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The rheological properties of rabbit blood fed high cholesterol diet at wide range of shear rates

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Abstract: Background: The rheological properties of rabbits blood fed high cholesterol diet (HCD) and HCD supplemented with high zinc (Zn) level have not been well documented. This study aimed to evaluate the rheological properties of rabbit's serum and whole blood fed on HCD and HCD supplemented with high Zn level at wide range of shear rates and for feeding period of 12 weeks. **Materials and methods:** Twenty four New Zealand white rabbits were divided into: control group, HCD group and HCD group supplemented with high Zn level. The HCD group was fed on normal Purina supplemented with 1.0% cholesterol plus 1.0% olive oil. The HCD + Zn group fed on NOR Purina plus 1.0% cholesterol and 1.0% olive oil supplemented with total estimate 470 ppm Zn for a period of 12 weeks. Several rheological parameters such as viscosity, shear stress and torque % at wide range of shear rates (s^{-1}) from 225 to 1875 s^{-1} for rabbit blood serum and from 75 to 900 $s^{-1}$for rabbit whole blood were evaluated. **Results:** The viscosity and shear stress of HCD rabbit's serum blood values significantly increased at all shear rate values from 225 to 1875s^{-1} compared with the control; while rabbits fed HCD + Zn, viscosity and shear stress values returned towards the control values at all shear rate values. The viscosity and shear stress of HCD rabbit's whole blood values significantly decreased at all shear rate values compared with the control; while rabbits fed HCD + Zn, viscosity and shear stress values returned towards the control. **Conclusions:** The Zn plays a major role as an endogenous protective factor against atherosclerosis, and Fe may accelerate the process of atherosclerosis Inducing anemia in HCD rabbits might delay the progression of atherosclerosis. The rheological properties changes in rabbit's serum and whole blood can be used as an important diagnostic tool during the progression of atherosclerosis. The changes in rabbit's blood serum viscosity might be attributed to changes in non-clotting proteins, glucose, nutrients, and trace elements; while the changes in rabbit's whole blood viscosity might be attributed to changes in hematocrit, hemoglobin, and erythrocytes count.
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Introduction

Blood serum and plasma are used frequently while performing blood tests and disease diagnostic purposes. Major determinants of whole-blood viscosity are hematocrit, plasma viscosity, and red cell aggregation and red cell deformation under conditions of low and high shear [1, 2]; while the blood serum is the blood plasma with the fibrinogens removed, and the blood serum includes all proteins (not used in blood clotting), electrolytes, antibodies, antigens, hormones, any exogenous substances (drugs and microorganisms), and group of lipids (fats, oils, triglycerides, and cholesterol).

The pathophysiological changes of atherosclerosis, arterial thrombosis, and stroke have received increasing attention. The whole-blood viscosity is a critical determinant of the mechanical stimulus, and is frequently abnormal in the conditions associated with hyperlipidemia, hyperglycemia, and high blood pressure [3, 4]. The whole-blood viscosity is a predictor of stroke, carotid intima-media thickening, and carotid atherosclerosis [5, 6].

There are also strong relationships between blood viscosity and blood lipids. In most studies, the whole-blood viscosity was measured at a few nonspecific shear rates, and these data do not reflect the complete rheological characteristics of the study subjects [7-9]. Hypertriglyceridemia is associated with an increased risk of coronary heart disease [10-12]; however, the causal relationship for triglycerides remains unclear because of the interactions between triglyceride rich lipoproteins and small dense, low density lipoprotein (LDL) [13-15].

The viscosity changes up to many pathologic conditions, but its importance has not been fully investigated because the current methods of measurement are poorly suited for clinical applications. Therefore, the rheological properties of blood can be helpful in detecting human diseases as well as designing suitable treatments. Obtained results in these fields can be helpful in better understanding of diseases and in medical diagnosis and therapy [16].

The rheological properties of blood can be used as an important diagnostic tool for several clinical disorders. The rheological properties of rabbits blood fed HCD and HCD supplemented with high Zn level have not been well documented. This study aimed to evaluate the rheological properties of rabbit's serum and whole blood fed on HCD at wide range of shear rates for feeding period of 12 weeks in addition to investigation the rheological properties of HCD supplemented with high Zn level for the same feeding period of time.

Materials and Methods

Animals and experimental design

The atherosclerotic model used in this study was the New Zealand white rabbit (male, 12 weeks old), obtained from the Laboratory Animal Center (College of Pharmacy, King Saud University). Twenty four New Zealand white rabbits (male, 12 weeks old) were used in this study. The rabbits were individually caged, and divided into: The 1st group is control group ((Purina Certified Rabbit Chow # 5321; Research Diet Inc., New Jersey, USA); n = 8), the 2nd group is high cholesterol diet (HCD; n = 8) group and the 3rd group is the HCD group supplemented with high Zn level (n =8). The chemical composition of the laboratory NOR rabbit diet (Purina Certified Rabbit Chow # 5321) is shown in Table 1 and Table 2 [16]. The control group was fed on (100 g/day) of normal diet for a feeding period of 12 weeks. The HCD group (100 g/day) was fed on normal Purina Certified Rabbit Chow # 5321 supplemented with 1.0% cholesterol plus 1.0% olive oil (100 g/day) for the same feeding period. The HCD + Zn group fed on NOR Purina Certified Rabbit Chow plus 1.0% cholesterol and 1.0% olive oil supplemented with additional 350 ppm Zn (total Zn estimate is about 470 ppm) for the same feeding period. The neither chemical structures of NOR, HCD and HCD + Zn rabbit diet is shown in Tables 1 and 2 [16].

Experimental set up and rheological parameters measurement

Several rheological parameters such as viscosity (cp), shear stress (dyne/cm^2) and torque% at wide range of shear rates from 225 to 1875s^{-1} for rabbit blood serum and from 75 to 900s^{-1} for rabbit whole blood were evaluated. These rheological parameters were measured using Brookfield LVDV-III Programmable rheometer (cone-plate viscometer; Brookfield Engineering Laboratory, Incorporation, Middleboro, USA) supplied with temperature bath controlled by a computer. The rheometer was guaranteed to be accurate within ±1% of the full scale range of the spindle/speed combination in use reproducibility is within ±.2%. Rheological parameters were measured at temperature of 37 ºC. The temperature inside the sample chamber was carefully monitored using a temperature sensor during the rheological parameters measurement [17].

A cone and plate sensor having a diameter of 2.4 cm with an angle of 0.8 was used. The rheometer was calibrated using the standard fluids. This viscometer has a viscosity measurement range of 1.5-30,000mPas. The spindle type (SC-40) and its speed combinations will produce results with high accuracy when the applied torque is in the range of 10% to 100% and accordingly the spindle is chosen.

0.5ml of each rabbit blood sample (serum and/or whole blood) was poured in the sample chamber of the rheometer. The spindle was immersed and rotated in these blood samples in the speed range from 20 to 180 RPM in steps of 20 minutes. The viscous drag of the blood samples against the spindle was measured by the deflection of the calibrated spring [17].

Statistical analysis

The results of this study were expressed as Mean ± Standard Error (Mean ± SE; n = number of rabbits). The significance of differences between the NOR group and the HCD group and HCD + Zn group was assessed at the 5% confidence level. The statistical analysis was performed using one-way variance (ANOVA) for repeated measurements.

Results and discussions

Rheological parameters measurement

This study is unique in examining relationships between blood cholesterol levels and serum and whole-blood rheology at wide range of shear rates using Brookfield LVDV-III Programmable rheometer supplied with temperature bath and controlled by a computer.

The viscosity (cp) against shear rate (s^{-1}) values for rabbit blood serum at wide range of shear rates from 225 to 1875s^{-1} and fixed temperature of 37 °C were shown in **Fig. 1**. **Fig. 1** shows that in case of rabbits blood serum, the viscosity of HCD values significantly increased at all shear rate values from 225 to 1875s^{-1} compared with the control; while rabbits fed HCD + Zn, the viscosity returned towards the control values at all shear rate values. This study suggests that the increase in rabbit blood serum viscosity might be attributed to changes in non-clotting proteins, glucose, nutrients, electrolytes, hormones, antigens, antibodies and other particles.

In case of rabbit's blood serum, the relationships between shear stress and shear rate values for control, HCD, and HCD + Zn were linearly related as shown in **Fig. 2.** The shear stress values of HCD significantly increased at all shear rate values from 225 to 1875s^{-1} compared with the control; while rabbits fed HCD + Zn, the shear stress returned towards the control values at all shear rate values.

The viscosity (cp) against shear rate (s^{-1}) values for rabbit's whole blood at wide range of shear rates from 75 to 900s^{-1} and fixed temperature of 37 °C were shown in **Fig. 3**. **Fig. 3** shows that the viscosity of HCD values significantly decreased at all shear rate values from 75 to 900s^{-1} compared with the control; while the rabbits fed HCD + Zn, the viscosity returned towards the control values at all shear rate values. The decrease in whole-blood viscosity might be attributed to decrease in hematocrit, hemoglobin concentration, erythrocytes counts, and to high erythrocyte deformability in addition to the lower pH of protein might has lower viscosity results.

In case of rabbit's whole blood, the relationships between shear stress and shear rate values for control, HCD, and HCD + Zn were linearly related as shown in **Fig. 4. Fig. 4** shows that the shear stress values of HCD significantly decreased at all shear rate values from 75 to 450s^{-1} compared with the control; while the rabbits fed HCD + Zn, the shear stress returned towards the control values at all shear rate values. Blood is a unique fluid, it exhibits non-Newtonian characteristics, and its viscosity is dependent on shear rate [1, 2].

Discussion

In this study, the rabbits were fed high cholesterol diet (HCD) for 12 weeks. On comparing HCD rabbits to control rabbits, we found that the viscosity of blood serum of HCD rabbits values significantly increased at all shear rate values from 225 to 1875s^{-1} compared with the control; while the viscosity of whole-blood of HCD rabbits values significantly decreased at all shear rate values from 75 to 900s^{-1} compared with the control. The rabbits fed HCD + Zn, the viscosity and shear stress for serum and whole blood returned towards the control values at all shear rate values. This study suggests that the increase in rabbit blood serum viscosity might be attributed to increase in non-clotting proteins, glucose, trace elements, hormones, antigens, antibodies and other particles; while the decrease in whole-blood viscosity might be attributed to decrease in hematocrit, hemoglobin concentration, erythrocytes counts, and erythrocyte deformability in addition to the lower pH of protein might induce lower viscosity results.

This study demonstrates that the rheological properties changes in rabbit's serum and whole blood can be used as an important risk factor during the progression of atherosclerosis. One of the factors responsible for increasing the viscosity of blood serum values of HCD rabbits is the increase in Fe and decrease in Zn levels in blood serum.

The oxidized lipids and proteins as well as the decreased antioxidant levels, have been detected in human atherosclerotic lesions, with oxidation catalyzed by Fe [18]. The dietary Zn supplementation in HCD + Zn rabbits decreases the extent of lesion lipid oxidation and attenuates atherosclerotic burden. The Zn is a co-factor of many enzymes and has been shown to have anti-inflammatory and anti-proliferatory properties. The Zn is vital to vascular endothelial cell integrity and Zn deficiency causes severe impairment of the endothelial barrier function [5]. The Zn is believed to have specific anti-atherogenic properties by inhibiting oxidative stress-responsive transcription factors which are activated during an inflammatory response in atherosclerosis [19]. In other works, the average lesion area was significantly reduced for the rabbits on the Zn-supplement diet [20]. Elevated levels of cholesterol oxidation products in aorta of rabbits fed cholesterol diet were significantly decreased by Zn supplementation [21].

The Fe concentration increased with percentage normalized changes of 25.09% in heart and 33.78% in aortic tissue of HCD rabbits compared with control rabbits. The Fe plays a major role in atherogenesis, probably through the production of free radicals, and that inducing anemia in HFD rabbits may delay or inhibit the progression of atherosclerosis [16]. The Fe has the potential to catalyze and generate hydroxyl radicals from superoxide and hydrogen peroxide via the Fenton reaction. The highly reactive hydroxyl radicals subsequently cause lipid peroxidation, degradation of other macromolecules, leading to cell damage or death, and that inducing mild anemia in HCD decreased the progression of atherosclerosis in conjunction with decreases in lesion Fe content [22].

The importance of whole blood viscosity lies in the fact that it appears to be an independent predictor of stroke, carotid intima-media thickening, and carotid atherosclerosis. The HDL prevents red cell aggregation by competing with LDL-induced red cell aggregation [23, 24], this effect was mediated via the outer coat of the HDL particle which is composed of apolipoprotein rather than the amount of cholesterol in HDL. A significant inverse relationship was demonstrated between whole blood viscosity and apolipoprotein A-1, not HDL cholesterol at shear rates (150 - 1000s^{-1}).

The whole blood viscosity was corrected to a standard hematocrit of 45% [5]. The whole blood viscosity and hematocrit were significantly related to common carotid intima-media thickening in men [9]. The men with carotid atherosclerosis had significantly greater values for whole blood viscosity and hematocrit compared with controls at shear rates of 225 and 450s^{-}1 [6].

A significant correlation between blood viscosity and LDL cholesterol, total cholesterol, and HDL cholesterol at shear rates of between 75 and 132s^{-1} in healthy adult volunteers was observed [25, 26]. The triglyceride, HDL cholesterol, fibrinogen, total serum protein, and hematocrit were significant predictors of uncorrected whole blood viscosity at a shear rate of 100s^{-1} in healthy adult subjects referred for cardiovascular risk assessment [7].

This study suggests that additional experiments should be performed taking into consideration lesion Fe content, production of free radicals, intracellular and extracellular lipids in the intima, connective tissue formation, and smooth muscle proliferation. In additional to additional experiments should be taken into consideration the rheological properties changes in whole blood viscosity of HCD and HCD + Zn rabbits as function of hematocrit and hemoglobin concentration, count of erythrocytes, and erythrocyte deformability; while for blood serum as function of non-clotting proteins, glucose, nutrients, trace elements, hormones, antigens, antibodies and other particles. Furthermore, inducing anemia in HCD rabbits may delay or inhibit the progression of atherosclerosis.

Conclusions

The present study aimed to evaluate the rheological properties of rabbit's serum and whole blood fed on HCD at wide range of shear rates for feeding period of 12 weeks in addition to investigation the rheological properties of HCD supplemented with high Zn level for the same feeding period of time.

Twenty four New Zealand white rabbits were used in this study. The rabbits were individually caged, and divided into 3 groups. Several rheological parameters such as viscosity (cp), shear stress (dyne/cm^2) and torque% at wide range of shear rates (s^{-1}) from 225 to 1875 s^{-1} for rabbit blood serum and from 75 to 900 $s^{-1}$for rabbit whole blood were evaluated.

In case of rabbits blood serum, the viscosity and shear stress of HCD values significantly increased at all shear rate values from 225 to 1875s^{-1} compared with the control; while the rabbits fed HCD + Zn, the viscosity and shear stress values returned towards the control values at all shear rate values. In case of rabbits whole blood, the viscosity and shear stress of HCD values significantly decreased at all shear rate values from 75 to 900s^{-1}

compared with the control; while the rabbits fed HCD + Zn, the viscosity and shear stress values returned towards the control values at all shear rate values.

This study demonstrates that the rheological properties changes in rabbit's serum and whole blood can be used as an important risk factor during the progression of atherosclerosis. The Zn plays a major role as an endogenous protective factor against atherosclerosis, and Fe may accelerate the process of atherosclerosis probably through the production of free radicals, deposition and absorption of intracellular and extracellular lipids in the intima, connective tissue formation, and smooth muscle proliferation. Furthermore, inducing anemia in HCD rabbits might delay or inhibit the progression of atherosclerosis.

The decrease in blood viscosity may be attributed to changes in the molecular weight, pH sensitivity and structure of protein. The protein of less spheroid shape is the higher molecular weight and aggregating capacity.

Competing interests

Authors declare that they have no competing interests

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