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dl-3-Hydroxybutyrate administration prevents myocardial damage after coronary occlusion in rat hearts

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Zou, Zhitian, Shiro Sasaguri, Katare Gopalrao Rajesh, and Ryoko Suzuki. *dl*-3-Hydroxybutyrate administration prevents myocardial damage after coronary occlusion in rat hearts. *Am J Physiol Heart Circ Physiol* 283: H1968–H1974, 2002; 10.1152/ajpheart.00250.2002.—To investigate the role of high concentrations of *dl*-3-hydroxybutyrate (DL-3-HB) in preventing heart damage after prolonged fasting, infarct size and the incidence of apoptosis caused by ischemia-reperfusion were determined in four groups of Wistar rats. Fed rats (±DL-3-HB group) and fasted rats (±DL-3-HB group) were subjected to 30 min of left coronary artery occlusion and 120 min of reperfusion. DL-3-HB was administered intravenously 60 min before the coronary artery occlusion. Infarct size, defined by triphenylyetrazolium chloride (TTC) staining, was reduced from 72 ± 3% (fed group), 75 ± 5% (fed + DL-3-HB group), and 70 ± 5% (fasting group), respectively, to 26 ± 4% ($P < 0.01$ vs. fasting + DL-3-HB group). Apoptosis, as defined by single-stranded DNA staining, was significantly reduced in the subendocardial region in the fasting + DL-3-HB group (9 ± 2%) compared with the other groups (39 ± 6% in the fed group, 37 ± 5% in the fed + DL-3-HB group, and 34 ± 3% in the fasting group; $P < 0.01$). In addition, levels of ATP in the fasting + DL-3-HB group were significantly higher compared with other groups after 30 min of ischemia and 120 min of reperfusion ($P < 0.01$). In conclusion, the present study demonstrates that high concentrations of DL-3-HB reduces myocardial infarction size and apoptosis induced by ischemia-reperfusion, possibly by providing increased energy substrate to the fasted rat myocardium.

infarct size; apoptosis; fasting

MYOCARDIAL ISCHEMIA initiates a sequence of cellular changes that culminates in irreversible injury and cell death. Initially, mitochondrial oxidative phosphorylation is severely reduced, followed by a decline in the levels of ATP and creatine phosphate (19, 28). Despite the cessation of contractile activity, continued cellular metabolism results in further ATP and creatine phosphate depletion (20). Coronary artery reperfusion enables an almost immediate restoration of myocyte creatine phosphate levels, whereas ATP repletion occurs over hours to days. This energy depletion appears to play a vital role in determining cell viability and whether contractile function resumes on reperfusion (5, 20). Consequently, drugs that either prevent the rapid decline in myocyte ATP levels during ischemia or that augment the rate of repletion of myocyte high-energy phosphate could be of potential therapeutic benefit to the ischemic myocardium.

dl-3-Hydroxybutyrate (DL-3-HB), one of the main ketone bodies, is available in the circulation for metabolic utilization by peripheral tissues, including the myocardium (23). When hearts were perfused with DL-3-HB as a sole exogenous substrate, the ketone body accounted for 50% of the energy needs of hearts from fed rats and 74% of the energy needs of hearts from fasted rats (26, 27). Tradif et al. (30) reported that DL-3-HB inhibits glucose utilization and oxidation when hearts are perfused with medium containing glucose and DL-3-HB. In the canine heart, DL-3-HB can modulate the cardiac phosphorylation potential in vivo (10). However, the effects of DL-3-HB on myocardial preservation in the prolonged fasted rat when DL-3-HB is the sole exogenous substrate have not been investigated. In this study, we investigated the role of high concentrations of DL-3-HB in preventing reperfusion injury of heart after prolonged fasting (84 h) using a rat model of 30 min of regional ischemia followed by 120 min of reperfusion.

MATERIALS AND METHODS

All animals in this study received humane care in compliance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (NIH Publication No. 86-23, Revised 1985).

Animals. Male Wistar rats weighing between 280 and 300 g were used. The animals were kept at a constant room temperature (22–25°C) with 12:12-h light-dark cycles. The fed animals were given food ad libitum, whereas fasted animals were deprived of food for 84 h but given free access to water.

General procedures. The experimental model was described previously (4) with slight modifications. After the animals were anesthetized (50 mg/kg ip pentobarbital sodium), the lungs were ventilated with 95% O_2-5% CO_2 with the use of a small rodent ventilator (Harvard) set at a tidal volume of 2.5 ml/stroke and at a rate of 70 breaths/min. Monitoring was performed throughout the procedure with a lead II electrocardiogram. Blood pressure was measured via a cannula introduced into the left carotid artery. In the

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Table 1. *Heart rate and blood pressure among study groups*

Groups	Preischemia	30-Min Ischemia	120 Min After Reperfusion
Fed			
HR, beats/min	433 ± 12	414 ± 16	411 ± 21
MAP, mmHg	106 ± 8	93 ± 5	91 ± 6
Fed + DL-3-HB			
HR, beats/min	429 ± 11	410 ± 15	407 ± 13
MAP, mmHg	105 ± 9	95 ± 4	96 ± 6
Fasting			
HR, beats/min	411 ± 15	394 ± 12	387 ± 13
MAP, mmHg	109 ± 9	95 ± 4	94 ± 5
Fasting + DL-3-HB			
HR, beats/min	414 ± 16	396 ± 9	390 ± 13
MAP, mmHg	107 ± 7	97 ± 6	96 ± 5

Values are means ± SE; $n = 8$ rats/group. HR, heart rate; MAP, mean arterial pressure; DL-3-HB, *dl*-3-hydroxybutyrate.

DL-3-HB (Sigma; St. Louis, MO)-treated groups, the drug (25 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) was administered intravenously using a syringe pump 60 min before left coronary artery occlusion.

Surgical procedures. The chest was opened by left thoracotomy at the fourth or fifth intercostal space, and the ribs were gently spread with a small retractor. The pericardium was carefully dissected and retracted, and the left main coronary artery was identified. A 7-0 prolene suture with an atraumatic needle was used to occlude the left main coronary artery. The needle was inserted ~0.5 mm into the myocardium, 2–3 mm away from the origin of the left coronary artery (just beneath the left auricle appendage), after the left auricle appendage was pushed aside with a small noncrushing microforceps. The suture was then connected to the snare and a special elastic arch. The snare was then tightened and coronary artery occlusion was confirmed by ST segment elevation in the electrocardiogram and the presence of regional cyanosis in the myocardium. Thirty minutes after occlusion, the snare was released, and reperfusion of the myocardium was visually confirmed. The heart was then reperfused for 120 min. The heart was arrested in diastole with an overdose of KCl and rapidly excised at the end of the experiment. To analyze the levels of plasma metabolites and hormones in the rats, 3 ml of blood were drawn from the abdominal aorta after 120 min of reperfusion. The blood samples were then centrifuged at 4°C, and the plasma was separated and stored at −80°C until further analysis. Plasma glucose was determined as described previously (3). The concentration of serum insulin was measured with a double-antibody radioimmunoassay kit (Insulin RIA Kit, Dinabbott RI Institute; Tokyo, Japan) using porcine insulin as a standard. Lactate was assayed with an immobilized enzyme technique (Yellow Springs Instruments 2300, STAT Plus). Serum free fatty acid (FFA) levels were determined using an acyl-CoA oxidase based colorimetric kit (NEFA-C; Wako Chemical; Osaka, Japan). Plasma concentrations of ketone bodies were analyzed enzymatically according to the method described by Uno et al. (31). Furthermore, plasma pH values were assessed with a pH meter (model F-8L; Horiba).

Definition of area at risk and area of infarction. Infarct size and ischemic risk area were determined as described previously (8). At the end of reperfusion, the left coronary artery was reoccluded and the heart was excised. Evans blue solution (0.5 ml) was slowly injected into the ascending aorta to delineate the risk area as a perfusion defect. The excised hearts were cut transversely into four 1.5-mm-thick slices. Each slice was then incubated in a 2% solution of triphenyltetrazolium chloride in phosphate buffer for 5 min at 37°C and pH of 7.4 to stain the viable myocardium a brick-red color. The slices were imaged in color at ×8 magnification by a charge-coupled device digital camera (Coolpix 990, Nikon) and stored on a Windows-based personal computer for image analysis. The area stained by the Evans blue dye (perfused area), the unstained area (area at risk; AR), and the area of infarction (AI) were defined with the use of image analysis software (IPLab/Mac Image). The AR normalized by the left ventricular area (AR/LV) and the AI normalized by the AR (AI/AR) were calculated. The heart slices were fixed with 20% formalin and embedded in paraffin for subsequent single-stranded DNA labeling (ssDNA).

Single-stranded DNA labeling. Paraffin sections were processed to determine apoptosis by the ssDNA method, as described earlier (2). In brief, after serial treatment with alcohol, the paraffin sections were incubated overnight with the primary antibody (Anti-single-stranded DNA, Dako), followed by a 30-min incubation with the secondary antibody (Anti-Rabbit Ig Fab, Dako). The sections were then incubated with peroxidase-labeled streptoavidin for 30 min, stained with DAB solution, and mounted to allow viewing of the apoptotic cells. Finally, the sections were counterstained with methyl green. After the procedures, border areas among the subendocardial regions in the risk area were examined by microscopy at ×200 magnification, and the ssDNA-positive myocyte nuclei were counted and expressed as a percentage of the total number of myocyte nuclei in each region (%AP).

Myocardial energy metabolic analysis. For the analysis of myocardial ATP, a full-thickness sample of the myocardium was taken from the risk area after reoccluding the left main coronary artery with already existing suture in the added experiment (each group contained 6 animals). After being weighed, the sample was immediately immersed in liquid nitrogen and stored at −80°C for subsequent analysis. The frozen sample was pulverized with a pestle and mortar precooled in liquid nitrogen and extracted with Tris·HCl buffer at pH 8.0. The supernatant extract was analyzed for ATP content in a bioluminescence luminometer (model LB9501,

Table 2. *Plasma metabolites and pH at end of experiments in rats*

Groups	Fed	Fed + DL-3-HB	Fasting	Fasting + DL-3-HB
Glucose, mg/dl	139 ± 7	137 ± 6	99 ± 4[a,b]	101 ± 3[a,b]
Lactate, μmol/l	1,356 ± 60	1,471 ± 51	2,595 ± 113[c,d]	2,761 ± 132[c,d]
Insulin, ng/ml	2.67 ± 0.20	2.45 ± 0.21	1.30 ± 0.14[c,d]	1.40 ± 0.17[c,d]
FFA, meq/l	0.47 ± 0.03	0.49 ± 0.03	1.31 ± 0.13[c,d]	1.41 ± 0.20[c,d]
β-Hydroxybutyrate, μmol/l	388 ± 31	851 ± 91[c]	802 ± 86[c]	1,735 ± 161[c,d,e]
Acetoacetate, μmol/l	124 ± 13	303 ± 28[c]	242 ± 24[c]	513 ± 42[c,d,e]
pH	7.39 ± 0.02	7.24 ± 0.01[c]	7.38 ± 0.02	7.22 ± 0.02[c,e]

Values are means ± SE. FFA, free fatty acid. [a]$P < 0.05$ vs. respective fed group value; [b]$P < 0.05$ vs. respective fed + DL-3-HB group value; [c]$P < 0.01$ vs. respective fed group value; [d]$P < 0.01$ vs. respective fed + DL-3-HB group value; [e]$P < 0.01$ vs. respective fasting group value.

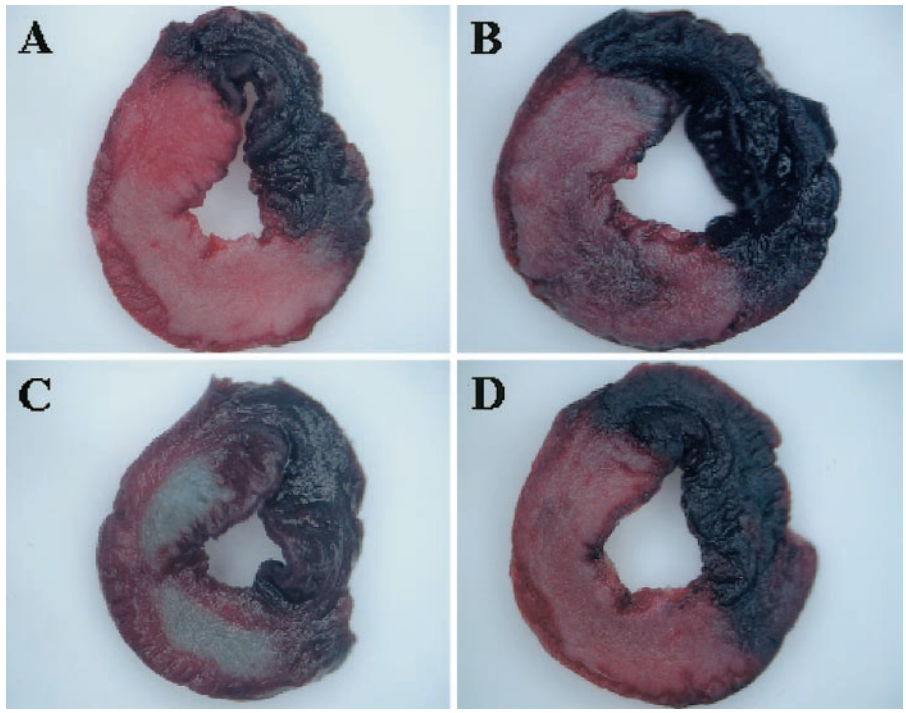

Fig. 1. Representative samples stained by Evans blue and triphenyltetrazolium chloride (TTC). Evans blue dye stains the nonrisk areas blue. The viable myocardium is stained red by TTC. The following groups are shown: fed group (*A*); fed + *dl*-3-hydroxybutyrate (DL-3-HB) group (*B*); fasting + DL-3-HB group (*C*); and fasting group (*D*).

Lumat) using a commercially available ATP bioluminescence assay kit (Promega) (11). All metabolite data are expressed as micromoles per gram of wet weight.

Myocardial tissue glycogen assay. In the fed group, the fasting 24-h group, and fasting 84-h group ($n = 3$), cardiac glycogen was measured from freeze-clamped tissue with the spectrophotometric method (25). Glycogen content is expressed as milligrams per gram dry weight.

Experimental protocols. Thirty-two rats were randomly assigned to one of four experimental groups. *1*) Fed group: fed rats ($n = 8$) were given 2 ml of saline as an intravenous infusion over 60 min before left coronary artery occlusion. *2*) Fed + DL-3-HB group: fed rats ($n = 8$) were treated with DL-3-HB (25 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ dissolved in 2 ml of saline) as an intravenous infusion over 60 min before left coronary artery occlusion. *3*) Fasting group: rats were fasted for 84 h ($n = 8$) and given 2 ml of saline as an intravenous infusion over 60 min before left coronary artery occlusion. *4*) Fasting + DL-3-HB group: rats were fasted for 84 h ($n = 8$) and treated with DL-3-HB (25 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ dissolved in 2 ml of saline) as an intravenous infusion over 60 min before left coronary artery occlusion.

Statistical analysis. All results are expressed as means ± SE. Differences in hemodynamics between groups were compared using two-way ANOVA. Other parameters were analyzed by one-way ANOVA, followed by the Tukey-Kramer multiple test. A P value of <0.05 was considered statistically significant.

RESULTS

Body mass and hemodynamics. The body mass of the rats used for the study was restricted to 280–300 g. The average weight of each group was the following: fed group, 296 ± 4 g; fed + DL-3-HB group, 293 ± 3 g; fasting group, 295 ± 3 g; and fasting + DL-3-HB group, 295 ± 4 g. After 84 h of starvation, the rats in the fasting group and the fasting + DL-3-HB group weighed 245 ± 4 and 249 ± 4 g, respectively. As expected, body mass decreased in the fasted rats, but there was no significant difference between fasting group and fasting + DL-3-HB group.

Detailed information regarding heart rate and mean arterial pressure during the experiment is summarized in Table 1. In all groups, there was no significant difference in heart rate, suggesting that a high concentration of DL-3-HB has no influence on heart rate. In addition, the mean arterial pressure did not significantly differ among the four groups at baseline and during ischemia and reperfusion.

Plasma metabolites and hormones. Plasma metabolite levels at the end of the experiment are summarized in Table 2. There were no significant changes in the concentrations of FFA, glucose, insulin, and lactate between the two groups of fed rats (fed group and fed + DL-3-HB group) or fasted rats (fasting group and fasting + DL-3-HB group). However, plasma concentrations of FFA and lactate were higher under the fasted condition (with and without DL-3-HB) than those in the fed group. Glucose and insulin levels in fasting groups and fasting + DL-3-HB group were lower when compared with those in the fed group. Concentrations of β-hydroxybutyrate and acetoacetate were significantly elevated in the fasting + DL-3-HB group when compared with those in three other groups. Futhermore, the levels of β-hydroxybutyrate and acetoacetate were also higher in fasting and fed + DL-3-HB groups when compared with fed groups. This indicates that endogenous ketone bodies are generated under starvation, and high concentrations of exogenous DL-3-HB contribute to the further increase in ketone bodies. pH decreased with DL-3-HB infusion, but there was no

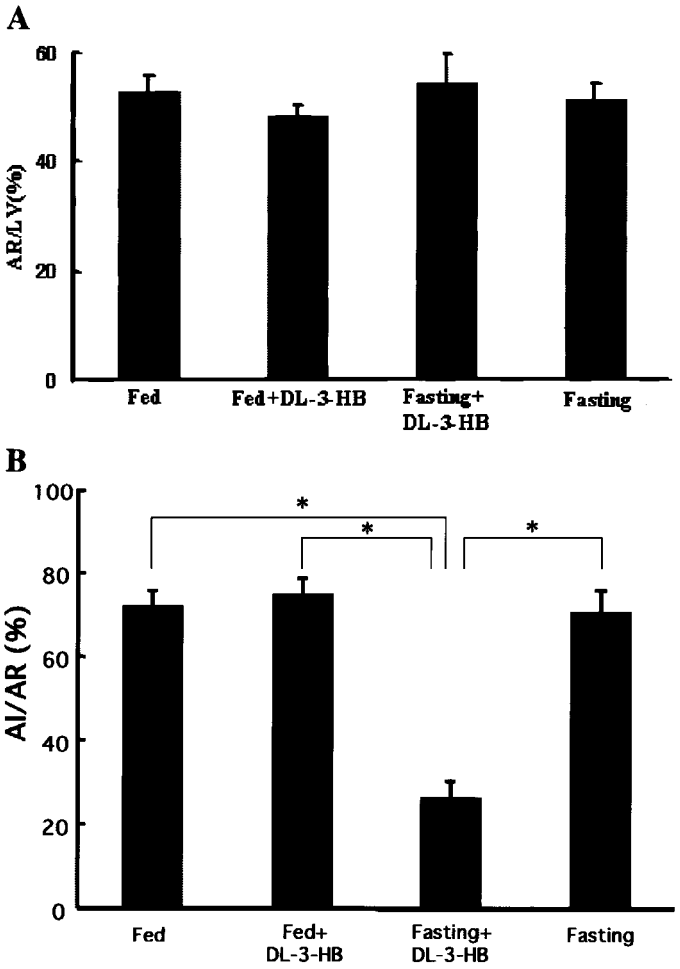

Fig. 2. Bar graphs represent area at risk (AR) by the whole left ventricular area (LV) (AR/LV; *A*) and area of infarction (AI) by the AR (AI/AR; *B*). AR/LV in all groups are identical. AI/AR in fasting + DL-3-HB group was significantly smaller than in other groups. $*P <$ 0.01 between groups. The error bar shows means ± SE.

significant difference between the fed + DL-3-HB and fasting + DL-3-HB groups.

Myocardial infarct size. Figures 1 and 2 illustrate AR/LV and AI/AR for these experiments. AR/LV did not differ significantly among the four groups (53 ± 3% in the fed group, 48 ± 2% in the fed + DL-3-HB group, 51 ± 3% in the fasting group, and 54 ± 6% in the fasting + DL-3-HB group). However, AI/AR was significantly smaller in the fasting + DL-3-HB group (26 ± 4%) than in the other three groups (72 ± 3% in the fed group, 75 ± 5% in the fed + DL-3-HB group, and 70 ± 5% in the fasting group; $P < 0.01$ vs. fasting + DL-3-HB group).

Prevalence of apoptotic cells. ssDNA staining was used to study apoptosis among the study groups. ssDNA-positive myocyte nuclei were counted in the histological sections. The normal myocardium did not reveal any apoptotic cells. The infarcted zone revealed very few apoptotic cells because most of the cardiomyocytes in the infarcted zone had already revealed necrotic changes. Hence, there was no difference in the percentage of apoptotic cells among all of the groups in the infarcted area ($P > 0.05$). Figure 3 illustrates ssDNA-positive nuclei at ×200 magnification. The ssDNA-positive nuclei were observed in the AR and were located mainly in the border area among the subendocardial region. Figure 4 illustrates %AP in the subendocardial region. The %AP was reduced significantly in the fasting + DL-3-HB group (9 ± 2%) compared with other groups (39 ± 6% in the fed group, 37 ± 5% in the fed + DL-3-HB group, and 34 ± 3% in the fasting group; $P < 0.01$ vs. fasting + DL-3-HB group).

Myocardial ATP. Figure 5 shows the levels of myocardial ATP in each group before and after 30 min of ischemia and after 120 min of reperfusion. Myocardial samples obtained at the preischemic period demonstrated that administration of DL-3-HB and/or fasting did not alter the normal physiological levels of ATP. After 30 min of ischemia, the levels of ATP in the fasted groups were increased when compared with the fed groups ($P < 0.01$). Furthermore, under the fasted condition, administration of DL-3-HB raised the myocardial ATP significantly ($P < 0.01$ vs. fasting group). After 120 min of reperfusion, the levels of ATP were significantly higher in the fasting + DL-3-HB group than in the other groups ($P < 0.01$). Although the increase of myocardial ATP was different between the fasting group and the fasting + DL-3-HB group after 30 min of ischemia, ATP levels were higher in the fasting group compared with the fed group. This suggests that the endogenous ketone may have an effect on ATP levels. However, after 120 min of reperfusion, there was no significant difference in ATP levels between the fasting group and the fed groups.

Myocardial glycogen. Table 3 summarizes changes in myocardial glycogen with a different fasting time. After 24 h of fasting, the levels of myocardial glycogen increased significantly when compared with those in the fed group. However, after prolonged fasting for 84 h, the levels of myocardial glycogen dropped, showing no difference with the fed group. This suggests that prolonged fasting gradually reduces myocardial glycogen concentrations.

DISCUSSION

The results of the present study suggest a new scheme of myocardial protection in the prolonged fasting state: prevention of myocardial necrosis and apoptosis by the exogenous acetyl CoA supplementation with the use of the short carbon derivative DL-3-HB.

Development of myocardial necrosis after a period of ischemia is directly related to the ATP stores (22). During ischemia, the cells maintain ATP levels via glycolysis, leading to an accumulation of lactic acid, a decrease in intracellular pH, and a subsequent inhibition of contraction and glycolysis. The Na^+/H^+ antiporter helps to correct this decrease in pH, resulting in high intracellular Na^+ concentrations. After a 30-min ischemic period, ATP stores decrease to <9% of the preischemic levels (22). Furthermore, Na^+-K^+-ATPase is inhibited, resulting in an inability drive out

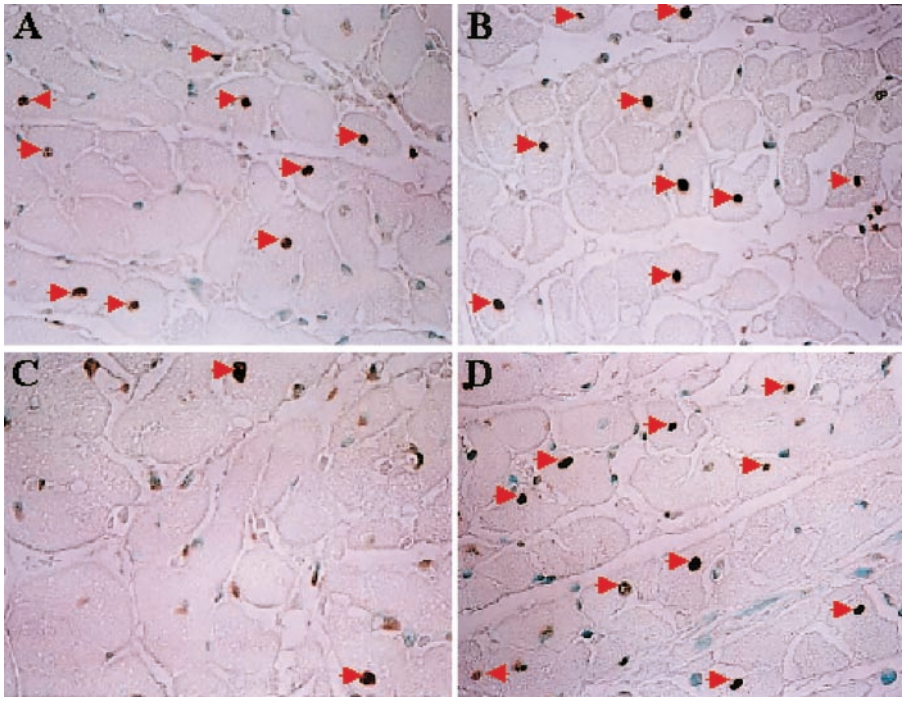

Fig. 3. Paraffin sections stained by ssDNA and methyl green are shown (×200 magnification). *A*: risk area in fed group. *B*: risk area in fed + DL-3-HB group. *C*: risk area in fasting + DL-3-HB group. *D*: risk area in fasting group. Red arrowheads point to myocyte nuclei stained by ssDNA in brown, which indicate apoptotic cells. ssDNA-positive nuclei are observed in subendocardial region.

the accumulated Na^+, thereby promoting cell death secondary to resulting Ca^{2+} accumulation (6).

Previous studies (25, 32) have demonstrated that fasting for 24 h can precondition the myocardium to ischemic injury by enhancing glycogen utilization and thus providing the ATP required for the cell to overcome the ischemic insult. However, this is the first study to evaluate the effect of prolonged fasting on ischemic injury. The glycogen stores that provide energy during short-term fasting is superceded with prolonged fasting by the ketone bodies. Endogenous levels of these ketone bodies can provide energy under normal conditions, but are insufficient to overcome ischemic insults. Hence, we investigated the hypothesis that supplementation of exogenous ketone bodies during a prolonged fasting state would result in maintenance of ATP levels and preserve the myocardium.

Our data, obtained after 30 min of ischemia and 120 min of reperfusion in the rats fasted for 84 h, demonstrated that reperfusion injury could be prevented by supplementation with DL-3-HB. We demonstrated sig-

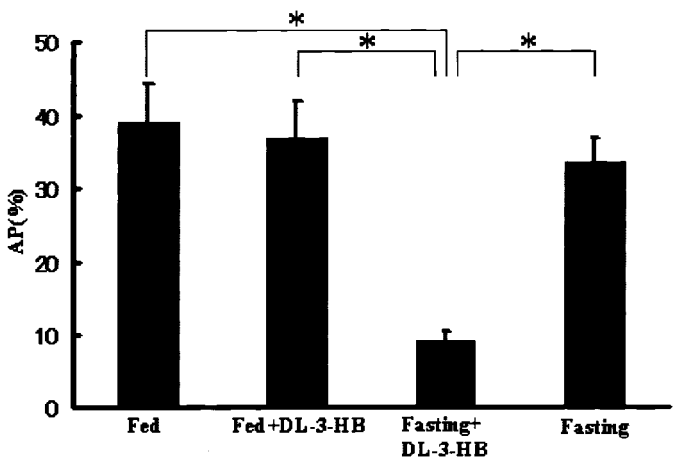

Fig. 4. %AP in risk area. Solid bars represent the effect of high-concentration DL-3-HB on %AP in risk area in the hearts of fasted rats. Fasting + DL-3-HB group significantly reduced ssDNA-positive myocyte nuclei in subendocardial region. *$P < 0.01$ between groups. The error bars show means ± SE.

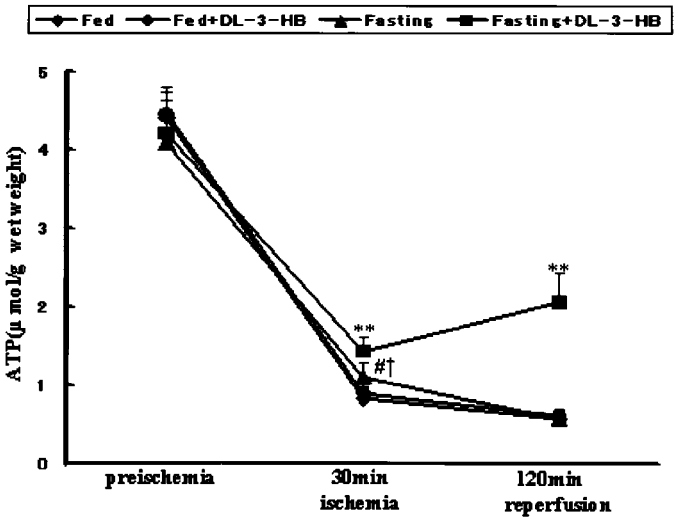

Fig. 5. Linear graphs show the effect of DL-3-HB on myocardial ATP levels in the fed and fasting rats before and after 30 min of ischemia and after 120 min of reperfusion ($n = 6$). After 30 min of ischemia, the levels of ATP were higher in the fasting group when compared with two fed groups. However, the levels of ATP in the fasting + DL-3-HB group were significantly higher than in the other three groups after 30 min of ischemia and 120 min of reperfusion. **$P <$ 0.01 vs. the other three groups, respectively; †$P < 0.05$ vs. the fed + DL-3-HB group; #$P < 0.01$ vs. the fed group. The error bars show means ± SE.

Table 3. *Changes of myocardial glycogen in different fasting times*

	Fed	Fasting for 24 h	Fasting for 84 h
Glycogen, mg/g dry wt	5.14 ± 0.18	6.51 ± 0.24*	4.59 ± 0.22†

Values are means ± SE. *$P < 0.01$ vs. respective fed group value; †$P < 0.01$ vs. respective fasting for 24 h group value.

nificant reduction in the area of myocardial infarction and maintenance of ATP levels in fasting + DL-3-HB. DL-3-HB is an alternative substrate for the energy production in the ischemic myocardium, and conversion of DL-3-HB to acetyl CoA is facilitated in cardiac cells due to high enzyme activities (29). Residual aerobic production of ATP by DL-3-HB may occur secondary to direct entry of DL-3-HB into the mitochondria (13, 15). In our study, the low concentration of endogenous ketone bodies failed to preserve the myocardial ATP levels whereas exogenous supplementation (to ~40 times the original concentration) prevented the loss of ATP by ischemic injury.

DL-3-HB has the ability to inhibit lipolysis (7), thereby inhibiting the production of the fatty acid that is implicated in extending the myocardial injury (18). In addition, inhibition of fatty acid utilization reduces oxygen demand of adjacent normal myocardial tissue (12), preventing the extent of cellular damage (16). Thus high concentrations of DL-3-HB may prevent myocardial damage by preventing the formation of damaging intermediates as well as by serving as an alternate energy source.

Myocardial ischemia triggers apoptosis (1, 9). During myocardial ischemia, ongoing cellular metabolism contributes to ATP depletion. With the loss of ATP in the ischemic tissue, myocytes reach an energy threshold that triggers apoptosis (21). Cell death can be altered in cultured cell experiments by changing ATP depletion: ATP loss ≥50% was invariably followed by apoptosis, whereas ATP loss ≥70% changed the mode of cell death from apoptosis to necrosis (14). In the present study, we demonstrated that myocardial apoptosis was reduced by intravenous DL-3-HB before coronary artery occlusion, consistent with increased ATP levels in the fasting + DL-3-HB group. Although the mechanism is not completely clear, we speculate that sufficient ATP provided by high concentrations of DL-3-HB may prevent apoptosis, particularly in the border zone between infarct area and noninjured myocardial tissue.

The role of DL-3-HB in preventing myocardial ischemia may be related to metabolic changes similar to fasting. During fasting, several metabolic adaptations occur, including increased concentrations of lipid-derived substrates. FFA and ketone bodies act as the energy sources for the normal myocardium. They regulate cardiac metabolism through inhibition of pyruvate dehydrogenase, a common enzyme required for the tricarboxylic acid cycle processing of glucose, pyruvate, and lactate. The ketone bodies are effective substrates for oxidative phosphorylation in the heart and are utilized over other metabolic substrates, including nonesterfied fatty acid and lactate in the canine heart (17, 24). Because of concentration-dependent dynamics, increases in ketone bodies during fasting can elevate the rate of DL-3-HB utilization (27). This is consistent with our data demonstrating elevated levels of ketone bodies after supplementation with DL-3-HB. Interestingly, the administration of high concentrations of DL-3-HB in fed animals (up to fourfold than the dose used in the fasted group) did not result in increased rates of DL-3-HB utilization (the infarct size was 69 ± 5%, not significantly different from fed group). This suggests that fasting stimulates utilization and oxidation of DL-3-HB, thereby supplying sufficient energy for an ischemic myocardium.

In conclusion, the present study demonstrates that high concentrations of DL-3-HB reduces myocardial infarction size and apoptosis induced with ischemia-reperfusion after prolonged fasting, possibly by increasing myocardial ATP levels. Further studies concerning the potential clinical use for DL-3-HB may be beneficial.

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