Rapid increase in fibrinolytic capacity of human blood after consumption of a blend of oral enzymes and anti-inflammatory fruit- and berry-extracts.

Steve G. Carter, Christian Drapeau, and Gitte S. Jensen.

Objective

To present a study design for evaluation of immediate changes in blood clot-lysing capacity after consumption of a blend of anti-inflammatory fruit- and berry-extracts and oral enzymes.

Introduction

Vascular disease is associated with inflammatory conditions, and is a major contributing factor to many health problems, including heart disease, stroke, and diabetic complications.

Part of a healthy vascular system includes a delicately balanced dynamic equilibrium between blood coagulation and fibrinolysis. A loss of equilibrium may result in atherosclerosis and thrombus formation, and natural strategies for improving a poorly reactive fibrinolytic system is of considerable interest in preventive health.

Certain nutritional and nutraceutical interventions have been shown to support improvement of peripheral vascular function. Such interventions include a fibrinolytic enzyme from the fermented soy cheese 'Natto', called Nattokinase [1, 2]. This enzyme reduces red blood cell aggregation and whole blood viscosity in vitro [3]. Oral consumption of Nattokinase has been shown to result in increased plasma fibrinolytic activity [4], and to provide a thrombolytic effect on chemically induced thrombosis in rats [5], and suppressed intimal thickening after vascular injury [6, 7]. Also, consumption of a few doses of Nattokinase prior to long-haul flights (7-8 hours) was shown to prevent venous thrombosis in humans, and resulted in a statistically significant reduction of edema [8].

Furthermore, antioxidant supplementation has been shown to indirectly favor vascular improvement due to a reduction of inflammatory conditions.

The removal of obstructions in the microvasculature can happen by at least 3 different, simultaneous mechanisms:

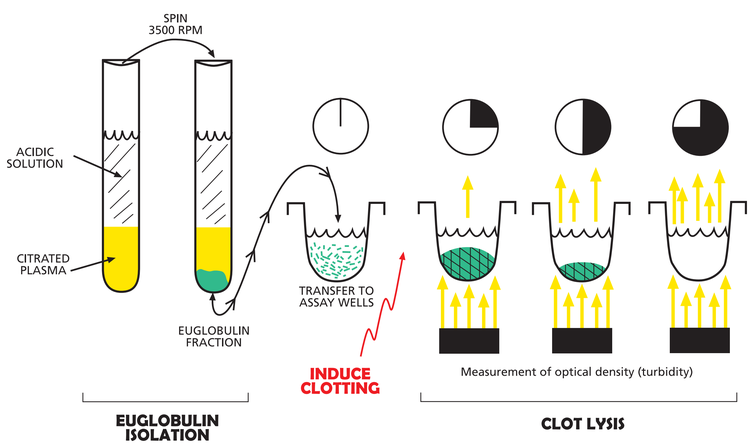
- Direct absorption of fibrinolytic enzymes from the diet;
- Production of innate fibrinolytic enzymes;
- Reduction of inflammation.

The goal of this study was to document whether consumption of the product StemFlo™ (SF) would lead to measurable improvements in blood fibrinolytic capacity. The dose was 1.575 grams, and was equal to 3 capsules.

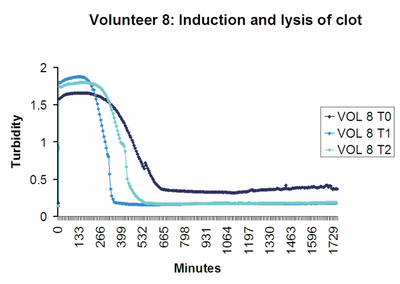
Study design

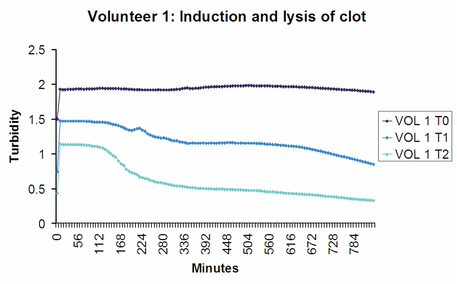
The study was performed on 12 healthy volunteers. Initially upon arrival the volunteer was asked to fill out a daily questionnaire pertaining to recent illness, meals, medications, sleep, and stress level, to help judge circumstances that would interfere with our measurements. Once the questionnaire was completed, blood pressure and heart rate was taken prior to the first blood draw. After a baseline blood draw, the consumable was given to the volunteer. Blood draws were taken at 1 and 2 hours after consumption. Blood pressure and heart rate was measured again immediately before the last blood draw. Citrated plasma was aliquoted and stored at -80°C for subsequent fibrinolytic testing.

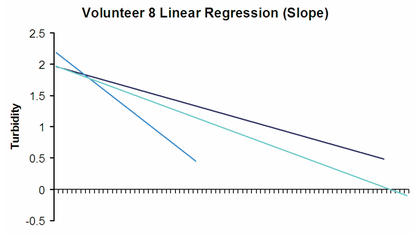
The classical euglobulin lysis method has been used since the 1950s for measuring overall blood clotting and clot lysing capacity, and remains one of the most widely used methods for studying clotting pathologies [9]. A modified euglobulin lysis method, described by Smith et al. (2004) [10], was used. In brief, 0.35 mL of citrated plasma was added to 6.3 mL 1% acetic acid. This was placed on ice for 10 minutes. The resulting precipitate was pelleted by centrifugation at 3500 rpm for 5 minutes at 25°C. The supernatant was discarded, and all remaining liquid on the inside of the vial carefully removed using a cotton tip. The precipitate was resuspended in 0.35mL NaCl (154mM)/NaB_3+ (2.6mM) solution. This was incubated for 3 minutes in a 37°C water bath. For each sample, duplicate aliquots of 0.14mL were pipetted into a pre-warmed 96-well U-bottom plate. The formation of a clot was induced by the addition of 0.14mL $CaCl_2$. The plate was placed in a pre-warmed 37°C microplate reader, where the turbidity caused by the formed clot was read at 405nm. The autolysis of the clot by plasma enzymes was followed by measuring the turbidity over the next 10-30 hours, with readings every 4-7 minutes.

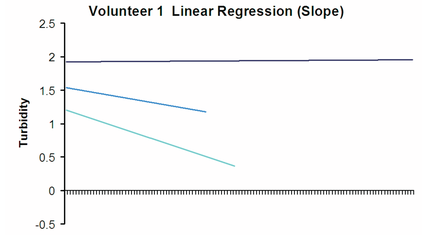


Results









Combined analysis

When all 4 parameters were analyzed together, to assess the overall improvement in fibrinolytic capacity, we found that even though 8 of the 12 study participants had a normal fibrinolytic capacity before consumption, the fibrinolytic capacity improved upon consumption. Data from Volunteer 8 is shown as an example, where already at 1 hour after consumption an improvement in clot lysing time, as well as a more complete lysis, was observed.

Among the 4 study participants that did not have a normal fibrinolytic capacity before consumption, they all showed some improvement within 1-2 hours after consumption. In the graph above is shown an example of a human subject, Volunteer 1, who had almost no fibrinolytic capacity at baseline (dark blue line), but who rapidly improved the fibrinolytic capacity to levels comparable to normal levels within 1 hour after consumption of a single dose of SF.

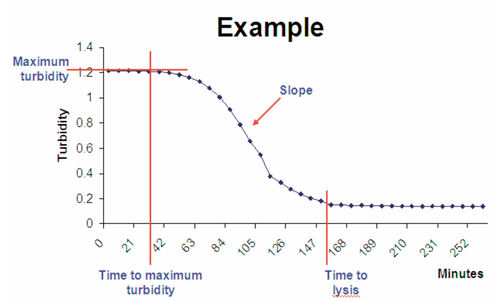
Table 1. Areas of improvement for each study volunteer.

Volunteer #	01	02	03	04	05	06	07	08	09	10	11	12
Reduced Maximum Turbidity	X		X	X		X			X		X	X
Reduced Time to Max	X	X	X			X	X	X	X		X	X
Increased Slope	X	X	X	X	X	X	X	X	X	X	X	X
Reduced Lysis Time			X	X	X	X		X	X	X		X

Data analysis:

We examined four different parameters:

- Maximum turbidity;
- Time to maximum turbidity (TM);
- Slope during optimal clot lysis;
- Lysis Time, i.e. the time to reach a plateau with no further clot lysis.



- **Maximum Turbidity:** Maximum turbidity is a measure of clot size when the clot reaches its most dense state, causing the highest absorbance reading. If SF results in an improved fibrinolytic capacity of the blood, then the Maximum Turbidity readings at T1 and T2 may be reduced compared to baseline (T0). Eight of the 12 volunteers showed a mild reduction in clot size after consuming SF. Of these, seven showed improved fibrinolytic capacity already at T1. Volunteers 2, 5, 7, and 8 did not show a reduced Maximum Turbidity after consumption of SF.

- **Time to Max:** Time to Max is the amount of time it takes to reach maximum turbidity. If SF consumption increases the fibrinolytic capacity, post-consumption blood samples should show a reduced time to max. Nine of the 12 volunteers showed an improved fibrinolytic capacity, as seen by a reduced time to max. Only three had an increase in the TM (Volunteers 4, 5, 10).

  In this assay the clot forms in the presence of clot lysing enzymes. An increased clot lysing capacity of a blood sample can result in either a lower maximum turbidity, a shorter time to max, or both. Therefore, even though we here present the different sets of data separately, an overall analysis must take both parameters into account.

- **Slope:** Slope was defined by performing a linear regression analysis on the area of the curve defined by the estimated Time to Max data point and the estimated Lysis Time point. The linear regression analysis was performed on both duplicate wells, and the results were averaged. A steeper downwards slope of the curve would reflect an increased fibrinolytic capacity. Among the 12 volunteers, 9 showed an improved fibrinolytic activity as measured by increased slope of the curve during the time of clot lysis.

  If a volunteer did not have a clearly identifiable Lysis Time, the slope could not be calculated by the method of linear regression of the data points between Time to Max and Lysis Time. Three volunteers did not have a Lysis Time (Volunteers 2, 7, and 11). However, even though complete clot lysis was not achieved in samples from these 3 volunteers, all three data sets had well-defined areas of the curve to the right of Time to Max, based on which linear regression analysis was performed.

  Based on the slope analysis, 100 % of the study population improved fibrinolytic capacity upon SF consumption. Volunteers 3, 6, 7, 8, and 10 showed the most improvement at T1, and volunteers 1, 2, 4, 5, 9, 11, and 12 showed the most improvement at T2.

- **Lysis Time:** The lysis time is the amount of time it takes to dissolve an artificially made clot over time. Lysis time can be represented as the start of a plateau phase after a significant drop from the maximum turbidity. Eight of the twelve Patients showed improved (i.e. shorter) Lysis Times after consumption of SF.

Conclusion

The study showed that the method, previously used primarily for pathological conditions, and secondarily for population studies including age, gender, and pregnancy, can be applied to fine changes in healthy individuals after consumption of a nutritional product.

Furthermore, the study showed that consumption of the test product SF resulted in improvement in the fibrinolytic capacity in the blood in 100% of the study population.

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