

## INHIBITION OF PARAOXONASE1 BY KETONE BODIES: AN INVITRO STUDY

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## ABSTRACT

**Objective:** In uncontrolled diabetes mellitus, concentration of ketone bodies is elevated to up to 25mM. This study aims to investigate the inhibitory action of ketone bodies on paraoxonase and arylesterase activity of Paraoxonase1. **Method:** The inhibition of Paraoxonase1 by ketone bodies was determined spectrophotometrically by using paraoxon and phenyl acetate as substrates. **Result:** Acetone decreased 32% of paraoxonase activity and 23% of arylesterase activity. Sodium-3-hydroxybutyrate showed 20% reduction in paraoxonase activity but it did not affect arylesterase activity. In contrast, neither of the enzyme activities was affected by sodium-acetoacetate. **Conclusion:** Higher concentration of acetone and sodium-3-hydroxybutyrate contribute to the reduction of Paraoxonase1 activity.

**Keywords:** Paraoxonase1, Arylesterase, High density lipoprotein, Ketone bodies, Sodium-acetoacetate, Acetone, Sodium-3-Hydroxybutyrate.

## INTRODUCTION

Human serum paraoxonase (PON1) is a high density lipoprotein (HDL) associated enzyme with diverse functions. It hydrolyzes aromatic carboxylic acid esters (phenyl acetate), organophosphates and carbamates [1]. It has been shown to protect lipoproteins from free radical mediated oxidation [2]. It can also hydrolyze the oxidized cholesterol esters and lipid peroxides [3]. Serum level of PON1 has been used as a predictor of cardiovascular risk [4].

PON1 levels can vary depending on physiological conditions and pathological states. It has been reported that, PON1 activity is significantly decreased in wide variety of human disorders which involve oxidative stress such as cardiovascular disease, diabetes mellitus, obesity, renal disease, liver cirrhosis, non-alcoholic steatohepatitis, and several mental disorders [5, 6].

In diabetes mellitus, the PON1 activity has been reported to be decreased [7, 8]. Since diabetes mellitus is a major risk factor for cardiovascular disorders, decreased PON1 activity in diabetes mellitus will be an indicator of cardiovascular risk. In diabetes mellitus, significant changes occur in various metabolites in blood [9, 10]. For example, in uncontrolled diabetes mellitus, levels of blood glucose and ketone bodies are elevated. In this study we have investigated the inhibitory action of ketone bodies on PON1 activity.

## MATERIALS AND METHODS

## Materials

Paraoxon (O,O-diethyl-O-p-nitrophenylphosphate), phenyl acetate, sodium-3-hydroxybutyrate were from Sigma Chemical Co, St. Louis, USA. All other chemicals and solvents used were of analytical grade.

## Methods

## Collection of blood and serum preparation

Blood was collected from healthy volunteers (n=4; age group 20-30 years; either gender). The blood was allowed to clot and centrifuged at 5000 rpm for 20 minutes. The serum obtained was pooled and used for subsequent experiments.

## Isolation of HDL by density centrifugation

HDL was isolated from serum by density gradient centrifugation according to the method of Redgrave et al. [11]. 6ml of serum was mixed with solid KBr such that the density was about 0.5 g/ml. 18 ml

of saline was layered on top of the serum sample. The tubes were centrifuged using ultracentrifuge in a fixed angle rotor for 3 h at 45000 rpm (2,00,000 g) at 4°C. After centrifugation, the tubes were placed in the vertical position. The fractions were aspirated from the top. HDL appears as an orange-layer at the bottom of the tube. HDL containing fractions were dialyzed in the dark for 6 to 8 h against 100mM phosphate buffer saline. PON1 activity of HDL was determined spectrophotometrically by using paraoxon and phenyl acetate as substrates.

## Paraoxonase Activity

Paraoxonase activity was measured (using paraoxon as substrate) according to method of Beltowski et al. with some modifications [12]. The activity was measured in Tris buffer (100mM, pH 8.0) containing 2mM CaCl<sub>2</sub> and 1mM paraoxon. HDL fraction (10µl) was added to initiate the reaction. The rate of generation of p-nitrophenol was determined at 412 nm at 25 °C, with the use of a continuously recording spectrophotometer (Shimadzu). One unit of enzyme activity was defined as the amount of enzyme that catalyzes the hydrolysis of 1µmol of substrate per minute.

## Arylesterase Activity (ARE'ase)

ARE'ase activity was measured using phenyl acetate as substrate. Diluted HDL fraction (1:10v/v, 10µl) was added to 10mM Tris HCl buffer, pH 8.0 containing 2mM CaCl<sub>2</sub> and 2mM phenyl acetate. The rate of generation of phenol was determined at 270 nm at 25°C with the use of a continuously recording spectrophotometer. One enzyme unit was defined as the amount of enzyme that catalyzes the hydrolysis of 1µmol of substrate per minute [13].

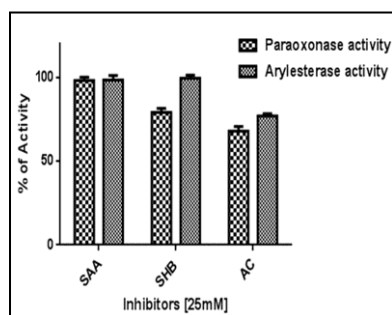
## Preparation of Sodium-acetoacetate.

Sodium-acetoacetate was prepared according to the method of Krebs and Eggleston [14]. To 2.6 ml of distilled ethyl-acetoacetate was added with 10.2 ml of 2N NaOH and were made up to 20 ml with water. The mixture was incubated for 1 h at 40°C, and then placed in ice-bath and neutralized (pH 7.0) with 1 N HCl. The mixture was poured into a distilling flask with 20 ml of water and lyophilized to 5 ml in order to remove ethanol. The solution was transferred to a measuring flask and made up to 20 ml. This stock solution (about 1 M) was stored in the refrigerator.

## RESULTS AND DISCUSSION

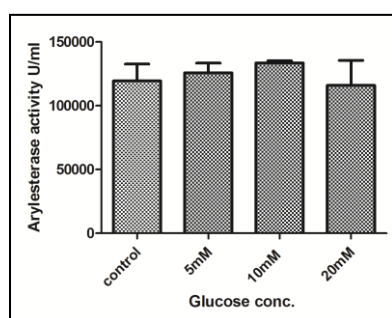
PON1 prevents lipid peroxidation in HDL [15]. Also, the ability of HDL to prevent LDL oxidation is attributed to the presence of PON1 [16]. Earlier studies have reported that PON1 activity in diabetes mellitus is reduced. There could be several factors responsible for the observed reduction in PON1 activity [17, 18, 19 and 20]. In uncontrolled diabetes mellitus, ketone bodies are elevated. Ketone bodies normally contribute modestly to energy balance, with serum levels of 2 to 3mM and in uncontrolled diabetes mellitus the levels may reach up to 25mM [21]. In cardiovascular disease, it has been reported that serum levels of ketone bodies are elevated in heart failure and are partly attributed to increased mobilization and degradation of free fatty acid [22, 23].

The present study reports that high concentration of ketone bodies exhibit preferential loss of paraoxonase and arylesterase activities of PON1. Acetone decreased 32% of the paraoxonase activity and 23% of arylesterase activity. Sodium-3-hydroxybutyrate showed 20 % reduction in paraoxonase activity but it did not affect arylesterase activity. Contrarily, both enzyme activities remained unaffected by sodium-acetoacetate (**Figure 1**). It has been reported that acetone moderately inhibit (30% to 70%) the PON1 activity [24].

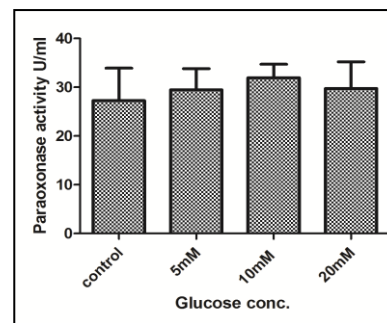


**Figure 1: It shows inhibitory action of acetone and sodium-3-hydroxybutyrate [at 25mM] on paraoxonase and arylesterase activity of PON1. [SAA: sodium-acetoacetate; SHB: sodium-3-hydroxybutyrate; AC: acetone]**

In cases of diabetes mellitus, the levels of glucose and ketone bodies are elevated substantially. The findings of PON1 inhibition by ketone bodies was further justified by determining the effect of glucose concentration on PON1 activity (**Figure 2** and **Figure 3**). We observed that  $\leq 20\text{mM}$  glucose (360mg/dl) does not alter PON1 activity.



**Figure 2: It shows the effect of glucose concentration on arylesterase activity of PON1**



**Figure 3: It shows the effect of glucose concentration on paraoxonase activity of PON1**

## CONCLUSION

In conclusion, higher concentration of ketone bodies may be responsible for the reduction of PON1 activity in routine assay system. Therefore, increasing concentration of acetone and  $\beta$ -hydroxybutyrate may be one of the reasons for decreasing PON1 activity.

## Abbreviations

PON1 - Paraoxonase 1  
ARE'ase - Arylesterase  
HDL - High density lipoprotein

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