Study of Blood Viscosity with Added Sodium Nitrate and Temperature Variance: A Potential Therapy to Regulate Blood Flow After Induced Hypothermia

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Abstract

The human body has natural systems for vasodilation which are fueled by nitric oxide production, but in cases of cardiac disfunction and stress nitric oxide can be inhibited. In this study, nitric oxide was studied as a mediator for the blood rush experienced in the warming process of induced hypothermia after a major cardiac event. Nitric oxide (NO) was introduced through sodium nitrate which was aimed to reduce the speed and turbulence of blood flow. A viscometer was used to examine the rate of blood flow, while the temperature was varied to simulate the conditions of induced hypothermia. Two temperatures were tested to simulate the warming process of induced hypothermia, and three concentrations of sodium nitrate were added to the samples. A control was used for comparison and for calculations. The results indicated that the 0.11 M $NaNO_3$ expressed constant blood viscosity for both temperatures, the 0.09 M $NaNO_3$ exhibited some mediation and the 0.10 M showed the least mediation.

Therapeutic Hypothermia

Therapeutic cooling has been employed in medicine for a very long time, but with varied results. In recent years, several studies have examined induced hypothermia as a treatment after cardiac arrest. These studies have concluded that cooling patients slows tissue damage caused from cardiac trauma [1,2]. Therapeutic hypothermia, however, contributes to a high mortality rate, and many patients who do survive the procedure are left with neurological deficits. A study conducted at the Central Lisbon Hospital Center, in Portugal, used induced hypothermia on ICU patients and yielded a 61% mortality rate [3]. Hemorrhage and increased blood loss were hypothesized as possible causes for this percentage [3,4]. Another study specifically examined blood effects from induced hypothermia in perioperative patients and indicated that blood loss

was increased by 16% [5]. The increased blood loss was likely the result of the blood rush during the hypothermia reversal process. In several cases, it was observed that as the blood was warmed, it reacts drastically due to blood shear thinning properties, leading to a blood rush [1,5]. This is a biological representation of the inverse relationship between viscosity and temperature, which is foreign to the human system. The blood rush can yield brain damage and added stress to an already pressured cardiac system. This blood rush has been noted to occur even when the warming and cooling processes are done at a very slow rate. In each of the studies referenced, the heating and cooling process took approximately 24 hours; however, the effects of the blood rush were still present [3,4]. If the rush could be controlled, it might make induced hypothermia a much more successful procedure.

Nitric Oxide

Nitric oxide plays a key physiological role in the cardiovascular, respiratory, digestive, immune, and nervous systems [6]. NO acts as a cardiovascular stimulant which relaxes the vessels and fuels the pacemaker [7,8, 9]. The NO molecule is produced in the endothelium of the blood vessels from the conversion of arginine to citrulline when the cardiac system is healthy [6]. Plaque buildup in the vessels can severely impair the ability of nitric oxide to be released [7, 10]. Since plaque is a major cause of cardiac disfunction, it is likely that many patients treated with induced hypothermia for cardiac arrest would have plaque hindering their ability to produce NO [7]. For this reason, an introduction of nitric oxide into the circulatory system during the warming process of induced hypothermia may work to normalize the blood flow and contribute to restoring the body’s natural homeostasis. As the core temperature is raised, vasodilation and sweating are required for heat to dissipate and the vasodilator responsible for this is NO. A study examining the blood flow to the skin noted that nitric oxide contributes 30% of the vasodilation reflex experienced in the body when heat is dissipated during core cooling [11]. In another study, scientists evaluated the amount of NO required by the body to achieve normal blood flow and discovered that patients with worse health or heart strain required more NO to propel normal circulation [7]. Nitric oxide also has been shown to work in the prevention of plaque in the circulatory system, which could aid in the long-term rehabilitation of cardiac arrest patients [7].

Pharmaceutical Use of Nitric Oxide

Sodium nitrate is an organic salt that is utilized in several medications to introduce nitric oxide into the circulatory system. Nitrodilators, which contain sodium nitrate, are currently used to relax the blood vessels of hypertensive patients [12]. Sodium nitrate itself has been used in patients to prevent cardiac arrest [13]. Sodium nitrate dissociates *in vivo* to release nitric oxide, sodium ions, and diatomic oxygen. Nitric oxide and sodium are diffused out of the bloodstream in milliseconds; the oxygen, however, is not [14]. Oxygen remains in the blood much longer than the nitric oxide because the NO molecule binds to the allosteric site of the heme group in hemoglobin reducing its ability to bind oxygen [15]. Saturating the blood with oxygen over a long period of time can be dangerous; therefore, a sodium nitrate treatment would only need to be only used in targeted cases for short durations [15,16]. Also, in many cases, patients treated with sodium nitrate quickly became accustomed to the treatment, and it no longer yielded an effect [12]. Therefore, employing sodium nitrate as a long-term medication would likely be fruitless. Studies have not yet explored the benefits of one-time intravenous treatments with sodium nitrate in therapeutic hypothermia. The controlled environment required for induced hypothermia would aid in controlling the oxygen levels of the patients. This could prevent saturation making the introduction of sodium nitrate safer.

Experimental Development

The current uses of sodium nitrate in medicine point towards a potential application in the blood warming process of induced hypothermia. While there are some negative associations connecting high levels of nitrates to cancer, the NO molecule only exists in the blood for milliseconds, therefore, the use of sodium nitrate is feasible in specialized procedures [14, 17]. Also, the controlled environment required for induced hypothermia would aid in controlling the oxygen levels of the patients in order to prevent oxygen saturation. This study hypothesized that sodium nitrate would regulate human blood flow when the blood was warmed to mitigate the blood rush and bring the blood flow back to homeostasis. The experiment was done to determine the interaction between the RBC and nitric oxide and its effect on blood flow as measured by viscosity. Blood flow was analyzed in this study using a Zahn cup viscometer at targeted temperatures to simulate induced hypothermia. Defibrinated sheep blood was used for the

experimentation and samples were tested with no added sodium nitrate as well as three concentrations of sodium nitrate.

Methodology

Sheep blood was obtained for testing and stored at approximately 1°C with a shelf life of 28 days. The blood was defibrinated mechanically by the manufacturer, Quad Five, and was verified for purity by the USDA [18]. Defibrinated blood is free of anticoagulants which could interfere with observation of the experimental induced effects on the blood's viscosity. Defibrinated sheep blood also has been certified to be a viable substitute for human blood in diagnostic testing and is used by many companies for experimentation [19].

The Zahn Cup size two was used to measure viscosity. The size two Zahn cup had an orifice size of 0.108 inches and a capacity of 44 milliliters which was used in the calculation of shear rate [20]. Zahn Cup viscometers measure the viscosity of a fluid using the time of efflux through the orifice. This brand of viscometer is used to measure the viscosity of paint. Paint is a non-Newtonian, shear thinning, fluid just like blood so the Zahn Cup viscometer should give applicable results for blood's viscosity. The design of the Zahn Cup employs gravity to determine viscosity which was ideal in reducing added turbulence in the samples [21].

The recommended temperature for use with Zahn cup viscometers is 25°C. However, a calibration curve can also be made with a control liquid and careful monitoring of the temperature as per the instructions of the manufacturer [20]. A calibration curve was constructed for this experiment using defibrinated sheep blood with no salt added. Three trials were taken for each degree tested with the average being used to form the curve. The range of temperature was set from 25°C to 37°C with trials beginning at the highest temperature. 37°C was set as the upper bound of temperature to account for the warming to normal human body temperature. 25°C was tested as the cooling temperature and was chosen from the recommendation of use for the Zahn Cup viscometer [20]. 25°C is approximately 5 degrees below the average temperature cooled to in induced hypothermia, but it was used to provide more accurate readings with the Zahn cup viscometer from the manufacturer's recommendations.

To begin, blood samples were raised from storage temperature to room temperature, approximately 22°C. The temperature was then raised slowly to 37°C using a warm water bath with temperature control. The warm water bath was raised in increments of 5°C allowing the blood to acclimate to the temperature slowly. This prevented the blood from denaturing. When the blood reached 37°C the first reading was taken. Readings were taken at each degree increment as the blood was cooled to 24°C using an ice bath for cooling.

Readings were taken by first submerging the Zahn Cup in the blood and allowing it to warm. Once the viscometer had warmed to temperature, the cup was raised to just under flush with the top of the blood. The cup was then raised vertically six inches and placed on a hook set over the center of the collection beaker. A stopwatch was started as the cup broke the surface of the blood and was stopped when the first break in the flow of the blood was observed [20]. Between each reading the Zahn cup was rinsed to prevent cohesion which could alter the flow time. The Zahn cup was cleaned completely in between each concentration to avoid contamination.

Sodium nitrate solutions were then made by diluting the salt in water. The amount of sodium nitrate to dilute was calculated using the concentration of salt administered in a typical IV bag of sodium chloride given for dehydration [22]. The calculated concentration was 0.10 M $NaNO_3$. 0.09 M and 0.11 M $NaNO_3$ were also tested to account for the difference in molecular weight of sodium nitrate and sodium chloride as well as to account for different rates of dissociation for each salt in the blood. Ten trials were run at each sodium nitrate concentration for 25°C and 37°C. The average of these ten trials, excluding major outliers, were considered in the final analysis of viscosity. Outliers were categorized as any values which were $\pm$ 0.20 away from the closest flow time.

Blood samples were also examined using the highest objective on a compound microscope to determine if the sodium nitrate solutions were causing any abnormalities in the structure of the blood. Samples of the defibrinated sheep blood as well as human blood samples were examined to determine the safety of the sodium nitrate. The human blood samples were drawn by a certified phlebotomist, Jennifer Love, from the experimenter, Brianna Munnich, with consent. The purpose of these qualitative observations was both to make sure that the

defibrinated sheep blood had not denatured and to determine if the sodium nitrate would affect the structure of the blood.

All blood and instruments which met blood were handled using medical grade gloves and goggles. These safety procedures also ensured that the blood was not contaminated during testing. Blood was disposed of properly using biohazard procedures. All beakers and utensils which touched the blood were also washed using biohazard procedures during each change of concentration and temperature in the experiment.

Results

TABLE 1. CONTROL TEMPERATURE CURVE DATA FOR BLOOD WITH NO SODIUM NITRATE

TEMP. (°C)	Flow Time (**sec**)	DV (PA·s)	Shear Rate (1/s)
25	15.18	4.4×10^{-3}	1.22
26	15.10	4.1×10^{-3}	1.23
27	14.96	3.6×10^{-3}	1.24
28	14.93	3.5×10^{-3}	1.24
29	14.94	3.5×10^{-3}	1.24
30	14.89	3.3×10^{-3}	1.26
31	14.80	3.0×10^{-3}	1.26
32	14.58	2.1×10^{-3}	1.27
33	14.64	2.4×10^{-3}	1.27
34	14.64	2.4×10^{-3}	1.27
35	14.80	2.9×10^{-3}	1.25
36	14.77	2.9×10^{-3}	1.26
37	14.60	2.2×10^{-3}	1.27

The results in Table 1 show the gathered data for the control curve. From 25°C to 32°C a decreased dynamic viscosity occurs as the temperature increases. From 32°C to 37°C a blood rush appears with an increase in dynamic viscosity followed by a quick reduction to the minimum dynamic viscosity.

Apparent Viscosity

The apparent viscosity is a comparison of the dynamic viscosity to the rate at which the fluid shears. Apparent viscosity is used to model non-Newtonian shear rate. Blood is a shear-thinning fluid so it should be modeled with apparent viscosity [21]. This is because blood aggregates due to its colloidal structure. For each concentration and temperature tested the apparent viscosity was tabulated and evaluated in Pascal seconds. The

ranges seen in the apparent viscosity across the trials indicate the influence of temperature on the shear stress causing turbulence in the sample.

Calculation of Dynamic Viscosity

Kinematic viscosity was calculated using the formula provided with the Zahn cup viscometer: KV=3.5 (t-14) [20]. To convert from kinematic viscosity to dynamic viscosity, the kinematic viscosity was multiplied by the density of blood, 1.06 [23]. Lastly, the dynamic viscosity was converted to Pa·s.

Sample Calculation of Dynamic Viscosity

Calculation was done using the flow time of 15.18 seconds from the 25°C control trial.

$KV = 3.5\ (t - 14)$

$KV = 3.5\ (15.18 - 14)$ $\quad KV = 4.13\ m\text{Pa} \cdot \text{s}$

$DV = \rho \times KV$ $\quad DV = 1.06(4.13)$ $\quad DV = 4.3778\ mPa \cdot s$

1 Pa · s = 1,000 m Pa · s $\quad DV = \frac{4.3778}{1{,}000}$ $\quad DV = 4.4\ x\ 10^{-3}\ Pa \cdot s$

Calculation of Control Shear Rate for Expressing Apparent Viscosity

The shear rate was calculated by dividing the velocity in m/s by the height the blood fell from the top of the Zahn cup to the blood's exit at the orifice. These measurements were obtained from the instructions provided with the Zahn cup size two viscometer [20].

Sample Calculation of Shear Rate

Calculation was done using the flow time of 15.18 seconds from the 25°C control trial.

$Velocity\ of\ Blood\ Flow = \frac{height\ of\ blood\ fall}{flow\ time}$ $\quad Velocity = \frac{0.0508}{15.18} = 3.3 \text{ x } 10^{-3}$

$Shear\ rate = \frac{velocity}{height\ of\ the\ zahn\ cup}$ $\quad Shear\ rate = \frac{3.3\ x\ 10^{-3}}{0.00274}$ $\quad Shear\ rate = 1.22\ 1/s$

Considerations for Result Interpretation

The data taken for the 0.09 M $NaNO_3$, 0.10 M $NaNO_3$, and 0.11 M $NaNO_3$ are

represented as averages taken from the ten trials taken at 25°C and 37°C. The averages were calculated while excluding major outliers. Outliers in this experiment are designated as any values which were within a ± 0.20 separation of flow time data. Outliers were removed to account for the human error caused from lifting the Zahn cup improperly or timing errors. Human error was also minimized by repeating the runs ten times until values were repeated or had very little deviation. Frequently values will be compared to normal human blood viscosity which is between 3.0 x 10^{-3} and 4.0 x 10^{-3} Pascals per second [24].

In Figure 1, the results of the control data by degree are plotted versus the calculated dynamic viscosity. The trendline obtained from the control curve was used to determine the deviation of the sodium nitrate trials. This plot was also constructed in order to determine if the experimental model exhibited the expected trend without a changed variable.

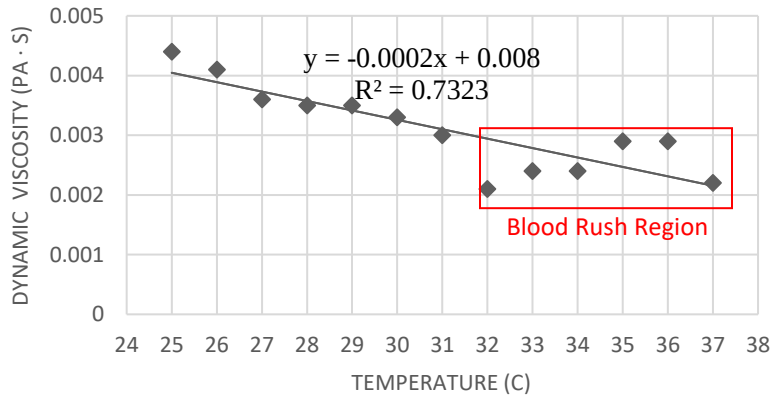

FIGURE 1. COMPARISON OF VISCOSITY AND TEMPERATURE FOR CONTROL

The data in Figure 1 exhibited an inverse relationship between the flow rate of the blood. A blood rush was also observed in the control trials from 32°C to 37°C as described in the literature. Between 31°C and 32 °C a dramatic decrease is observed in the dynamic viscosity which brings the dynamic viscosity to nearly 0.001 below the range of normal human blood viscosity. From 32 °C to 36°C an increase in dynamic viscosity is observed, but it does not return to the normal range of human blood

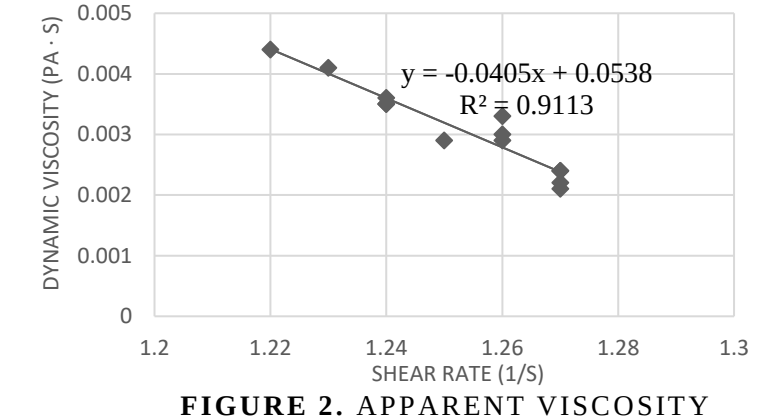

FIGURE 2. APPARENT VISCOSITY COMPARISON FOR CONTROL

viscosity. Another, dip is seen from 36°C to 37°C. The dynamic viscosity at 37°C lies below the range for normal human blood viscosity and is likely a result of two factors. These are the lack of vessel induced friction in the experimental model as well as the blood rush phenomenon increasing shear stress and eddies in the blood samples. Figure 2 expresses the apparent viscosity through the comparison of the dynamic viscosity and the shear rate of the blood flow for the control temperatures. The trendline on this plot was used in further comparisons of the trials employing sodium nitrate to evaluate deviations.

In Figure 3, each concentration of sodium nitrate is compared by apparent viscosity for the 25°C trials. The minima, maxima, and averages for each concentration are depicted along with a trendline to express the turbulence of the blood sample. The 0.09 M NaNO3 had the highest viscosity range due to a larger maximum, minimum, and average value. The trendline does not include the average marker indicating that this concentration of sodium nitrate experienced turbulence. The data points for the 0.09 M concentration do not lie within the normal range for human blood viscosity. The trials employing 0.10 M NaNO3 and 0.11 M NaNO3 had similar ranges and averages with little deviation and each trendline contains all three data points. The 0.10 M slope is steeper than the 0.11 M NaNO3 with a more consistent slope to the 0.09 M concentration. The averages for the 0.10 M and 0.11 M concentrations fell within the range for normal human blood viscosity. The ranges for the 0.10 M and 0.11 M trials were much narrower than the 0.09 M trial with the 0.11 M being the narrowest of the concentrations of sodium nitrate. The ranges for each of the concentrations exceed the limits for normal human blood viscosity on the upper and lower bound indicating that turbulence is still experienced in each sample.

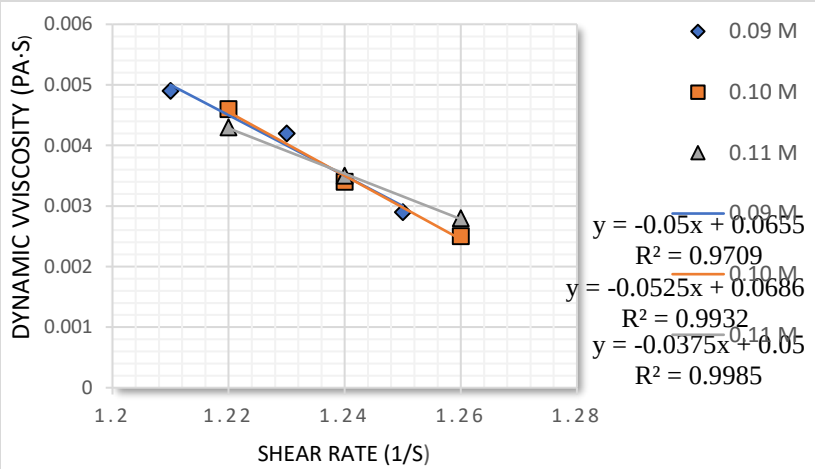

FIGURE 3. APPARENT VISCOSITY FOR VARYING CONCENTRATIONS OF $NANO_3$ AT 25°C

Table 2. Apparent Viscosity of Various Concentrations of $NaNO_3$ at 25°C

	Avg. Flow Time (s)	Avg. DV (Pa·s)	Shear rate (1/s)
0.09 M $NaNO_3$	15.12	4.2×10^{-3}	1.22
0.10 M $NaNO_3$	14.87	3.3×10^{-3}	1.25
0.11 M $NaNO_3$	14.93	3.5×10^{-3}	1.24

In Table 2, the trends from Figure 3 are clearly defined. It is clear from the Figure 3 as well as Table 2 that the 0.09 M concentration of sodium nitrate was the only concentration to lie outside of the normal range for human blood viscosity at 25°C at both temperatures as well as the average. Table 2 illustrates the inverse relationship between the shear rate and the dynamic viscosity as well as clarifies the averages seen in the 0.10 M and 0.11 M concentration which are tightly packed in Figure 3. The average dynamic viscosity for the 0.10 M concentration of sodium nitrate is the lowest of the three trials at 25°C, but it still very close to the value calculated for the 0.11 M concentration. The 0.10 M and 0.11 M concentrations are very close in values while the 0.09 M concentration is separated from the rest of the data.

In Figure 4, the apparent viscosity is compared for each concentration of sodium nitrate at 37°C representing the warming phase of induced hypothermia. The minima, maxima, and averages for each concentration are depicted along with a trendline to express the flow of the blood sample. The 0.09 M concentration had a very narrow range with all but the maximum value within the range for normal human blood viscosity. The 0.10 M concentration had more deviation between its maximum and minimum than the 0.09 M concentration and only its maximum was within the

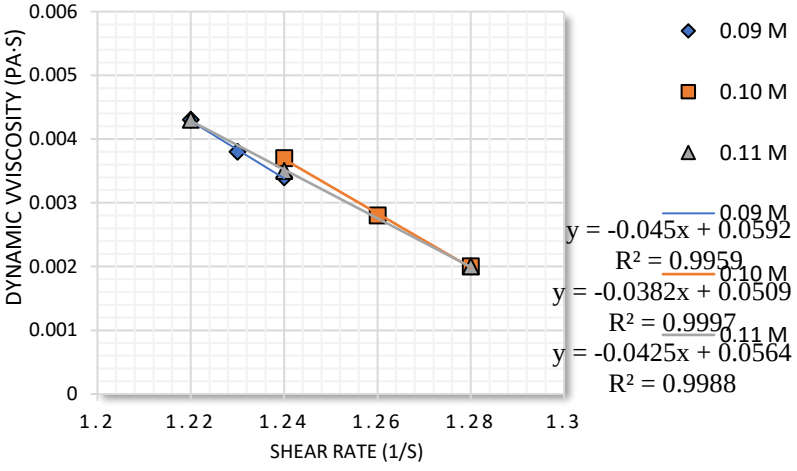

FIGURE 4. APPARENT VISCOSITY FOR VARYING CONCENTRATIONS OF $NANO_3$ AT 37°C

normal range for human blood viscosity. The 0.11 M concentration had the largest deviation in its range, but its average was within the area for normal human blood viscosity. The trendlines for the 0.09 M and 0.11 M concentrations are very similar. The 0.10 M trendline is lower in value than the other two concentrations tested. The maximum and average portions of the ranges were within normal human blood viscosity for the 0.09 M and 0.11 M concentrations. The minimums for the 0.10 M and 0.11 M fell below the normal range for human blood viscosity likely due to similar differences in the experimental model as discussed for the control data.

In Table 3, the average values at 37°C are expressed to clarify between close values in Figure 4. Table 3 shows that the 0.09 M and 0.11 M concentration were closer in value than the 0.10 M concentration. The 0.10 M concentration had the lowest dynamic viscosity and was also the only concentration that was below the normal value for human blood viscosity.

Table 3. Apparent Viscosity of Various Concentrations of $NaNO_3$ at 37°C

	Avg. Flow Time (s)	Avg. DV (Pa·s)	Shear rate (1/s)
0.09 M $NaNO_3$	15.03	3.8×10^{-3}	1.23
0.10 M $NaNO_3$	14.76	2.8×10^{-3}	1.26
0.11 M $NaNO_3$	14.93	3.5×10^{-3}	1.24

In Figure 5, the average dynamic viscosities were plotted to show the change between

25°C and 37°C for each of the concentrations of sodium nitrate as well as the control. The slopes of each sample indicate whether the viscosity change was mediated during the warming of the

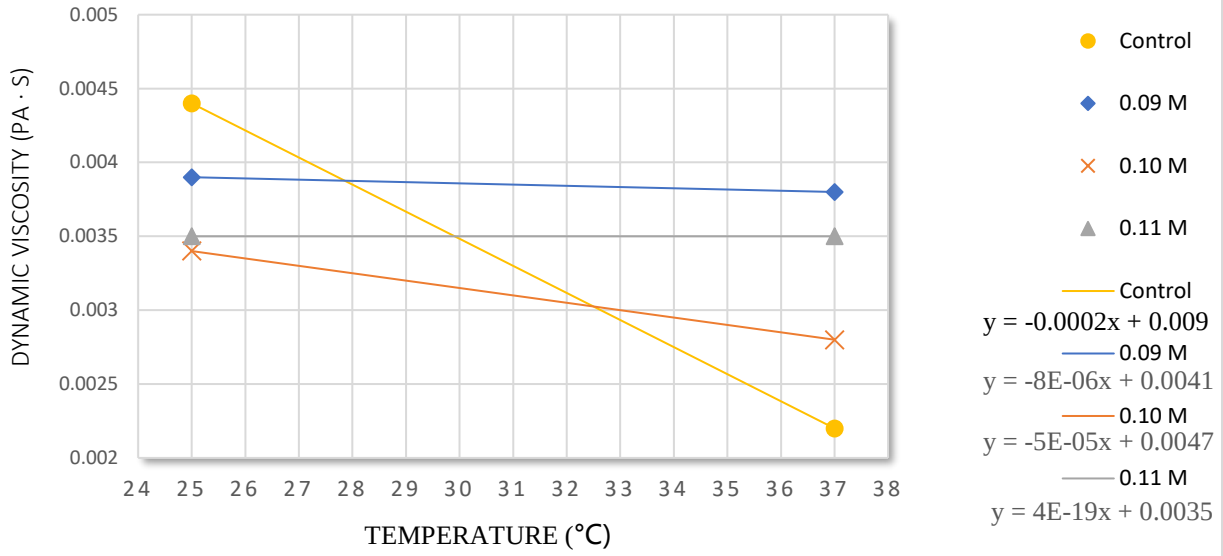

FIGURE 5. VISCOSITY CHANGE DURING WAMING FOR VARYING CONCENTRATIONS OF SODIUM NITRATE

blood. The control sample had the largest variance in dynamic viscosity with a range from 4.4×10^{-4} to 2.2×10^{-4} and a net change of 2.2×10^{-3}. The 0.10 M $NaNO_3$ had the next largest change in dynamic viscosity over the two temperatures with a change of 6.0×10^{-4}. This was the largest variance of the sodium nitrate trials and it ended with a dynamic viscosity that is not within the range of normal human blood viscosity. The 0.09 M and 0.11 M concentrations have relatively flat slopes with very low change over the two temperatures. The 0.09 M concentration had a net change of 1.0×10^{-4} and the 0.11 M concentration showed no change over the two temperatures. The 0.09 M and 0.11 M concentrations of sodium nitrate both lie within the range for normal human blood viscosity at 25°C and 37°C.

In Table 4, the results of the graph are tabulated into calculations to more precisely differentiate between the different trials over the course of the two temperatures tested.

Table 4. Deviation of Dynamic Viscosity (DV) from the Control as the Samples were Warmed

	Δ DV (Pa·s)	Deviation of Slope	Deviation of DV at 25°C	Deviation of DV at 37°C
0.09 M $NaNO_3$	1.0×10^{-4}	1.92×10^{-4}	5.0×10^{-4}	1.6×10^{-3}
0.10 M $NaNO_3$	6.0×10^{-4}	1.50×10^{-4}	1.0×10^{-3}	2.8×10^{-3}

0.11 M $NaNO_3$	0	1.99×10^{-4}	9.0×10^{-4}	1.3×10^{-3}
Control	2.2×10^{-3}	n/a	n/a	n/a

The 0.09 M and 0.11 M concentrations had a larger deviation in slope from the control with the values 1.92×10^{-4} and 1.99×10^{-4} respectively. A smaller deviation was observed in the 0.10 M concentration of 1.50×10^{-4}. At 25° C the deviation for the 0.10 M concentration is much larger than for the 0.09 M and 0.11 M concentration. The 0.09 M and 0.10 M concentrations exhibit a smaller change at the 25°C temperature as shown by their higher values when cooled.

Conversely the 0.10 M concentration experienced the lowest deviation at 37°C due to its steeper slope in comparison to the other concentrations of sodium nitrate tested. The 0.09 M concentration of sodium nitrate was the furthest deviated from control with a value of 1.6×10^{-3} followed closely by the 0.11 M concentration at a value of 1.3×10^{-3}. These samples experienced more deviation because they did not experience the dip in dynamic viscosity due to the increase in temperature in the blood rush region.

Analysis

The control curve displayed in Figure 1 exhibited the expected pattern of decreased temperature and increased viscosity. At 32°C variation seen in the viscosity illustrates the blood rush phenomenon which was the focus of observation in the experiment. The viscosities at 25°C and 37°C are out of the range of normal human blood viscosity so the results of the control are not optimal for comparison to the sodium nitrate trials therefore slopes were examined more closely for comparison. The steep decrease in dynamic viscosity may have been caused by the lack of friction from the Zahn cup in comparison to a blood vessel.

The 0.09 M $NaNO_3$ showed some signs of mediation with a very small decrease in dynamic viscosity between the 25°C and 37°C measurements. At both tested temperatures the dynamic viscosity for the 0.09 M $NaNO_3$ stayed within the range of normal human blood viscosity. In comparison to the other trials the 0.09 M concentration of sodium nitrate was the second most effective at maintaining the constant viscosity during the warming of the blood.

The 0.10 M $NaNO_3$ showed varied results with a much steeper decrease in dynamic

viscosity when the temperature was raised than the other two concentrations of sodium nitrate tested. The dynamic viscosity at 25°C was within the range of normal human blood viscosity, but over the warming process the dynamic viscosity was lowered to below the normal range for human blood viscosity. The dynamic viscosity for the 0.10 M $NaNO_3$ was expected to lie between the 0.09 M and 0.11 M concentrations of sodium nitrate when measured at 25°C, but it did not indicating a possible error in the experimental model.

The 0.11 M $NaNO_3$ expressed the greatest amount of mediation with an unchanging dynamic viscosity over the two temperatures tested. Both dynamic viscosity measurements taken lie within the range for normal human blood viscosity. In comparison to the other measurements taken for each concentration it was hypothesized that more nitric oxide may be released for the higher concentrations of sodium nitrate which lead to greater mediation. The 0.10 M $NaNO_3$ did not follow the pattern of this hypothesis, but it possibly due to error in the 0.10 M $NaNO_3$ trial.

Several errors occurred over the course of this experiment which affected the results obtained. The friction caused by the metal of the Zahn cup is much different than the friction caused by a blood vessel. This limits the full potential of the blood reactivity to temperature to be realized so the inhibition may not be seen in a living organism. The Zahn cup is also not reactive to vasodilation which means the nitric oxide likely would have a very different result in vivo due to the high permeability of nitric oxide and its role as a neurotransmitter for vasodilation. Also, the procedure in this experiment employed gravity to promote blood flow whereas normal circulation is propelled even against gravity. The experimental model also does not account for the presence of plaque in the circulatory system inhibiting blood flow which is a more accurate depiction of flow for a patient undergoing induced hypothermia. Finally, since the experiment was conducted ex vivo it cannot be known with certainty that this method is safe to conduct or if there are any adverse effects to the treatment.

Human error is also present in this experiment due to the extensive amount of multitasking and timing in order to conduct the experiment. When analyzing the data, error was assumed to have made the apparent viscosity values lower than they would be under normal circulatory conditions. This is prominent in the control curve which has values which lie outside of the normal range of human blood viscosity.

The use of sheep blood itself may also account for several errors. One inconsistency may be faster flow times due to the tightly packed structure of the sheep blood in comparison to human blood. This structure may also affect the for the amount of the nitric oxide bound to heme groups. This is because when examined the sheep blood appeared to have more RBCs per unit of area than the human blood so nitric oxide could bind in more locations effectively slowing the blood more than in a human sample. Sheep blood may also have a higher affinity for nitric oxide than human blood which could skew the results of the experiment.

Degradation of the blood samples overtime also effected the extent of experimentation and possibly the results of the 0.10 M $NaNO_3$ **s**ample. The shelf life of the blood when stored and properly cared for is approximately 28 days however, it lasts longer when it is frozen and treated with anticoagulants. The blood employed in this experiment was not frozen and was defibrinated for testing purposes due to this the RBCs likely degraded quicker than expected. The 0.10 M concentration was the final concentration tested due to the several changes in the procedure which called for retesting. It is possible that the RBCs had denatured causing it to flow faster than the other concentrations examined skewing the results. This trial also could have been denatured during heating of the blood to 37°C too quickly. This risk of degradation also stopped testing lowering the extent of accuracy and limiting improvements to the experimental model.

Conclusion

In this experiment sodium nitrate was examined as a method to introduce nitric oxide into the circulatory system of patients who are undergoing induced hypothermia. The viscosity of the blood was measured in order to determine how the sodium nitrate would affect the flow rate. In order to lower the mortality rate associated with induced hypothermia, the flow rate of the blood as it is warmed would need to be slowed to avoid the effects of the blood rush. To examine the sodium nitrate's ability to slow the flow rate of blood two temperatures were tested, 25°C and 37°C. 25°C was used to simulate the temperature patients are lowered to for induced hypothermia and 37°C models the temperature patients are raised to after treatment. Each concentration was compared at each temperature to determine if the blood rush was impeded.

Upon analyzing the data, the 0.11 M $NaNO_3$ was the most effective at maintaining the

blood's viscosity after raising the blood's temperature. This concentration had concurrent values for both temperatures. The 0.09 M concentration of $NaNO_3$ also expressed some mediation with a low amount of variation in dynamic viscosity as the blood was warmed from 25°C to 37°C. The 0.10 M $NaNO_3$ did not show mediation as effectively as the other two samples tested. There was a large comparative disparity between the two temperatures tested which more closely resembled the control. Neither the initial nor final temperature for the 0.10 M concentration were found between the 0.09 M and 0.11 M concentration of sodium nitrate as they were expected to be. These odd results were likely caused from the degradation of the blood as discussed previously as an experimental error.

Due to these results no solidified conclusions can be made as to whether sodium nitrate mediated the effects of the blood rush during the warming process of induced hypothermia. The skew seen in the 0.10 M $NaNO_3$ trial's data produced doubt as to whether the mediation seen in the other nitrate trials were valid. I do believe that a correlation is present which relates nitric oxide to a decreased blood flow due to an interaction in the blood between the hemoglobin and bound nitric oxide. The correlation is likely because none of the trials lowered to the level of the control at 37°C even the 0.10 M $NaNO_3$. Error induced from the rerun of the 0.10 M $NaNO_3$ close to the expiration date of the blood possibly lead to the degradation of the RBC's reducing the ability of the hemoglobin to interact with the nitric oxide which could account for the decreased viscosities in this trial.

In order to verify that there is a correlation between decreased viscosity and intake of nitric oxide more trials need to be taken and the experimental design should also be improved to obtain more accurate results. One of the large unknowns in this experimental design is that the amount of nitric oxide introduced into the blood is not certain because it can only be assumed that higher concentrations of sodium nitrate release more nitric oxide into the blood. In future experiments the levels of ornithine, citrulline, and arginine should be monitored in the blood before and after the addition of nitric oxide to determine the relative release of nitric oxide in the blood. More improvements can be made to the experiment by testing each degree in the warming process from 32°C and 37°C for each concentration to create a better curve for correlation. The correlation between nitric oxide introduction and dynamic viscosity would be better verified using only one concentration of nitric oxide to narrow the focus of the experiment. Finally, a

more precise viscometer should be used in future experiments that better models blood flow as well as reduces the human error associated with the fast flow times. Much more information could be gathered if the experiment was carried into a more realistic ex vivo model.

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