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ORIGINAL ARTICLE

24-h storage of pig livers in UW, HTK, hydroxyethyl starch, and saline solution: is microdialysis an appropriate method for the continuous graft monitoring during preservation?

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Summary

Recent studies demonstrate the feasibility of microdialysis to monitor metabolism in ischemic livers. Whether these parameters correlate with markers of liver cell integrity in an experimental model using pig livers and different preservation solutions was an aim of this study. Pig livers were flushed with either 4 °C Histidine-Typtophan-Ketoglutarate solution (HTK) (Custodiol®), University of Wisconsin solution (ViaSpan®), and hydroxyethyl starch, or 12 °C saline solution. After 24-h storage, the livers were rinsed with saline to measure liver enzymes and lactate from the effluate. Utilizing microdialysis, intraparenchymal lactate, pyruvate, glucose, and glycerol was monitored. Tissue biopsies were taken for histological examinations. Cold preservation resulted in a decrease of metabolic activity measured by intrahepatic glucose, lactate, and pyruvate levels, as well as lactate in the effluate, independently of the solution used. Of particular interest, glycerol levels partially reflected the extent of hepatocellular damage and liver enzyme release. Glycerol levels partially discriminated preservation of different quality and were in accordance to histological findings and liver enzyme release. Lactate, pyruvate, and glucose levels were not appropriate as markers during cold storage. Whether or not glycerol monitoring could represent an additional and rational complementation to the current practice of macroscopic, microscopic and donor evaluation has to be clarified by further studies.

Introduction

Liver transplantation is currently considered to represent the primary therapy of end-stage liver disease and acute liver failure. Despite improvements in liver preservation and surgical techniques, some degree of clinical and biochemical dysfunction following orthotopic liver transplantation occur almost invariably. Perception and knowledge of reliable parameters being predictive for the outcome of liver transplantation is still major issues of the liver transplant community [1]. With a growing disparity between the demand for and the supply of organs for transplantation, the use of marginal organs, with a higher susceptibility for hypoxia, cold storage, and reperfusion, is justified [2,3]. Underlying high-risk factors include *(1)* donor age more than 70 years or <3 months, *(2)* moderate (30–60%) or severe (>60%) macrovesicular fat content, *(3)* intensive care unit hospitalization of 5 days and longer, *(4)* high doses of positive inotropic drugs, and *(5)* extension of cold ischemia time above 14 h [4]. It is well documented that these single factors, as well as their combination, predict the appearance of moderate-to-severe graft preservation injury [5].

The assessment of a donor liver before transplantation has been the subject of much research. However, the transplant surgeon still has to rely on a subjective interpretation of donor data and the macro- and microscopic appearance of the liver to decide whether to use the graft. This decision will only be proved to have been right if the liver is

transplanted and the recipient discharged from hospital. The possibility of graft monitoring during the storage period could give further information of grafts condition before transplantation and could therefore close the gap of a time period always associated with organ transplantation, which has been inaccessible for diagnostic thus far.

Recently, microdialysis was suggested as a tool to evaluate quality of the donor liver [6–9]. This technique offers the opportunity for continuous monitoring of metabolic changes within the tissue and cell membrane integrity. The general principle of microdialysis is to sample fluids with a semipermeable membrane catheter introduced directly into the tissue. While being slowly perfused with Ringer solution, an equilibration takes place through the membrane, resulting in a composition of the collected effluate identic to the extracellular fluid. The metabolic markers of interest for microdialysis monitoring of ischemia are based on the intermediates of the glycolytic pathway, which is employed for the breakdown of glucose to provide energy. With an adequate oxygen supply, glucose is metabolized to pyruvate, which in absence of oxygen is further reduced to lactate. Another marker of ischemia is released by phosphoglycerides from the cell membrane. Calcium ion influx as a result of ATP depletion activates phospholipases, leading to phospoglycerides breakdown, resulting in an efflux of glycerol. Glycerol may therefore indicate cell membrane disintegrity [10].

The aim of this study was to continuously monitor hepatic metabolic changes and cell membrane damage over a preservation period of 24 h by using the microdialysis technique. Within the experimental protocol, pig livers were stored at 4 °C in UW, HTK, and hydroxyethyl starch solution and opposed to mild hypothermic storage at 12 °C in physiologic saline solution. After a subsequent flush of the livers, a comparison was made between the microdialysis data and liver enzyme levels from the flush solution effluate, as well as to histological findings.

Materials and methods

Study protocol

Sixteen male pigs (German Landrace) with a body weight of 22–24 kg, divided into four groups ($n = 6$ per group), were used as liver donors. The pigs were group-housed in the experimental facility, fasted for 24 h before organ retrieval with free access to water. The study protocol was approved by the local Ethics Committee.

Pigs were premedicated with intramuscular premedication of Azaperone (Stresnil, Janssen Pharmaceutica, Beerse, Belgium) and ketamine hydrochloride plus atropin sulfate, whereas anesthesia was maintained by 0.5–1.5% halothane inhalation and continuous fentanyl infusion. The external carotid artery was cannulated for blood pressure measurements during the retrieval operation. The liver was freed from surrounding ligaments and 10 000 IE heparin was administered before cannulation of the infrarenal aorta with a 20 Ch catheter. Aortic perfusion was started with either 4 °C UW (Viaspan™; DuPont, Bad Homburg, Germany) (group I), HTK (Custodiol™; Köhler Chemie, Alsbach, Germany) (group II), hydroxyethyl starch solution (HES) 6% 130/04 (Voluven™; Fresenius Kabi Deutschland GmbH, Bad Homburg, Germany) (group III) or with 12 °C physiologic saline solution (NaCl 0.9%; B. Braun AG, Melsungen, Germany) (group IV) at a perfusion pressure of 120–150 mmHg. After graft retrieval, the livers were then consecutively stored for 24 h at 4 °C (UW, HTK, HES) or 12 °C (saline solution). Microdialysis samples were collected continuously, and sequential tissue biopsies were taken for histological examination. After 24 h, the livers were flushed via the portal vein with 4 °C nonoxygenated saline solution at a perfusion pressure of 100 cm H_2O and a flow rate of approximately 500 ml/min for the measurement of cumulated liver enzymes and lactate in the effluate.

Microdialysis

The microdialysis catheter (CMA 61 Liver MD Catheter; CMA Microdialysis AB, Stockholm, Sweden) is a double lumen tube that has a semipermeable outer membrane with a 20 000 Da cut-off at the tip, which has been described in detail elsewhere [11]. After organ retrieval, two catheters were inserted into the liver through the right liver segments V/VIII and the left liver segments II/III, respectively [12]. The catheters were connected to the microinjection pump (CMA 106; CMA Microdialysis AB) and perfused with isotonic sterile solution (sodium 147 mM, potassium 4 mM, calcium 2.3 mM, osmolality 290 mOsm/kg, pH6; perfusion fluid T1, CMA Microdialysis AB). The initial flow rate of 15 μl/min was automatically maintained for 6 min to remove cellular debris resulting from the catheter insertion, and its effluate was withheld from analysis. The microdialysis was then performed with a given perfusion rate of 0.3 μl/min. The dialysate fluid was sampled into microvials at intervals of 30 min and automatically analyzed as batches for pyruvate, lactate, glucose, and glycerol using enzymatic reagents and colometric measurements (CMA 600 Microdialysis Analyser; CMA Microdialysis AB). The validated measurement ranges for the standard reagents were specified by glucose 0.1–25, lactate 0.1–12, pyruvate 10–1500, and glycerol 10–1500 mM.

Laboratory analysis

Liver enzyme activities of aspartat-aminotransferase (AST) und lactic dehydrogenase (LDH) in the effluate

were assessed by photometrical analysis using commercialized standard kits.

For evaluation of histopathological changes, tissue biopsies were taken at 6, 12 and 24 h. Liver tissue was cut into small blocks (3-mm thickness) and fixed by immersion in 4% buffered formalin overnight at 4 °C. The blocks were embedded in paraffine and cut into 2-µm sections using a microtome. After hematoxy-eosin staining, histological changes with a focus on hepatocyte necrosis and endothelial swelling were evaluated by a semiquantitative score from 0 to 3 (Hepatocellular apoptosis/necrosis: *0* no damage, *1* 1–10 apoptotic/necrotic hepatocytes per 10 lobules, *2* 11–20 apoptotic/necrotic hepatocytes per 10 lobules, *3* more than 20 apoptotic/necrotic hepatocytes per 10 lobules; Endothelial damage: *0* no damage, *1* endothelial swelling, *2* endothelial swelling, vacuoles in endothelial cells, single detached endothelial cells, *3* detachment of sheets of endothelial cells).

Graphs and statistics

All data were expressed as box plots with the median (line within the box), framed by the 25th and 75th percentile. In addition, the mean was expressed by the dotted line within the box. Although the microdialysis data were measured continuously over the whole storage period, the box plot illustration accounts for the presentation of data at just five time points out of 48. Stochastical significance of differences was assessed using the Kruskal–Wallis analysis of variance on ranks and appropriate *post hoc* test (Dunn's method). Statistical significance was assumed for *P*-values <0.05.

Results

Effluate measurements

Median effluate levels of AST were lower after UW, compared with HTK preservation, and did not reach statistical significance, whereas the difference to the HES preservation group did. As expected, mild hypothermic saline storage differed significantly to the other groups (Fig. 1a). The measurements of LDH were similar to the course of AST, with UW median 193 U/l (range 135–216 U/l), HTK 246 U/l (170–531 U/l), HES 415 U/l (283–891 U/l), and 12 °C saline 2266 U/l (684–3493 U/l) (Fig. 1b).

For effluate lactate, significant higher concentrations were seen only in the mild hypothermia saline group. Independently of the solution used, the lactate levels did not differ between the groups with cold storage at 4 °C (Fig. 1c).

Histology

Mild hypothermia in saline solution resulted in a significant hepatocellular damage after 6 h (Fig. 2a), 12 h (Fig. 2b), and 24 h (Fig. 2c), when compared with all cold-stored livers. Almost no hepatocellular damage was detectable in the cold storage UW and HTK groups, whereas the livers after cold HES storage showed an intermediate injury with significance to the UW groups to all time points. The differences to the HTK group were less pronounced with significance after 12 h ischemia, but not after 6 h and 24 h.

Livers preserved in UW and HES solution revealed minor endothelial changes over the time period of cold ischemia. The endothelial impairment of the livers stored in cold HTK and mild hypothermic saline solution was significantly worse after 6 h (Fig. 3a), 12 h (Fig. 3b), and 24 h (Fig. 3c), when compared with UW storage, and cold HTK storage was superior to mild hypothermic saline solution after 12-h cold storage.

Microdialysis

Continuous measurement of glycerol during the 24-h storage period showed a significant increase in both the HES and mild hypothermic saline solution groups after

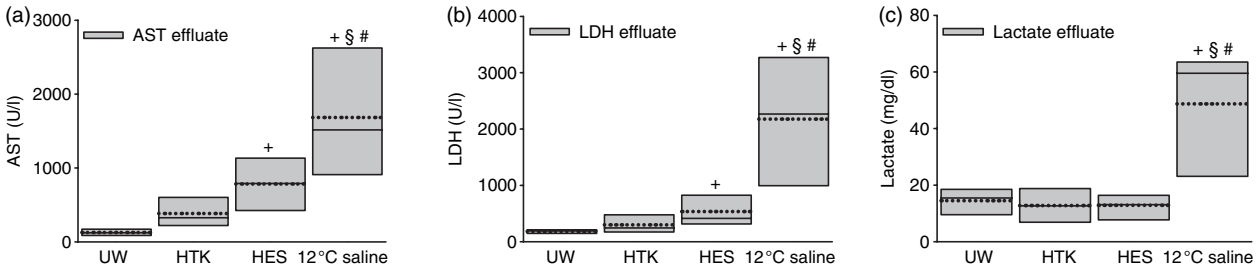

Figure 1 Cumulative liver enzymes and lactate levels following 24-h cold storage (4 °C) in standard UW, HTK, and HES solution, as well as in mild hypothermic (12 °C) physiologic saline solution with subsequent graft rinse. The increase of (a) AST and (b) lactic dehydrogenase levels in both saline-stored groups indicate a distinct hepatocellular injury. (c) Lactate levels were accelerated only after mild hypothermic storage at 12 °C, indicating a decrease of glycolytic activity because of cold storage, even in HES solution. $P < 0.05$ vs. †UW, §HTK, #HES.

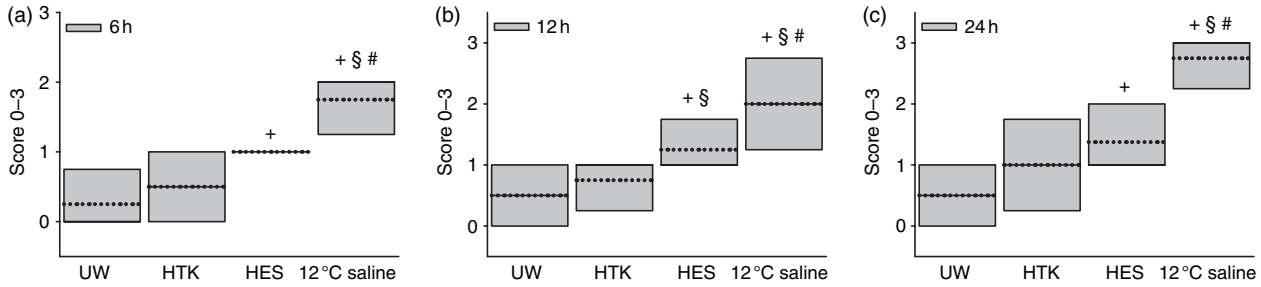

Figure 2 Degree of hepatocellular cell death during 24 h of ischemic storage at 4 °C (UW, HTK and HES solution) or 12 °C (saline solution) assessed by means of a semiquantitative score from 0 to 3 (score: *0* no damage, *1* 1–10 apoptotic/necrotic hepatocytes per 10 lobules, *2* 11–20 apoptotic/necrotic hepatocytes per 10 lobules, *3* more than 20 apoptotic/necrotic hepatocytes per 10 lobules). (a) 6 h, (b) 12 h, and (c) 24 h histological changes were opposed with superiority of UW solution over the whole storage period and of HTK storage until 12-h storage. $P < 0.05$ vs. †UW, §HTK, #HES.

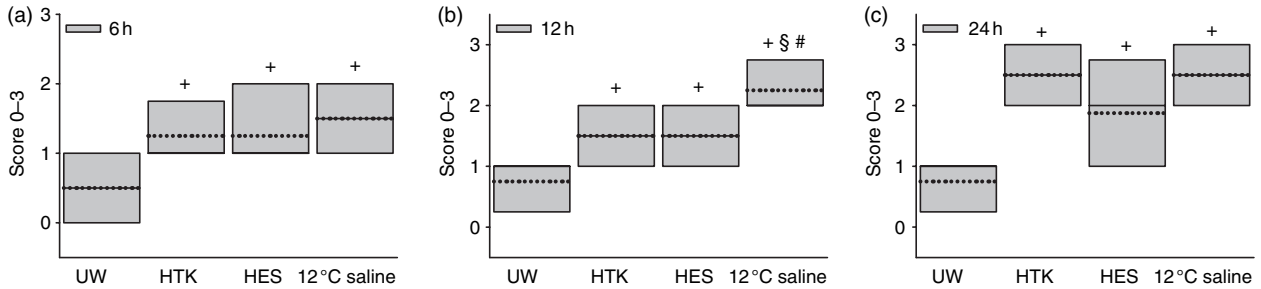

Figure 3 Degree of structural endothelial changes during 24 h of ischemic storage at 4 °C (UW, HTK and HES solution) or 12 °C (saline solution) assessed by means of a semiquantitative score from 0 to 3 (score: *0* no damage, *1* endothelial swelling, *2* endothelial swelling, vacuoles in endothelial cells, single-detached endothelial cells, *3* detachment of sheets of endothelial cells). (a) 6 h, (b) 12 h, and (c) 24-h histological changes demonstrate the superiority of UW for preservation of endothelial cell integrity. $P < 0.05$ vs. †UW, §HTK, #4 °C HES.

6 h, when compared with the UW and HTK groups (Fig. 4a). Glycerol levels in the HTK group were significantly higher compared with the UW group after 12 h (Fig. 4b) and 18 h (Fig. 4c). The difference of HTK storage to the HES and saline solution storage equalized after 18 h, whereas no differences of intrahepatic glycerol levels between all groups were detectable after 24 h (Fig. 4d), which, in terms of the validated measurement range for this parameter, has to be interpreted with caution.

In regard to the metabolic status, intrahepatic lactate levels increased with the onset of cold and mild hypothermic ischemia. A steady state condition was reached after 6 h with a significant difference between the cold and mild hypothermia, but not between the different preservation solutions in the cold storage groups. Pyruvate levels were under the detection limit over the whole observation period; therefore, the calculation of the lactate/pyruvate ratio was not performed. The intrahepatic glucose levels demonstrated a persistent glycogenolysis in the HES and the mild hypothermic saline storage groups, with highest levels in early phase of the ischemia in the mild hypothermic and the late phase in the HES storage group. The glucose levels remained unchanged in the UW and HTK group (Table 1).

Discussion

In this study, a continuous graft monitoring during 24-h cold storage (4 °C) in standard UW and HTK solution, as well as in cold HES and the mild hypothermic (12 °C) physiologic saline solution with subsequent graft rinse, was performed by using the microdialysis technique. The experimental protocol was amended by the estimation of liver enzyme release and lactate accumulation derived from the graft rinse solution, as well as of histological examinations. As measured by all parameters, mild hypothermic preservation in saline solution was worse compared with the other groups. Cold preservation resulted in a decrease of metabolic/glycolytic activity, as measured by intraparenchymal glucose, lactate, and pyruvate levels, and lactate in the rinse solution, independently of the solution used. Of particular interest, the dynamics of glycerol levels, which reflect cell membrane disintegration, were in accord with the extent of hepatocellular damage

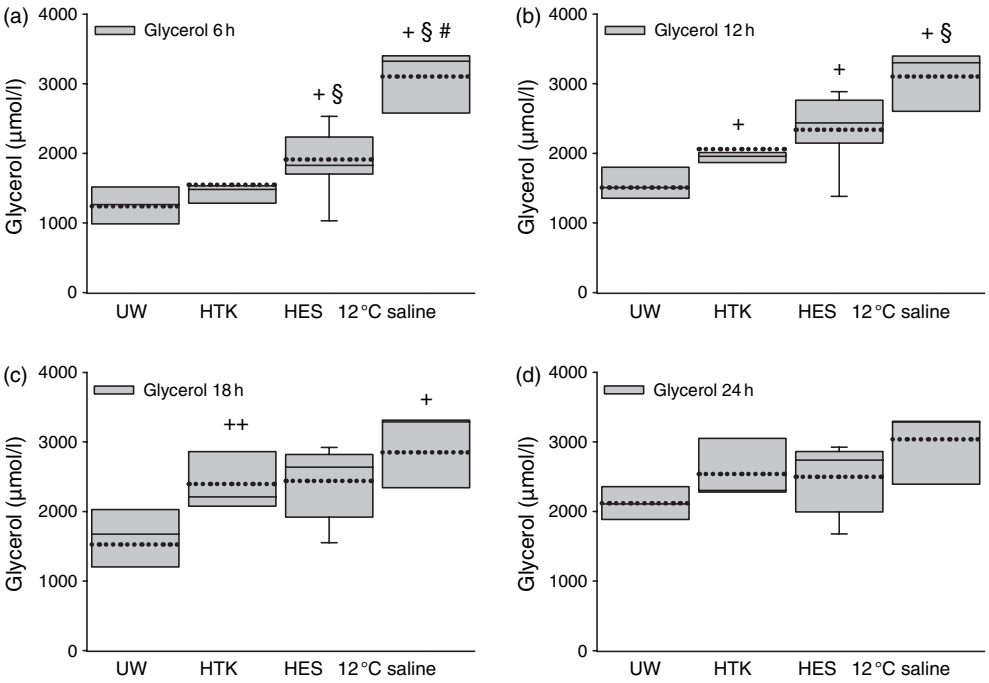

Figure 4 Intrahepatic glycerol levels during 24 h ischemic storage at 4 °C (UW, HTK and HES solution) and 12 °C (saline solution). After (a) 6 h ischemia, glycerol levels in both HES and saline solution were significantly accelerated compared with standard preservation solution, indicating membrane disintegration. Compared with UW solution, the glycerol levels in the HTK group significantly differed after (b) 12 h and (c) 18 h, demonstrating the superiority of UW for long-term preservation. After (d) 24 h of ischemia, the differences were equalized. Glycerol levels beyond 18 h have to be interpreted with caution, as far as the glycerol levels exceeded the validated measurement range. $P < 0.05$ vs. †UW, §HTK, #HES.

Table 1. Intrahepatic glucose (mmol/l) and *lactate (mmol/l)* levels as measured by continuous microdialysis during 24-h storage.

Group	0 h	3 h	6 h	12 h	18 h	24 h
UW	12 (7–13)	14 (9–21)	25 (6–33)	24 (10–35)	21 (10–37)	19 (13–36)
	1 (0–1)	3 (2–7)	6 (2–8)	6 (3–11)	6 (3–10)	6 (4–10)
HTK	12 (10–15)	16 (11–23)	28 (12–33)	25 (11–33)	23 (11–30)	23 (10–31)
	0 (0–3)	4 (2–9)	7 (4–10)	7 (4–10)	6 (4–11)	7 (5–11)
HES	11 (9–15)	20 (18–76)	30 (18–73)	33 (18–76)	36 (19–70)†§¶	31 (17–73)†¶
	0 (0–2)	6 (3–11)	6 (4–10)	6 (5–12)	7 (4–12)	7 (3–13)
12 °C saline	13 (10–18)	25 (16–73)	43 (19–81)	25 (16–73)	13 (4–21)	11 (3–19)
	1 (0–3)	21 (12–33)	19 (11–27)†§#	19 (13–30)†§#	20 (2–32)†§#	18 (14–29)†§#

Statistical significance with $P < 0.05$ vs. †UW, §HTK, #HES, ¶12 °C saline.

and liver enzyme release in these groups. Combined with the predominant endothelial cell integrity, the superiority of UW for longer storage (12–18 h) was accentuated. In addition, the data suggest a less effectiveness of HTK for preservation longer than 12 h.

The study of Nowak [6] demonstrated the correlation between cold ischemia time and glycerol levels following 5 and 15-h cold storage of pig livers in UW solution and suggested glycerol as a suitable parameter for the monitoring during cold storage. Even though they decided for the extensive pig liver transplantation model, they restricted their monitoring to the evaluation of glycerol until 1 h after graft reperfusion and gave no information about graft function or the animal survival. The intention of our study was to assess, whether or not continuous microdialysis during ischemia might be able to distinguish between different qualities of organ preservation. With a storage period of 24 h, we decided for an *ex situ* examination during a 24-h cold storage period, as far as 24-h cold ischemia represents a lethal condition in swine livers, even after storage in UW solution. For that reason, we actively decided against a reperfusion model, which

would have blurred the results of the storage examinations.

The metabolic markers of interest for microdialysis monitoring in this study were reduced to parameters, which typically reflect changes caused by ischemia. High lactate levels are encountered in ischemic and hypoxic states when cells depend on anaerobic glycolysis, resulting in an increase in lactate levels, and a decrease in pyruvate levels [13,14]. This may be the situation at normothermia [15] and was reproducible during 12 °C storage in saline solution, but not in the cold-stored livers. Pyruvate levels were undetectable during the entire cold storage, whereas lactate levels in the liver after cold perfusion did increase within the first 6 h with a stable steady state thereafter. This may be the result of reduced enzyme activities because of lowering the temperature to 4 °C, which has already been the assumption of Nowak *et al.* [8]. This hypothesis was confirmed by the findings of the cold HES group that cooling down the graft to 4 °C alone was obviously sufficient for this enzymatic inactivity, as far as there were no differences between the cold HES, compared with the HTK and UW groups. The assessment of lactate levels from the effluate after flushing the grafts exactly displayed the situation of the microdialysis measurements on the level of the vascular compartment. This is in a clear contradiction to the knowledge of the anaerobic metabolism during cold storage [17] and cannot be explained on the basis of available data. Another plausible, but hypothetical explanation for the lack of lactate accumulation in the microdialysis effluate could be a temperature-related diffusion block across the membrane [16]. Although reported by Nowak *et al.* [8], the intraparenchymal glucose levels did not increase during cold storage, but during mild hypothermic storage, as well as beyond 12 h cold storage in HES solution.

Glycerol is an integral component of the cell membrane, and increased levels in the interstitium reflect cell injury [10,18]. Loss of energy leads to an influx of calcium into the cell and activation of phospholipases, which split glycerol from the phospholipids in the cell membrane, resulting in the morphological correlate of membrane blebbing [19]. Nowak *et al.* could demonstrate the value of glycerol monitoring during cold storage of livers, with a correlation between the length of cold ischemic time and increasing glycerol levels [6]. In this study, cold ischemia effectively reduced the metabolic activity but could not prevent the time-dependent membrane damage as emerges from continuous increasing tissue glycerol levels. Glycerol levels started to increase after cold perfusion, being highest in the cold HES and the mild hypothermic saline solution groups after 4, 6, and 12 h. The further course of glycerol levels beyond 18 h has to be interpreted with caution, as far as the glycerol levels exceeded the validated measurement range. Regarding the histological findings of hepatocellular damage, as well as the liver enzymes released into the effluate, glycerol levels at about 3000 mM obviously represented the detection limit for this parameter and method. The real differences for glycerol release between the different preservation procedures were assumed to be even higher, especially for the HES and mild hypothermic saline solution group. Unfortunately, the microvials and the sample volume per measurement did not allow the further dilution, once the measurement was already performed. One possible modification could have been to increase the microdialysate flow to achieve an *in situ* dilution, which is not possible by using the standard equipment because of the technical configuration of the microdialysis pump system.

Nevertheless, by the means of glycerol levels, it was possible to distinguish between different qualities of organ preservation, even to discriminate the effectiveness of UW compared with HTK until 18 h of cold preservation. Combined with the predominant endothelial cell integrity, the superiority of UW for longer storage (12–18 h) was accentuated. In addition, the data suggest a less effectiveness of HTK for preservation beyond 12 h, which is well in accordance with experimental studies on liver preservation [20–22].

Real-time monitoring of metabolic changes by using microdialysis is possible, even under conditions of cold storage. From these experiences, we conclude that the assessment of intraparenchymal glycerol levels can give additional information of the dynamic process of damage in the time course of graft preservation. Despite the dynamics of lactate, pyruvate, and glucose levels during reperfusion, which reflect an increase of metabolic activity because of rewarming and restoration of blood flow [8,9,23], the estimation of these parameters is not appropriate to describe the injury during the cold storage. Whether glycerol monitoring could represent an additional and rational complementation to the current practice of macroscopic, microscopic, and donor evaluation has not been clearly demonstrated by this study.

The potential of microdialysis to exclude severely damaged livers from transplantation or to bring so-called marginal donor livers to transplantation has to be clarified by further studies, but expectations to further studies utilizing microdialysis for cold ischemia monitoring should be scaled down against the background of these results.

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References

1. D'Alessandro AM, Hoffmann RM, Knechtle SJ, *et al.* Liver transplantation from controlled non-heart-beating donors. *Surgery* 2000; **128**: 579.
2. Busuttil RW, Tanaka K. The utility of marginal donors in liver transplantation. *Liver Transplant* 2003; **9**: 651.
3. Lopez-Navidad A, Caballero F. Extended criteria for organ acceptance. Strategies for achieving organ safety and for increasing organ pool. *Clin Transplant* 2003; **17**: 308.
4. Melendez HC, Rela M, Murphy G, Heaton N. Assessment of graft function before liver transplantation. *Transplantation* 2000, **74**: 560.
5. Brinceno J, Marchal T, Padillo J, Solorzano G, Pera C. Influence of marginal donors on liver preservation injury. *Transplantation* 2002, **74**: 522.
6. Nowak G, Ungerstedt J, Wernerson A, Ungerstedt U, Ericzon BG. Hepatic cell membrane damage during cold preservation sensitizes liver grafts to rewarming injury. *J Hepatobiliary Pancreat Surg* 2003; **10**: 200.
7. Nagayama M, Katsuramaki T, Kimura H, *et al.* Prediction of graft viability from non-heart-beating donor pigs using hepatic microdialysate hypoxanthine levels. *J Surg Res* 2002; **107**: 210.
8. Nowak G, Ungerstedt J, Wernerman J, Ungerstedt U, Ericzon BG. Metabolic changes in the liver graft monitored continuously with microdialysis during liver transplantation in a pig model. *Liver Transplant* 2002; **8**: 424.
9. Nowak G, Ungerstedt J, Wernermann J, Ungerstedt U, Ericzon BG. Clinical experience in continuous graft monitoring with microdialysis early after liver transplantation. *Br J Surg* 2002; **89**: 1169.
10. Hillered L, Valtysson J, Enblad P, Persson L. Interstitial glycerol as a marker for membrane phospholipids degradation in the acutely injured human brain. *J Neurol Neurosurg Psychiatry* 1998; **64**: 486.
11. Rasmussen I, Hillered L, Ungerstedt U, Haglund U. Detection of liver ischemia using microdialysis during experimental peritonitis in pigs. *Shock* 1994; **1**: 60.
12. Zanchet DJ, Montero EF, de Souza D. Pig liver sectorization and segmentation and virtual reality depiction. *Acta Cir Bras* 2002; **17**: 381.
13. Sommer T, Larsen JF. Intraperitoneal and intraluminal microdialysis in the detection of experimental regional intestinal ischemia. *Br J Surg* 2004; **91**: 855.
14. Jansson K, Ungerstedt J, Jonsson T, *et al.* Human intraperitoneal microdialysis: increased lactate/pyruvate ratio suggests early visceral ischemia. A pilot study. *Scand J Gastroenterol* 2003; **38**: 1007.
15. Mehrabi A, Golling M, Busch C, *et al.* Experimental monitoring of hepatic glucose, lactate, and glutamate metabolism by microdialysis during surgical preparation of the liver hilus. *J Surg Res* 2002; **105**: 128.
16. Kennergren C, Mantovani V, Strindberg L, Berglin E, Hamberger A, Lonnroth P. Myocardial interstitial glucose and lactate before, during, and after cardioplegic heart arrest. *Am J Physiol Endocrinol Metab* 2003; **284**: E788.
17. Churchill TA, Cheetham KM, Fuller BJ. Glycolysis and energy metabolism in rat liver during warm and cold ischemia: evidence of an activation of the regulatory enzyme phosphofructokinase. *Cryobiology* 1993; **31**: 441.
18. Nilsson OG, Brandt L, Ungerstedt U, Saveland H. Bedside detection of brain ischemia using intracerebral microdialysis: subarachnoidal hemorrhage and delayed ischemic deterioration. *Neurosurgery* 1999; **45**: 1176.
19. Nadig SN, Periyasamy B, Shafizadeh SF, *et al.* Hepatocellular ultrastructure after ischemie/reperfusion injury in human orthotopic liver transplantation. *J Gastrointest Surg* 2004; **8**: 695.
20. Straatsburg IH, Abrahamse SL, Song SW, Hartman RJ, van Gulik TM. Evaluation of rat liver apoptotic and necrotic cell death after cold storage using UW, HTK, and Celsior. *Transplantation* 2002; **74**: 458.
21. Janssen H, Janssen PH, Broelsch CE. UW is superior to Celsior and HTK in the protection of human liver endothelial cells against preservation injury. *Liver Transplant* 2004; **10**: 1514.
22. Wilson CH, Stansby G, Haswell M, Cunningham AC, Talbot D. Evaluation of eight preservation solutions for endothelial in situ preservation. *Transplantation* 2004; **78**: 1008.
23. Silva MA, Richards DA, Bramhall SR, Adams DH, Mirza DF, Murphy N. A study of metabolites of ischemia-reperfusion injury and selected amino acids in the liver using microdialysis during transplantation. *Transplantation* 2005; **79**: 828.

