

## Effect of Experimentally Induced Hyper- and Hypocalcaemia on Myocardial Function in Goats as Assessed by the Serum Concentration of Cardiac Troponin I

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**Abstract:** The objective of this study was to determine the effect of induced hyper- and hypocalcaemia on cardiac cell damage in goats as assessed by the serum concentration cardiac troponin (cTnI). For this purpose, 24 clinically healthy does were used, divided into two equal groups (G1, hypercalcaemia; G2, hypocalcaemia). Hypercalcaemia was induced in G1 by slow IV infusion of 10% calcium gluconate (2 ml/kg BW, speed 1 mL/5 sec). Hypocalcaemia was induced in G2 goats by slow IV infusion of 5% Na<sub>2</sub>EDTA. From both groups, eleven blood samples (T0-T10) were then collected from each goat. The first blood sample was collected immediately before induction (T0). Four blood samples (T1-T4) were collected 30, 60, 120 and 240 min after injection. Blood samples 6 to 11 (T5-T10) were collected 1, 2, 3, 4, 5 and 6 days after induction. In G1, the serum concentration of calcium had increased significantly at time points T1-T4, while in G2, the serum concentration of calcium had decreased significantly ( $P < 0.0001$ ) at time points T1-T3. Compared to the pre-injection mean values of  $0.01 \pm 0.01$  ng/mL, the serum concentration of cTnI had increased significantly in G1 at T1-T4 and had maximized to a value of 2.10 ng/mL 24 h after induction (T5). Thereafter, the serum concentration of cTnI declined gradually until it returned to normal at 6 days post-induction (T10). In G2, the serum concentration of cTnI had increased significantly ( $P < 0.0001$ ) at T1-T4, then decreased at T5-T6 and at T7-T10, the serum concentration of cTnI was normal. With the exceptions of times T0, T1 and T10, the serum concentration of cTnI differed significantly between the two groups. The results of this study clearly show that a reversible cardiac injury occurred following experimentally induced hyper- and hypocalcaemia.

**Key words:** Cardiac Troponin I • Cardiomyocytes • Goat • Hypercalcaemia • Hypocalcaemia

### INTRODUCTION

Cardiac troponin I (cTnI), the “gold standard” for the non-invasive diagnosis of myocardial injury, is a protein found in the myocardial cells that initiates tropomyosin contraction [1]. Its serum concentration elevates after acute myocardial injury because of leakage from the damaged myocardial cells [2]. A persistent increase of cTnI blood concentration suggests ongoing, irreversible damage to the cardiomyocytes [2] and the degree of increase has been shown to be correlated with the extent of myocardial damage and, in humans, with survival. In veterinary medicine, cardiac troponins, especially cTnI, are highly sensitive and specific markers of myocardial injury [1]. In goats, the importance of cTnI

as a cardiac biomarker with cardiac nutritional muscular dystrophy, pregnancy toxemia and dystocia has been recently established by our group [3, 4].

In the goat, hypocalcaemia (“parturient paresis,” “lambsickness”) is an acute or subacute pathological condition. The salient features of the disorder include reduced serum concentrations of calcium, progressive paralysis of smooth and striated muscles, recumbency and loss of consciousness [5]. In contrast to “milk fever” in cows, which always occurs at calving, hypocalcaemia can develop in ewes and does several weeks before parturition, when the fetal skeletons are mineralizing and also during the first two weeks post parturition [6]. On the other hand, in intensively managed dairy flocks/herds the disease is more frequent after kidding, coinciding with the

time of peak milk production [6]. In goats, uncomplicated hypocalcaemia responds immediately (within 5 min) to the IV administration of calcium; this can also be used to confirm the diagnosis [7]. An IV administration of 30 to 60 mL of 20% calcium borogluconate solution is usually sufficient. Subsequently, an additional dose of 60 mL of calcium borogluconate, without dextrose, can be administered subcutaneously to ensure a more prolonged absorption. In view of the potential dangers associated with IV administration of calcium solutions, the SC route has been advocated as preferable to the IV route [8].

The role calcium plays in heart muscle contraction and relaxation is well established. Inflow of extracellular calcium together with that from the stimulated sarcoplasmic reticulum raises the levels of cytosolic, resulting in muscle contraction [9]. Severe extracellular hypocalcaemia impairs cardiac contractility because the sarcoplasmic reticulum is unable to maintain sufficient calcium content to initiate myocardial contraction [10]. On the other hand, hypercalcaemia affects atrial and ventricular activity and is involved in the production of arrhythmia [11]. The present study was designed to examine the effect of experimentally induced hyper- and hypocalcaemia on cardiac cell damage as assessed by the serum concentration of the cardiac biomarker cTnI.

## MATERIALS AND METHODS

**Animals and Physical Examination:** The experimental protocol was approved by the Animal Ethical Committee, Deanship for Scientific Research, Qassim University, Saudi Arabia. Twenty-four clinically healthy does (age  $38.4 \pm 6.4$  mo; weight  $47.2 \pm 3.5$  kg) were used, divided into two groups of twelve each (G1, hypercalcaemia; G2, hypocalcaemia). Enrolment of the goats in the study was based on normal findings after complete physical examination (general behaviour and condition, auscultation of the heart, lungs, rumen and intestine, measurement of heart and respiratory rates and rectal temperature), laboratory evaluation (complete blood count, chemistry profile and cTnI concentration) and echocardiography. The goats were maintained in a free-stall barn and kept under the *Laboratory Animal Control Guidelines* of Qassim University, which basically conform to the *Guide for the Care and Use of Laboratory Animals* of the National Institutes of Health in the USA (NIH publications No. 86 to 23, revised 1996).

**Induction of Hyper- and Hypocalcaemia and Blood Sampling:** Hypercalcaemia was induced experimentally in the normocalcemic goats by a slow IV infusion of 10%

calcium gluconate (14 mg calcium/mL) for about 10 min (2 ml /kg BW, speed 1 mL/ 5 sec). Hypocalcaemia was induced experimentally in the second group of normocalcemic goats by a slow IV infusion of 5% Na<sub>2</sub>EDTA (sodium salts of ethylenediamine tetra acetic acid) (BDH Chemicals Ltd., Poole England) at a dose of 1.2 mL/kg. Throughout this study, from each goat, 11 blood samples (T0-T10) were collected in plain tubes to obtain serum for cTnI, calcium, phosphorus and magnesium analyses. The first blood sample was collected immediately before calcium or EDTA injection (T0). Four blood samples (T1-T4) were collected 30, 60, 120 and 240 min after injection. Blood samples 6 to 11 (T5-T10) were collected 1, 2, 3, 4, 5 and 6 d after injection.

**Cardiac Troponin I Assay and Serum Chemistry:** Cardiac troponin I was analysed in the serum of the goats as has been recently reported [3, 4], using a point-of-care analyser (VetScan i-STAT® 1, Abaxis, California, USA) according to the manufacturer's instructions. This analyser employs a two-site enzyme-linked immunosorbant assay. All results are expressed as nanograms per millilitre (ng/mL) with intra- and inter-assay coefficient of variances of less than 5%. The lower limit of detection of cTnI for this assay was 0.02 ng/mL. The i-STAT cTnI test reports 0.00 to 50.00 ng/mL. Samples above the reportable range will yield ">50.00 ng/mL" on the analyser display screen. However, the performance characteristics of the i-STAT cTnI measurement have not been established for cTnI values above 35.00 ng/mL. Values < 0.02 ng/mL cannot be discriminated; however, the analyser provides a specific point estimate of 0.00, 0.01 or 0.02 ng/mL. The serum concentrations of calcium, phosphorus and magnesium were determined using an automated biochemical analyser (VetScan VS2, Abaxis, California, USA).

**Statistical Analysis:** Data are presented as means  $\pm$  SD and were analysed statistically using the SPSS statistical package, version 18 [12]. A repeated measures analysis of variance was used as the statistical model to evaluate the differences among T0 through T10. The Duncan test was used to calculate multiple comparisons. Results were considered significant at  $P < 0.05$ .

## RESULTS

In G1, slight shivering was observed in three goats at T0-T4, teeth grinding and groaning in five of the goats was noted at T0-T4 and stargazing in four of the goats at T2-T3. The rectal temperature and cardio-respiratory rates

Table 1: Serum concentrations of calcium, inorganic phosphorus and magnesium in the goats with experimentally induced hyper- (group 1, G1) and hypocalcaemia (group 2, G2).

	Calcium (mmol/L)		Phosphorus (mmol/L)		Magnesium (mmol/L)	
	G1	G2	G1	G2	G1	G2
T0	2.2±0.1 <sup>a</sup>	2.2±0.1 <sup>a</sup>	2.0±0.1 <sup>a</sup>	2.8±0.3 <sup>a</sup>	1.04±0.09 <sup>a</sup>	0.7±0.1 <sup>a</sup>
T1	3.1±0.3 <sup>b</sup>	1.7±0.2 <sup>b</sup>	1.8±0.2 <sup>b</sup>	2.6±0.1 <sup>b</sup>	0.96±0.05 <sup>b</sup>	0.5±0.1 <sup>b</sup>
T2	3.0±0.2 <sup>b</sup>	1.9±0.1 <sup>b</sup>	1.6±0.2 <sup>b</sup>	2.0±0.3 <sup>b</sup>	0.93±0.04 <sup>b</sup>	0.6±0.1 <sup>b</sup>
T3	3.1±0.3 <sup>b</sup>	2.0±0.1 <sup>b</sup>	1.5±0.2 <sup>b</sup>	2.0±0.3 <sup>b</sup>	0.92±0.04 <sup>b</sup>	0.7±0.1 <sup>b</sup>
T4	2.7±0.2 <sup>b</sup>	2.2±0.1 <sup>a</sup>	1.4±0.2 <sup>b</sup>	2.1±0.2 <sup>b</sup>	0.95±0.04 <sup>b</sup>	0.7±0.1 <sup>b</sup>
T5	2.2±0.1 <sup>a</sup>	2.3±0.1 <sup>a</sup>	2.0±0.1 <sup>a</sup>	2.3±0.5 <sup>b</sup>	0.93±0.05 <sup>b</sup>	0.9±0.1 <sup>b</sup>
T6	2.2±0.1 <sup>a</sup>	2.3±0.1 <sup>a</sup>	2.1±0.1 <sup>a</sup>	2.3±0.3 <sup>b</sup>	0.93±0.05 <sup>b</sup>	1.0±0.1 <sup>b</sup>
T7	2.2±0.1 <sup>a</sup>	2.2±0.2 <sup>a</sup>	2.1±0.3 <sup>a</sup>	2.4±0.2 <sup>b</sup>	0.93±0.05 <sup>b</sup>	0.9±0.1 <sup>b</sup>
T8	2.2±0.1 <sup>a</sup>	2.3±0.1 <sup>a</sup>	2.2±0.7 <sup>a</sup>	2.2±0.2 <sup>b</sup>	0.91±0.05 <sup>b</sup>	1.0±0.1 <sup>b</sup>
T9	2.2±0.1 <sup>a</sup>	2.3±0.1 <sup>a</sup>	2.2±0.7 <sup>a</sup>	2.2±0.3 <sup>b</sup>	0.90±0.04 <sup>b</sup>	1.1±0.1 <sup>b</sup>
T10	2.2±0.1 <sup>a</sup>	2.3±0.1 <sup>a</sup>	2.4±0.9 <sup>a</sup>	2.1±0.4 <sup>b</sup>	0.92±0.03 <sup>b</sup>	1.1±0.1 <sup>b</sup>

G1, group 1; G2, group 2; T0, immediately before calcium or EDTA injection; T1-T4, 30, 60, 120 and 240 min after induction; T5-T10, 1, 2, 3, 4, 5 and 6 day after induction. <sup>a,b</sup>Differ significantly ( $P < 0.05$ ) in the same column.

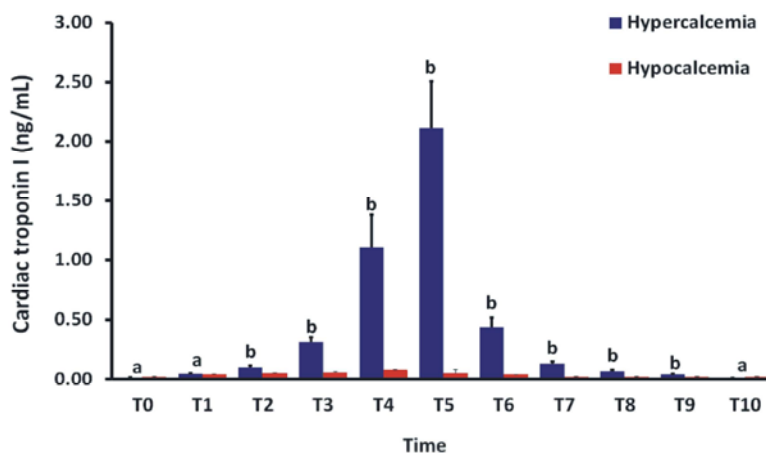


Fig 1: Mean  $\pm$ SD serum concentrations of cardiac troponin I in goats with experimentally induced hyper- and hypocalcaemia. T0, immediately before calcium or EDTA injection; T1-T4 at 30, 60, 120 and 240 min after injection; T5-T10 at 1, 2, 3, 4, 5 and 6 day after injection. <sup>a,b</sup>Differ significantly between goats with induced hyper- and hypocalcaemia.

did not change significantly. On the contrary, the rumen contractions decreased significantly at T1-T4 ( $P < 0.0001$ ) and at T5-T10 returned to normal values. In G2, three goats showed frequent urination and defecation at T1-T4, one had diarrhoea at T4 and three temporarily staggered and fell repeatedly. All of the goats showed signs of dullness, ruminal stasis and decreased heart rate intensity at T1-T4. Two of the goats did not display any abnormal signs. The rectal temperature and respiratory rates did not change significantly.

A summary showing the serum concentrations of calcium, phosphorus and magnesium in G1 and G2 can be seen in Table 1. In G1, the serum concentration of calcium had increased significantly ( $P < 0.0001$ ) at time points T1-T4. In contrast, the serum concentration of inorganic phosphorus had decreased significantly ( $P < 0.001$ ) at the

same time points. The serum concentration of magnesium remained significantly lower ( $P < 0.05$ ) at all time points after calcium injection (T1-T10). In G2, the serum concentration of calcium had decreased significantly ( $P < 0.0001$ ) at time points T1-T3. The serum concentrations of inorganic phosphorus and magnesium remained significantly low ( $P < 0.05$ ) at all time points after calcium injection (T1-T10).

Figure 1 summarizes the mean serum concentrations of cTnI in the goats in response to experimentally induced hyper- and hypocalcaemia. In G1, compared to pre-injection mean values of  $0.01 \pm 0.01$  ng/mL, the serum concentration of cTnI had increased significantly ( $P < 0.0001$ ) at 30, 60, 120 and 240 min to values of 0.05, 0.10, 0.30 and 1.10 ng/mL, respectively. Twenty-four h post-induction, the serum concentration of cTnI

maximized to a value of 2.10 ng/mL ( $P < 0.0001$ ). Subsequently, the serum concentration of cTnI declined gradually to values of 0.44, 0.13, 0.08 and 0.04 ng/mL on days 2, 3, 4 and 5, respectively. By day 6, the serum concentration of cTnI had decreased (0.01 ng/mL) with no statistically significant difference compared to pre-injection values ( $P = 0.17$ ). In G2, compared to pre-injection values of  $0.02 \pm 0.01$  ng/mL, the serum concentration of cTnI had increased significantly ( $P < 0.0001$ ) at 30, 60, 120 and 240 min to values of 0.04, 0.05, 0.06 and 0.08 ng/mL, respectively. The serum concentration of cTnI had decreased to a value of 0.05 and 0.04 ng/mL on days 1 and 2 post-injection. On days 3 to 6, the serum concentration of cTnI was stable at 0.01 ng/mL; this value did not differ significantly compared to pre-injection values ( $P > 0.05$ ). With the exceptions of times T0, T1 and T10, the serum concentration of cTnI differed significantly ( $P < 0.01$ ) between the two groups.

## DISCUSSION

To the authors' knowledge, this is the first study to evaluate the effect of experimentally induced hyper- and hypocalcaemia on the cardiovascular function in goats as assessed by the serum concentration of the cardiac biomarker cTnI.

In humans and in several animal species, measurement of cTnI serves as a useful biomarker of myocardial injury and has been shown to be both highly sensitive and specific for cardiomyocyte damage LaVecchio *et al.* [13]. The unique aspect of cTnI as 100% tissue-specific for the heart makes it an excellent marker to serve as a biochemical tool for detecting myocardial injury. We have reported recently that, in goats, cTnI is elevated with prolonged birth, pregnancy toxemia and in those with cardiac nutritional muscular dystrophy [3, 4].

In the present study, the cTnI concentrations increased significantly in both induced hyper- and hypocalcaemia groups. However, in the hypercalcaemia group, the increases of serum cTnI were remarkable compared to baseline values and maximized (2.1 ng/mL,  $P < 0.0001$ ) at T5 (24 h post-induction). It remained significantly elevated for the following 5 days. In contrast, in the group with induced hypocalcaemia, although significant compared to baseline values, the elevations were mild and maximized (0.08 ng/mL) at T4 (240 min post-induction). Serum elevations remained high for only 2 days post-induction of hypocalcaemia.

Cardiac troponin I is reported to have a very high specificity for the myocardium and it is therefore unlikely that these increases are related to cross-reactivity with

skeletal muscle [2]. Because the goats used in this study were clinically healthy and had no cardiac disease to cause the increases in cTnI seen post-induction, it is therefore likely that the increases in cTnI seen in the goats are related to hyper- and/or hypocalcaemia. Recently, our group reported that cTnI elevations in goat kids with nutritional muscular dystrophy were accompanied histologically with severe myocardial degeneration and Zenker's necrosis of the heart muscle [4]. Unfortunately, in the present study, histopathological examination of cardiac specimens was not performed.

Ionized calcium has a central role for regulating myocardial contraction. During activation of the cardiac action potential, ionized calcium enters intracellularly through depolarization-activated calcium channels. This ionized calcium triggers the release of calcium from the sarcoplasmic reticulum. The  $\text{Ca}^{2+}$  binds to the myofilament proteins such as troponin C, thus initiating contractions of the myocardium [10]. There are two main ways to change the contractility of the myocardium. One is by altering the amplitude or duration of transient  $\text{Ca}^{2+}$ ; another is by altering the sensitivity of the myofilaments to  $\text{Ca}^{2+}$  [10]. Therefore, hypocalcaemia-induced DCMP is developed by the altering of the amplitude or duration of transient  $\text{Ca}^{2+}$ .

The reasons behind the increases found in cTnI concentrations after induction of both hyper- and hypocalcaemia are not clear. Hypocalcaemia causes not only heart failure, but also elevating cardiac enzymes [14]. It is explained that, in the condition of hypocalcaemia, cell membrane potential is lower, which increases cell membrane permeability and muscle enzyme leakage from the cells [15]. In the Pallidis [14] study, the elevated cardiac enzyme usually returned to normal after treatment for hypocalcaemia. In humans, hypocalcaemia has been documented as a reversible cause of heart failure and dilated cardiomyopathy [16]. Although the kinetics of intracellular calcium is clearly related to muscle contraction and relaxation, the mechanism of myocardial dysfunction secondary to hypocalcaemia is not fully understood [9].

In cows with clinical hypocalcaemia, IV treatment with calcium rapidly increases blood calcium concentrations to extremely high and potentially dangerous levels [17]. Extremely high blood calcium concentrations may cause fatal cardiac complications [18, 19]. In cows not responding to calcium therapy, necropsy revealed that invariably, multifocal myocardial necrosis was found in the heart. The lesion was often found to be accompanied with cellular infiltration and interstitial fibrosis [20]. In goats with hypocalcaemia,

IV administration of calcium must be performed slowly, over 5 to 7 minutes, while the clinician monitors the animal's heart rate and rhythm; administration should be stopped at once if there is evidence of arrhythmia [6].

In conclusion, the results of this study clearly show that a reversible cardiac injury occurred in the goats following experimentally induced hyper- and hypocalcaemia, as assessed by serum concentrations of cTnI. In the goats with induced hypercalcaemia, the cTnI elevations were remarkable and long-lasting compared to the short-lived mild elevations in the goats with induced hypocalcaemia.

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