

# The changes in serum and whole blood rheological properties of rabbits during the progression of atherosclerosis

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**Abstract:** This study aimed to evaluate the role of zinc (Zn)-supplemented with high cholesterol diet (HCD) on the serum and whole blood rheological properties of rabbits fed a HCD. Twenty-four New Zealand white rabbits were divided into three groups. The HCD group was fed a diet with 1.0% cholesterol and 1.0% olive oil. The HCD + Zn group was fed a diet with 1.0% cholesterol, 1.0% olive oil, and Zn. Blood viscosity, shear stress, and torque (%) were measured at shear rates ranging from 225 to 1875 s<sup>-1</sup> for serum and 75-900 s<sup>-1</sup> for whole blood. Serum viscosity and shear stress in HCD rabbits were significantly higher at all shear rates compared to controls; while whole blood viscosity and shear stress in HCD rabbits were significantly lower at all shear rates compared to controls. Viscosity and shear stress in both serum and whole blood from rabbits in the HCD + Zn group returned to normal values at all shear rates. The Zn supplemented to HCD rabbits, delays the progression of atherosclerosis. Changes in blood serum viscosity could reflect changes in non-clotting proteins, glucose, nutrients and trace elements; while changes in whole blood viscosity could result from changes in hematocrit, hemoglobin, and erythrocyte count. One of the factors responsible for increasing the serum viscosity values of HCD rabbits might be attributed to increase in Fe and decrease in Zn levels in the blood serum.

**Keywords:** Rheological properties; high cholesterol diet; Zn supplementation; rabbits; shear rates; diagnostic tool; atherosclerosis.

## INTRODUCTION

Increased attention has been focused on atherosclerosis, to explore its pathogenesis as well as to diagnose and treat the disease. The dimensional and morphological changes of the arterial wall of rabbits during initiation of atherosclerosis have been well documented.

The mechanical properties of rabbits arterial wall fed HCD have extensively been studied (Abdelhalim *et al.*, 1992; Abdelhalim *et al.*, 1994; Abdelhalim and Al-Ayed, 2007). In these studies, we found that the cholesterol concentration of blood plasma significantly increased throughout the cholesterol-feeding period. In cholesterol fed rabbits, the aortic wall had a tendency to thicken; while the ratio of wall thickness to radius of aortic ring specimens was significantly increased, compared with the normal-fed rabbits (Abdelhalim *et al.*, 1992; Abdelhalim *et al.*, 1994; Abdelhalim and Al-Ayed, 2007; Abdelhalim and Alhadlak, 2008). Moreover, the cholesterol concentration in the blood plasma of rabbits fed a HCD significantly increased throughout the cholesterol-feeding period of 12 weeks (2111.1mg/dL± 440.1; Mean ±SE) compared with the control (163 mg/dL±73.19; Mean ±SE) (Abdelhalim and Al-Ayed, 2007; Abdelhalim *et al.*, 2010).

Blood serum and plasma are frequently used for

diagnostic purposes. The whole blood viscosity depends on the following: hematocrit, plasma, red blood cells aggregation and deformation at shear rate values (Dutta and Tarbell, 1996; Goldsmith and Turitto, 1986). Blood serum (blood plasma without fibrinogen) contains non-clotting proteins, electrolytes, antibodies, hormones, lipids, and several exogenous substances.

Atherosclerosis, arterial thrombosis, and stroke cause pathophysiological changes, which have garnered increasing attention recently. Whole blood viscosity is frequently abnormal in patients with hyperlipidemia, hyperglycemia, and high blood pressure (Rosenson, 1993; Malek *et al.*, 1999). Thus, blood viscosity is considered as an indicator to carotid atherosclerosis, stroke, and carotid intima-media thickening (Lee *et al.*, 1998; Carallo *et al.*, 1998). Blood viscosity is also strongly associated with lipids; for example, hypertriglyceridemia increases the risk for coronary heart disease (Jeppesen *et al.*, 1998). However, the mechanisms underlying this relationship remain complicated by the close association of triglyceride-rich lipoproteins with small low-density lipoproteins (LDLs) (Gardner *et al.*, 1996).

The Zn is essential for the normal cell function as well as the metabolism. The Zn functions as a complex antioxidant and inhibits the oxidative stress (Brocardo *et al.*, 2005). Moreover, the Zn deficiency results in an increase in oxidative damage of the cells (Yousef *et al.*,

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2002) as well as the alterations in the components of the antioxidant defense system (Virgili *et al.*, 1999). It has the ability to form coordinating bonds with electronegative atoms (Prasad *et al.*, 2004). It regulates the metallothionein synthesis and can interact with the radical of several compounds.

Most studies have measured the whole blood viscosity at a single shear rate value, which fail to generate a complete picture on the rheological characteristics. The rheological properties can help to detect the disease as well as can lead to new treatments (Abdelhalim, 2010).

The high-density lipoprotein (HDL) prevents the aggregation of red blood cells, through the mediation of the outer coat of the HDL particle. A significant inverse relationship was demonstrated between the whole blood viscosity and the apolipoprotein at range of shear rates from 150 to 1000s<sup>-1</sup> (Abdelhalim, 2011; Abdelhalim *et al.*, 2013).

The Fe increased and the Zn decreased in the most HCD rabbit's tissues compared with the control. The Fe plays a major role during the progression of atherosclerosis; through the production of free radicals, deposition of lipids in the intima, and proliferation of smooth muscle cells (Abdelhalim, 2011; Abdelhalim *et al.*, 2013; Abdelhalim and Alhadlak, 2008).

Rheological properties can be used as an important tool for several clinical disorders; Zn may reduce the Fe content in the lesion, reduce the lipids of the intima and prevents the proliferation of the smooth muscle cells (Abdelhalim, 2011; Abdelhalim *et al.*, 2013). The effects of zinc (Zn)-supplemented to HCD on the serum and whole blood rheological properties of rabbits fed only a HCD have not been documented before. Thus, we used a rabbit model of atherosclerosis to evaluate the role of Zn-supplemented HCD on the serum and whole blood rheological properties of rabbits fed a HCD.

## MATERIALS AND METHODS

### *Animals and experimental design*

Twenty-four male New Zealand white rabbits (aged 12 weeks old) were obtained from the Laboratory Animal Center of the College of Pharmacy at King Saud University. The rabbits were individually caged and divided into control, HCD, and HCD + Zn. The rabbits were fed 100g of Purina Certified Rabbit Chow #5321 (Research Diet Inc., New Jersey, USA) daily for 12 weeks. The control group (n=8) was fed a normal, unmodified normal diet. The HCD group (n=8) was fed a NOR diet that also contained 1.0% cholesterol and 1.0% olive oil. The HCD + Zn group was fed a NOR diet that contained 1.0% cholesterol, 1.0% olive oil, and Zn (total of 470 ppm Zn over the 12-week feeding period). The

chemical compositions of the NOR, HCD, and HCD + Zn diets has been reported by Abdelhalim *et al.*, 2013. All experiments were conducted in accordance with guidelines approved by the Local Animal Care and Use Committee of King Saud University (Abdelhalim *et al.*, 2010).

### *Rheological measurements*

Blood viscosity (cp), shear stress (dyne/cm<sup>2</sup>), and torque (%) were measured at shear rates of 225 - 1875s<sup>-1</sup> and 75 - 900s<sup>-1</sup> for rabbit serum and whole blood, respectively. These rheological parameters were measured using the Brookfield LVDV-III Programmable cone-plate rheometer (Brookfield Engineering Laboratory, Incorporation, Middleboro, USA).

The rheometer measures viscosities between 1.5 to 30,000m Pas and is accurate within ±1% with reproducibility of ±0.2%. A cone and plate sensor with a diameter of 2.4cm and angle of 0.8 and a SC-40 spindle, which has a high accuracy at torques of 10% to 100%, were used. The temperature inside the sample chamber was carefully monitored using a temperature sensor during measurements [20] and measurements were taken in a computer-controlled water bath at 37°C.

A 0.5ml aliquot of each sample (serum or whole blood) was poured into the rheometer sample chamber. Samples were then immersed, rotated by the spindle at steadily increasing speeds (20-180 RPM) for 20 minutes (Abdelhalim, 2011).

## STATISTICAL ANALYSIS

Results were expressed as mean ± standard error (Mean ± SE). Statistical analyses were performed using a was performed using one-way analysis of variance (ANOVA) for repeated measurements, with significance assessed at 5% confidence level for repeated measurements (Abdelhalim, 2011).

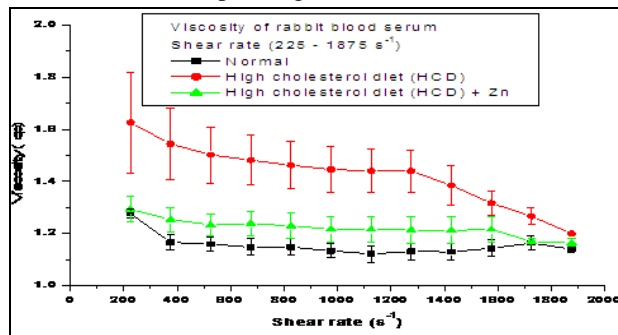
## RESULTS

### *Rheological parameters measurement*

Fig. 1 shows that in case of rabbits serum, the viscosity of HCD values significantly (p<0.05) increased at all shear rate values from 225 to 1875s<sup>-1</sup> compared with the control; while in case of rabbits fed HCD + Zn, the viscosity values became closer to the control values at all shear rate values.

In case of rabbit's 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 (p<0.05) increased at all shear rate values from 225 to 1875s<sup>-1</sup> compared with the control; while in case of rabbits fed HCD + Zn, the

shear stress returned towards their control values at all shear rate values. Moreover, the viscosity values for whole blood were higher than those for blood serum at the measured corresponding shear rate values.



**Fig. 1:** The viscosity (cp) against shear rate ( $s^{-1}$ ) for rabbits blood serum ( $p < 0.05$ )

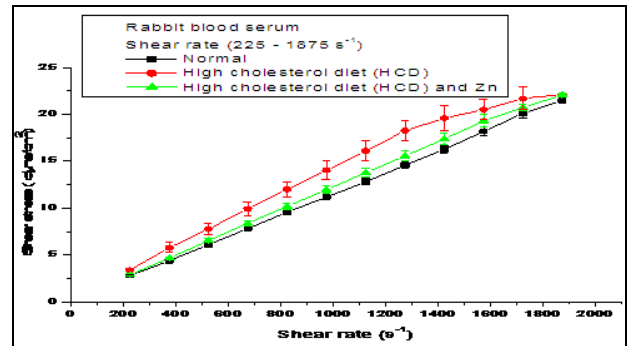
To get real viscosity values from Brookfield rheometer, we found that at the upper shear rate value was around  $900s^{-1}$  at the highest %torque value while at the lower shear rate value was found around  $75s^{-1}$  at the lowest %torque value. The viscosity (cp) against shear rate ( $s^{-1}$ ) values for rabbit's whole blood at shear rates from 75 to  $900s^{-1}$  was 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 in case of rabbits fed HCD + Zn, the viscosity became closer to the control values at all shear rate values. Moreover, the viscosity values for whole blood were higher than those for blood serum at the measured corresponding shear rate values.

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 ( $p < 0.05$ ) 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 their control values at all shear rate values.

To get real viscosity values from Brookfield rheometer, the % torque should be greater than % and lower than %100. In this study, we found that the upper shear rate value was around  $1875s^{-1}$  at the highest % torque value while the lower shear rate value was found around  $225s^{-1}$  at the lowest % torque value for rabbits' blood serum; while the upper shear rate value was around  $900s^{-1}$  at the highest %torque value and  $75s^{-1}$  at the lowest % torque value for whole blood.

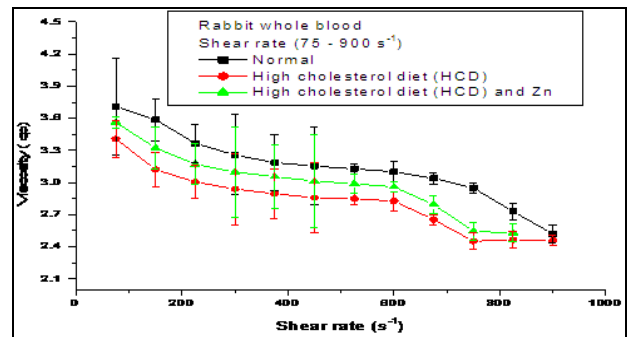
It has been reported that the changes in whole-blood viscosity are attributed to changes in hematocrit, hemoglobin concentration, erythrocytes counts and deformability values (Abdelhalim and Alhadlak, 2008; Carallo et al., 1998; Ren et al., 2005; Sloop and Garber, 1997).

Blood fluid exhibits non-Newtonian characteristics, and its viscosity is dependent on shear rate (Dutta and Tarbell, 1996; Goldsmith and Turitto, 1986).



**Fig. 2:** The shear stress (dyne/cm<sup>2</sup>) against shear rate ( $s^{-1}$ ) for rabbits blood serum ( $p < 0.05$ )

It has been reported that aortic sections of rabbits fed HCD and stained with hematoxylin and eosin, shows dilated lumen of rabbit aortic sections, vascular occlusion of the lumen and subintimal deposition of fibro fatty tissue associated with smooth muscle proliferation. The rabbits fed a HCD marked aortic wall intimal thickening with focal loss of medial architecture (Abdelhalim et al., 1994; Abdelhalim et al., 2013).



**Fig. 3:** The viscosity (cp) values against shear rate ( $s^{-1}$ ) values for rabbit whole blood ( $p < 0.05$ )

In regards to the biochemical analysis, the total cholesterol (TC) significantly decreased from  $658.95 \pm 95.27$  (Mean  $\pm$  SE) in HCD to  $93.12 \pm 5.5$  in HCD + Zn rabbits; the low density lipoproteins (LDL) significantly decreased from  $677.2 \pm 11.1$  in HCD to  $95.0 \pm 24.1$  in HCD + Zn rabbits; the high density lipoproteins (HDL) significantly decreased from  $17.32 \pm 1.5$  in HCD to  $13.0 \pm 1.6$  in HCD + Zn rabbits; the triglyceride significantly decreased from  $197.25 \pm 32.25$  in HCD to  $109.00 \pm 10.3$  in HCD + Zn rabbits (Abdelhalim and Alhadlak, 2008).

In regards to the hematological analysis, it has been observed different changes in the measured complete blood count of the HCD rabbits compared with the control; the zinc supplementation to the HCD rabbits improved and returned all the blood indices to become

comparable with their normal values. Moreover, the dimensional parameters and the PLTs count are important predictor factors during the progression of atherosclerosis and the cardiovascular diseases (Pearse, 19865).

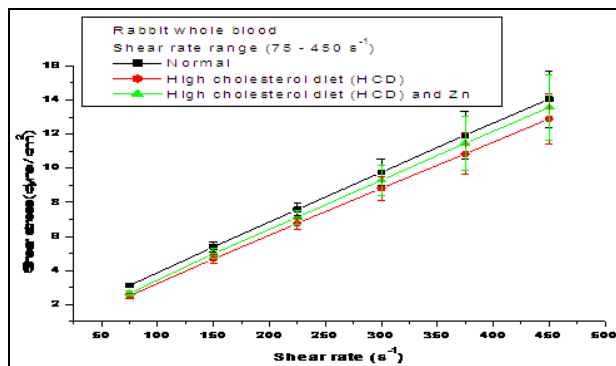


Fig. 4: The shear stress (dyne/cm<sup>2</sup>) against shear rate (s<sup>-1</sup>) for rabbits whole blood (p<0.05)

On comparing the high cholesterol to control rabbits, the viscosity of blood serum of HCD rabbits values significantly increased at all shear rate values from 225 to 1875s<sup>-1</sup> compared with the control; while the viscosity of whole-blood of HCD rabbits significantly decreased at all shear rate values from 75 to 900s<sup>-1</sup> 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.

The oxidized lipids and proteins and the decreased antioxidant levels, has been observed in the atherosclerotic lesions of the humans (Watt *et al.*, 2006).

The Zn supplementation to HCD rabbits decreased the lesion lipid oxidation and attenuated the atherosclerotic portion. The deficiency of Zn causes severe damage to the endothelial function (Lee *et al.*, 1998; Abdelhalim, 2011; Abdelhalim *et al.*, 2013). Our study demonstrated that increasing the Zn levels in the HCD rabbits, returned the whole blood viscosity values towards their normal values.

The Zn inhibits the oxidative stress-responsive factors, activated during the development of atherosclerosis (Lamb *et al.*, 1999). The supplementation of Zn to HCD rabbits significantly decreased the oxidative products in the rabbits aorta (Beattie and Kwun, 2004; Ren *et al.*, 2005).

The whole blood viscosity and hematocrit in men with carotid atherosclerosis were significantly greater compared with the control at shear rates of 225 and 450s<sup>-1</sup> (Carallo *et al.*, 1998). A significant correlation was observed between blood viscosity and LDL cholesterol and total cholesterol at shear rates between 75 and 132s<sup>-1</sup> in healthy adult volunteers (Sloop and Garber, 1997; Crowley *et al.*, 1994).

Experiments with Fe-supplemented to HCD rabbits have not designed in our schedule work, for the following reasons: 1) it has been reported that Fe accelerates the process of atherosclerosis. % change of Fe was significantly increased, but with different % values in the different rabbits tissues compared with the control ones; while %change of Zn significantly decreased in the different rabbits tissues compared with the control ones (Abdelhalim *et al.*, 2010).

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