Blood samples were collected from the antecubital vein, between 8:00 and 9:00 AM, with subjects in a supine position, after an overnight fast. Blood was anti-coagulated with 3.8% tri-sodium citrate (9:1 v/v) and centrifuged for 10 minutes at 240g, at room temperature to produce platelet rich plasma.

Platelet aggregation was measured in response to four agonists, arachidonic acid, collagen, adenosine diphosphate (ADP) and epinephrine, using a PAP-4 Aggregometer (Bio/Data Corporation). The following concentrations of agonists were used.

1. Arachidonic acid – fixed concentration (1.9mg/ml)
   Data collected;
   • aggregation; yes/no
   • aspirin effect; yes/no

2. Collagen – fixed concentration (5 mg/ml)
   Data collected; lag time until aggregation began

3. ADP and epinephrine – 0.01, 0.03, 0.05, 0.1, 0.5, 1.0, 3.0, 5.0, 10.0, 15.0 mmol/L
   Various concentrations of ADP and epinephrine were used in order to identify the threshold concentration. The threshold was defined as the lowest concentration of the agonist that produced 50% aggregation in four (ADP) or five (epi) minutes
   Data collected;
   • Slope of primary wave of aggregation
   • Maximum aggregation at set time (ADP – four minutes, epinephrine – five minutes)
   • Threshold

September 12, 2008