

# Clinical motivation for $^{31}\text{P}$ MRS studies on the myocardial energy metabolism of brain dead cats

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**Abstract.** Hemodynamic instability of the brain dead potential heart donor is an exclusion criterion for heart donation for transplantation. Based on the results of myocardial biopsies it has been reported that brain death-related catecholamine induced damage of the heart causes depletion of high-energy phosphates which could explain contractile dysfunction. Our group has shown in a series of  $^{31}\text{P}$  MRS experiments in cats that neither the onset of brain death, nor the hemodynamic deterioration which follows, nor its treatment with high dosages of dopamine affect the heart energetically as expressed by PCr/ATP ratios. However, after cardioplegic arrest and explantation, an initial and prolonged lower ATP content and an anomalous higher PCr/ATP ratio in the brain death group was found when compared with controls during long-term unperfused cold storage of the hearts. During subsequent reperfusion of the hearts, ATP and PCr levels in the brain death group were lower than in controls but equal partial recovery of PCr/ATP ratios was observed in both groups. It was concluded that PCr/ATP ratios need to be interpreted with great caution. Secondly, brain death-related hemodynamic instability is not related to significant changes of myocardial energy metabolism. Thirdly, brain death does affect the myocardial energy metabolism but the impact became apparent only during hypothermic storage and subsequent reperfusion of the donor heart.

## 1. Introduction

In heart transplantation, the heart of a hemodynamically unstable brain dead donor is often rejected because survival of the recipient is reduced [1–3]. The precise mechanisms of brain death-related hemodynamic instability remain unknown. One of the reported contributing factors is myocardial injury resulting from the acutely increased discharge of endogenous catecholamines during the onset of brain death [4,5]. This injury has been supposed to change aerobic to anaerobic energy metabolism, causing depletion of myocardial high-energy phosphates and contractile dysfunction [6–8]. Energy depletion appeared to be even more pronounced when high dosages of inotropic agents were used to treat contractile dysfunction of the canine donor heart [9]. Notwithstanding these results, the presence of anaerobic metabolism is disputed by others [10,11,13].

Explantation, preservation, and storage of hearts are essential aspects of transplantation. Their harmful impact on the myocardial integrity has been related to the applied method of cardioplegic arrest and conditions of storage [14–16]. Up to now, static information on the myocardial energy status was derived

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principally from small biopsies of the left ventricle. In addition, biopsies are liable to PCr and ATP breakdown during the assessment procedure. These factors may, in part, explain the contradicting results.

$^{31}\text{P}$  MRS provides continuous information of a large area of the left ventricle or the whole isolated heart and has contributed, up to now, mainly data of animal experiments to current controversies in human donor issues [17]. Up to now, assessment of the myocardial energy metabolism by applying  $^{31}\text{P}$  MRS on the human donor is too complicated. The ultimate possible goal now is to correlate myocardial energy status of the isolated donor heart during preservation with post transplantation heart performance [18].

In this review we have combined the results of a series of experiments to address the impact of brain death on *in vivo* and *ex vivo* high-energy phosphate metabolism of the feline donor heart during the onset of brain death, on the hemodynamic instability which follows up to 6 h afterwards, on the treatment of hemodynamic instability with an inotropic agent, and during preservation and reperfusion after explantation.

## 2. Materials and methods

Male European shorthair cats were sedated and placed in supine position. They were assigned alternately to three brain death groups (each  $n = 6$ ) or three control groups (each  $n = 6$ ). The animals were intubated and mechanically ventilated. Anesthesia was maintained during the entire experiment. Ventilation was adjusted according to analysis of arterial blood gases.

### 2.1. Assessment of hemodynamic alterations

Catheters in the superior caval vein and the abdominal aorta served for continuous recordings of central venous pressure (CVP) and arterial blood pressure (and mean arterial blood pressure (MAP)), respectively. The latter catheter was also used for arterial blood sampling. CVP was kept constant. A catheter in the femoral vein served for continuous intravenous administration of anesthetics and Geloplasma. Heart rate (HR) was measured from three-lead surface ECG electrodes. Central venous pressure was kept constant. Myocardial workload was expressed as rate-pressure product ( $\text{RPP} = \text{HR} \times \text{systolic arterial blood pressure}$ ).

### 2.2. Induction of brain death

The cats were subsequently placed in the prone position. A trephine hole was drilled in the right dorso-parietal cranial area and a balloon catheter was inserted epidurally [19]. The animal was repositioned into the supine position. At the start of the experiment ( $t = 0$  min), brain death was induced by inflation of the balloon, 120 min after premedication. It was observed that electrocerebral activity disappeared within 30 s after inflation of the balloon. Brain death was confirmed by histologic study of the brain stem [19]. In the control group the intracranial balloon was not inserted.

### 2.3. Assessment of myocardial energy metabolism

#### 2.3.1. *In vivo* $^{31}\text{P}$ MRS assessment of myocardial energy metabolism

The cat was repositioned in the supine position. The heart was exposed through a distal midsternal sternotomy. A circular, single turn 20 mm  $\varnothing$  RF surface coil was sutured to the free wall of the left ventricle. The cat was fixed in a cradle and positioned in an Oxford 400 mm bore 4.7 T horizontal magnet,

interfaced to a SISCO spectrometer. The surface coil was pretuned to 80.9 MHz and field homogeneity was optimized using the  $^1\text{H}$  FID after adaptive tuning and matching of the  $^{31}\text{P}$  coil. Data acquisition was gated both to the midexpiration phase of the ventilation cycle (frequency set at approximately 25/min) and to the upstroke of the systolic arterial blood pressure curve. After excitation by applying adiabatic 90° BIR-4 pulses with 2 msec width,  $^{31}\text{P}$  MR spectra were obtained. Two *in vivo* studies were performed.

*In vivo  $^{31}\text{P}$  MRS assessment of myocardial energy metabolism during the onset of brain death and during hemodynamic instability (240 min protocol).* In this first study,  $^{31}\text{P}$  MRS spectra were obtained from 12 or 24 FIDs with a repetition time ( $T_R$ ) of approximately 2.4 s. For assessment of the myocardial energy metabolism during the short period of hemodynamic alterations following the induction of brain death and shortly afterwards, 30 sec spectra (12 FID's) were acquired between 2.5 min before and 15 after the induction of brain death. For assessment of the basal condition of the myocardial energy metabolism and during the period of hemodynamic instability, 60 s (24 FID's) spectra were acquired 5 min before and from 15 min till 240 min after the induction of brain death.

*In vivo  $^{31}\text{P}$  MRS assessment of myocardial energy metabolism during treatment of hemodynamic instability with high dosages dopamine (360 min protocol).* In the second study,  $^{31}\text{P}$  MRS spectra were obtained from 60 FID's (5 min) with a  $T_R$  of approximately 4.8 s during 5 min before the induction of brain death until 360 min afterwards. At 210 min dopamine was infused intravenously at 5  $\mu\text{g}/\text{kg}/\text{min}$  for 30 min. The dosage was consecutively increased to 10, 20, and 40  $\mu\text{g}/\text{kg}/\text{min}$  every 30 min. Dopamine infusion was discontinued during the last 30 min of the 360 min protocol.

### 2.3.2. *Ex vivo $^{31}\text{P}$ MRS assessment of myocardial energy metabolism (24 h protocol)*

In the third study, sternotomy was performed in a state of progressive hemodynamic deterioration at 360 min after the induction of brain death. The aorta was crossclamped and the heart was arrested by infusing St. Thomas' Hospital Solution (STHS) at 4°C. The heart was explanted as in standard clinical practice, submerged in STHS at 4°C, and mounted to a Langendorff perfusion system, as reperfusion was to follow the period of storage. Still submerged in STHS, the heart was placed in a glass MR tube, 44 mm internal diameter. The tube was lowered into a vertical 150 mm bore 4.7 T magnet interface to a Bruker MSL200 spectrometer. Field homogeneity was optimized now using the  $^1\text{H}$  FID after adaptive tuning and matching of the 44 mm  $^{31}\text{P}$  volume coil. After excitation with 165  $\mu\text{s}$  90° block pulses  $^{31}\text{P}$  MRS spectra were obtained from 128 FID's with a  $T_R$  of approximately 2.35 s. The first 5 min spectra were obtained 1 h after crossclamping of the aorta. A spectrum was collected every hour during 17 h unperfused storage at 4°C. During the following 60 min reperfusion at 38°C and 100 cm H<sub>2</sub>O perfusion pressure with a modified Krebs–Henseleit solution, 5 min spectra were collected continuously. In both *in vivo* studies and in the *ex vivo* study, peak areas in all spectra were determined using a time-domain fitting routine VARPRO. PCr/ATP ratios were calculated. Although no  $^{31}\text{P}$   $T_1$  relaxation times in cat hearts have been published, we assume that partial saturation will have occurred. However, the changing conditions in all protocols did not allow proper measurement of  $T_1$ . Therefore, no correction for partial saturation was applied.

### 2.4. Statistical analysis

All results were expressed as mean  $\pm$  SEM. The unpaired, two-tailed Student's *t*-test was used to determine the significance of differences between the means of the brain death group and the control group. Analysis of variance (ANOVA) for repeated measurements was applied when the values obtained within each group at various times were compared to the basal value. If significant differences were present the Bonferroni test was applied. A difference was considered significant when  $p < 0.05$ .

### 3. Results

#### 3.1. Ventilatory and metabolic variables

No significant differences in metabolic or ventilatory variables were observed between the two groups at  $t = -5$  min and at  $t = 360$  min after induction of brain death. (Data not shown.)

#### 3.2. Hemodynamic response

A biphasic response was noted in HR whereas the response in MAP was represented by a triphasic profile upon the inflation of the intracranial balloon (Fig. 1A and B). Heart rate increased from a basal value of  $143 \pm 7$  to a maximum value of  $241 \pm 10$  beats/min ( $p < 0.0001$  vs control group) at 2 min, returned to normal levels 20 min after inflation of the balloon and remained so for the rest of the experimental period. MAP initially increased from  $135 \pm 5$  to a maximum value of  $260 \pm 8$  mmHg ( $p < 0.0001$  vs control group) at 2 min, and returned to basal values at 20 min. Subsequently, MAP deteriorated progressively, became significantly different from the control group at 150 min ( $93 \pm 11$  vs  $128 \pm 7$  mmHg ( $p < 0.05$ ) and was  $53 \pm 8$  mmHg ( $p < 0.001$  vs control group) at 360 min. The response in RPP was similar to the response in MAP (data not shown). However, the deterioration in RPP in the brain death group appeared 30 min later, at  $t = 180$  min, than the deterioration of the MAP at  $t = 150$  min. This was caused by the

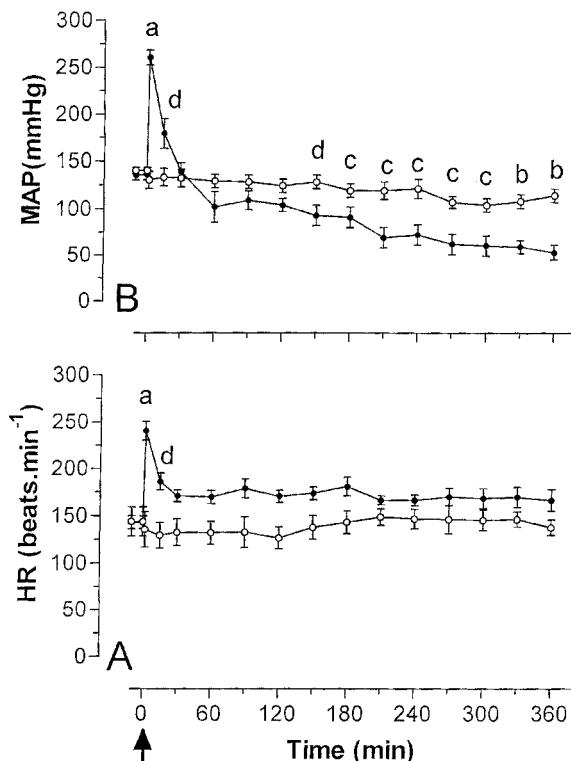


Fig. 1. The typical course of heart rate (HR) in  $\text{beats} \cdot \text{min}^{-1}$  (A) and mean arterial pressure (MAP) in mmHg (B) during 360 min after induction of brain death. The arrow indicates the onset of brain death by inflation of the intracranial balloon at  $t = 0$  min. Brain death group (●,  $n = 6$ ), control group (○,  $n = 6$ ). (a:  $p < 0.0001$ ; b:  $p < 0.001$ ; c:  $p < 0.01$ ; d:  $p < 0.05$ .)

fact that HR in the brain death group remained somewhat higher after inflation of the balloon compared with controls during the duration of the protocol (Fig. 1A).

### 3.3. Myocardial energetic response to brain death

#### 3.3.1. Myocardial energetic response during onset of brain death and hemodynamic instability

Although HR, MAP, and RRP were significantly higher than in controls during the hyperdynamic phase (2 to 15 min after  $t = 0$  min) and significantly lower after 150 min (MAP) to 180 min (RRP), no significant variation in the PCr/ATP ratio's was seen during the hyperdynamic phase or during the hemodynamic deterioration which followed. The average PCr/ATP ratio was  $1.61 \pm 0.12$  in the brain death group and  $1.61 \pm 0.08$  in the control group [20].

#### 3.3.2. Myocardial energetic response during treatment of hemodynamic instability with dopamine

In this study, MAP progressively decreased and became significantly lower compared with controls at 90 min after inflation of the intracranial balloon (Fig. 2). This appeared to be 60 min earlier than the response in MAP as presented in Section 3.3.1. However, the response in RPP was identical as mentioned in the Section 3.3.1. At 240 min, when dopamine was infused at  $5 \mu\text{g}/\text{kg}/\text{min}$  for 30 min, MAP (and RPP) increased in both groups and the differences disappeared between the groups (Fig. 2). During the stepwise increase of dopamine to 10, 20, and  $40 \mu\text{g}/\text{kg}/\text{min}$  a further increase in MAP (and RPP) was seen without significant differences between the two groups (Fig. 2). However, when dopamine infusion was discontinued at  $t = 330$  min, MAP deteriorated to  $35 \pm 3 \text{ mmHg}$  in the brain death group and to  $74 \pm 7 \text{ mmHg}$  in the control group ( $p < 0.01$ ) at  $t = 360$  min. RPP decreased to  $(9.5 \pm 0.3) \times 10^3 \text{ mmHg} \cdot \text{min}^{-1}$  in the brain death group and to  $(14.8 \pm 2.3) \times 10^3 \text{ mmHg} \cdot \text{min}^{-1}$  in the control group ( $p < 0.05$ ). Again, no significant changes of the PCr/ATP ratio were observed during the period of hemodynamic deterioration or during the period of treatment with increasing dosages dopamine or during the discontinuation of dopamine. The average PCr/ATP ratio was  $2.06 \pm 0.10$  in the brain death group and  $2.14 \pm 0.08$  in the control group [21].

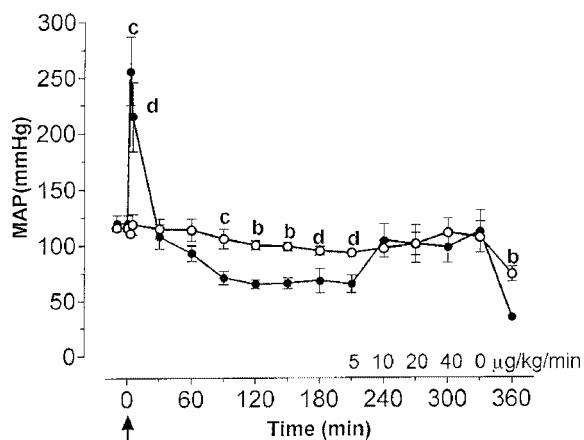


Fig. 2. Mean arterial pressure (MAP) in brain dead cats (●,  $n = 6$ ) and control cats (○,  $n = 6$ ), before, during, and after the dopamine protocol (5 to  $40 \mu\text{g}/\text{kg}/\text{min}$  from 210 to 330 min) following the induction of brain death at  $t = 0$  min (arrow). (a:  $p < 0.0001$ ; b:  $p < 0.001$ ; c:  $p < 0.01$ ; d:  $p < 0.05$ .)

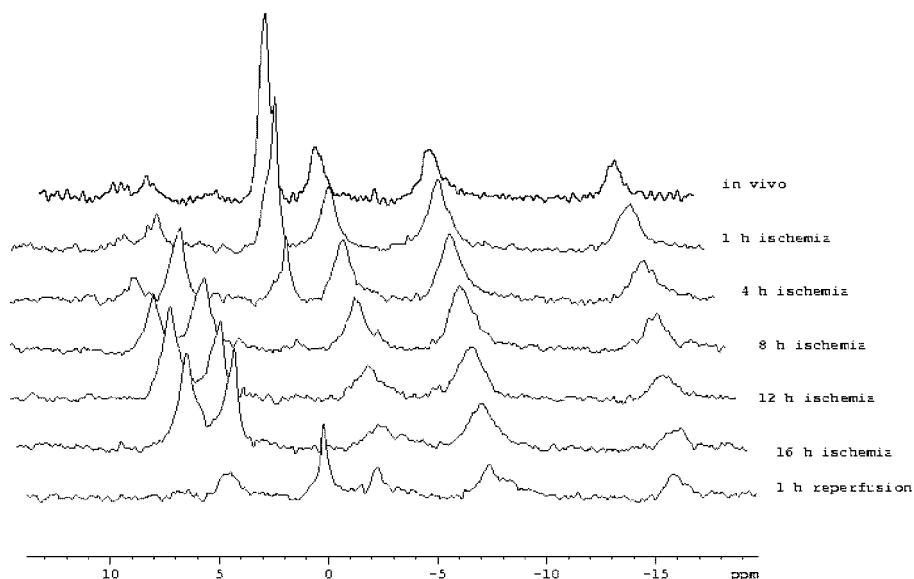


Fig. 3. *In vivo* and *ex vivo* myocardial  $^{31}\text{P}$  MR spectra of a brain dead cat. *In vivo* spectrum in the rear, followed by spectra at 1, 4, 8, 12, and 16 h ischemia during hypothermic unperfused storage, and in the front an *ex vivo* spectrum after 1 h normothermic reperfusion of the isolated heart.

### 3.3.3. Myocardial energetic response during preservation and reperfusion

Figure 3 shows  $^{31}\text{P}$  MR spectra of an explanted feline heart during hypothermic unperfused storage (17 h) and normothermic reperfusion (60 min). During hypothermic storage, a progressive decrease in PCr was observed which was not only paralleled by an increase of inorganic phosphate (Pi) but also by an increase of phosphomonoesters (PME), while during reperfusion only a partial recovery occurred. The first PCr/ATP ratio (at 1 h storage) was  $1.08 \pm 0.17$  in the brain death group and  $0.56 \pm 0.07$  in the controls. A subsequent decrease of the PCr/ATP ratio was observed, which was significantly sharper in controls than in the brain death group, until PCr/ATP ratio was practically zero in both groups at 6 h storage. However, this observation does not indicate a better metabolic condition of the donor hearts of the brain death group, but is the result of a sharper decrease of ATP in this group which already occurred before the first spectrum was acquired. During reperfusion PCr/ATP increased to approximately 1.4 in both groups. As mentioned earlier ATP at 1 h storage was significantly lower in the brain death group than in controls and ATP decreased gradually in both groups during the rest of the period of storage and reperfusion. PCr levels at 1 h storage were similar in both groups and decreased to practically zero at 6 h storage. During reperfusion PCr in controls recovered to a level similar to the level of 1 h storage but in the brain death group recovery was only approximately 50% of that level. Pi and PME showed an increase during storage, which was much sharper in controls than in the brain death group. Upon reperfusion Pi and PME normalized in both groups. No difference in intracellular pH (pHi) between the groups was observed during storage. The pH of the applied STHS cardioplegic solution was 7.8. This resulted in a pHi of approximately 7.6 at 1 h storage which decreased to 6.2 at 17 h storage. During reperfusion, using a modified Krebs–Henseleit solution with a pH of  $7.35 \pm 0.05$ , Pi-peaks had a broadened appearance, which indicated an average pHi of 6.7. Only one heart, from the brain death group, showed restoration of sinus rhythm and contractile recovery upon reperfusion [22].

#### 4. Discussion

The studies in the cat as described in this review showed that neither the hyper-hemodynamic state following the onset of brain death, nor the subsequent hemodynamic deterioration in the hours afterwards, nor the treatment of this deteriorated hemodynamic state with high dosages dopamine affect the heart energetically. However, the impact of brain death to myocardial energy metabolism became apparent during storage and subsequent reperfusion. It should be kept in mind that PCr/ATP ratios were not corrected for partial saturation. In particular during the hyperdynamic phases during the onset of brain death and during the period of dopamine infusion, an increased contribution of the chemical shift to overall relaxation can be assumed, thus lowering the saturation factor and leading to overestimation of PCr/ATP ratios. The differences in PCr/ATP ratios between Sections 3.3.1 and 3.3.2 can be attributed to differences in partial saturation too, caused by different repetition times. The results described in Section 3.3.3 emphasize the importance of correct interpretation of PCr/ATP ratios. The higher PCr/ATP ratio in the brain death group at 1 h storage compared with controls was not a representation of a better energetic status of these hearts but was merely due to the fact that myocardial ATP content in the brain death group was already lower than in the controls. This is supported by the lesser extent of recovery of PCr and ATP in the brain death group compared with controls during reperfusion, whereas the PCr/ATP ratios showed equal partial recovery in both groups. Due to preparatory procedures we were not able to collect spectra within the first hour of storage which may have produced important data. In retrospect, as functional recovery of the hearts was very poor, except for one in the brain death group, it may indicate that the period of storage was chosen too long. Nevertheless, our findings in these <sup>31</sup>P MRS studies are in contrast with reported results of myocardial biopsies [4–9]. In the search for causative mechanisms of brain death-related hemodynamic instability not only the myocardial energetic status should be studied but we suggest that other factors, for instance the influence of non-adrenergic non-cholinergic substances, should be investigated too [23].

In conclusion, by applying <sup>31</sup>P MRS it was demonstrated that brain death-related hemodynamic instability is not related to significant changes of myocardial energy metabolism. However, brain death does affect myocardial energy metabolism but the impact became apparent only during hypothermic storage and subsequent reperfusion of the feline donor heart. We recommend further clinical <sup>31</sup>P MRS studies to evaluate the energy metabolism of donor hearts before procurement, during storage, and after implantation.

#### Acknowledgements

The authors express their gratitude and respect to T.J.C. Ruigrok, PhD, and J.J. Bredée, MD, PhD, for their great contributions to the studies described in this review.

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