

# The effects of alloxan-induced diabetes on plasma creatine kinase and cardiac creatine kinase isoenzyme in rabbits

YEĞİN E.<sup>1</sup>, ERCAN M.<sup>2</sup>, ÇELİK İ.<sup>1</sup>, SÜZEK H.<sup>3</sup>, TUNCER İ.<sup>4</sup>

Departments of Biology and Science<sup>1</sup>, Physiology<sup>2</sup>, Biochemistry<sup>3</sup> and Internal Medicine<sup>4</sup>, School of Medicine, Yüzüncü Yıl University, Van

**Objective** The aim of this study is to examine the effects of diabetes on plasma creatine kinase and cardiac creatine kinase isoenzyme levels.

**Methods** This investigation was performed on male NZW rabbits. Twenty-four rabbits were studied; diabetes and control groups of twelve animals each. Diabetes was induced by IV injection of ALX monohydrate (100 mg/kg). Plasma CK and CKMB levels were measured by autoanalyser and with ready to use kit.

**Results** There were statistically significant differences in plasma creatine kinase ( $t=2.18$ ,  $p=0.042$ ) and plasma cardiac creatine kinase isoenzyme ( $t=5.11$ ,  $p=0.00007$ ) values between alloxan induced diabetes and control groups.

**Conclusion** Our data succeeded that the plasma creatine kinase and cardiac creatine kinase isoenzyme levels decrease in alloxan induced diabetes mellitus.

**Key words** Diabetes mellitus, CK, CK-MB, alloxan, rabbits.

## Introduction

Several of the ATP ase enzymes that are responsible for muscle contraction rely on high-energy phosphatases supplied by the creatine kinase (CK) system. Three isoenzymes of creatine kinase have been identified in human tissues: MM from skeletal muscle (CK-MM), MB from myocardial tissue (CK-MB) and BB from nervous tissue (CK-BB). Plasma from healthy humans contains MM almost exclusively (1). Experimental diabetes mellitus has been shown to cause a decrease in the contractile performance of muscles and especially heart's muscle (2,3). Decrease in contractile performance of muscles in diabetes mellitus may be due to CK and CKMB decreases (4,5,6). So, in this study we investigated CK and CKMB levels in experimental diabetes mellitus in rabbits.

## Material and Method

### Animals

This investigation was performed on male NZW rabbits (*Oryctolagus cuniculus huxleyi*) weighing 2.5 to 3 kg. Twenty-four rabbits were studied; two groups of twelve animals each. They were maintained under conditions of  $20 \pm 3^\circ\text{C}$  temperature and daily light/dark cycle and supplied with normal food and water ad libitum. Studies were carried out two months after ALX or control treatment.

### Induction of diabetes

Diabetes was induced by IV injection of alloxan monohydrate (ALX) (100 mg/kg). ALX was dissolved in 0.9% NaCl to constitute a 10% (w/v) solution immediately before injection. After an

overnight fast, ALX was injected via a marginal ear vein using a 22 gauge needle. Control animals received an equal volume of 0.9% NaCl solution only (7,8).

Venous blood samples for glucose analyses were taken from the contralateral ear. Blood glucose measurements were routinely determined within 24 hours following each dose of ALX. These measurements were made to detect potentially life threatening hypoglycaemia (blood glucose  $< 50$  mg/dl), a complication previously reported following alloxan administration (7). Post ALX hypoglycaemia was occurred in two rabbits was treated by immediate injection of 5 cc of 50% dextrose given subcutaneously (9).

The efficacy of ALX in inducing diabetes was confirmed by measurement of nonfasting blood glucose levels exceeding 250 mg/dl by dextrostix (Reflex S - BOEHRINGER MANN HEM) at the end of the first week and for two months after its administration. In contrast, control animals had mean blood glucose concentrations of less than 180 mg/dl in these periods (7,10).

All animals were maintained for two months, then the rabbits anaesthetised and killed by cervical dislocation. Five millilitres of blood were collected by cardiac puncture and immediately put into tubes containing EDTA. The plasma samples were obtained by centrifuging the blood samples at 3,000 rpm for 10 minutes at  $4^\circ\text{C}$  for measurement of CK and CKMB.

### Measurement of CK and CKMB

Plasma CK and CKMB levels were measured by autoanalyser (Hitachi 705) and with ready to use kit (DPC Diagnostic Products Corporation Los Angeles CA 90045. Lot no: TKDSI 575).

Accepted for publication: 20 January, 1996.

## Analysis of data

Statistical analysis was performed using unpaired, two-tailed Student *t* tests. Statistical significance was indicated by  $P < 0.05$ . Data expressed as mean  $\pm$  SD.

## Results

In this study, the effects of diabetes on plasma CK and cardiac CK isoenzyme (CK-MB) levels have been revealed in diabetic rabbits compared with control rabbits. Mean plasma CK value was found to be  $1898 \pm 586$  U/L and mean CK-MB value was  $1756.1 \pm 197$  U/L in control rabbits, whereas mean CK value was found to be  $1442.2 \pm 307$  U/L and mean CK-MB value was  $1218.4 \pm 267$  U/L in diabetic rabbits. As shown in Table 1, Figure 1 and Figure 2, there were significant decreases in CK and CK-MB values in ALX-induced diabetic rabbits.

In comparing the plasma CK and CK-MB values between diabetic and control group rabbits, we found that there were statistically significant differences as ( $t = 2.18$ ,  $p < 0.0434$ ) in CK and ( $t = 5.11$ ,  $p < 0.001$ ) in CK-MB values concerning effects of ALX-induced diabetes.

## Discussion

Creatine kinase particularly susceptible to oxidative inactivation is a sulphhydryl containing enzyme. CK is important for energy transfer that catalyses the reaction of ATP + creatine to yield ADP + phosphocreatine. The reaction stores ATP energy as phosphocreatine in muscle and brain tissue and holds the muscle concentration of ATP constant during the initiation of exercise. This enzyme is potentially vulnerable to inactivation under conditions when it would be used as a diagnostic marker of tissue damage or other oxidative tissue injury. Elevated MB creatine kinase activity is a possible indicator of myocardial injury (1,11,12).

The concentrations of plasma CK and CK-MB have been shown to decrease in diabetes mellitus (13). Also there are a few studies showing transient increase of plasma CK and CK-MB in diabetes mellitus in the literature (14). Our results provided for evidence statistically significant decreases in CK and CKMB plasma levels between ALX induced diabetes and control groups. This decreases are probably due to a decrease in CK mRNA that is found in diabetes mellitus (13). A lowering of CK mRNA suggests that decrease in the synthesis of the CK subunit protein may be responsible for the decreased CK activity or CK plasma level. These changes are reversible by 4 weeks of insulin treatment (15).

Table 1. Comparison of plasma CK and CK-MB values in ALX-induced diabetic and control rabbits.

	CK (U/L)	CK-MB (U/L)
Diabetes (n=10)	1442 $\pm$ 307*	1218 $\pm$ 267**
Control (n=10)	1898 $\pm$ 586	1756 $\pm$ 197

Results are expressed as mean  $\pm$  SD. \*  $t < 0.0434$ .

\*\*  $p = 0.0001$  compared with control rabbits.

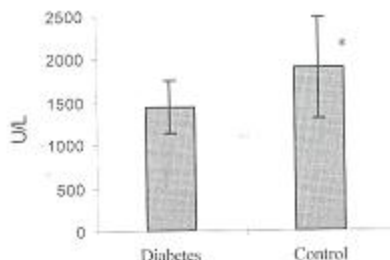


Figure 1. CK values of diabetes and control rabbits. \* $t = 2.18$ ,  $p < 0.0434$ .

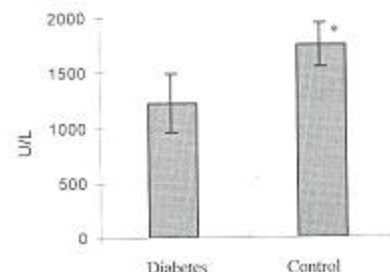


Figure 2. CK-MB values of diabetes and control rabbits. \* $t = 5.11$ ,  $p < 0.001$ .

## References

- Burtis CA, Ashwood ER: Tetz textbook of clinical chemistry. Pennsylvania, 1994.
- Ng YC, Tolerico PH, Book CB: Alterations in levels of Na(+)-K(+)-ATPase isoforms in heart, skeletal muscle, and kidney of diabetic rats. *Am J Physiol*. 265: E243-251, 1993.
- Yu Z, Tibbits GF, McNeill JH: Cellular functions of diabetic cardiomyocytes: contractility, rapid-cooling contracture, and myosinase binding. *Am J of Physiol*. 266: H2082-2089, 1994.

4. van Deursen J., Heerschap A., Oerlemans F., Ruitenbeek W., Jap P., ter Laak H., Wieringa B.: Skeletal muscles of mice deficient in muscle creatine kinase lack burst activity. *Cell* 74: 621-631, 1993.
5. Jones, MG., Swaminathan R.: The clinical biochemistry of creatine kinase. *J. Int. Fed. Clin. Chem.* 2: 108-114, 1990.
6. Lang H.: Creatine kinase isoenzymes: Pathophysiology and Clinical Application, Berlin: Springer Verlag, 1981.
7. Basow CD., Zhao ZH., Benovic F., Chan S., Friedman EA.: Effects of alloxan-induced diabetes on hematology in rabbits. *Horm. Metab. Res.* 24: 254-257, 1992.
8. Zhao ZH., Watschinger B., Brown CD., Monica M, Boyer EA.: Variation of Susceptibility to alloxan induced diabetes in the rabbit. *Horm. Metabol.* 19: 534-537, 1987.
9. Bailey CC., Bailey OT., Losch RS.: Alloxan diabetes with diabetic complications. *New Eng. J. Med.* 230: 533-536, 1994.
10. Gözül B., Kaz M., Ersoz G., Kaplan B.: Effect of EGF on the corneal wound healing of alloxan diabetic mice. *Exp. Eye. Res.* 54: 519-524, 1992.
11. Painter PC., Van Meter S., Dobbs RL., Clement GE.: Analytical evaluation and comparison of Dupont ace lactate dehydrogenase-1 (LD1) isoenzyme assay diagnostic efficiency for acute myocardial infarction detection with other LD1 methods and ace CK-MB. A two-site study. *Angiology.* 45: 585-595, 1994.
12. Chattington P., Clarke D., Neithert WD.: Creatine kinase isoenzym electrophoresis for the early confirmation of myocardial infarction detected by fixed sequential CK slope analysis. *Postgraduate Medical Journal.* 70: 805-808, 1994.
13. Bett KP., Kenneth RB., Wolfgang HD.: Diabetes decreases creatine kinase enzyme activity and mRNA level in the rat heart. *Am. J. Physiol.* 257: E573-E577, 1989.
14. Luzarov G., Damev S., Manolov D., Dobrev S.: Creatine kinase in patients with diabetes mellitus. *Vutr. Boles.* 29: 77-83, 1990.
15. Savahi F., Kirsch A.: Alteration of the phosphocreatine energy shuttle components in diabetic rat heart. *J-Mol-Cell-Carbol.* 23: 1323-1333, 1991.

#### Correspondence to:

Yrd.Doç.Dr. Muhterem Ercan  
 Yüzüncü Yıl Üniversitesi Tıp Fakültesi  
 Fizyoloji Anabilim Dalı Van