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Characterization of organ quality of donation after cardiac death, brain-dead donors and living donors A review of current literature

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Graz, am 28.5.2013 *Julia Reisinger*

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Zusammenfassung

Für Patienten mit Krankheiten, die zu einem irreversiblen Organversagen führen, ist eine Transplantation die Therapie, die Chance auf ein neues Leben. Um den Organmangel bekämpfen zu können und einen größeren Spenderpool zur Verfügung zu haben, greift man wieder auf DCD und immer marginalere Spender zurück. Immer grenzwertigere Spenderorgane liefern aber schlechtere Ergebnisse nach Transplantation. In den Spenderorganen kommt es zu Schäden durch Ischämie und Reperfusion. ROS bzw. RNS Entstehung führen zu einer Veränderung in der Gen- oder Proteinausprägung. Veränderungen die durch Ischämie/Reperfusion entstehen, können Gene in Richtung Apoptose beeinflussen. Dadurch führt ein starker Ischämie/Reperfusionsschaden zu einer geringeren funktionierenden Zellmasse im Spenderorgan, was die Organfunktion und das Ergebnis nach Transplantation beeinträchtigt.

Um die Organqualität zu messen und in weiterer Folge zu verbessern, werden Primer wie Bcl-2, Bax, GSH, GPx (Gpx3), OXSR-1, NF-κB, SOD (SOD2), Heat Shock proteins (Hsp70), PPARα, HPRT, Nitrotyrosine (3-NT), Myeloperoxidase (MPO) und Caspase-3 gemessen.

Zusammenfassend kann gesagt werden, dass Bax, NF-κB, MPO, 3-NT und Caspase-3 während Ischämie hochreguliert werden und zu schlechteren Ergebnissen und Apoptose führen können. Andere Gene oder Proteine, wie Bcl-2, GSH, GPx-3, OXSR-1, NF-κB, SOD2, Hsp70, PPARα oder HPRT-1 haben schützende Eigenschaften.

Abstract

For patients with diseases leading to irreversible organ failure, transplantation is the therapy and the chance for a new life. To combat the shortage of organs and to have a larger pool of donors available, it falls back to DCD and always more marginal donors. Still marginal donor organs are providing poorer results after transplantation. Ischemic and reperfusion damage occur in donor organs. ROS or RNS formation lead to a change in gene or protein expression. Changes caused by ischemia/reperfusion affect genes leading to apoptosis. Therefore, a strong ischemia/reperfusion injury ends up in apoptosis and lower functioning cell mass in the donor organ, which affects the function and the result in the recipient.

In order to measure the body and to improve quality subsequently, primers such as Bcl-2, Bax, GSH, GPx (Gpx3), OXSR-1, NF-κB, SOD (SOD2), Heat Shock proteins (Hsp70), PPARα, HPRT, Nitrotyrosine (3-NT), Myeloperoxidase (MPO) and Caspase-3 are measured.

In summary, can be said that Bax, NF-κB, MPO, 3-NT and Caspase-3 are up regulated in ischemia and determine worse results or lead to apoptosis. Other genes and proteins, such as Bcl-2, GSH, GPx-3, OXSR-1, NF-κB, SOD2, Hsp70, PPARα, HPRT-1 have protective properties.

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Glossar und Abkürzungen

3-NT – 3-Nitrotyrosin

ACC – N-Acetylcystein

ALT – Alanin-Aminotransferase

AST – Aspartat-Aminotransferase

ATG – Anti-Thymocyte Ig

BAX – Bcl-2 Associated X-protein

BCL-2 – B-cell lymphoma 2

BD – Brain Death

BMI – Body Mass Index

CIT – Cold Ischemia Time

CKD – chronic kidney disease

CRP – C-reactive protein

DCD – Donation after cardiac death or donation after circulatory death

DGF – Delayed graft function

DNA – Deoxyribonucleic Acid

ECD – Expanded Criteria Donors

ET – Plasma Endothelin

GMP – Guanosine Monophosphate

GPx – Glutathione Peroxidase

GPx3 – Glutathione Peroxidase 3

GSH-S – Glutathione synthetase

H_2S – Hydrogen Sulfide

HES – Hydroxyethyl starch

HIF1α – Hypoxia-inducible factor 1

HLA-System – Human Leukocyte Antigen-System

HTKC – Histidine-Tryptophan-Ketogluterate Solution

HPRT1 – Hypoxanthine-phosphoribosyl-transferase 1

HGPRT – Hypoxanthine-guanine phosphoribosyltransferase

HSP – Heat Shock Protein

IAPs – Inhibitors of apoptosis protein

IC – Intrahepatic Cholestasis

IMP – Inosine Monophosphat

IPC – Ischemic Preconditioning

I/R – Ischemia/Reperfusion

MELD – Model for End Stage Liver Disease

MPO – Myeloperoxidase

MTP – Mitochondrial Transmembrane Potential

NOS – Nitric Oxide Synthase

- eNOS – endothelial Nitric Oxide Synthase
- iNOS – inducible Nitric Oxide Synthase
- nNOS – neuronal Nitric Oxide Synthase

NF-κB – Nuclear Factor kappa-light-chain-enhancer of activated B cells

- I-κB – Inhibitor kappa-light-chain-enhancer of activated B cells
- RelB – Family member of NF-κB Protein Family

OS – Oxidative Stress

OXSR1 – Oxidative Stress Receptor 1

PNF – Primary Non Function

pONS – Preconditioning Oral Nutritional Supplement

PFC – Perfluorocarbon

PPARα – Peroxisome proliferator-activated receptor alpha

PRA – Panel Reactive Antibody

PRPP – Phosphoribosyl Pyrophosphate

ROS – Reactive Oxygen Species

RNS – Reactive Nitrosative Species

SOD – Superoxide dismutase

- SOD2 – Superoxide dismutase 2

SPAK – STE20/SPS1-related proline/alanine-rich kinase

TNF – Tumor Necrosis Factor

UW – University of Wisconsin solution

WIT – Warm Ischemia Time

Abbildungsverzeichnis

1 Introduction

1.1 Historical overview

The aim of this review was to give an overview of the current literature of characterization of organ quality on molecular basis of brain death versus donors after cardiac death with living donation.

Initial trials of transplants were made early on. In the 17th Century, attempts to replace damaged human skin by animal tissues were made. Later attempts were to transplant endocrine tissue. In 1902 Emerich Ullmann (1861-1937) carried out the first experimental kidney transplantation (autotransplantation) in Austria on a dog. (1)
After advances in vascular surgery a lot of experimental transplantations were performed, as a prerequisite for human transplantation. However, most grafts were lost due to the lack of knowledge about rejection and immunosuppression. In 1954 the team of Joseph E. Murray managed a successful kidney transplant. However, in this case donor and recipient were monozygotic twins, therefore no rejection occurred. First trials to prevent rejection have been made by irradiation. In the 1960s prevention of rejection was achieved with newly developed drugs (Azathioprin) as a milestone in transplantations history. (2)
After some unsuccessful trials, the addition of steroids showed better results in the inhibition of rejection. The next milestone in immunosuppression was the introduction of cyclosporine in 1978. (3, 4)
Over the years experience in transplantation of nearly all organs such as lung, liver and heart was gained and parallel advances in immunosuppression ameliorated graft and patient survival. (4)

Nonetheless, because of the problems of rejection, the immune system was studied extensively. In 1958 and in 1963 Dausset and Van Rood described the HLA system. (5) Results of these experiments showed that the antigens on leucocytes are different for each individual and if a high degree of donor and recipient match of the HLA system is given, the probability of rejection is low. (6)
Kidneys from living donors with HLA-mismatch have a better outcome than kidneys from brain dead donors with HLA-match. Organs from brain dead donors or donation after

cardiac death (DCD) are damaged by the circumstances before removal. Warm ischemia damages the donor organs and the diseases a subsequent DCD donors has, can increase the ischemic damage. DCD organs have a lower functioning cell mass, which results in dysfunction and chronic rejection. (6 - 9)

Since transplantation is used as treatment for many diseases and end-stage organ failure, there are more patients needing an organ, than available donor organs. With increasing number of patients registered for transplantation and a limited number of donor organs, many patients die while waiting for an organ. (4)
In certain cases, such as in kidney, liver and bone marrow sources of donor organs may be living donors. (4)
Yet with living donors, the growing demand cannot be met. Thus it is important to try to expand the organ donation pool continuously. Possibilities to increase the numbers of organs transplanted are among others, the use of so-called “marginal donors” as well as organs from donors after cardiac death. Due to the problem of meeting demands, the access to even more marginal donors and DCD is increasing. However, among other problems ischemia/reperfusion injury and early graft failure can occur because of an active apoptotic process (4) and changing in gene expression (10) during BD.
Over time, the waiting time has ever extended to get an organ, because there are more and more patients who need an organ. Adults wait longer for an organ than children. And the waiting time is also dependent on the age and blood group. Elderly patients on the waiting list have a greater chance of dying. The outcome of transplantation is also affected by whether it is the first transplantation or even a subsequent transplantation. (11)
The quality of so-called marginal donor organs is on the border between acceptability and unacceptability. (12)
Inflammation and oxidative stress (OS) affect organ function after transplantation. If too many oxygen radicals are formed, the antioxidant defense system cannot compensate and therefore there will be disturbances or damage to the tissue. Patients with chronic kidney disease have a higher risk of forming potential oxidative stress. This increased formation of oxidative stress leads to further damage to the kidneys. Patients with chronic kidney disease have oxidative stress, which is blamed for the fact that we age and for chronic diseases. Because OS, as an imbalance between anti-oxidative defense system and reactive oxidative substances, damages tissue. (13, 14)

As part of renal transplantation ischemia/reperfusion phase leads to further damage by building oxidative stress leading to a deterioration of the graft survival and to primary non-function (PNF). (7, 14)

1.2 Oxidative Stress and Nitrosative Stress

Reactive oxygen species (oxygen, superoxide, hydrogen peroxide and hydroxyl radicals) are produced by cells during normal metabolic processes such as cell cycle, cell differentiation and reaction to stimuli, such as ischemia. They have an unpaired electron, thus connect easily and can thereby triggering a series of reactions. (15 - 18)

To compensate for the damaging effects of ROS, there are repair mechanisms and antioxidant substances such as, superoxide dismutase, glutathione or glutathione peroxidase. If ROS predominates and acts in a harmful manner, it destroys membranes, DNA and proteins. (19) Oxidative stress leads to destruction, beginning with a loss of cell integrity and resulting in cell death. (17)

ROS formation and effects develop into a vicious circle, because primarily in oxidative stress, mitochondria are damaged. Yet, at the same time, they themselves are a major producer of ROS. (20)

Reactive nitrosative species (RNS), including nitric oxide (NO) and nitrogen compounds also occur in the cell life and in inflammation as well as ischemia. Low NO values extist to protect cells, high concentrations of NO and nitrogen compounds damage by affecting the respiratory chain, which inhibits protein synthesis, leading to lipid peroxidation. (17, 19) RNS also can lead to apoptosis. (19)

RNS is produced because of the oxidation of L-arginine by nitric oxide synthase (NOS). There are three isoforms of NOS: endothelial NOS (eNOS), neuronal NOS (nNOS) and inducible NOS (iNOS). (13) In the kidneys there can be found all three isoforms of NOS. In the liver and the heart, there is only eNOS and iNOS. (16)

1.3 Warm Ischemia

Warm ischemia or warm ischemia time (WIT) occurs when blood flow to the organs no longer exists, but cooling has not yet taken place. At donors, there is warm ischemia from functional cardiovascular arrest to organ removal. (46) During this period there is a need for more energy. When the metabolism is changing, energy reserves are consumed, harmful metabolites are formed and the membrane potential can no longer be maintained. This results in cell swelling and cell death. (4) WIT occurs in BD donors due to circulatory instability. (60) But the longest warm ischemia time is usually in uncontrolled DCD organs in category I (see below). Those donor organs provide inferior results. (21, 22)

1.4 Cold Ischemia

After organ removal, all organs go through cold ischemia during storage and transport. The organ is cooled to 1 to 4 ° C. (16) This is supposed to keep the ischemic damage as low as possible. (4) At normal temperatures all metabolic processes continues. At lower temperatures, the metabolic process is slowed. But even during the cooling, oxidative stress is produced. (23) And the longer cold ischemia lasts, the greater the ischemia/reperfusion injury. In addition to that reperfusion exacerbates the damage. (24, 25)

1.5 Pathophysiology of Apoptosis

Apoptosis is a necessary cell function for the removal of unnecessary, damaged or infected cells in normal development, tissue homeostasis, aging, protection against pathogens and occurs as well in transplantation during ischemia and reperfusion. (25, 26)
Apoptosis can be induced through two major pathways, an extrinsic and an intrinsic one. The extrinsic pathway can be divided into at least two parts. The first part of the extrinsic pathway is regulated via an activation of cell death receptors on the cell surface, whereas the other is triggered by cytotoxic stress. Various forms of cellular stress activate the latter pathway. The activation mechanism leads to altering mitochondrial permeability by loss of membrane potential, leading to subsequent cytochrome c release. The release of cytochrome c is regulated by anti-apoptotic Bcl-2 family members. (27) The pro-apoptotic Bcl-2 family member Bax is also localized on the outer mitochondrial membrane. In order to activate apoptosis by Bax, it has to translocate into mitochondria (20) and either directly form pores, or bind to domains including Bcl-2 and antagonize anti-apoptotic genes. (26, 71) Bax also activates the release of cytochrome c. Released cytochrome c leads to an activation of caspase-3, which damages DNA and thereby causes cell death. (27)
Damage in the mitochondrial membrane or change in membrane potential, additionally caused by ischemia, is also responsible for further ROS production. (20)
The intrinsic pathway to apoptosis is triggered by events in the cell, such as hypoxia or DNA damage. P53 is a sensor of cellular stress and an activator of the intrinsic pathway. P53, occurring during ischemia, activates pro-apoptotic Bcl-2 family members such as Bax. Apoptosis is necessary for the development and tissue balance but apoptosis can also be caused by disturbances. (25, 28)

At the time of transplantation apoptosis occurs during brain death or even more in DCD setting, during cold ischemia (CIT) as well as during reperfusion phase. The ischemic damage can be aggravated by the reperfusion phase. (12, 16, 21) An increasing amount of apoptosis can be detected, the longer the ischemia lasts. (25) Shortly after transplantation (day 1 after transplantation), there are no differences in the degree of apoptosis in organs with short and long ischemia. From day three after transplantation on, differences can be observed. In the organs with prolonged ischemia more apoptotic cells can be found. (29)

The various types of donors are damaged to varying degrees. In DCD, kidney damage could be found: among others, acute renal tubular lesions, lesions of the proximal tubule, loss of the brush border and isolated necrotic cells occur in DCD kidney. In comparison, the donor organs from living donors show only slightly damaged cells. The highest levels of apoptosis show the cells of the distal tubules. Occasionally interstitial cells were damaged. Damage to the glomeruli could not be detected. There was also a correlation between the occurrence of apoptosis and the duration of ischemia. (30)

Bax was detectable in all apoptotic tubular cells of DCD kidneys. In comparison, detectable Bax was more than 5 times lower in kidney cells from living donors. During ischemia in DCD kidneys the proapoptotic Bax is activated. Bax is a mediator in the mitochondrial pathway of apoptosis. (30)

Cytochrome c detection in cells from living donors was lower than in DCD kidneys. Cytochrome c release is typical for an activation of the mitochondrial apoptosis pathway. (30)

1.6 Donor types

1.6.1 Brain dead donors

In 1956 the concept of brain death was described for the first time. In 1968, guidelines for defining brain death were established by the Harvard Medical School. (31, 32) Since then the largest source of donor organs are brain-dead patients. (32)

According to the definition from brain death, a patient must be unaware of and unresponsive to external stimuli, show no spontaneous movements or breathing, absent reflexes and a flat encephalogram. Causes such as hypothermia, hypotension, or intoxication, which can cause similar symptoms must be ascertained and ruled out. (33)

Brain death is a clinical diagnosis. Brain death is the irreversible loss of all brain function, including the cerebellum and brain stem. (34) Brain-dead patients are ventilated mechanically, with the heart beating. (4)

In daily clinical routine most organs are from brain-dead donors, therefore it is important to get insights how brain death influences organ quality, (32) subsequently contributing to the improvement of donor quality. (35)

Initially, after brain death, large amounts of catecholamines lead to severe hemodynamic changes and swings in blood pressure. (32) In the early stage there is hypertension, but then gradually a normotension or a hypotension can be observed. Finally, hypotension can persist. Due to the fact that catecholamines are distributed which leads to vasoconstriction, an insufficient blood supply of organs is caused, despite increased blood pressure. (32)

Further tissue ischemia leads to changes in energy supply and an associated rise of oxygen free radicals. The electrolyte balance is changing. Among others, the cytosolic calcium, potassium (36) and nitric oxide synthase increases. (38)

Brain death affects hormonal homeostasis, although there is controversy in the role of endocrine changes after brain death in animals and humans. (32) After brain death, residual blood flow can remain in the brain. The function of the hypothalamus can be maintained and a massive amount of catecholamines is released, mostly leading to hypertension. By the catecholamine release there is a systemic hypoperfusion and endothelial cells are damaged. Endothelial defects lead to destruction of the blood-brain barrier. (39) During and after brain death a release of adrenocorticotropic hormone and anti-diuretic hormone may result. If then a failure of the circulation manifests, support is needed. An impaired release of anti-diuretic hormone leads to an impaired osmotic balance and diabetes

insipidus. Thyroid hormones and blood sugar levels become unbalanced. Combined with increased gluconeogenesis and insulin resistance it may lead to hyperglycemia, which requires insulin therapy. The hormonal dysregulation should be responsible for the faulty work of the mitochondria and their added production of oxidative stress. (33, 39)

A difference in graft survival between grafts from brain-dead donors and those from living donors can be observed. Inflammation and an increased number of apoptotic cells have been found in liver grafts after brain death. (37) Shortly after brain death inflammation may be detected at different levels. ROS are produced during ischemia/reperfusion, leading to oxidative stress and cell damage. (40) Alterations gene expression in BD are not (only) affected by hypoxia. Hypoxia can lead to apoptosis. Damage can be detected by hypoxia through HIF1α, which is up regulated during hypoxia. During and after BD no HIF1α is detectable, so it is assumed that changes in gene expression and activation of apoptosis is mainly made by BD. In addition the intrinsic apoptotic pathway is stimulated by hypoxia. But BD activates much more the extrinsic apoptotic pathway, although both are activated. (41)

However it was shown, that there are not only detrimental changes during brain death, but also favorable, such as an up regulation of cell protecting proteins, like Hsp 70. (39, 42)

It was found that the longer the brain death takes the transplantation outcome is improved. Hemodynamic instability after brain death affects organ function. A longer time of removal of the donor organ has apparently the possibility to adapt and protect itself with relevant up-regulation systems. (43, 44)

Treatment of brain-dead donors to compensate for disturbances (for example electrolyte balancing and hormone replacement) brings a benefit for organ function and the results after transplantation. (10)

1.6.2 Donation after Cardiac Death (DCD)

Since the *First International Workshop on DCD* in Maastricht in 1995, four categories of donation after cardiac death or donation after circulatory death (DCD) have been identified (Figure 1). (45) Until the brain death guidelines were issued, DCD were the main source for organ transplants. (22) From the time of cardiac or circulatory arrest until initiation of organ preservation there is a period of warm ischemia or WIT. (46) During this period, ischemic damage occurs and reduces the graft quality. In kidneys, warm ischemia leads to an acute kidney injury, increased incidence of acute tubular necrosis (4), resulting in delayed graft function (DGF) and primary nonfunction (PNF). Organs from Category I DCD have potentially the longest time of warm ischemia, Category IV potentially have the shortest one. (22, 47)

Maastricht category II and I are so-called "uncontrolled" DCD donors. Category III and category IV are "controlled" DCD donors. In contrast to uncontrolled DCD donors where no preparations were made for a transplant, at controlled DCD donation the removal of organs is prepared and life support will be terminated. (46)

Maastricht category	Description
I	Dead on arrival (uncontrolled)
II	Unsuccessful resuscitation (uncontrolled)
III	Awaiting cardiac arrest (controlled)
IV	Cardiac arrest while brain dead (controlled)

Figure 1 Categories of donors after cardiac death (DCD) (47)

While the incidence of PNF and one-year graft survival in controlled versus uncontrolled kidney DCD is not significantly different, there is a higher incidence in delayed graft function in uncontrolled DCD. (45) The incidence of PNF is higher in DCD than in donation after brain death (46), which is a result from the ischemic injury before organ recovery. In spite of higher incidence in PNF, the survival from DCD kidneys and those from brain dead donors seem to be equal. (4)

Also livers retrieved from DCD donors show a higher incidence of PNF and poorer survival than livers retrieved from DB donors. (4, 48)

Each organ tolerates warm and cold ischemia differently, because of their natural metabolic processes. (4) DCD organs are exposed to prolonged warm ischemia time (46),

as mentioned above. Kidneys retrieved from BD donors should be transplanted within 18 hours and a liver or pancreas within 12 hours. DCD organs have a poorer tolerance and need to be transplanted within 12 for kidneys and 6 hours for livers and pancreas, because they suffer from longer ischemia. (4)

Intrahepatic cholestasis (IC) is also more common in "controlled" DCD than in DBD. It is believed that using the University of Wisconsin solution the higher viscosity of this preservation solution as compared to the use of histidine-tryptophan-ketogluterate solution (HTKC), plays a part for the formation of an IC. A higher viscosity results in a poorer perfusion in the small vessels, which characterizes that there is a poorer supply of the biliary system. The bile-access system is less tolerable over hypoxia. IC is actually no problem in DBD but in DCD. (46)

But there are also results showing that DCD organs are not significantly worse than the BD or living donor organs. (7, 50, 51) Donor organs from Maastricht category II and III had longer warm ischemia times than organs from BD and live donor organs. If WIT was longer than 40 minutes, organs failed. Despite the poor donor category there were approximately, the results are obtained as with BD and living donor organs by keeping the WIT as small as possible. (51)

The number of DCD transplantations organs rise in the U.S. and in Europe. However, there are a lot of legal and ethical issues that have to be discussed before DCD donors will become more accepted in society. (46)

From the time of cardiac arrest, there is still a certain waiting time (which is different from country to country), to make sure that no organs are taken from living. During the period after cardiac arrest, and actually already in the circulation insufficient time shortly before cardiac arrest, organs suffering form hypoxia and ischemia. Therefore, there exists time pressure to keep the ischemic time as low as possible to minimize damage to the organs. (52)

1.6.3 Expanded criteria donors, so-called “Marginal donors”

Increasingly, so-called “marginal” donor organs are used to shorten the waiting time for an organ transplant. (46) An optimal donor with expanded criteria (ECD) is a young “controlled” DCD patient who succumbs to a traumatic brain injury. The WIT and CIT in “controlled” groups of DCD can be estimated and thus be reduced. Arrangements for withdrawal can be made. (49) Thus, the remaining organs in the young patient are well preserved, usually little predamaged and re-start operation immediately after transplantation. In older patients, the organs can be damaged already by the age alone, pre-existing conditions such as hypertension, diabetes mellitus, and vascular damage caused by disease, infection or malignancy. (53)
ECD organs have a higher risk of organ failure, acute rejection and reduced long-term survival. Responsible may be a longer CIT, a smaller amount of functioning starting mass and less repair capacity. (53, 54)

Patients who are listed for an ECD organ and get ECD organs are usually older, belong to a certain race, disease groups and gender. As a patient at ECD waiting lists, you still have the possibility of non-ECD organ to receive. ECD organs are more often HLA-mismatched than standard organs. There is a trend towards more transplants with fewer HLA match but lower CIT, both with non-ECD and ECD organs. Poorer outcome with and between different races or genders can be attributed to panel reactive antibodies (PRA). PRA antibodies are formed by previous transplants, transfusions or pregnancies that are directed against HLA antigens. If you have a great amount of such antibodies, the patient is sensitized, so to speak, to respond with a higher probability against an alien organ. (11, 53)

The marginal donors, focusing on liver transplantation include those ECD organs from elderly patients. But the age of the recipient influences the results negatively. The older the donor, the worse is the result of transplantation. However, an ideal liver transplant recipient for an ECD organ of elderly patients (over 70 years) is under 45 years old, has a body mass index (BMI) less than 35 and no viral hepatitis. Transplantation within 8 hours CIT brings results near transplantation results of standard organs. Steatosis in donor livers is a criterion for ECD liver organs and steatotic livers are more sensitive to ischemia and reperfusion. (12)

There is not only steatosis in DCD donors, but also in living donors. Steatosis occurs with increasing age and BMI more frequently. Steatosis is divided into microvesicular and macrovesicular (with alcohol abuse, obesity and hyperlipidemia) forms. Microvesicular steatotic livers are of little concern for transplantation. In macrovesiculare types, the possibilities for transplantation depends on the severity of steatosis. The severity of steatosis is divided into three grades: mild, up to 30% of hepatocytes are afflicted, moderate, 30 to 60% are affected and the severe form with an infestation of more than 60% of hepatocytes. If there are no other risk factors in transplantation of mild steatotic livers, the results are similar to those of standard grafts. Livers with severe steatosis have often PNF and should not be used. But it is possible to take very fattened livers, if it is a microvesicular steatosis. Livers with moderate steatosis are treated as marginal donor organs. There may be worse results than with standard organs, especially if additional risk factors such as extended CIT are added. (49)

Also organs of patients with infectious diseases are meant to be classed as marginal donor organs. In the case of, for example, HCV positive organs, recipients are usually infected, therefore positive livers should be transplanted into HCV negative recipients only in exceptional cases HCV. For HCV positive recipients who received HCV positive organ, there are similar results in HCV negative recipients who received HCV negative organs. Generally, HCV positive organs show a stronger fibrosis, more organ failure after transplantation in the same age group as compared to HCV negative organs. (49)

For kidney transplantation ECD organs are DCD organs over 60 years of age or over 50 years and have at least two of three risk factors, such as chronic hypertension, serum creatinine above 1.5 mg/dl or death from a cerebrovascular event. (54) In the case of kidney transplants, each transplant is better despite with ECD transplant organs, than staying in dialysis therapy, waiting for a standard organ. Thus, elderly patients over 60 years, diabetics older than 50 years, patients with vascular problems or patients whose life expectancy is shorter than the anticipated waiting time for a standard organ should be taken into consideration for ECD organ. (53)

1.6.4 Living donors

Organs from living donors have a lower ischemic time and more healthy functional tissue. WIT and CIT can be estimated and are kept short. (43) But "It still remains controversial whether organs from living donors are superior to organs from brain dead donors or DCD in terms of organ function and graft survival." (55)

In Japan, liver donations for adults stem mainly from living donors and split liver grafts are frequently used for children. (22) Results of split liver transplants can cause different results. (56) Liver size of donor and recipient need to match. If the right lobe of the liver is transplanted, the amount of liver tissue will be too small. Even when a part from the left liver lobe is transplanted, no adequate amount of functional hepatocytes are obtained. (57)

Living donor organs for kidney transplantation provide better results than DCD organs, especially if the transplantation can take place before the start of dialysis therapy. (9, 58)

In living donor organs, for the best results blood group, HLA and PRA accordance are chosen. Organ quality is affected by gender, race, age, BMI, and medical status. Poor health causes a risk of poor graft quality. Additionally, it is a risk for the donor. (58)

For living donors, the best results after transplantation are between HLA-identical twins' and siblings' donations. In the next place, there are the results of unrelated living donors and parent donations. All of these results mentioned above are still better than transplantations with DCD organs. Applies to living donation, the better the organs match, the better are the results. While the long-term survival at non-related living donor organs and parent donations approach to the results of DCD donations, however, depend on the HLA-matching status. When parents donate, the age difference between donor organ and recipient, probably causes worse long-term survival. Organs from living donors have a higher amount of functional initial tissue mass. In spite of that, functional tissue mass in living donors is nevertheless reduced by cell death. However, using organs from living donors remains a better option. (9)

1.7 Donor organs

1.7.1 Kidney donor organs

Patients with chronic kidney disease have an exposure to oxidative stress. Thus, not only the kidneys are damaged, but also other organ systems in the body. Furthermore, unfavorable oxidative stress status in the receiver can also affect the new organ. Despite of that, if the new organ is not exposed to an additional load of OS by transplantation, the OS status can recover. Due to a new kidney, antioxidants are in a better position to counter OS. After kidney transplantation, patients have a better OS status than patients who need hemodialysis, and the better OS status also protects the new organ. (14)

Brain-dead donors are the largest source of kidney transplanted. However, donor kidneys from brain-dead donors often yield poor results. Organs from brain dead donors are different by systemic and hormonal changes connected brain death. And even the age of the donor, cause of death, pre-existing diseases play a role. Results in kidney transplantation from living donors have better results than those with organs from deceased donors. (33) In renal transplantation, patients on the waiting list have a higher chance of dying, the longer a necessary dialysis has lasted. (11) One is able to judge the quality of a kidney by measuring renal functional markers and histological damage. After brain death serum creatinine, urea (38) and apoptosis (37) increases.

Ischemia, especially cold ischemia affects results of transplants. Organs from deceased donors have a longer ischemic time (CIT as well as WIT). If a kidney transplant suffers more than 24 hours cold ischemia time, poorer results are reached. (33)

Pretreatment of kidneys from brain-dead donors with steroids reduces ischemia/reperfusion injury. Brain death leads to changes in inflammatory status. A treatment with anti-inflammatory steroids brings a benefit for the transplant outcome. (33, 38)

1.7.2 Liver donor organs

Liver transplants are the therapy for patients with end-stage liver failure. As with all organs, there are more patients on the waiting list than there are donor organs. Although you can rely on organs in liver transplantation from living donors, there are also increasingly requested marginal donor organs to gain the donor pool and improve their quality and therefore the outcome. (12) Today the majority of the organs comes from BD donors, but already the first liver transplant was from a DCD donor. (48)

1.7.3 Heart donor organs

The first heart transplant, performed by Barnard in 1967 in South Africa, was a DCD organ. (4, 7) Through the recognition of brain death as legitimized form of organ donation and the ensuing better results, moving away from DCD donors. But BD and the associated changes and disturbances of the circulation, affect the organ function and the results after transplantation. Changes in the donor heart are due to high catecholamine levels. High catecholamine levels appeare when BD sets on fast, tending to circulatory disorders. In contrast, catecholamine levels remain relatively low when the insertion of brain death happens slowly, having less impact on the circulatory stability. Infiltration with neutrophils in the heart of BD donors is higher as compared to organs from living donors. And cell death in the heart of BD donors is due to an ischemic injury, and circulatory instability. (59)

1.7.4 Pancreas donor organs

Pancreas transplantation for diabetics with kidney damage, can be made jointly with kidney transplants. Single pancreas transplantation is carried out for patients with diabetes mellitus without kidney damage or kidney was transplanted before. (11)

The results after pancreas transplantation from DCD donors and BD show no statistically significant differences, even though the results of DCD are slightly worse than after transplantation of BD organs. The endocrine function of pancreas after transplantation of DCD and BD are comparable. (60)

1.8 Improving Organ quality

To expand the available donor pool, beside living donors and the classical BD donor, also DCD and ECD organs are used. Unfortunatelly, these donor organs are linked to worse results. (46) An improvement of the donor organs may be achieved by treatments prior, during or post to transplantation. (38, 54)

In order to improve the quality and the results of kidney transplants, it is necessary to achieve a high starting value and functioning cell mass obtained. The CIT should be reduced, donor-recipient matching and age adjustments need to be made. There are worse outcomes for young patients if they do not get a body with glomerular sclerosis and a donor in their age group. Organs from elderly patients may provoke a stronger immune response in young patients. Improving in storage and transport can protect against I/R injury, improves or prevents this. (54) In order to achieve an improvement in long-term survival after DCD, chronic rejection has to be combated. Chronic rejection leads to loss of function of organs after at least 90 days after transplantation. Damage after cold ischemia can lead to malfunctions. This function disorders, such as delayed graft function (DGF), also has an impact on a chronic rejection and long-term outcomes after transplantation. (30) An activation and infiltration of the tissue by neutrophil granulocytes and apoptosis is induced by cold ischemia. Activation and infiltration of neutrophil granulocytes and apoptosis is caused by ROS. In turn, ROS is produced during ischemia. By addition of SOD this damage can be reduced. A modified form of SOD should also be more stable and better able to retain and therefore can perform its useful activity. With the addition of SOD, organs endure long periods of CIT. Damage to endothelial cells due to ischemia lead to activation of neutrophil granulocytes. If there is enough SOD, less neutrophil activation and infiltration, and thus, a better organ function can be seen. The modified SOD binds to endothelial cells and may well live out its antioxidant effect during ischemia and improve organ function. (29)

The better the processes that occur during brain death are understood, the better they can be fought. Good care of brain-dead donors and a treatment against possible disturbances (for example, electrolyte or hormonal) brings a benefit in organ function and in the outcome after transplantation. (10) Initially, after brain death a large amount of catecholamins leads to vasoconstriction and hypertension, a reduction of blood flow,

followed by a period of hypotension. Therefore brain death impairs microcirculation. Brain-death-induced disturbance of hepatic microcirculation occurs together with recruitment of leukocytes in sinusoids. Heat shock preconditioning prevents the recruitment of leukocytes during ischemia-reperfusion injury via suppressing NF-κB activation. (61) NF-κB is also responsible for the formation and activation of inflammatory mediators. Before ischemia or reperfusion, no signs of inflammation or stress, as well as nitrosative stress, are detectable. Heat shock pretreatment can prevent stress damage in which NF-κB is inhibited, and Hsp is up regulated. One way that heat shock treatment reduces ischemic damage, causing I-κB to stay bound to NF-κB, in this way it continoues to function as a blocker. After heat shock pretreatment, even higher levels of I-κB can be detected. These larger amounts of I-κB are thereby preventing activation of NF-κB. The up regulation of Hsp 72 helps to obtain I-κB, with its chaperone function. Besides, after heat shock pretreatment up regulated Bcl-2 can inhibit NF-κB. (62)

To obtain improved results after transplantations and to reduce ischemia/reperfusion injury, the duration of storage can be used, in order to positively influence the organ quality with the solution used during the storage.

DCD organs show worse results than DB organs. Perfluorocarbon (PFC), added to UW-solution, is used to improve organ quality. PFC is an inert substance. Due to its high solubility and ability to transport gases, it is used to improve the gas exchange and oxygen uptake during storage. At DCD donor organs, the benefit from the addition of PFC is shown. Also, reduction of necrosis can be seen. Apoptosis could not be detected. But vacuolization is regarded as a precursor of apoptosis and less vacuolization was detected after treatment of PFC. Although necrosis and apoptosis occur in various ways, both can be caused by hypoxia. Caspase-3 is upregulated when apoptosis happens. (23) Caspase-3 was detected only in 50 percent after treatment with PFC, as compared without. (46)

The PFC group showed a higher oxygen transport capacity. The additionally possible transport of oxygen creates a longer maintenance of aerobic metabolism. Although during organ preservation hypothermia is achieved and thereby the metabolism slows down, there is a depletion of energy resources, stress occurs and thereby tissue destruction. PFC can delay emptying the energy storage and therefore achieve less ROS, less cell destruction in DCD. During DCD, there is a decreased mitochondrial membrane potential, resulting in facilitated escape of cytochrome c into the cytosol. (46, 63)

If another substance is added to the storage solution, that can affect the organ quality. Usually we know carbon monoxide (CO) as a poisonous gas. Mammalian cells produce small amounts of carbon. This is usually done at the reaction when hemeoxygenase degrades heme. In these physiological quantities, CO is good for cell integrity. Stress creates pores in the mitochondrial membranes. If CO is added to the UW solution, the damages to the mitochondrial membrane are fewer than in an UW solution without CO. This was demonstrated by measurements of caspase-3. Caspase-3 in kidney was increased when no CO-UW solution was used. Compared to the previous example, caspase-3 was decreased when the CO was interred UW solution. Kidneys after transplantation without the addition of CO showed a greater increase in serum creatinine as kidneys in CO-UW solution. The addition of CO caused fewer apoptotic cells in the kidneys after transplantation. CO also has a positive effect on the release of inflammatory mediators. (64)

Hydrogen sulfide (H_2S) can also be added to the UW solution. H_2S has protective effects on many tissues, including liver tissue in kidney tissue or heart muscle tissue. H_2S may protect against ischemia/reperfusion injury. It acts as a vasodilator, prevents apoptosis and inflammatory responses, and it is also anti-oxidative. This addition to UW-solution protects cells from warm and cold ischemia and reperfusion injury. Treated tissues show less cell damage and less cell death. Also be detected MPO can in the less treated tissues. (65)

An additional benefit can be achieved with a cooling solution, when substances are added. Hydrogen can be added to histidine-tryptophan-ketogluterate Solution (HTK solution). HTK solution is used for cardioplegia and myocardial keeping. Hydrogen has cytoprotective properties. It is effective against oxidative stress, inflammation and cell death. The more hydrogen is supplied to HTK solution, the better the result. In myocardial cells it was able to obtain the SOD activity and there was an increase of Bcl-2. In contrast, Bax and caspase-3 was reduced. This leads to less apoptosis. (23)

The liver can be affected by many things such as heart failure, trauma, vascular occlusions and during transplantation. Ischemic damage leads to the activation of inflammatory mediators, ROS genesis and cell damage. The extent of the damage depends on NF-κB.

Currently you cannot prevent the development of damage in transplants and the corresponding deterioration of results.
Triptolide gained from Chinese herbs have been used for the anti-inflammatory, antifertility, immunosuppressive, and anti-neoplastic activity. It is already used in autoimmune diseases, the prevention of transplant rejection and graft-versus-host disease. Triptolide also act as an inhibitor of NF-kB and can potentially act to prevent damage caused by ischemia in transplants. The extent of liver damage has been measured by liver enzymes. After treatment with triptolide, not only liver enzymes decreased significantly, also the antioxidative status is improved. This effect was demonstrated in both, livers after ischemia and in normal livers of mice.
SOD and GPx in the liver tissue after ischemia are, as a result of damage, usually lowered. However, after treatment with Triptolide the values of SOD and GPx were increased. (66)
Even oral antioxidants (pONS) are tested to improve organ quality. Measured by serum transaminases ALT and AST liver damage is lower after pONS. This is also reflected in the MPO and caspase-3 levels, which are also lower. (67)

L-arginine has protective properties to organs from DCD. Among others hypoxia disrupts the balance between vasoconstriction and vasodilatation. Nitritic oxide (NO), acting as a vasodilator, and plasma endothelin (ET), influencing the vascular contraction, are opponents. Due to ischemia, the relationship between vasodilatation and vasoconstriction may be disturbed and result vasoconstriction. After DCD, NO levels sink compared to normal organs. In contrast, ET is higher in DCD organs than in normal comparison organ, leading to vasoconstriction and insufficient tissue supply and favors ischemia reperfusion injury. The longer the ischemia lasts, the lower are the NO values.
L-arginine is the substrate for NO synthesis. If there is sufficient substrate available, a decrease of NO in DCD organs is not so severe and there is less damage. Organs treated with L-arginine show higher NO values after than DCD without treatment. ET and organ damage could be reduced by L-arginine treatment. (68)

Even anti-thymocyte Ig (ATG) can help to influence the quality of organ donor kidneys. ATG influences the expression of surface antigens to immune cells and subsequent reactions. If donor kidneys are treated with ATG, the serum creatinine remains significantly lower. It does not show any differences in the urea content. ATG also causes a beneficial effect on the occurrence of necrotic cells. These occur less after ATG

treatment. ATG-treated kidneys after brain death show lower increases of MPO. ATG treatment may influence donor kidneys from brain dead patients positive. (38)

To prepare the tissue in living donation prior to ischemia, there is an ischemic preconditioning (IPC) method, which can be used. Thereby, the organ is inflicted to short intervals of ischemia and reperfusion. It has been shown that IPC has a protective effect on liver tissue. In the model with living liver donation IPC application shows less damage, as measured by ALT and AST values after Reperfusion. MPO was reduced by IPC also.
Shortly after reperfusion sinusoidal endothelial cells and hepatocytes show apoptosis. Apoptosis in hepatocytes occurs later than in cells sinusoidal cells. The apoptotic changes are associated with changes in liver enzymes (ALT and AST increase). So in liver transplant in case of apoptosis caspase-3 increases. In Liver Transplantation there is a higher increase of caspase-3 in sinusoidal endothelial cells than in hepatocytes. Most apoptosis was localized around the central vein. According to IPC treatment caspase-3 increase was lower afterwards. Because IPC reduces the MPO and the leukocyte activation and infiltration play a role in the damage mechanism, it is assumed that IPC affects the ROS formation. (69)

2 Genes influencing organ quality after transplantation

Bcl-2, Bax, GSH, Gpx3, OXSR-1, NF-κB, SOD2, Heat Shock proteins (Hsp, HSP70), PPARα, HPRT, Nitrotyrosine, Myeloperoxidase and Caspase-3, among other genes or proteins, serve to measure molecular changes in organs during BD, DCD and living donation.

Genes or proteins with stable expression in many tissues are well suited to serve as a reference value. By changes of these genes or proteins, which occur, for example, after brain death. Owing to the changes, which have been mentioned, organs can be compared. Gene expression in the donor organs associated with the results of the organ function and the result of the transplantation to the recipient. (10)

2.1 BCL-2

Bcl-2 family

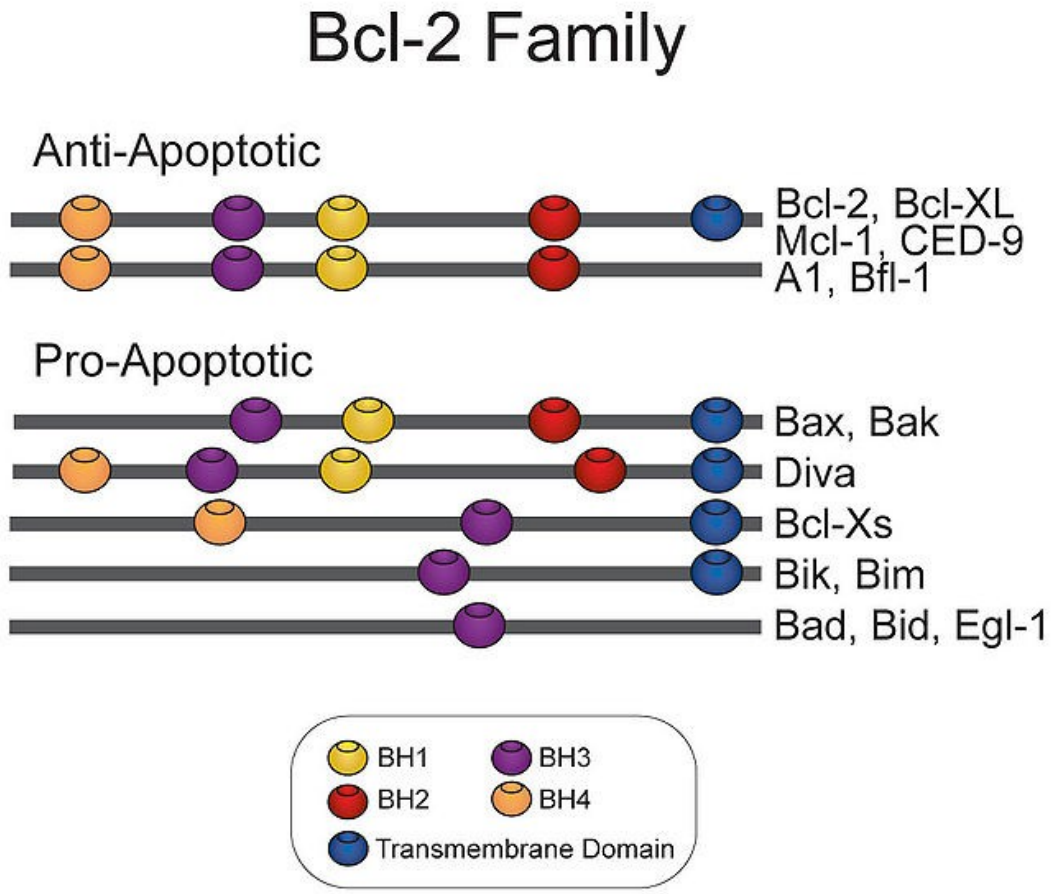

Figure 2 Bcl-2 Family
There are four domains (BH1-4). The family members have different combinations of these domains. This equipment results in the function of individual family members. (70)

The Bcl-2 protein is the founding member of the Bcl-2 family. The Bcl-2 family consists of 25 pro-and antiapototic members. These proteins are situated in different components in the cell, e.g. mitochondria, smooth endoplasmatic reticulum. Bcl-2 family members can be divided into three subfamilies. Bcl-2, Bcl-xl and others belong to the anti-apoptotic subfamily that suppress apoptosis and contain all four Bcl-2 homology domains. The pro-apoptotic Bax belongs to the second subfamily. The second subfamily contains Bcl-2 homology 1-3 domains. Other pro-apoptotic proteins, such as Bim, Bad and Bid contain only the BH3 domain. (26, 72)

Bcl-2 itself and Bax are assumed to be regulated by NF-κB. (37) BCl-2 and Bax have been described to build the balance between cell death and survival. (26)

Anti-apoptotic and pro-survival Bcl-2 family members

Bcl-2, Bcl-xL, Bcl-w, Mcl-1 and A1 inhibit apoptosis during development as well as in response to cellular stress. (71)

Bcl-2

Bcl-2 is identified due to chromosomal alterations in follicular lymphomas and diffuse B-cell lymphomas. It has been found that over expression of Bcl-2 leads to follicular hyperplasia and to T-cell lymphomas. (72)

Bcl-2 protects cells against diverse cytotoxic impulses for example, γ- and ultraviolet-irradiation, glucocorticoides, staurosporine, and cytotoxic drugs. Bcl-2 can protect cells after a lot of cytochrome c has been released. (26)

High levels of Bcl-2 can prevent superoxide production and cytochrome c release. Bcl-2 maintains the membrane potential of mitochondria and therefore prevents cytochrome c release. Because of that, there is lower apoptosis. (27, 63) But therefore high levels of Bcl-2 have been associated with more aggressive malignant phenotype in hematologic malignancies and solid tumors. (72)

A difference in graft survival between grafts from brain-dead donors and those from living donors has been seen. Inflammation and an increased number of apoptotic cells have been found in liver grafts after brain death. There are found more apoptotic cells in brain-dead liver grafts, but no changes in mRNA level of anti-apoptotic Bcl-2 or even pro-apoptotic Bax could be observed. (37)

In heart muscle tissue Bcl-2 levels are higher in brain-dead donors than in living organs. But in brain-dead donors proapoptotic proteins, such as Bax, clearly predominated to anti-apoptotic family members. (41)

Bcl-2 values can also be influenced by the duration of cold ischemia. The longer the CIT takes, the higher Bcl-2. The Bcl-2 is higher when the CIT lasts longer than 24 hours. (44)

Pro-apoptotic Bcl-2 family members
Bax, Bad, Bak, Bcl-xS, Bik, Bim, Bid (27, 71)

2.2 BAX

The pro-apoptotic Bax belongs to the second subfamily of the Bcl-2 family, containing BH1, BH2 and BH3 domains. (72) Bax is located in the cytosol and also localized on the outer mitochondrial membrane before a stimulus activating apoptosis arrives the cell. (26) To activate apoptosis by Bax, it has to translocate into mitochondria and either directly form pores or bind to domains including Bcl-2, building a heterodimer with Bcl-2, and antagonize anti-apoptotic genes.
After perforating the mitochondrial membrane there is a release of cytochrome c leading to apoptosis, mediated by Bax. (20, 26, 41)

In myocytes from brain-dead donors in comparison to living donors increased Bax levels were found. (41)
In liver tissue an increased number of apoptotic cell are found in brain-dead rats. But there were no changes in Bax levels. (37)
After ischemia and reperfusion Bax increases in tubular cells of the kidney. (20)

2.3 GSH-S

Glutathione synthetase (GSH-S or GSH) catalyses the production of glutathione from γ-glutamylcysteine and glycine consuming ATP. The process is located in the cytosol. Glutathione (γ-glutamylcysteinylglycine, GSH) is an intracellular antioxidant. It protects cells, such as erythrocytes, against damage by oxidative stress. Glutathione is involved in the amino acid transport, participates in the detoxification of foreign compounds and the biosynthesis of DNA and leukotrienes, as well as neurotransmission and neuromodulation. (73)

In humans, defects in GSH-S are inherited in an autosomal recessive way and cause acidosis due to accumulation of 5-oxoproline and hemolytic anemia, electrolyte imbalance and convulsions, because of defective function of the central nervous system. In severe cases progressive neurological symptoms (e.g. mental retardation, seizures, spasticity, ataxia) and recurrent bacterial infections due to defective granulocyte function can appear. (74)

In brain-dead rats a reduced concentration of hepatic GSH has been found, consequently leading to enhanced lipid peroxidation. Protective GSH is lower in organs from brain dead donors than in non-brain-dead donors. In already damaged liver by steatosis, the combination of steatosis and brain death led to the lowest GSH levels. (75) Steatotic livers are more vulnerable to ischemia/reperfusion injury than non-steatotic livers. In steatotic livers lower levels of GSH in liver tissue has been found. (76) There is a difference how the fat composition in the liver is. Macrovesiculare steatotic livers shall be more sensitive to ischemia. Steatosis is characterized by an accumulation of fats such as triglycerides, free fatty acids and cholesterol. Microsteatosis caused by overriding triglycerides and free fatty acids, is less sensitive to noxious ischemia.
Macrovesiculare steatosis occurs through cholesterol. High cholesterol leads to deposits in mitochondria and reduced mitochondrial GSH. This is responsible for making the liver sensitive to ischemic damage. High cholesterol changes the membrane permeability of the mitochondria leading to a loss of GSH. Treatment with statins, inhibits a key enzyme in the biosynthesis of cholesterol, lowering cholesterol dose-dependent. Additionally, there is again a higher protective GSH. (77)

A reduction of hepatic GSH levels was accompanied with increased liver injury after ischemia/reperfusion. Hepatozytes with low levels of GSH become susceptible to damage by oxidative stress. (75)

Oppositely high values of GSH, as found in hypothyroid rats, protect against oxidative stress during storage and reperfusion. In brain death, the release of thyroid hormones is compromised, high levels of GSH are measured. Little GSH allows greater injuring of liver cells by ischemia. Hypothyroid metabolism with a high GSH in donor organs provides protection against oxidative stress. (78) Kidney of hypothyroid animals showed less damage during anoxia. Products of lipid peroxidation during hypothyroidism are decreased. In these kidneys high levels of GSH are found. (79)

There are controversies concerning the measurement of GSH levels after kidney transplantation. Increased GSH levels in erythrocytes after kidney transplantation are described, with the highest values after six months, whereas (14) others showed no significant changes in erythrocyte GSH level. (80)

2.4 GPX 3

GPx3 belongs to the family of glutathione peroxidases. (81) The glutathione peroxidase (GPx) families are widely expressed in various tissues. This cellular antioxidant system provides the balance of the production of reactive oxygen species and the removal, protecting cells against oxidative damage. It oxidizes reduced glutathione to eliminate hydrogen peroxide. GPx3 occurs in a secreted form, located in plasma and mucosal surfaces. But there is a second form of GPx3 found in the cytoplasm. The extracellular GPx3 functions as an antioxidant enzyme and reduces ROS. The intracellular version of GPx3 leads to the production of ROS and cell death. (81)

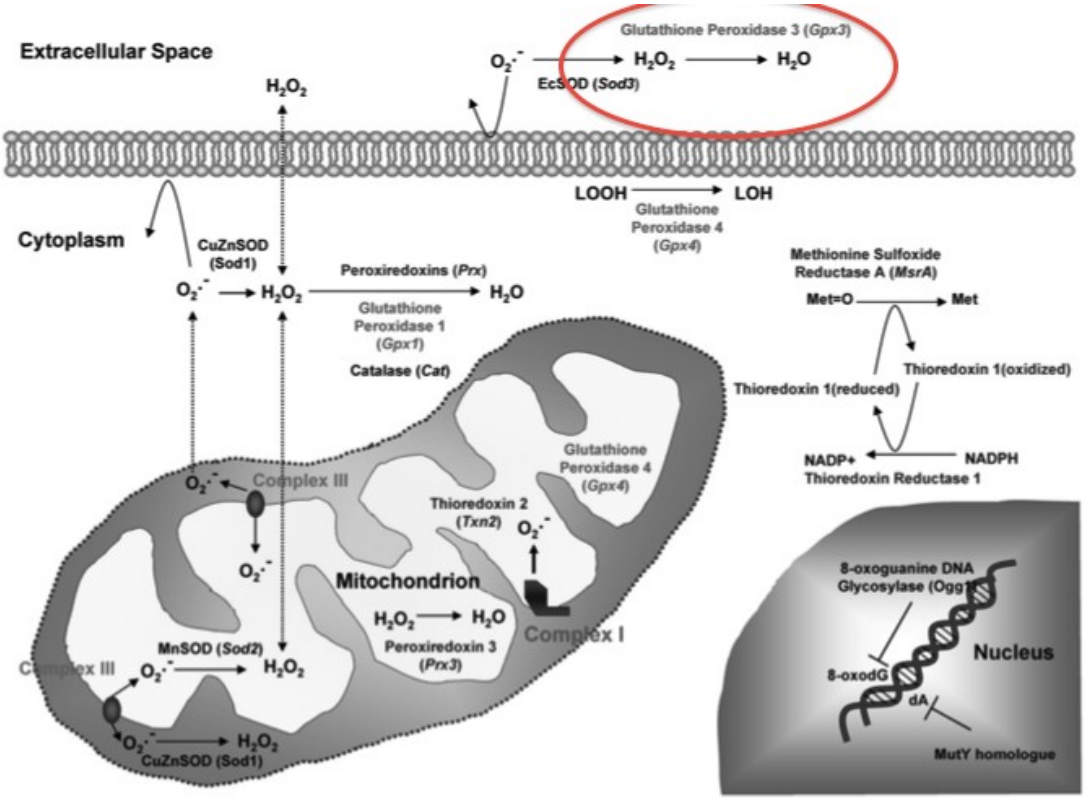

Figure 3 GPx-3
GPx-3 turns hydrogen peroxide into water. (82)

The GPX family consists of 4 selenoproteins. GPX catalyzes the reduction of hydrogen peroxide to water and simultaneously glutathione is oxidized. Kidney cells, liver cells, skeletal muscle cells, pancreatic cells, lung and heart cells can produce GPX. GPX-3 is found in plasma. (83)

The GPX-3 gene has two areas responding to oxidative stress. These two areas are called antioxidant response element and metal response element. This is intended to perceive

damage and injury by trigger mechanisms like hyperglycemia. High regulation of GPX-3 works to protect cells. Hyperglycemia should trigger oxidative stress. In cardiac cells by hyperglycemia, an increase of GPX-3 was achieved. (83)
If GPX-3 is released, it will protect cells by preventing the transfer from hydroperoxides into the cell. (83)

In various cancers, such as lung, breast, bladder, esophageal, prostate and gastric cancer, alterations and down regulation in GPx3 expression has been observed, assuming that the inactivation of this reactive oxygen species scavenger plays a role in human tumorgenesis. (84) Low levels of GPx3 are found associated with cancer progression. However, a reduction of tumor growth has been associated with high levels of GPx3.
GPx3-mediated cell death occurs in cancer cells as well as normal epithelium and increased expression of intracellular GPx3 results in cell death. Low levels of the enzyme prevent cell death even under stress. (81)

In chronic kidney disease and during kidney transplantation oxidative stress occurs. GPx plays an important role in detoxification of reactive oxygen species. But GPx levels show no changes after kidney transplantation. (14)

The liver can be affected by many things such as heart failure, trauma, and vascular occlusions and during transplantation. Ischemic damage leads to the activation of inflammatory mediators, ROS genesis and cell damage. The extent of liver damage, measured by liver enzymes, significantly decreased after treatment with triptolide.
Enzymes such as GPx have a positive effect against ischemic damage. With an increase in liver damage after ischemia GPx decreases. After treatment with triptolide, less liver damage and higher GPx values have been measured. (66)

2.5 OXSR-1

Oxidative Stress Responsive 1, OSR1 or OXSR1 is a serine/threonine protein kinase that is far spreading in mammalian cells, including in the heart, spleen, liver, kidney, lung, testes, intestines and stomach. Human OSR1 has 58kDa and 527 amino acids. OXSR1 is activated by osmotic stress, sorbitol and through natriumchloride. OXSR1 is part of the Ste20p ((sterile 20)-like kinases) family. Members of this protein family do also influence the process of apoptosis.

OXSR1 has a binding site for caspase-3. Caspase-3 activates apoptosis. OXSR1 is involved in stress-related processes and activates further regulatory processes. (85, 86)

Beside the involvement to the regulation of the pheromone response, pseudohyphal-growth pathway and also osmotic response Ste20p is additionally involved to the signaling cascade of stress response.

Level measurements of OXSR1 show overlaps because of structural similarity to SPAK (STE20/SPS1-related proline/alanine-rich kinase). SPAK has a structural identical C-terminal end plus an additionally part, which is not necessarily needed. C-terminal end is important for function. The C-terminal end of SPAK and OXSR1 is in charge of caspase-connection and involvement in apoptosis.

Ste20p is involved in ion transport mechanisms, ion balancing and the control of cell volume. (86)

2.6 NF-κB 1

NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) was first identified in the nucleus of B-leukocytes The NF-κB family consists of five proteins. (87)
NF-κB is located in the cytoplasm of almost all cell types. The inactivated form is bound in a complex in the cytosol. After stimulation of cells to activate NF-κB, the inhibitor (I-κB) (62) is phosphorylated and degraded. NF-κB translocates from the cytosol to the nucleus and activates the transcription of specific target genes. NF-κB is stimulated by hypoxia and endotoxin in systemic inflammation. A common mechanism for the activation of NF-κB is an increase of reactive oxygen species (ROS). But endotoxin activates NF-κB DNA binding activity independently of an increase in ROS. Hypoxia stimulates mitochondrial ROS production. Hypoxic induction of NF-κB DNA-binding activity requires mitochondrial ROS generation. Hydrogen peroxide can activate NF-κB and an antioxidant like N-acetylcystein can block NF-κB activation triggered by hypoxia. (88)

NF-κB regulates the expression of anti-apoptotic genes and proteins. Prohibition of NF-κB activation leads to a shift towards apoptosis. (24)
It has been shown that the expression of family members of the IAPs (inhibitors of apoptosis protein) is regulated by NF-κB. IAP family members bind and inhibit the activation of caspases. Caspases are proteins involved in both pathways, intrinsic and extrinsic, leading to apoptosis. It could have been shown that survival of hepatocytes is depending on NF-κB activation. Is there an activation of NF-κB regulated IAP family member (cIAP2), caspase-3 activation is inhibited and apoptosis can be prevented. (89)
It also has been found that iNOS (inducible nitric oxide synthase) may act as anti-apoptotic gene, which is regulated by NF-κB. (89)

The extrinsic pathway of apoptosis begins at the cell membrane at the death receptors. This leads to activation of TNF receptors on one side to apoptosis, on the other hand also anti-apoptotic signals via NF-κB are activated. At the cell membrane a complex is formed, responsible for the activation of NF-κB and thus for the activation of anti-apoptotic pathway. If NF-κB is not able to initiate anti-apoptotic proteins, the apoptotic complex is formed in the cytosol and initiated the apoptosis pathway. (90)

In livers (40) and kidneys (36) of brain dead donors the NF-κB pathway is up regulated. The expression of pro-apoptotic proteins in livers of brain-dead donors is increased and it has also been shown that NF-κB-regulated proteins such as anti-apoptotic Bcl-2 family members (Bcl-xl) were elevated in livers of brain-dead donors. (37)

In various studies (62, 66), it is shown that the inhibition of NF-κB is better for the quality of organs for transplantation. Therefore, in attempts to improve the quality of organs, NF-κB is inhibited. Damage to organs through transplantation is caused by the ischemia and reperfusion. This leads to an inflammatory response with the release of inflammatory mediators, cytokines, chemokines and the infiltration of monocytes and neutrophils. NF-κB family regulates the expression of different genes in different cells. The RelB protein is a family member of the NF-κB family. Ischemia/reperfusion in the liver leads to among others, edema, necrosis and infiltration of neutrophils. By inhibiting RelB the harmful changes in the liver were less pronounced. Myeloperoxidase (MPO) as a marker of the index for the infiltration of neutrophils, it was lower after treatment with RelB and the inhibition of RelB protein prevented a decrease of SOD levels after ischemia. Silenced RelB has a positive effect on I/R injury. In order to counteract the liver damage a lot of is attempted. Modified perfusion solutions, pre-treatments and gene therapy show a positive effect on the reduction of ischemic damage. (87)

Several signaling pathways are activated in response to ischemia/reperfusion injury, such as NF-κB. Heat shock preconditioning showed a protective effect on I/R injury by suppressing the activation NF-κB in the liver. (62)

As mentioned above triptolide gained from Chinese herbs have been used for its anti-inflammatory, antifertility, immunosuppressive, and anti-neoplastic activity. Use was found to be beneficial in autoimmune diseases, the prevention of transplant rejection and graft-versus-host disease because triptolide also act as an inhibitor of NF-κB and can potentially act to prevent damage caused by ischemia in transplants. After treatment with triptolide the liver enzymes decreased significantly in comparison to ischemic damage without triptolide treatment.
Triptolide caused a significant reduction of NF-κB distribution. This effect was demonstrated in both, liver after ischemia and in normal livers of mice. After ischemia in

livers a significant increase of NF-κB was detected. However, in the with Triptolide pre-treated livers a significantly smaller amount of NF-κB was found. (66)

On the other hand, there are also reports showing that high NF-κB is good for the organs. (87)
NF-κB activation is a result of stress and to protect cells from apoptosis. (24)
There are two isoforms of the NF-κB inhibitor: I-κBα and I-κBβ. With a modified form of IκBα, NF-κB cannot be activated. The altered IκBα cannot be phosphorylated and thus not cleaved of NF-κB. If NF-κB is disabled more apoptosis occurs in the liver. (24)
Numerous substances can inhibit NF-κB activation, such as glucocorticoids, immunosuppressants, N-acetylcysteine (ACC) and SOD. (76) SOD distribution has a positive effect on transplanted livers. (91) So SOD represents a possibility for malicious actors, like NF-κB, to block in transplantations to improve the quality organ. (24)
By ischemia and reperfusion in transplantations organs are damaged. The longer the cold ischemia lasts, the greater the reperfusion injury. (24)

2.7 SOD2

This gene is a member of the iron/manganese superoxide dismutase family. It encodes a mitochondrial protein. SOD2 protects cells against reactive oxygen species. (18, 21) This mitochondrial enzyme is responsible for scavenging superoxide radicals originated in mitochondria. (27) It transforms toxic superoxide into hydrogen peroxide and diatomic oxygen. (21, 63) Is a cell lacking of SOD2 hyperoxide anions are free to react and damage cells. Heterozygote SOD2 mice showed mitochondrial damage and alteration in function. (92)

It is assumed that SOD prevents the release of cytochrome C and thus apoptosis. In SOD2 50% deficient mice, low levels of mitochondrial transmembrane potential (MTP) is found. Overproduction of ROS can reduce the MTP, leading to an easy release of cytochrome c. Thus enough SOD2 protects the MTP and complicates cytochrome c release. (63)

Because superoxide anions are produced after the start of reperfusion phase and harm tissue, an addition of SOD just before reperfusion can reduce damage. Available SOD in reperfusion can eliminate produced superoxide anions. SOD administration before ischemia cannot cause a change in tissue damage. (21)

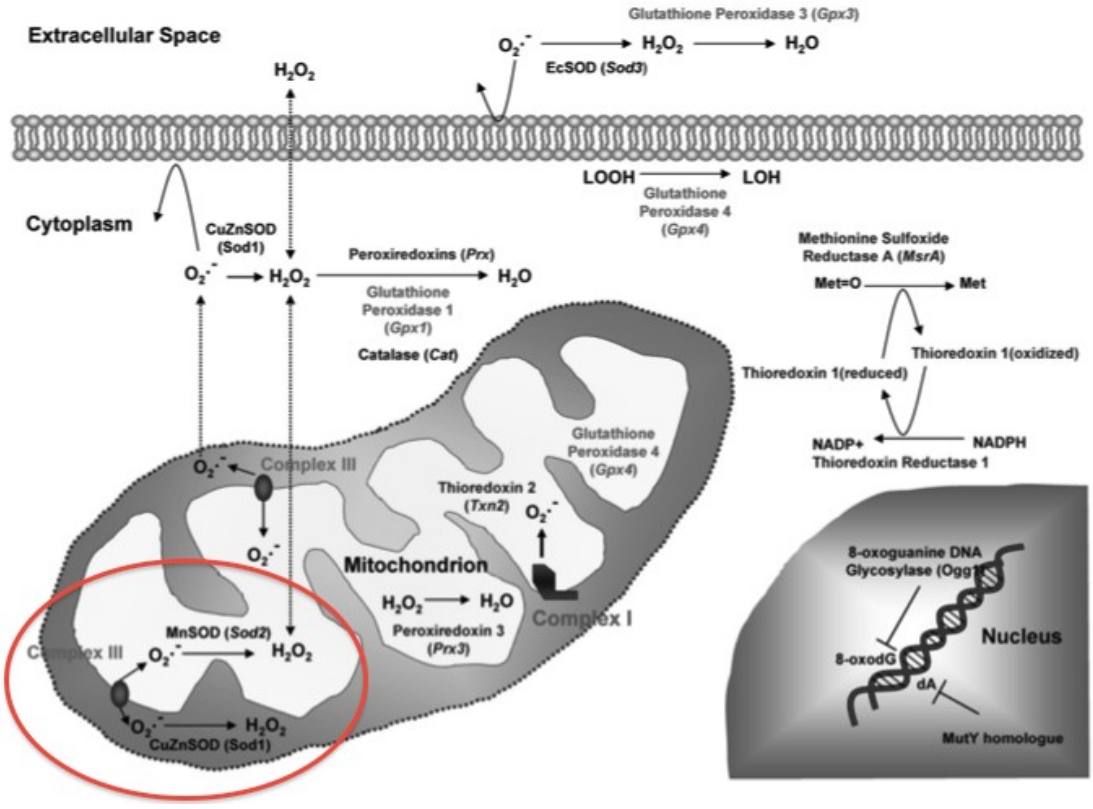

Figure 4 SOD2

Oxygen radicals arise in the mitochondria in three complexes. SOD is the main scavenger in mitochondria and reacts with the unstable superoxides. (82)

In chronic kidney disease and during kidney transplantation oxidative stress occurs. SOD plays an important role in detoxification of reactive oxygen species. But SOD levels show no changes after kidney transplantation. Controversially, a decrease of SOD two days after kidney transplantation is described. Some time after kidney transplantation, SOD values rebounded and were almost at the level before transplantation. Although others observed an increase in SOD after kidney transplantation. (14)

ROS is generated by tissue damage. To counteract ROS, antioxidant defense systems were formed. Enzymes such as SOD have a positive effect against ischemic damage. With an increase in liver damage after ischemia, SOD decreases. After treatment with Triptolide less liver damage and higher SOD values have been measured. (66)
The RelB protein is a family member of the NF-κB family (as mentioned in chapter NF-κB). Inhibition of RelB protein prevented a decrease of SOD levels after ischemia. With the higher SOD values, the liver can protect itself better and silenced RelB has a positive effect on ischemia/reperfusion injury. (87) SOD is one of many substances that can inhibit NF-κB. Thus SOD can be used to improve the quality of organs. (24, 91)

2.8 HSP

Heat shock proteins (Hsp) were originally identified in Drosophila melanogaster cells exposed to elevated temperatures. Proteins with similar structure exist in virtually all living organisms. These proteins, also expressed under normal physiological conditions support ATP-dependent correct folding, transport and localization of the mature proteins, and regulation of immune system. They are divided into groups according to their molecular weight. Members of the Hsp70 family are constitutively expressed or inducible. The inducible Hsp70 takes part in processes of protection and repair of stress-induced protein damage. (93)
An Up regulation of Hsp 70 can protect cells from apoptosis. Thereby it can promote the development of tumor cells. On the other hand it has been shown that a down regulation of Hsp 70 can kill tumor cells or, respectively, lead to apoptosis of tumor cells. Hsp 70 inhibits the cytochrome dependent pathway and the apoptosis-inducing factor dependent pathway of apoptosis. (94)

There are forms of Hsp 70 that are ubiquitously expressed in all cells, whereas Hsp 70.2 is expressed at high levels in testis and lower or non-detectable in other tissues. Oxidative stress in testicular germ cells can cause high levels of heat shock proteins, protecting against damage. Hsp 70.2 is mainly expressed in male germ cells during prophase of the first meiotic division. (95) In Hsp70.2 deficient mice apoptosis of spermatogenic cells occur. (96)

It was shown that there are not only detrimental changes during brain death, but also favorable, such as an up regulation of Hsp 70. (42)

Induction of molecular chaperones, such as Hsp72, prevents ischemia- reperfusion injury of the liver. (61)

Cells with low levels of Hsp 70 are more sensitive to stress signals. It is presumed, that low levels of Hsp 70 in cells are detected after a massive stress, leading to damage that could not be repaired. Hsp 70 shifts into extracellular space. (97, 98)

Brain death is considered to be a massive stress factor. Kidneys from brain death donors have inferior graft survival and an increased loss of graft function in comparison to kidneys from living donors. In kidneys a stress response can be shown in Hsp70 up regulation during brain death. (99) Rats treated with hydroxyethyl starch (HES) for blood pressure, show even higher values of renal Hsp 70. (100) HES is used as a plasma expander in brain dead donors. But it has been shown that treatment with HES leads to a worse organ function. After treatment with HES tubule lesions were found, higher serum creatine values are measured and more often dialysis is used. (101)

2.9 PPARα

The Peroxisome proliferator-activated receptor α (PPARα) is a type II nuclear receptor. Type II nuclear receptors are located in the nucleus, whereas type I nuclear receptors are cytosolic and translocate into the nucleus after ligand binding. Other members of this family are PPAR γ and PPAR β/δ. All family members play a role in energy metabolism. PPARα is mainly expressed in hepatocytes, enterocytes, vascular and immune cell types such as monocytes, endothelial cells, smooth muscle cells, lymphocytes, non-neuronal cells like microglia and astroglia. (102, 103) PPARα is activated by synthetic peroxisome proliferators and saturated and polyunsaturated fatty acids and plays a role in lipoprotein synthesis and fatty acid oxidation systems. (102) Synthetic ligands for PPARα are fibrates and glitazone. (104)

PPARα seem to be also involved in cell proliferation, cell differentiation and apoptosis. (104) PPARα influences inflammatory response, reduce inflammation and regulate the duration of inflammatory response. PPARα ligands reduce levels of pro-inflammatory cytokines by inhibiting a subunit of NF-κB. PPARα deficit leads to increased NF-κB. PPARα plays a role in the development of hepatic steatosis and cancer. (102) It is regulated by various physiological conditions such as stress or hormones and its expression could be related to aging. Under conditions of hunger PPARα is activated and expression levels are increased. It up regulates fatty acid oxidation systems and reduces hepatic steatosis. PPARα deficient mice develop hepatic steatosis. PPARα activation leads to suppression of apoptosis. (28) PPARα level in humans can differ among individuals and are supposed to be lower than in rodents.

In livers of brain dead animals PPARα signaling pathway is down regulated. During brain death inflammatory pathways modify the expression of metabolic genes, including those involved in fatty acid oxidation. PPARα levels are reduced in livers from brain dead donors compared to non-brain dead controls. (40)

PPARα plays a role in lipid metabolism, but it also has anti-inflammatory properties in which it affects the regulation of NF-κB. PPARα is to act via a negative control of the inflammatory response. (105)

Statins reduce not only the blood lipids, but also the microvascular function is improved, inflammation can be favorably influenced because of down regulating NF-κB, which in

turn leads to a reduction of cytokines and chemokines. Simvastatin may also cause an increase in the expression PPARα. (105)
In the cytoplasm NF-κB is bound to its inhibitor IκB. If NF-κB is activated, there is a measurable IκB decrease and an increase of p65. After damage, NF-κB pathway is activated and a high amount p65 can be measured. The higher increase of p65 occurred in the absence of PPARα. In the case of activated and stimulated PPARα, a reduced amount of p65 was present. (105)
In case of inflammation, the values of PPARα decline. Activation of PPARα can cause an increase of normal value in healthy tissue and in return prevent a drop in inflamed tissue. (105)

To assess the pancreatic function amylase and lipase were measured. When the pancreas is damaged there is an increase of amylase and lipase. If PPARα was not active or blocked, this results in more damage to the pancreas. In comparison, amylase and lipase not rise as much when PPARα is effective. The activation of PPARα by simvastatin, can prevent an increase of amylase and lipase. (105)

Renal function was measured by creatine. In kidney damage, creatine increases. If PPARα was active simvastatin could cause an increase in the PPARα expression and protect the kidney. Measured by the liver values, the presence of PPARα also protected liver cells. PPARα is involved in the anti-inflammatory process. A deactivation of PPARα resulted in damage to organs. Simvastatin reduced the organ injury when PPARα was active. In case of treating organs such as lungs, liver or kidneys with simvastatin, PPARα expression increases. If PPARα gene was deactivated, simvastatin could not prevent organ damage.
Active PPARα is leading to a smaller NF-κB activation. (105)

PNF (primary non-function) occurs through massive cell damage of the transplanted organ. PPARα regulates fatty acid metabolism. If the activity of PPARα in the liver is promoted, there is less likely steatosis. More often steatosis occurs when PPARα is turned off. Then the ability to oxidize fatty acids is reduced.
PPARα has an anti-inflammatory effect by the inhibition of NF-κB. (105) After liver transplantation PPARα is lower than before. This change could be a response to metabolic changes during and after the transplantation. PPARα actually has preventing effects and a down regulation has negative effects. If this down-regulation of PPARα is prevented after transplantation for example by fibrates (clofibtrate), although the PPARα level is higher,

markers showing cell damage, also increases. It is assumed that NF-κB is responsible for this regulation. (106)
When use is made, as an extension of the donors, on steatotic livers, dysfunction may occur more frequently. Through the accumulated fat in the liver cells activate PPARα. (106)

Overexpression of PPARα in mouse hearts delayed recovery after ischemic damage. PPARα overexpression results in increased fatty acid oxidation in the heart and reduces the regeneration. For the recovery a lower fatty acid oxidation seems to be better. (107)

2.10 HPRT

The HPRT gene encodes for the enzyme Hypoxanthine phosphoribosyl transferase (HPRT), also known as Hypoxanthine-guanine phosphoribosyltransferase (HGPRT). (108) The enzyme catalyzes the conversion of hypoxanthine to inosine monophosphate (IMP) and guanine to guanosine monophosphate (GMP). The energy-efficient reaction plays a central role in the salvage pathway. HGPRTase functions primarily to salvage purines from degraded DNA to reintroduce into purine synthetic pathways. In this role, it catalyzes the reaction between guanine and phosphoribosyl pyrophosphate (PRPP) to form GMP.
Partial HGPRT deficiency causes in high levels of uric acid in the blood, which leads to the development of gouty arthritis and the formation of uric acid stones in the urinary tract. This condition has been named the Kelley-Seegmiller syndrome. Lesch-Nyhan syndrome is due to HPRT mutations resulting in extremely ineffective enzyme activity. Some mutations have been linked to gout, the risk of which is increased in hyperuricemia. (109)

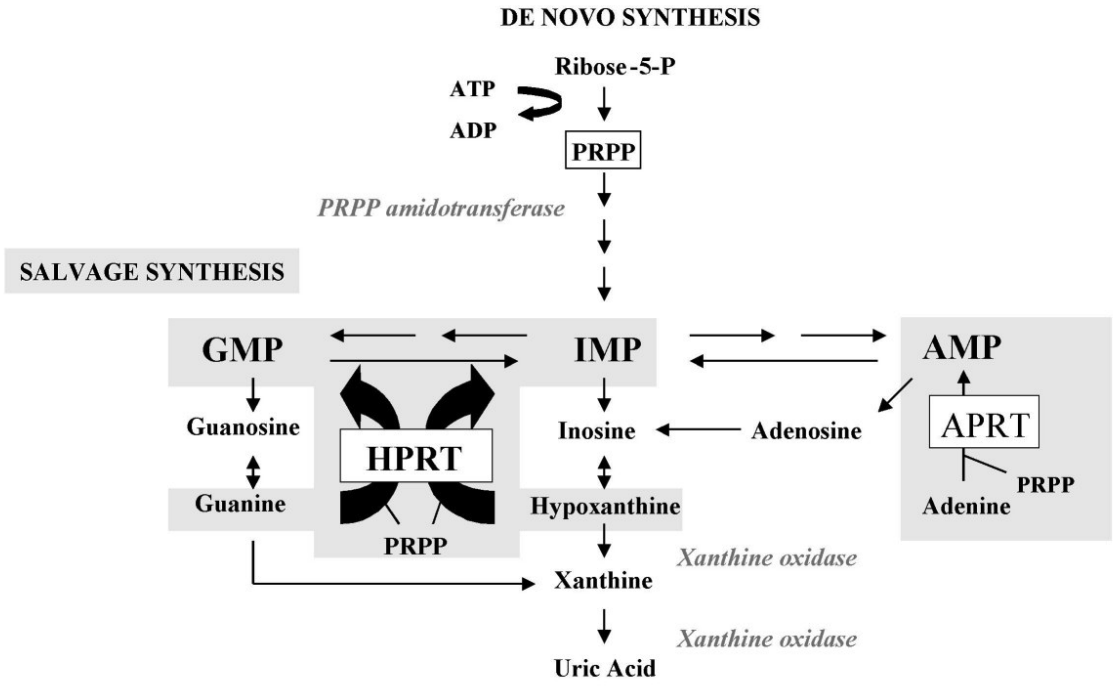

Figure 5 De novo purine synthesis
The synthesis of purine nucleotides and also pyrimidine nucleotides takes a lot of energy. Therefore, the incurred purines from food or degradation are recycled (salvage pathway). The salvage pathway is controlled by HPRT. By HPRT guanine and hypoxanthine are converted to IMP and GMP. In an HPRT deficiency, the substrates accumulate, which then determine the clinical picture. (109)

HPRT shows stability tissue-specific, with values in heart muscle cells higher than in skeletal muscle cells. This is probably dependent on metabolism in individual tissues. HPRT increases shortly after cardiac death, but falls off again after some time. (110)

Expression of HPRT1 seems to be dependent on the age. The HPRT1 expression is not changed between normal and hypoxic conditions. (111)

2.11 Nitrotyrosine

RNS occurs in ischemia and damage organs. RNS produced by nitric oxide synthase (NOS), which has three isoforms: endothelial NOS (eNOS), neuronal NOS (nNOS) and inducible NOS (iNOS). (13, 17)

In order to detect reactive nitrosative species (RNS), nitrotyrosine (3-NT), as an indicator for cell damage, is measured. RNS include nitric oxide (NO) and nitrogen compounds, and peroxynitirit. Peroxynitrite is formed from nitric oxide and superoxide radicals. Peroxynitrite and the resulting radicals can cause damage to enzymes, DNA, and mitochondrial membranes and lead to apoptosis. 3-NT is a reaction product of peroxynitrite. (20, 112)

Nitrosative stress damages kidneys. Increased 3-NT can be found in tubular cells after I/R. If iNOS is inhibited, tubular structures in the kidney are damaged less. (17)

Physiological amounts of NO are necessary to maintain the normal glomerular function in the kidney. Large amounts of NO, produced by activated iNOS, damage. INOS and thus the production of RNS have an increased activity after I/R. If ROS or RNS is produced, thereby also antioxidants such as superoxide dismutase, glutathione and glutathione peroxidase become active to counteract a predominance depleting substances. (13)

A stimulation of PPARα reduced nitrotyrosine in the lungs, liver and kidney. (105)

2.12 Myeloperoxidase

Myeloperoxidase (MPO) is a protein in neutrophils and is released during activation. MPO itself has inflammatory properties and therefore can damage tissue. (69)

MPO is an enzyme in neutrophils. MPO is, in smaller amounts, also present in monocytes and macrophages. In liver cells, it normally is not distributed, and therefore is a good MPO measured value for the accumulation and activation of neutrophils. (113)

MPO is a marker of the index for the infiltration of neutrophils. (87) An accumulation of neutrophils in the tissue leads to an enhancement of tissue damage. It was shown that myeloperoxidase is increased in damaged tissue. MPO was reduced at elevated PPARα activity. (105)

MPO levels in liver after ischemia/reperfusion were increased. However, MPO was lower after inhibiting the NF-κB family member RelB. (87)

Leukocyte infiltration in the sinusoids is higher in organs from brain dead donors. If the donor liver was steatotic before, leukocyte infiltration after brain death is still higher than in normal livers. (75)

Oral antioxidants (pONS) are tested to improve organ quality. Measured liver values, ALT and AST, showed less damage after pONS administration. MPO was also lower after pONS. Warm ischemia of the liver was better tolerated than treatment without pONS. (67)

2.13 Caspase-3

Caspases (cysteine-aspartic acid protease) are proteins involved in both pathways, intrinsic and extrinsic, leading to apoptosis. (89) Caspase-3 is a key mediator in apoptosis. (23)
By cytochrome c release the caspase sequence is activated and caspase-3 leads to the destruction of DNA.
Active caspase-3 can be inhibited by inhibitor of apoptosis protein family (IAP). By inhibition of caspase-3, apoptosis can be prevented. If IAP itself is inhibited the caspase-3 activity is not inhibited and leads to apoptosis (27)

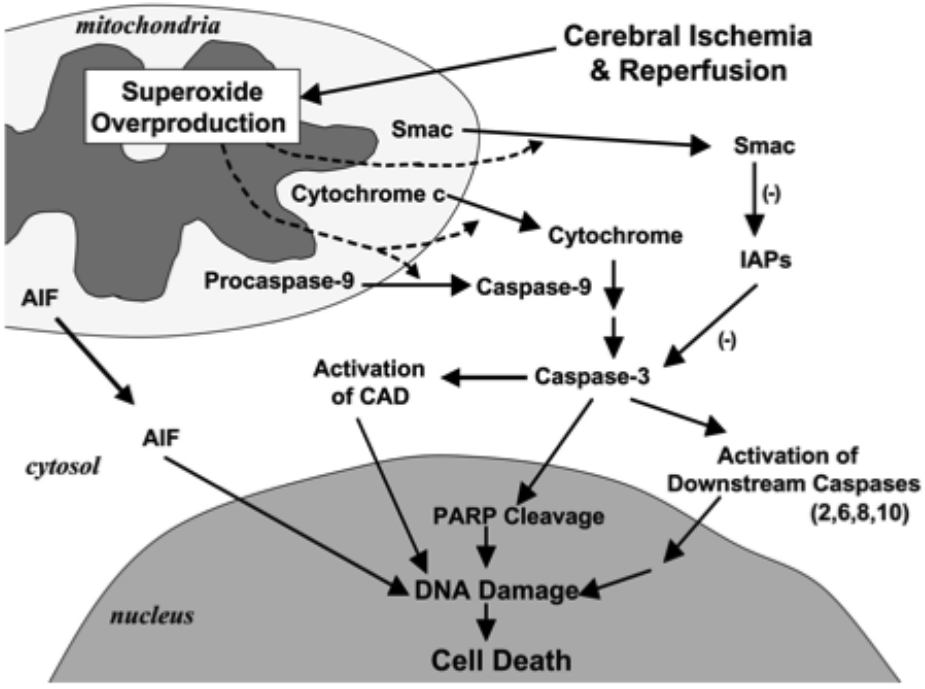

Figure 6 Caspase-3 Activation
After cytochrome c release from mitochondria sequence of caspases is activated. The activation of caspase 3 results in the destruction of DNA. Active caspase-3 can be inhibited by inhibitor of apoptosis protein family (IAP). By inhibition of caspase-3, apoptosis can be prevented.
However, if IAP itself is inhibited the caspase-3 activity is not inhibited and leads to apoptosis. (27)

Prolonged ischemia can lead to DGF, and can be associated with higher creatinine levels after transplantation. Human studies and animal studies have shown that long cold ischemia leads to apoptosis. In rat livers and kidneys and human kidneys prolonged cold ischemia leads to increased apoptosis. Caspases are cysteine proteases and play a role in cell death and inflammation development, where they activate pro-inflammatory cytokines. Caspases act in both pathways of apoptosis. Caspase-3 is also called the executor caspase. Caspase-3 inhibitors protect against ischemic damage in various organs. An addition of caspase inhibitors to storage solutions can reduce the development of apoptosis. This

allowed a better survival of rats after liver transplantation. The kidneys exposed to any cold ischemia, show no tubule apoptosis, in comparison to the kidneys exposed to cold ischemia (48h). Kidneys exposed to cold ischemia (48h) clearly showed apoptosis. If a caspase inhibitor is added to University of Wisconsin (UW) solution, significantly less apoptosis occurs in kidneys. (114)

Perfluorocarbon (PFC) improves organ quality. There were fewer caspase-3 positive cells after treatment PFC. In comparison, the only with UW solution treated cells show more caspase-3 release. (46)

Caspase-3 is higher in BD donors than that in living donors. (41) The duration of the brain death affects caspase-3. Caspase-3 is higher after a shorter time of brain death. If organs are harvested after a period shorter than 10 hours of BD, they showed larger caspase-3 levels than organs removed after 10 hours. Responsible for this change could be a repair mechanism starting after some time. (44)
High caspase-3 levels are also observed in DGF, therefore, a longer brain death time, which leads to lower caspase-3 values could positively influence the result to the receiver. And Caspase-3 values can also be influenced by the duration of cold ischemia. Caspase-3 is higher when the CIT lasts longer than 24 hours. (44)

Caspase-3 was increased in kidneys exposed to cold ischemia showing apoptosis. In kidneys after cold ischemia with high caspase-3 levels, processed form of caspase-3 was detected. In the kidneys without relevant caspase increase, in the control group without cold ischemia and kidneys treated with caspase inhibitors, only the precursor of caspase-3 can be verified. A pancaspaseinhibitor was used, following that other caspases, such as caspase-8 and caspase-9 were also inhibited. However, caspase-3 is also activated by these caspases. Most caspase-3 distribution in the kidneys was found in the nucleus of tubular epithelial cells and in the brush border of the proximal tubules cells. There was a loss of microvilli in the brush border cells. In the kidneys treated with caspase inhibitor, this damage to the brush border was not that severe. (114)
As mentioned above oral antioxidants (pONS) are tested to improve organ quality. Caspase-3 was also lower after pONS. Warm ischemia of the liver was better tolerated than treatment without pONS. (67)

3 Aims of the Study and Methodology

The aim of this study was to give an overview on molecular changes that might occur during brain death, donation after cardiac death and living donation and their possible effect on transplantation outcome and graft survival after whole organ transplantation.
Therefore, a literature review in PubMed was carried out for the key words brain death, DCD, living donation and the parameters: Bcl-2, Bax, GSH (GSH-S), GPx (Gpx3), OXSR-1, NF-κB, SOD (SOD2), Heat Shock proteins (Hsp, HSP70, sBAB), PPARα, HPRT, Nitrotyrosine, Myeloperoxidase and Caspase-3.

Thereafter, substantial reading of the different literature was performed and the survey presented above was given. Then, an interpretation of the information gained from the literature research was drawn as shown in the discussion.

4 Results and Discussion

Since the first attempts to transplant organs and tissues, some time has passed. Obstacles, such as organ matching and thereby rejection has been fought. Due to other reasons, organ dysfunction and organ failure still limit results. (1-9) With increasingly more people who need an organ as a therapy for end-stage disease, the need for organs can no longer be covered.

The classic donor for solid organs is a brain dead donor. (41) In some cases, such as kidney, liver, bone marrow, living donors can be used. (4)

In order to have access to a larger pool of donors, also donation after cardiac death (DCD) donors can be used. But in Germany, for example, the largest country of Euro Transplant DCD is prohibited. (52)

Besides DCD donor organs, there are also so-called marginal or extended or expanded criteria donors (ECD). ECD are basically DCD, but are generally older and sicker. (49, 53, 54)

In varying extent, in all donor organs ischemia and thus ROS or RNS occur. Ischemia leads to increased apoptosis in the organs. (29) After reperfusion the ischemic damage is even stronger. (12, 16, 21) The longer warm and cold ischemia take, the worse the organs work in the receiver. The longest ischemic times, and thus the worst results after transplantation have (uncontrolled) DCD and ECD organs. (21, 22)

In addition, ECD are damaged not only by to ischemia, though by disease condition, age, race, already undergone transplants, transfusions or pregnancies. (49)

In brain-dead donors the particular state of brain death damages already before and in addition to ischemic damage. (41)

After brain death, large amounts of catecholamines can lead to severe hemodynamic changes and swings in blood pressure. (32) Further tissue ischemia leads to changes in energy supply and an associated rise of oxygen free radicals. (38) Hormonal dysregulation should be responsible for the faulty work of the mitochondria and their added production of oxidative stress. (33, 39) Kidneys from living donors with HLA-mismatch have a better outcome than kidneys from brain dead donors with HLA-match. (6)

Although some of the same outcomes between DCD and describe BD donors. (51)

To get access to the largest possible pool of donors it is necessary always looking for ways to use marginal donor organs and improve the results for the receiver. (46)

The ischemia time should be kept short. (54, 65) The effect of brain death should be minimized by optimal care of the donor (10) and adjustment of the time to organ removal. (43, 44) The formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) can be reduced and primers specifically influenced and improved. (10)
To measure changes before and after ischemia and reperfusion, to compare them and to make statements about organ quality not only functional markers of organs, but also genes or proteins that change due to ischemia / reperfusion or brain death are determined. (111)
By changes of ischemia energy loss, which lead to changes in cell metabolism, ROS and RNS, appear. Genes or proteins such as Bcl-2, Bax, GSH, GPx (Gpx3), OXSR-1, NF-κB, SOD (SOD2), Heat Shock proteins (Hsp70), PPARα, HPRT, Nitrotyrosine (3-NT), Myeloperoxidase (MPO) and Caspase-3 can be influenced and changed. Changes caused by ischemia/reperfusion affect genes leading to apoptosis. Therefore, a strong ischemia/reperfusion injury ends up in apoptosis and lower functioning cell mass in the donor organ, which affects the function and the result in the recipient. (25)
To get meaningful parameters to compare with each other, uniform protocols should be introduced for organ donation. Common protocols in animal experiments and human transplantation for all donor types are to create a situation in which the organ quality can be assessed and further can be improved. Or better starting positions can be created to ensure the highest possible organ quality. (35, 115)
In summary, it can be said that Bax, NF-κB, MPO, 3-NT and Caspase-3 are up regulated in ischemia and determine worse results or lead to apoptosis. Other genes and proteins, such as Bcl-2, GSH, GPx-3, OXSR-1, NF-κB, SOD2, Hsp70, PPARα, HPRT-1 have protective properties.

Of the Bcl-2 family anti-apoptotic Bcl-2 can prevent ROS production and cytochrome c release by stabilizing mitochondrial membrane, even protect cells after a lot of cytochrome c already has been released and is able to reduce apoptosis. (26, 27) There are no changes in Bcl-2 between BD and living donors are not, but Bcl-2 is lower in DCD than in BD. Bcl-2 is influenced by cold ischemia and more produced.
Also from the Bcl-2 family is proapoptotic Bax. It affects the mitochondrial membrane, leading to cytochrome c distribution and apoptosis. Bax may antagonize anti-apoptotic Bcl-2. BD and ischemia can raise Bax expression. (20,41)
GSH is an intracellular antioxidant, thus protecting cells against stress. In BD donors GSH is reduced, therefore ischemia reperfusion injury is greater. (75)

OXSR1 protects against stress. (86)

About the effect of NF-κB there is a disagreement. NF-κB is activated by ROS and regulates its part processes such as activation of inflammatory mediators. Some achieved less apoptosis and better results by activation (24, 89, 90) of NF-κB, others show a benefit by inhibition (37, 62, 66, 87, 91) of NF-κB.

SOD protects against ROS (27), reduces the release of cytochrome c and thus apoptosis. (63) In ischemia SOD2 level decreases. To better protect organs, SOD2 can be up regulated.

Hsp protects against apoptosis and is up regulated in stress. BD organs show lower Hsp 70 as organs from living donors. (99) It is believed that after massive damage Hsp get extracellular and can no longer repair damage. (97, 98)

PPARα seem to be also involved in cell proliferation, cell differentiation and apoptosis. (104) PPARα influences inflammatory response and its (102) activation leads to suppression of apoptosis. (28) During brain dead PPARα signaling pathway is down regulated. (40) HPRT increases shortly after death, (110) the expression of HPRT1 seems to be dependent on the age and do not change between normal and hypoxic conditions. (111)

Nitrosative stress damages organs after I/R and increased 3-NT can be detected. (17) If ROS or RNS is produced, thereby also antioxidants such as superoxide dismutase, glutathione and glutathione peroxidase become active to counteract a predominance depleting substances. (13)

MPO is a marker of the index for the infiltration of neutrophils. (87) MPO itself has inflammatory properties and therefore can damage tissue (69) and there is an increase in I/R damaged tissue, but it can be reduced if antioxitative agents are active. (87, 105)

Caspase-3 is a key mediator in apoptosis. (23) Caspase-3 increases after cold ischemia and is higher in BD donor organs than in living donors, but it can be inhibited and thus limit apoptosis after cold ischemia. (27, 41, 114)

In summary, can be said that the use of more marginal donor organs can be increased, if ischemia will be kept short, measurement of parameters can help to assess the organ quality and in the future, the favorable influence of genes or proteins can secure the graft quality for the receiver and exploit the donor pool to send patients a good therapy. (10)

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