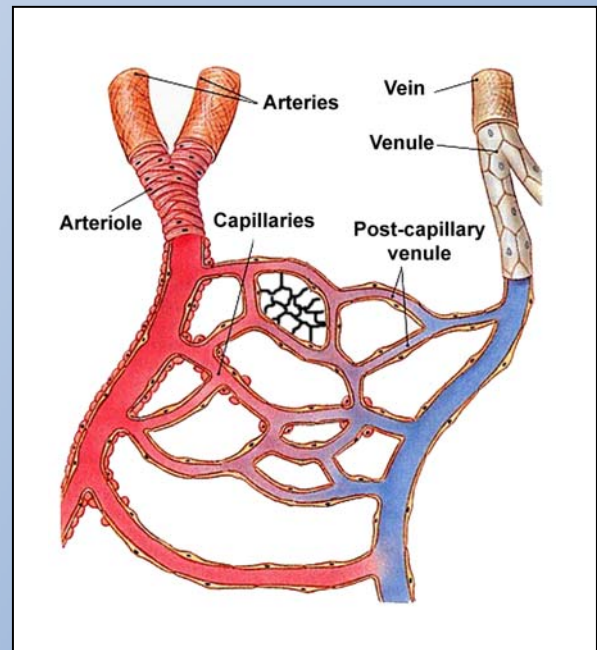


StemFLO™ improves vascular functions and hemodynamics

Introduction

The microvasculature (microscopic blood vessels) consists of small arterioles carrying the blood from away the heart to the tissues and venules carrying the blood from away the tissues back to the heart. Between arterioles and venules are the capillaries where O₂ and CO₂ exchange take place, as well as nutrient delivery.

A portion of the capillary referred to as the post-capillary venule is a highly significant site for cellular migration from blood into tissue. It is at the level of the post-capillary venule that stem cells can migrate out of the blood into tissues. Therefore, it has been suggested that blocking of capillaries, which hinders blood flow to the post-capillary venules, may reduce migration of stem cells into tissue. If this hypothesis is correct, then it follows that the removal of obstructions in the microvascular system, particularly the capillaries, may facilitate stem cell migration into tissues.



Certain nutritional and nutraceutical interventions were shown to support improvement of peripheral vascular function. For example, a fibrinolytic enzyme extracted from the fermented soy cheese 'Natto', called Nattokinase, was shown to increase plasma fibrinolytic activity of the blood and to promote lysis of blood clot after induced

thrombosis. Consumption of Nattokinase prior to long-haul flights (7-8 hours) was shown to prevent venous thrombosis in humans, as well as edema. Similar effects have been reported with other fibrinolytic enzymes such as Serrapeptase extracted from the enterobacteria *Serratia E15*.

Antioxidant nutrition can also improve micro-circulation. Transformation of fibrinogen into fibrin mesh can be triggered through the so-called Bradford-Allen Coagulation Pathway. This pathway involves activation of sialidase enzyme by reactive oxygen species. Once activated, sialidase would cleave sialic caps on fibrinogen disulfide bridges, triggering the agglomeration of fibrin monomers into fibrin mesh. Antioxidant supplementation has also been shown to indirectly favor vascular improvement due to a reduction of inflammatory conditions.

Therefore optimal blood circulation in the microvasculature, which is essential for optimal migration of stem cells into tissues, can be achieved by:

- Digesting fibrin mesh in the blood by consumption of fibrinolytic enzymes such as Nattokinase and Serrapeptase, and
- Reducing oxidative stress and inflammation by antioxidant nutrition, which prevents the formation of fibrin mesh.

StemFLO™, a blend of Nattokinase, Serrapeptase™, and antioxidant nutrition including berry extracts, mangosteen, curcumin, and cat's claw was investigated to see if consumption of this product could lead to any measurable improvements in vascular function and hemodynamics.

Methods & Results

In brief, the study was comprised of two independent phases using a total of 19 volunteers. The first phase was a short-term study that investigated the fibrinolytic capacity of blood samples obtained immediately before and within 2 hours after consumption of StemFLO. The second phase was a long-term investigation (4 weeks) of

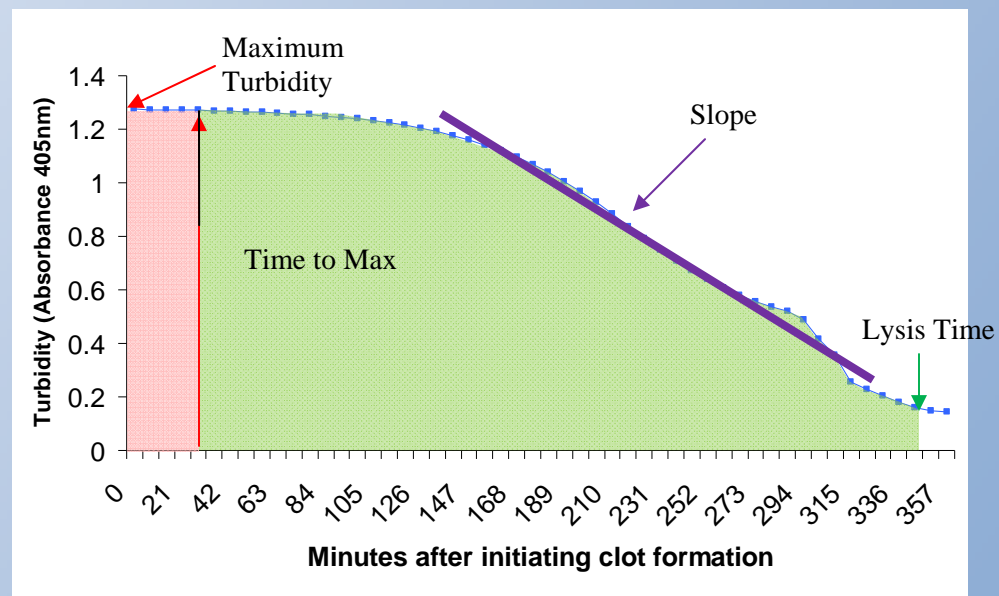
the effect of StemFLO on microvascular functions.

PHASE 1

The short-term phase was carried using 12 volunteers. Blood samples taken before (T0) as well as 1 hour (T1) and 2 hours (T2) after consumption of StemFLO. Blood samples were prepared and then pipetted into a pre-warmed 96-well U-bottom plate where blood clots were induced by the addition of CaCl_2 . The plate was placed in a pre-warmed 37°C microplate optical reader, where the turbidity caused by the formed clot was read at 405nm. Lysis of the clot by fibrinolytic enzymes present in the plasma was followed by measuring the turbidity of the sample over the next 29 hours, with readings every 7 minutes.

We examined four different parameters, as described in the below:

- 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 the time point at which the clot reaches its most dense state, causing the highest turbidity and absorbance reading. If StemFLO results in an improved fibrinolytic capacity of the blood, then the Maximum Turbidity readings at T1 and T2 would be reduced compared to baseline (T0). This being said, it is important to mention that in cases of very low baseline fibrinolytic activity, the formation of larger clots may lead to reduced turbidity, as smaller clots agglomerate into larger clots. Eight of the 12 volunteers showed a reduction in clot formation after consuming StemFLO. Of these, seven showed improved fibrinolytic capacity already after one hour.

Time to Max: Time to Max is the amount of time it takes to reach maximum turbidity, and consequently the time it takes before initiation of fibrinolysis. If StemFLO consumption increases the fibrinolytic capacity, blood samples taken after consumption should show a reduced TM. Nine of the 12 volunteers showed an improved fibrinolytic capacity, as seen by a reduced Time to Max.

Slope: Slope was determined by linear regression analysis on the area of the curve defined by the estimated Time to Max data point and the estimated Lysis Time point. If a volunteer did not have a clearly identifiable Lysis Time, the slope was estimated on the curve to the right of Time to Max, based upon which linear regression analysis was performed. Based on the slope analysis, 100 % of the volunteers showed improved fibrinolytic capacity after StemFLO consumption. Five volunteers showed the most improvement at T1, while seven volunteers showed the most improvement at T2.

Lysis Time: The Lysis Time is the amount of time it takes to dissolve an artificially made clot. Lysis Time can be determined as the start of a plateau phase after a significant drop from the Maximum Turbidity. Nine of the 12 volunteers showed improved (i.e. shorter) Lysis Times after consumption of StemFLO. While six volunteers showed maximum improvement at 1 hour after consumption, 3 volunteers showed greatest improvement at 2 hours after consumption.

The blood pressure and heart rate of all volunteers were taken before and after the study. These readings were taken prior to the first blood draw and prior to the last blood draw, to minimize the effect of stress from a blood draw on the readings. Eight of the 12 volunteers showed lower systolic blood pressure 2 hours after StemFLO consumption. Overall average change in systolic blood pressure in all volunteers was -4%.

PHASE 2

Seven volunteers with some minor vascular problems (varicose veins, circulatory complaints) were recruited. Vascular assessment was performed at study start, and after 2 and 4 weeks of StemFLO consumption, and involved measurement of blood pressure, ankle-brachial index (bilateral), and assessment of microvascular function. Ankle-brachial index was determined using Laser Doppler and was used to quantify Post Occlusive Reactive Hyperemia (PORH), while assessment of microvascular function was made through the measurement of transcutaneous oxygen pressure (tcpO₂).

Blood pressure

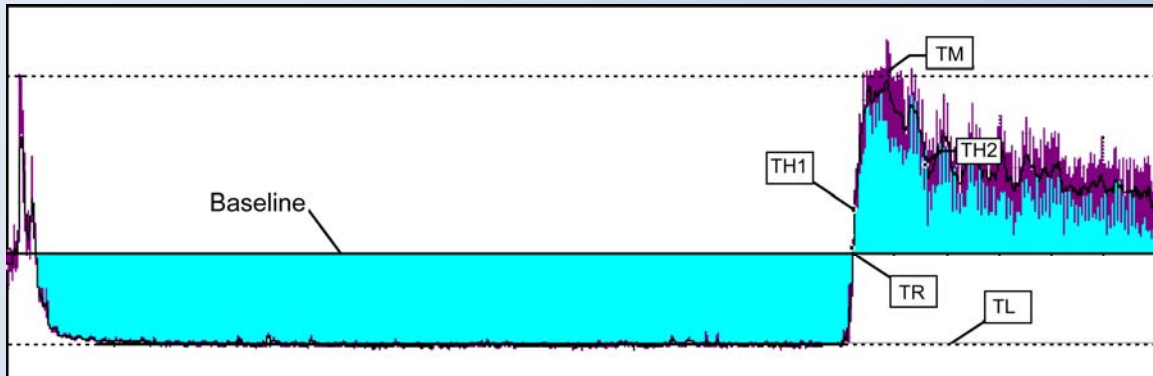
Consumption of StemFLO over a period of 4 weeks led to healthier blood pressure in all volunteers, as shown by a reduced systolic pressure. Overall, average change in systolic blood pressure after 4 weeks was -7%.

Post Occlusive Reactive Hyperemia (PORH)

PORH is a test whereby a limb, arm or leg, is deprived of oxygen for four minutes using a pressure cuff, and then fluctuations in blood flow are quantified immediately after releasing the pressure cuff. Laser Doppler data is analyzed using a PORH analysis software (PeriFlux 5000, PeriMed, Stockholm, Sweden).

The vascular system is subject to many types of immediate physiological regulation to maintain relatively constant blood pressure, temperature, oxygen supply, and blood perfusion. The health of the microvasculature can be assessed by looking at a number of parameters. **Time to recovery (TR)** is the time it takes for blood flow to reach baseline

level after the occlusion is released. **Time to half before hyperemia (TH1)** is the time it takes after the release of the occlusion for perfusion to reach the midpoint between no-flow and peak flow. **Time to Max (TM)** is the time it takes after the release of the occlusion for perfusion to reach peak flow. **Time to half after hyperemia (TH2)** is the time it takes after the release of the occlusion, post-hyperemia, for perfusion to reach the midpoint between peak flow and baseline.



Consumption of StemFLO over a period of 4 weeks led to improvements in microvascular functions in all volunteers. Improvements in TM and TH2 were seen in all volunteers with decrease of 31% and 40%, respectively. Improvements in TR and TH1 were seen in some of the volunteers.

Discussion

StemFLO is a combination of fibrinolytic enzymes and antioxidant nutrition aimed at improving blood circulation in small capillaries. Improvement in fibrinolytic activity of the plasma was shown to be significant in all participants when measured 1 and 2 hours after consumption. Reducing the presence of fibrin in the blood, which increases the volume of the overall blood vasculature by providing greater access to capillaries, would be expected to reduce overall blood pressure. As expected, blood pressure was reduced (-

4%) in half of the participants 2 hours after consumption and in all participants (-7%) after 4 weeks of daily consumption.

In this study, actual circulation in small capillaries was assessed using post Occlusion Reactive Hyperemia (PORH). Using this method, two of the four parameters measured (TM and TH2) were improved in all participants whereas the other two parameters were improved in only half the participants.

Overall, consumption of StemFLO led to improvements of at least one parameter in every participant and of most parameters in nearly half of the participants. Therefore, we can conclude that consumption of StemFLO is likely to improve capillary circulation in most individuals, supporting the migration of stem cells into tissues, as well as the delivery of nutrients and oxygen throughout the body.