLL↓	AA↓ ADP↓	AA↓ ADP↓ COLL↓	AA↓ ADP↓ COLL↓	AA↓ ADP↓ COLL↓ THROMB↓	AA↓ ADP↓ COLL↓ TRAP↓
Intra-individual variability (range of median CVs among agonists used)	8.6–13.8	17.2–40.4	4.8–12.2	19.0–26.8	21.4–35.1	14.6–25.3
Profiles abnormal with:						
1 agonist	21%	19% (28%*)	13%	15%	25% (30%*)	6%
>1 agonist	9%	5% (10%*)	2%	4%	10% (14%*)	0

* profiles including both aggregation and ATP release

Table IV

Reference ranges (median and 2.5–97.5 percentiles) for aggregation and ATP release in platelet-rich plasma (PRP) and whole blood (WB) with collagen, arachidonic acid (AA), adenosine diphosphate (ADP), epinephrine (EPI), thrombin, thrombin receptor activating peptide (TRAP), and ristocetin in drug-free normal subjects

Agonist	Platelet Aggregation Method				ATP Release	
	BioData PRP n=36	Chrono-Log PRP n=40	Chrono-Log WB n=41	Multiplate WB n=24	PRP n=40	WB n=41
Collagen	80 (73–88)	72 (55–89)	21 (14–33)	74 (25–118)	0.84 (0.49–1.44)	1.32 (0.68–2.12)
AA	82 (59–89)	76 (48–98)	17 (7–31)	80 (27–108)	0.69 (0.2–2.0)	0.98 (0.47–2.0)
ADP	85 (61–92)	62 (48–88)	14 (10–21)	59 (29–96)	0.57 (0–1.22)*	1.05 (0.55–2.13)
EPI	79 (8–90)	63 (10–86)	--	--	0.66 (0–1.28)*	--
Thrombin	--	--	--	--	1.0 (0.6–1.7)	1.47 (0.71–2.16)
TRAP	--	--	--	111 (72–141)	--	--
Ristocetin						
1.5 mg mL^{-1}	85 (69–91)	73 (16–97)	--	--	--	--
1.25 mg mL^{-1}	85 (65–112)	62 (2–93)	--	--	--	--
1.0 mg mL^{-1}	84 (69–93)	7 (0–78)*	--	--	--	--
0.75 mg mL^{-1}	--	--	6 (2–12)	--	--	--
0.3 mg mL^{-1}	--	--	--	170 (62–239)	--	--

*More than 2.5% of results are 0

Table V

Test profiles by method with tests scored as abnormal if outside the reference ranges and profiles scored as abnormal if results with 1 agonist or >1 agonist are abnormal in platelet-rich plasma (PRP) and whole blood (WB).

Method		*Test Profiles*		
		Number	*Abnormal n (%)*	
			1 Agonist	**>1 Agonist**
Aggregation Alone:	Chrono-Log PRP	81	17 (21)	7 (8.6)
	Chrono-Log WB	81	12 (15)	3 (3.7)
	BioData PRP	60	8 (13)	1 (1.7)
	Multiplate WB	49	3 (6.1)	0
ATP Release Alone:	Chrono-Log PRP	81	15 (19)	4 (4.9)
	Chrono-Log WB	81	20 (25)	8 (10.0)
Aggregation and ATP Release:	Chrono-Log PRP	81	23 (28)	8 (9.9)
	Chrono-Log WB	81	24 (30)	11 (13.6)

Table VI

Medians of the coefficients of variation calculated for each individual subject over time by method and agonist in platelet-rich plasma (PRP) and whole blood (WB). AA=arachidonic acid, ADP=adenosine diphosphate, EPI=epinephrine.

Method	Collagen	AA	ADP	EPI	Thrombin
BioData PRP	4.8	5.7	8.9	12.2	--
Chrono-Log PRP	8.6	12.0	12.2	13.8	--
Chrono-Log WB	25.0	26.8	19.0	--	--
Multiplate WB	25.3	14.6	18.0	--	17.7
Chrono-Log PRP REL	21.0	30.0	40.4	32.6	17.2
Chrono-Log WB REL	24.2	22.5	35.1	--	21.4

Table VII

Exposures to flavonoid-rich foods prior to blood draw for specimens with aberrant results and non-aberrant results

Exposures	Non-aberrant Results (94 specimens) n (%)	Aberrant Results (28 specimens) n (%)	Significance
Morning of blood draw only*	9 (9.6)	1 (3.6)	
Evening before blood draw only**	32 (34.0)	9 (32.1)	χ^2=23.50,
Both Morning and Evening	10 (10.6)	14 (50.0)	P<0.0001
None	43 (45.7)	4 (14.3)	
All Exposures within 18 hours	51 (54.3)	24 (85.7)	P=0.0035
Exposures within 6 hours with or without earlier exposure	19 (20.2)	15 (53.6)	P=0.0004

* Within 1–6 hours of blood draw

** Within 12–18 hours of blood draw

Table VIII

Specimens with aberrant results for aggregation and/or ATP release with arachidonic acid (AA), adenosine diphosphate (ADP), collagen, thrombin, and thrombin receptor activating peptide (TRAP) in platelet-rich plasma (PRP) and whole blood (WB)in the Chrono-Log (CL), BioData (BD), and Multiplate (MP) instruments

Subject/Specimen	Agonist and Test System				Flavonoid-rich Food Exposures	
	AA	ADP	Collagen	Thrombin/TRAP	MORNING	EVENING
4/5	CL-PRP, CL-WB	CL-PRP, BD-PRP	CL-WB		none	cola
12/6	CL-PRP		CL-WB		raspberries	red wine
15/3	CL-PRP	BD-PRP, CL-WB			chocolate	tomato, garlic
16/1				CL-WB	orange juice	orange juice
16/6	BD-PRP		BD-PRP		none	none
18/4			BD-PRP		none	chocolate, tea
18/5		CL-PRP			tea	coffee, chocolate
24/3				CL-WB	cola	tomato
25/5		CL-PRP			none	tomato, grape juice
27/5		CL-WB	CL-WB	CL-WB	none	none
27/6		CL-WB			none	beer
29/2	CL-PRP	CL-PRP, CL-WB	CL-PRP	CL-WB	none	chocolate, red wine
33/1	CL-PRP	MP-WB	CL-WB		cranberry juice	tea, cranberry juice
33/2		CL-PRP, MP-WB			cranberry juice	chocolate
33/5	CL-PRP, CL-WB				peanut butter	fish
33/6		BD-PRP			none	none
34/1				MP-WB	orange juice	none
34/6		BD-PRP			none	none
35/1	CL-PRP	CL-PRP			tomato, orange juice	avocado, tomato, orange
40/1			CL-WB	CL-WB	apple	chocolate, fish
40/4		CL-WB	CL-WB		blueberries	tomato, onion, cranberries
48/1	CL-PRP				none	peanut butter
48/4		CL-WB			strawberries	soy, garlic, chocolate
49/2		CL-PRP, BD-PRP			orange juice	tomato

Subject/Specimen	Agonist and Test System				Flavonoid-rich Food Exposures	
	AA	ADP	Collagen	Thrombin/TRAP	MORNING	EVENING
50/2	BD-PRP, CL-WB	CL-PRP	MP-WB		none	chocolate, tomato, cola
50/4	CL-PRP, CL-WB	CL-PRP	MP-WB		none	tomato, tea
53/2		BD-PRP	BD-PRP		none	fish, sake
56/6	CL-PRP				tomato	tomato