Brain death: from inflammation to metabolic changes
Rebolledo Acevedo, Rolando

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2016

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the “Taverne” license. More information can be found on the University of Groningen website: https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment.

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.
NON INVASIVE ASSESSMENT OF TISSUE OXYGENATION AND ORGAN PERFUSION IN BRAIN DEAD RATS

Rebolledo R.$^{1,2}$, Van Erp AC.$^1$, Laustsen C.$^3$, Nørregaard R.$^4$ Pedersen M.$^3$, Leuvenink HG.$^1$, and Jespersen B.$^5$

$^1$Department of Surgery, University Medical Center Groningen, Groningen, The Netherlands
$^2$Physiopathology Program, Institute of Biomedical Sciences, Faculty of Medicine, University of Chile, Santiago, Chile.
$^3$MR Research Center, Clinical Institute, Aarhus University, Aarhus, Denmark.
$^4$Department of Clinical Medicine, Aarhus University, Aarhus, Denmark.
$^5$Department of Nephrology, Aarhus University Hospital, Aarhus, Denmark.

*In preparation*
ABSTRACT

Introduction: Brain death (BD) triggers hemodynamic impairment and leads to global changes in energy production, thereby negatively affecting organ quality. Despite the clinical efforts made in the last decades, no clear improvement in organ care has been achieved. A deeper understanding of the metabolic status of the organ grafts could be the key to improve transplant outcomes. Therefore, we studied oxygen consumption and tissue perfusion in brain-dead rats using a noninvasive strategy based on Magnetic Resonance Imaging.

Methods: BD was induced in mechanically-ventilated rats by inflation of a Fogarty catheter in the epidural space. Sham-operated rats served as controls. An Agilent 9.4 T pre-clinical MRI system was used to obtain BOLD and T1 sequences throughout the experiment. After 4 h of BD, plasma, kidney and liver tissue were collected. Blood gas analyses and routine biochemistry were performed.

Results: In plasma of brain-dead animals, renal (creatinine, urea) and liver injury markers (AST) were increased. Also, plasma lactate significantly increased while glucose levels decreased in brain dead animals compared to sham animals. Oxygen consumption in the kidney remained stable but increased in the liver of brain dead animals. Yet, relative blood flow in the liver did not change while flow through the kidney decreased during BD compared to sham animals.

Conclusions: Using a non-invasive MR assessment, increased oxygen consumption suggests increased metabolic activity in the liver during BD. On the contrary, renal perfusion becomes increasingly reduced during BD, which could pre-dispose the kidney to ischemic injury. These results point out that even though both organs are facing increased injury during BD, the underlying etiology could be different, highlighting the need for an organ-specific approach to improve graft quality prior to transplantation.
INTRODUCTION

Organs from deceased brain dead donors have inferior quality and higher rejection rates compared to organs from living donors [4].

Brain death (BD) is known for causing hemodynamic instability [2, 6, 12, 25], hormonal impairment [5, 8, 13], and a strong inflammatory reaction as well as activation of the innate immune system [1, 9, 14, 15].

Until now, donor treatment has focused on improving each of these BD-induced injury pathways [27]. However, our previous results showed that despite stable hemodynamics and reduced inflammation, kidney and liver quality from brain-dead animals is still poor compared to living donors [20]. Moreover recent advances in donor treatment have not led to increasing the donor pool [28].

Driven by the need for improved knowledge about BD pathophysiology, we investigated the metabolic status during BD. Global changes in human BD donor energy metabolism have been described by Sztark et al. [21] as seen by an increase of anaerobic metabolism in BD donors with an increase in lactate plasma levels. Novitzky et al. [16] reported similar findings in an animal model of BD. Our recent results show that glycogen storage as well as plasma glucose are decreased in the livers of brain dead rats. Moreover, changes in glucose metabolism were evident as seen by up-regulation of glycolytic pathways in both kidney and liver. Interestingly, the liver is able to maintain an aerobic energy production whilst the kidney is producing energy anaerobically, via fermentation pathways (data not published).

This shift was confirmed in the human setting, where our recent unpublished results from kidney biopsies of BD donors also show this upregulation of glycolytic-anaerobic pathways. To explain this shift in energy production we propose three hypotheses, pointing towards (1) an ischemic response due to a decrease in organ perfusion or (2) a stress response due to inflammatory environment and/or (3) a primary metabolic impairment (e.g. mitochondrial dysfunction) during BD.

Until now, research from the relatively unexplored branch of metabolic changes in the field of organ transplantation cannot support either one of these hypotheses. However, based on the shift to anaerobic energy production, we are inclined to believe that a study of organ perfusion and tissue oxygen consumption could elucidate this matter. The current study investigated the hypothesis that brain death induced metabolic changes are caused by a change in organ perfusion and oxygen consumption, using real-time, in vivo magnetic resonance imaging (MRI).

Briefly, the MRI technique is based on the interaction of atomic nuclei with an external magnetic field. Blood oxygen level–dependent (BOLD) MR imaging is a subset of techniques that utilizes the magnetic properties of deoxyhemoglobin to reflect alterations in blood oxygenation, blood flow, and blood volume. Although BOLD MR imaging methods are widely used for functional activation studies of the brain, they are increasingly
being used for functional imaging of tumors, cardiac and skeletal muscle, renal tissue, and the liver [17].

Alternatively, Arterial Spin Labelling (ASL) imaging uses spatially selective inversion of inflowing arterial blood as a way to label and monitor blood flow in the organs. With this technique, arterial blood becomes magnetically labeled and the flow of this so-called inverted blood can than be visualized. To account for already present, static tissue, an image of the tissue containing un-inverted blood is subtracted from the subsequent image with inverted blood. Thus, subtraction of the labeled image from the unlabeled control image gives a measure of the amount of labeled blood, which flowed into the tissue. This quantity in turn is closely related to the tissue perfusion [26].

In this study we investigated changes in renal and hepatic perfusion as well as oxygen consumption using real-time, in vivo MRI techniques in brain-dead rats, to elucidate whether alterations in organ perfusion could be responsible for the BD-induced changes in metabolic status of donor.

Methods Animals

Nineteen male adult Fisher F344 (Harlan, UK) rats (250-300 g) were used. The Danish Inspectorate of Animal Experimentation approved the study. All animals received humane care in compliance with the Principles of Laboratory Animal Care formulated by the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources and published by the National Institutes of Health, Publication No. 86-23, revised 1996).

Brain-dead (BD) was induced as described previously [11]. Animals were anesthetized using sevoflurane with 100% O2. Animals were intubated via a tracheostomy and ventilated (MR-compatible Small Animal Ventilator. SA Instrument, Inc. NY. US) using a Tidal Volume: 7 ml/BW (Kg) per stroke, PEEP of 3 cm of H20 initial respiratory rate of 120 and corrected base on ETCO2 throughout the experiment. Two cannulas were inserted in the femoral artery and vein for continuous mean arterial pressure (MAP) monitoring and volume replacement. Through a frontolateral hole drilled in the skull, a no. 4 Fogarty catheter (Edwards Lifesciences Co, Irvine, CA) was placed in the epidural space and inflated with saline using a syringe pump (Model 980-733, Harvard Apparatus, Harvard Bioscience Co., Massachusetts, US). A second hole was drilled contra-laterally for Intracranial pressure monitoring, a 24G cannula was used for this propose. Induction of BD was achieved by inflating the catheter at a speed of 16 ml/hr. Inflation of the balloon was terminated once a rise in the MAP above 80mmHg was noted; reflecting the autonomic storm at the beginning of BD. BD was confirmed by the absence of corneal reflexes. Following confirmation of BD, ventilation was continued and anesthesia was terminated. As an internal control for the BD model, declaration of BD was re-confirmed when the intracranial pressure (IP) was higher than the mean arterial pressure (MAP) and cerebral perfusion pressure (CPP) below 0 mmHg. Animals were kept ventilated and
monitored for 4 hours after declaration of brain death. Mean arterial pressure (MAP) was maintained above 80 mmHg. If necessary, colloid infusion with polyhydroxyethyl starch (HAES) 10% (Fresenius Kabi AG, Bad Hamburg, Germany) was started (at a maximum rate of 1 ml/hr) to maintain a normotensive MAP. Unresponsiveness to HAES administration indicated the start of an intravenous noradrenaline (NA) drip (1 mg/mL). A homeothermic blanket control system was used throughout the experiment. For Sham operated animals the surgical procedure was the same but the Fogarty catheter was not placed. These animals were kept alive for four hours under anesthesia and mechanical ventilation. A drip of Rocuronium (0.1 mg/ml at 0.3 ml/hr) was used in sham and brain-dead animals in order to avoid movements during MR scanning. A bolus of Rocuronium (0.1 mg) was used before aortic puncture preventing cramps during the procedure.

After the experimental time, blood and urine were collected and all abdominal organs flushed with cold saline. After the flush out, organs were harvested and tissue samples were snap frozen in liquid nitrogen and stored at -80 °C or fixated in 4% paraformaldehyde. Plasma samples and urine were also snap-frozen and stored.

Animals were randomly assigned to one of the experimental groups:

1. Brain Death (n = 8)
2. Sham (n=8)

Plasma determination

Plasma levels of creatinine, aspartate transaminase (AST), alanine transaminase (ALT), urea, as well as urine creatinine were determined at the clinical chemistry laboratory of University Medical Center Groningen according to standard procedures.

Blood gas analyses were performed immediately after aortic puncture using ABL725 analyzers (Radiometer Medical Aps, Brønshøj, Copenhagen, Denmark) to determine the pH, partial pressure of oxygen (PO_2), partial pressure of carbon dioxide (PCO_2), hemoglobin (Hb), oxygen saturation (SO_2), lactate and glucose. Samples containing blood clots were excluded from the analyses.

MR protocol

Animals were placed in an MRI holder (Rapid Biomedical, Würzburg, Germany). Rectal temperature, intraarterial blood pressure, intracranial pressure, ETCO_2 and pulse oxymetry were controlled. MRI data were collected using an Agilent 9.4 T preclinical MRI system (Agilent Technologies, Yarnton, UK); with a 72-mm volume coil (Rapid Biomedical).

A high-resolution coronal spin-echo sequence was employed for anatomical description, acquired using the following sequence parameters: matrix 256×192, field of view (FOV) of 107×90 mm^2, repetition time (TR) of 3000 ms, echo time (TE) of 22.8 ms, and 1 mm thickness. An oxygenation-dependent sequence (T2*-weighted) was
performed using an axial 1H-multi-echo gradient-echo sequence, covering the entire abdomen with 32 slices. The sequence parameters were: matrix of 128×128, FOV of 80×80 mm², flip angle of 90°, TR of 800 ms, TE of 2,4,6,8,10,12,14, and 16 ms, number of transients of 2, and 2 mm thickness. For T1-measurements, a single-slice segmented Look-Locker sequence with a gradient-echo readout was used to acquire T1-weighted data, using the sequence parameters: matrix of 128×128, FOV of 80×80 mm², flip angle of 8°, TR of 3 ms, TE of 2 ms, inversion times (TI) of 150, 250, 400, 600, 900, 1200, 2500, 4000 ms, and 2 mm thickness.

Image reconstruction and volumetric analysis were performed in VnmrJ (Agilent Technologies). Manually drawn regions of interest were encompassed of the liver and kidneys. Quantitative T2* maps were calculated from pixel-by-pixel analysis using a nonlinear least-squares fit to the logarithmic magnitude vs. TE. Quantitative T1 was calculated from a three-parameter fit applied to the inversion recovery Look-Locker sequence, using the mathematical approach given by Ramasawmy et al [18].

Statistical Analyses
For plasma determinations, *R2 baseline (0 hrs), and induction data a Mann-Whitney test was performed, with p < 0.05 regarded as significant to compare between two groups individually, despite the small group size. For *R2 BOLD and T1 data the Two-way repetitive measurements ANOVA was performed, with p < 0.05 regarded as significant to compare between groups, followed by Sidak's multiple comparisons test to compare between groups in each time point (Prism 6.0. GraphPad Software, Inc. CA. US). Data are presented as mean ± SD (standard deviation).

RESULTS
Induction of BD showed a consistent and uniform pattern of alterations of the MAP, with mean time to declare brain death of 29.29 ± 5.91 min. All animals were kept with a MAP above 80 mmHg throughout the experiment. No HAES or noradrenaline was needed in any of these animals (Sham nor BD). CCP was below 0 mmHg during the experiment in 7 brain-dead animals. However, in one animal, the CPP was higher than 0, but we decided to continue and include this animal in the study, since MAP profile and absence of corneal and pupillary reflexes confirmed BD. When the experiment was finished we confirm an obstruction in the ICP catheter (Figure 7.3).

Blood gas analyses were performed as internal controls for the ventilation and surgical procedure. The pH was slightly alkaline in brain dead animals compared to sham animals. Carbon dioxide partial pressure was lower in brain dead animals compared to sham animals. Hemoglobin was significantly decreased in the sham group compared to the BD group. However, oxygen saturation and oxygen partial pressure were not different when comparing the two groups. (Table 7.1).
Plasma levels of LDH, AST and ALT were measured as cellular liver injury markers. No difference was found in LDH nor ALT plasma levels. AST levels were found increased in BD animals (94.14 ± 9.25 vs 69.00 ± 8.04 U/L, p = 0.0012) compared to sham animals. Urea, plasma, and urine creatinine levels were measured as renal injury markers. BD animals showed an increase in urea (13.14 ± 1.94 vs 9.04 ± 0.75 mmol/L, p = 0.0006) and plasma creatinine (59.67 ± 13.95 vs 22.86 ± 2.55 mmol/L, p = 0.0012) levels compared to sham. Urine creatinine levels were lower in BD animals compared to sham animals (4.90 ± 0.58 vs 10.11 ± 2.06 mmol/L, p = 0.0003. Figure 7.4).

To estimate the metabolic status, levels of plasma metabolites lactate and glucose were measured. Lactate was found increased in BD animals (2.50 ± 0.49 mmol/L) compared to sham animals (1.45 ± 0.49 mmol/L p = 0.0065). In contrast, glucose plasma levels were higher in sham animals (11 ± 1.4 mmol/L) compared to BD animals (9.3 ± 0.86 mmol/L, p = 0.0163, Figure 7.5).

Deoxyhemoglobin concentration is directly proportional to the spin-spin relaxation rate (R2*) and can thus be calculated based on T2* signal (R2* = 1/T2*) (Figure 7.1). As an internal control, the R2* in kidney and liver tissue was calculated at time 0 h between groups. No difference in R2* was found comparing between groups at this time point (Table 7.2).

Next, R2* was calculated before and after BD induction in liver and kidney tissue. In the liver, the R2* values increased in the liver when comparing the signal after induction with the signal before induction of BD (p = 0.0499). In the kidney, no difference was found comparing the signal before versus after induction. (Table 7.3)

Following, R2* values were calculated every hour during the BD period. In the liver, R2* values increased after 2 hours until the end of the experimental time in BD animals compared to sham (Figure 7.6). In the kidney, the R2* was calculated per individual kidney. Throughout the BD period, no differences in R2* values were found comparing BD and sham animals (Figure 7.7).

T1-weighted data measured hourly throughout the BD period was analyzed to estimate relative changes in tissue perfusion. These relative changes were calculated using the baseline T1 signal as a reference (Figure 7.2). No differences in liver perfusion were found in this experiment when comparing BD to sham animals at the different time points. (Figure 7.8). In the kidney, a decrease in perfusion was found after 3 h in both kidneys when comparing BD to sham animals (Figure 7.9).

DISCUSSION
Recently, alterations in metabolism and energy production have been observed in both human as well as rodent BD donors. This study was performed to assess whether these changes in metabolism could be explained by BD-induced changes in organ perfusion and/or oxygen consumption in the liver and kidney of BD rats. We confirmed a change in metabolism after 4 h of BD, suggesting a diminishing glucose supply with concurrent
utilization of alternative energy sources. When attempting to understand what causes these changes, we found an interesting duality in the organ-specific response in the BD setting. In the liver, BD animals show increasing levels of deoxygenated hemoglobin. Alternatively, the kidney does not experience changes in deoxyhemoglobin levels but is faced with diminishing tissue perfusion as the BD period is longer than 3 hours.

In order to determine if, and understand how, this duality between the kidney and liver response fits in with our current knowledge about BD pathophysiology, a deeper analysis of the acid-bases status is required. Besides the global changes in plasma metabolites, blood gas analyses reveal that BD animals were hyperventilated throughout the experiment, in attempt to maintain a normal ETCO₂ and adequate pH values. This could reinforces the idea that BD animals suffered from a metabolic acidosis. This metabolic acidosis could reflect poor renal function and as a consequence lack of proper acid removal, or could point to an increase in anaerobic metabolism. An increase in anaerobic metabolism can subsequently explain the lactate build-up, caused by a primary metabolic dysfunction, poor oxygen delivery to the tissue, or a combination of both plus a decreased hepatic lactate clearance as was describe in sepsis [22]. Organ function, oxygen consumption and tissue perfusion were, therefore, measured to elucidate the observed alterations in donor metabolism.

BD is known to negatively affect hepatic and renal function and ultimately be associated with higher rejection rates after transplantation [23, 24]. This was confirmed by altered plasma and urea creatinine levels in brain dead animals, suggesting poor renal function in brain dead animals compared to sham treated animals. Similar pattern was show by AST plasma levels as liver injury marker. However now, sham animals underwent the same experimental period as the brain dead animals, which is in sharp contrast with the short, 0.5 h period of sham animals in previous works [19]. This may suggest that the duration of the sham procedure can influence hepatic injury markers.

The observed changes in plasma metabolites could reflect a shift from aerobic energy metabolism to anaerobic energy metabolism. This can be explained by a primary metabolic dysfunction of the primary players of cellular energy production, the mitochondria. Alternatively, diminished oxygen supply and subsequent tissue ischemia can be responsible for a shift to anaerobic metabolism. This lack in oxygen supply is happening in the renal tissue and according to our results could take place in others parenchymas as well. Concomitantly in the liver we did not found any change in relative blood flow, but we did found an increase in deoxyhemoglobin levels, which could be explain by a relative increase in oxygen consumption due an (i) increased demand or a (ii) decrease in oxygen delivery with a sustained consumption.

Perfusion in the liver arise from the portal vein, representing 75% of the total hepatic flow, and hepatic artery representing 25% [10]. We measured the relative blood flow change in the liver, which represented a mixed flow from portal and arterial systems. We acknowledge that we cannot evaluate portal nor arterial contribution separately with this
method. Thus, we postulate two possible explanations for conserved flow and increased deoxyhemoglobin in the liver of BD animals. First, assuming that flow and oxygen delivery stay stable, the increase in deoxyhemoglobin could be secondary to an increased oxygen consumption due to BD related increased energy demand. An alternative explanation could be that the relative contribution from the artery flow (oxygenated blood) is decreasing while Portal flow (poor-oxygenated blood) is increasing or keeps stable. This could imply that the relative amount of oxygenated blood is decreased as well.

In the kidney, deoxyhemoglobin levels are not altered. However, the kidneys do experience a decrease in relative blood flow and secondary oxygen supply, following BD induction. This connotes that the kidneys, despite normal mean arterial pressure, are suffering from decreased blood flow and perhaps even ischemic injury. A known phenomenon in brain-dead donors is the so-called catecholamine storm, a surge of catecholamines in response to strong sympathetic activation following BD onset. These catecholamines are capable of triggering strong peripheral vasoconstriction, especially in highly sensitive areas like the renal vasculature [7]. Consequently, ischemia in the kidneys could explain the general lactate build-up in BD donors and might be an underlying mechanism causing renal inflammation and subsequently, renal damage.

We think that the assessment of mitochondrial function and tissue metabolites could help to understand the physiology behind BD induced tissue injury. Mitochondrial function will determine if this shift to anaerobic metabolism is related with a lack of oxygen delivery or primary mitochondrial dysfunction in liver and kidney tissue. This could give us an interesting clue about tissue vitality and organ quality during BD. Such metabolite study could help understanding which metabolic pathways are active and how we can treat the organ (or donor) in order to improve graft quality before transplantation.

Concluding, BD triggers altered oxygen utilization, hemodynamic problems and a metabolic shift in the donor. Yet, the etiology of and organ-specific response to these challenges is not homogenous. Therefore, treatment of brain-dead donors should be adjusted to organ-specific needs. As the liver consumes oxygen probably to restore body homeostasis, an organ-based treatment should include the restoration of the energy status using physiological perfusion solutions. In the case of the kidney, treatment should address the ischemic injury should focus on oxygen supply restoration but also providing protection against reactive oxygen species.
REFERENCES


FIGURES

Figure 7.1: BOLD sequence. An example of T2 map for liver tissue. On the left a grayscale of T2 signal. On the right T2*-weighted signal represented in a color map.

Figure 7.2: ASL sequence. An example of T1 signal for liver tissue at different inversion times. From the top left to bottom right we can see the signal pass from the hepatic vessels to the hepatic tissue.
Figure 7.3: Cerebral Perfusion Pressure (CPP) at MR sequence time.

Figure 7.4: Plasmatic levels of kidney and liver injury markers.
Figure 7.5: Plasma levels of Glucose and Lactate.

Figure 7.6: R2* BOLD signal in Liver tissue.

Figure 7.7: R2* BOLD signal in kidneys.
Figure 7.8: Relative change of liver perfusion during brain death

Figure 7.9: Relative change of kidney perfusion during brain death
## TABLES

### Table 7.1: Blood Gases Analyses and Acid-Base status

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Brain Death</th>
<th>Sham</th>
<th>Normal Values (FiO₂ 21%) [3]</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.48 ± 0.04*</td>
<td>7.34 ± 0.08</td>
<td>7.466 - 7.472</td>
</tr>
<tr>
<td>PO₂</td>
<td>29.7 ± 6.51</td>
<td>31.3 ± 2.40</td>
<td>9.73 - 13.9 kPa</td>
</tr>
<tr>
<td>PCO₂</td>
<td>3.58 ± 0.55*</td>
<td>6.67 ± 2.19</td>
<td>3.47 - 5.73 kPa</td>
</tr>
<tr>
<td>Hb</td>
<td>9.27 ± 0.69*</td>
<td>7.95 ± 0.40</td>
<td>8.1 - 10 mmol/L</td>
</tr>
<tr>
<td>SO₂</td>
<td>97.0 ± 0.23</td>
<td>97.2 ± 0.33</td>
<td>92 - 99%</td>
</tr>
</tbody>
</table>

*p < 0.05

### Table 7.2: R²* BOLD Signal base line (0 hrs)

<table>
<thead>
<tr>
<th>Organ</th>
<th>Sham</th>
<th>Brain Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>0.18 ± 0.046</td>
<td>0.14 ± 0.032</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.14 ± 0.069</td>
<td>0.13 ± 0.087</td>
</tr>
</tbody>
</table>

### Table 7.3: R²* BOLD Signal before and after Brain Death Induction

<table>
<thead>
<tr>
<th>Organ</th>
<th>Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>0.14 ± 0.021</td>
<td>0.16 ± 0.011*</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.13 ± 0.066</td>
<td>0.12 ± 0.066</td>
</tr>
</tbody>
</table>

*p < 0.05