LITHUANIAN UNIVERSITY OF HEALTH SCIENCES
MEDICAL ACADEMY

Arslan Mamedov

IMPACT OF CARDIOPLEGIC SOLUTIONS ON IMMATURE MYOCARDIAL TISSUE: A COMPARATIVE STUDY OF DEL NIDO, CUSTODIOL HTK, AND ST. THOMAS SOLUTIONS

Doctoral Dissertation
Medical and Health Sciences,
Medicine (M 001)

Kaunas, 2026

The dissertation has been prepared in the Heart Centre, Medical Academy of the Lithuanian University of Health Sciences, during the period 2020–2026.

Consultant

Assoc. Prof. Dr. Tadas Lenkutis (Lithuanian University of Health Sciences, Medical and Health Sciences, Medicine – M 001).

Dissertation is defended at the Medical Research Council of the Lithuanian University of Health Sciences:

Chairperson

Prof. Dr. Andrius Macas (Lithuanian University of Health Sciences, Medical and Health Sciences, Medicine – M 001).

Members:

Prof. Dr. Gintarė Šakalytė (Lithuanian University of Health Sciences, Medical and Health Sciences, Medicine – M 001);

Prof. Dr. Ramunė Morkūnienė (Lithuanian University of Health Sciences, Medical and Health Sciences, Pharmacy – M 003);

Prof. Dr. Kęstutis Ručinskas (Vilnius University, Medical and Health Sciences, Medicine – M 001);

Prof. Prakash P Punjabi (Imperial College London, Medical and Health Sciences, Medicine – M 001).

Dissertation will be defended at the open session of the Medical Research Council of the Lithuanian University of Health Sciences on 26 June 2026 at 9:00 a.m. in the Heart Center Conference Hall of the Lithuanian University of Health Sciences.
Address: Eivenių St. 2, LT-50161 Kaunas, Lithuania

LIETUVOS SVEIKATOS MOKSLŲ UNIVERSITETAS
MEDICINOS AKADEMIJA

Arslan Mamedov

MIOKARDO LĄSTELIŲ FUNKCIJOS IR STRUKTŪROS POKYČIŲ PALYGINIMAS NESUBRENDUSIOSE ŠIRDYSE, NAUDOJANT SKIRTINGUS APSAUGOS TIRPALUS (DEL NIDO, CUSTODIOL HTK, ŠV. TOMO)

Daktaro disertacija
Medicinos ir sveikatos mokslai,
medicina (M 001)

Kaunas, 2026

Disertacija rengta 2020–2026 metais Lietuvos sveikatos mokslų universiteto Medicinos akademijos Širdies centre.

Konsultantas

doc. dr. Tadas Lenkutis (Lietuvos sveikatos mokslų universitetas, medicinos ir sveikatos mokslai, medicina – M 001).

Disertacija ginama Lietuvos sveikatos mokslų universiteto Medicinos mokslo krypties taryboje:

Pirmininkas

prof. dr. Andrius Macas (Lietuvos sveikatos mokslų universitetas, medicinos ir sveikatos mokslai, medicina – M 001).

Nariai:

prof. dr. Gintarė Šakalytė (Lietuvos sveikatos mokslų universitetas, medicinos ir sveikatos mokslai, medicina – M 001);

prof. dr. Ramunė Morkūnienė (Lietuvos sveikatos mokslų universitetas, medicinos ir sveikatos mokslai, farmacija – M 003);

prof. dr. Kęstutis Ručinskas (Vilniaus universitetas, medicinos ir sveikatos mokslai, medicina – M 001);

prof. Prakash P. Punjabi (Imperatoriškasis Londono koledžas, medicinos ir sveikatos mokslai, medicina – M 001).

Disertacija bus ginama viešajame Medicinos mokslo krypties tarybos posėdyje 2026 m. birželio 26 d. 9:00 val. Lietuvos sveikatos mokslų universiteto Širdies centro konferencijų salėje.
Disertacijos gynimo vietos adresas: Eivenių g. 2, LT-50161 Kaunas, Lietuva

CONTENTS

ABBREVIATIONS

AKI – Acute kidney injury
AP-1 – Activation protein 1
ATP – Adenosine triphosphate
BAX – A protein that promotes programmed cell death
BcL-2 – An anti-apoptotic protein that regulates programmed cell death (apoptosis)
BCP – Blood cardioplegia
BNIP2 – A protein-coding gene (known as BCL2-interacting protein 2)
CCP – Crystalloid cardioplegia
CK-MB – Creatine Kinase-Myocardial Band
CP – Cardioplegia
CPB – Cardiopulmonary bypass
cTnT – Cardiac troponin T
DN – Del Nido
DNA – Deoxyribonucleic acid
EF – Ejection fraction
ETC – Electron transport chain
FCCP – Carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone
FOS – FOS proto-oncogene, AP-1 transcription factor subunit
GRK2 – G protein-coupled receptor kinase 2
HIF 1A – Hypoxia-Inducible Factor-1
HM – Homogenization medium
HTK – Histidine-tryptophan-ketoglutarate solution (known as Custodiol)
I/R – Ischemia/reperfusion
ICU – Intensive care unit
IL-10 – Interleukin-10
IL-4 – Interleukin-4
IL-6 – Interleukin-6
CON – Control group
LAD – Left anterior descending artery
LCOS – Low cardiac output syndrome
LV – Left ventricular
MI – Myocardial infarction
MIR – Mitochondrial respiration medium
MIT – Mitochondrial basal respiration

MMP – Matrix metalloproteinase
mRIPC – Modified remote ischemic preconditioning
NADH – Nicotinamide adenine dinucleotide
NCX – Na^{+}/Ca^{2+} exchanger
NO – Nitric oxide
OCR – Oxygen consumption rate
OXP – Oxidative phosphorylation
PAF – Platelet-Activating Factor
PCR – Polymerase chain reaction
PDA – Posterior descending artery
PTP – Permeability transition pore
RCA – Right coronary artery
ROS – Reactive oxygen species
RV – Right ventricle
SR – Sarcoplasmic reticulum
ST – St. Thomas
TNF-α – Tumor necrosis factor alpha
VEGF – Vascular endothelial growth factor

INTRODUCTION

Cardioplegia (CP) is essential in cardiac surgery because it protects the myocardium from ischemic injury during purposefully created cardiac arrest. Myocardial ischemia is a progressive process in which metabolic and structural changes accumulate with increasing ischemia duration. Without timely reperfusion, these changes may become irreversible and ultimately lead to cell death [1].

Since the inception of cardiac surgery, various strategies for myocardial protection have been developed based on two fundamental principles: hypothermia and modulation of electrochemical gradients with cardioplegic solutions. CP causes cardiac arrest by altering electrochemical processes, resulting in a motionless operative field and lower energy requirements for cardiomyocytes. Although all cardioplegic solutions share the common goal of arresting the heart and limiting ischemic damage, no universal consensus exists regarding the optimal solution for all patient groups. As a result, numerous formulations and delivery strategies continue to be employed in clinical practice [2].

Cardioplegic solutions are typically classified as depolarizing or hyperpolarizing, based on their effects on the myocardial cell membrane potential. Depolarizing CP, predominantly achieved through hyperkalaemia, remains the most widely used approach. Hyperpolarizing solutions, on the other hand, rely on hyponatremia and hypocalcemia to reduce sodium and calcium levels outside of cells, resulting in diastolic arrest through membrane hyperpolarization.

Del Nido cardioplegia, developed in the early 1990s for pediatric cardiac surgery, is an extracellular-based, potassium-rich solution that includes lidocaine to stabilize the cell membrane. This combination reduces excitability and prolongs the duration of myocardial arrest, eliminating the need for frequent dosing [1, 3, 4]. St. Thomas' solution, traditionally applied in adult cardiac surgery, contains potassium and magnesium and typically requires repeat application every 20–40 minutes to maintain myocardial protection [5]. Both Del Nido and St. Thomas solutions primarily act via membrane depolarization [6, 7].

Developed by Bretschneider in 1964, the histidine-tryptophan-ketoglutarate (HTK) solution is widely used as a cardioplegic medium and for organ preservation. Its formulation contains low sodium and calcium concentrations, which allow for metabolic suppression, intracellular pH buffering, membrane stabilization, and the provision of an energy substrate. These features provide extended myocardial protection, often up to 3 hours, which is beneficial in

long, technically complex operations [8, 9]. The low extracellular sodium concentration in HTK leads to membrane hyperpolarization and diastolic arrest, distinguishing it mechanistically from depolarizing solutions [10, 11]. Despite their widespread use, there is not much comparative data on the molecular and metabolic effects of these cardioplegic media in the immature myocardium.

Taking these factors into account, we conducted a study to simulate clinically relevant conditions and evaluate the effects of Del Nido, Custodiol HTK, and St. Thomas solutions on an immature rat myocardium subjected to ischemia/reperfusion (I/R) injury using the Langendorff perfused heart preparation. CP effectiveness was assessed not only through functional parameters but also through molecular markers associated with cell cycle regulation, proliferation, apoptosis resistance, and hypoxia response. The expression of several key genes was examined. The hypoxia-inducible factor *HIF-1α*, a central regulator of cellular adaptation to reduced oxygen availability, plays a crucial role in maintaining metabolic homeostasis during ischemic stress [12]. The *FOS* gene, a component of the AP-1 transcription factor complex, supports cellular responses related to proliferation and survival under surgical or ischemic stress [13]. *BNIP2*, a regulator of apoptotic pathways, may influence cardiomyocyte susceptibility to ischemic injury and, therefore, represents an important indicator of cell survival capacity [14]. Additionally, levels of pro- and anti-inflammatory cytokines were measured to characterize inflammatory responses associated with different cardioplegia solutions.

Furthermore, we aimed to assess the effect of the previously mentioned cardioplegic solutions on mitochondrial function in an immature myocardium. The respiratory functions of mitochondria were evaluated in permeabilized fibres by gradually introducing various substrates that supply electrons via different parts of the electron transport chain (ETC).

These investigations aimed to provide a comprehensive evaluation of how Del Nido, Custodiol HTK, and St. Thomas CP influence subcellular signalling pathways and mitochondrial bioenergetics in the immature myocardium. Combining functional, molecular, and mitochondrial data, the study aims to deepen understanding of cardioprotective mechanisms relevant to pediatric cardiac surgery and to identify strategies to enhance myocardial preservation during ischemia and reperfusion.

AIM AND OBJECTIVES

The aim of the study

The study aimed to evaluate the impact of different cardioplegic solutions (Del Nido, Custodiol HTK, St. Thomas) on mitochondrial respiratory pathway, gene expression, and cytokine responses in an immature rat heart model.

The objectives of the study

1. To evaluate and compare the influence of Custodiol HTK, St. Thomas, and Del Nido cardioplegic solutions on mitochondrial respiratory function in immature myocardial tissue.
2. To compare gene expression responses to Del Nido, Custodiol HTK, and St. Thomas cardioplegic solutions in immature myocardial tissue under ischemia/reperfusion conditions using the Langendorff model.
3. To evaluate the differential modulation of pro-inflammatory and anti-inflammatory cytokines in immature myocardial tissue in response to Del Nido, Custodiol HTK, and St. Thomas cardioplegic solutions.

Scientific novelty of the study

The search for an ideal cardioplegic solution has persisted for over a decade. During this period, numerous scientific studies have analyzed the effects of various cardioplegic solutions on the myocardium. However, most of these studies have focused on comparing the effects of traditional blood cardioplegia with Custodiol and Del Nido solutions or other crystalloid solutions on heart muscle cells.

Although many studies have documented findings in this field, the majority of the data come from adult patients and are often directly applied to pediatric cardiac surgery, which may increase postoperative complications and mortality [15-17].

In contrast, our study aims to investigate the effects of cardioplegic solutions on immature myocardium, for which data on their protective properties are lacking.

Even the most recent articles from 2019 to 2024 do not provide definitive answers and sometimes present contradictory findings. Thus, the debate regarding the optimal cardioplegic solution continues. It is also important to note that the physiology of pediatric cardiac muscle differs significantly from that of the adult myocardium. There have been contrasting descriptions of the immature heart, with some studies suggesting it is more tolerant to ischemia

[18-20], while others indicate it is less tolerant [21, 22]. Cardioplegic solutions are essential for protecting the immature myocardium during cardiac surgery by inducing temporary cardiac arrest, minimizing ischemia–reperfusion injury, and preserving mitochondrial and overall cardiac functions.

This study aimed to evaluate the impact of three cardioplegic solutions. Myocardial protection can be particularly challenging in certain cases, such as prolonged, complex procedures or pediatric patients with preoperative myocardial damage [23]. In such situations, selecting an optimal cardioplegic solution poses more questions than answers. Experimental studies have demonstrated a preference for single-dose CP in neonatal hearts [24], while other studies have found no significant difference when compared with multidose approaches [25].

Our research focuses on subcellular alterations in immature cardiomyocytes, as these represent the earliest detectable changes following cardioplegic intervention.

To assess whether specific cardioplegic formulations optimally protect the immature myocardium during cardiac surgery, a focused preclinical investigation is warranted that interrogates mitochondrial respiratory pathways, gene-expression profiles pertinent to cardioprotection and cellular stress, and the spectrum of pro- and anti-inflammatory cytokine responses.

This aspect of the study is timely because the international literature on the immature heart is methodologically diverse and frequently contradictory, leaving critical evidence gaps. The new detailed information obtained in immature myocardium using various cardioplegic solutions will be useful both globally and in Lithuania. Although the dissertation's research was conducted on an animal model, the new knowledge gained could also be applied in clinical practice.

1. LITERATURE REVIEW

1.1. Anatomy of the heart

The heart is a muscular organ. It is in the middle part of the lower mediastinum, just behind the sternum, between the two pleural cavities, surrounded by the pericardium and attached to the large blood vessels. It is roughly the size of a clenched fist and weighs about 250–350 g in an adult.

The heart has four chambers: two upper atria, the receiving chambers, and two lower ventricles, the discharging chambers. The atria open into the ventricles via the atrioventricular valves, present in the atrioventricular septum.

The fibrous cardiac skeleton forms the atrioventricular septum, which separates the atria from the ventricles, and the fibrous rings, which serve as bases for the four heart valves. It gives structure to the heart. The cardiac skeleton also serves as an important boundary in the heart's electrical conduction system because collagen cannot conduct electricity. The interatrial septum separates the atria, and the interventricular septum separates the ventricles. The interventricular septum is much thicker than the interatrial septum because the ventricles contract with more pressure.

The coronary arterial system comprises the right and left coronary arteries, which arise from the right and left aortic sinuses of the ascending aorta, immediately superior to the aortic valve cusps. The right coronary artery (RCA) courses within the right atrioventricular (coronary) sulcus, giving rise to the sinoatrial nodal artery (in ~60% of cases), right marginal branch, and, typically, the posterior interventricular artery (posterior descending artery, PDA) in right-dominant circulation. The left coronary artery is generally short and bifurcates into the anterior interventricular artery (commonly referred to as the left anterior descending artery, LAD) and the circumflex artery. The LAD descends within the anterior interventricular sulcus, supplying the anterior wall of the left ventricle, the anterior two-thirds of the interventricular septum, and the apex. The circumflex artery courses within the left atrioventricular sulcus, giving off obtuse marginal branches to the lateral wall of the left ventricle and, in left-dominant circulation (~10-15% of individuals), the PDA [26].

The posterior descending artery runs in the posterior interventricular groove and supplies the inferior wall and inferior third of the interventricular septum. The artery that supplies the PDA and a posterolateral branch determines the coronary dominance, so there can be three situations: right-dominance (approximately 70% of the cases) (supply from the RCA), left-dominance (10%) (supply from the circumflex artery), and codominance (20%), the

situation in which PDA and posterolateral branches arise from both right and left systems [27].

1.2. History of cardioplegic solutions

The concept of cardioplegia with a hyperkalemic solution was introduced by Melrose et al. [28] in 1955. It enabled better post-ischemic recovery of myocardial function compared with mere aortic cross-clamping. The concentration of the applied potassium citrate solution was 77 mmol/L [29]. This strategy was introduced into cardiac surgery as the Melrose technique, with the potential to provide a bloodless field for surgical maneuvers while preserving heart function [30]. Nevertheless, further examinations suggested that this type of hyperkalemic solution predisposed the heart to contractile dysfunction, ventricular fibrillation, and consequently cardiomyocyte death. These post-operative complications limit clinical usage. In 1975, Tyers et al. discovered that complications with the Melrose technique were due to the high potassium ion concentration [31].

In 1975, the St. Thomas Hospital (ST) solution was introduced by Hearse and Braimbridge in open-heart surgery in London. ST represents one of the widely used extracellular cardioplegic solutions worldwide [32]. Several years later, an improved formulation of this solution, known as the modified ST solution (ST 2), was developed. While both types of solutions contained increased magnesium (16 mmol/L) and normal calcium ion concentrations, they differed in potassium ion concentrations. The first ST contained potassium ions at a concentration of 20 mmol/L while the second contained a lower content, i.e., 16 mmol/L [33].

In 1983, Hearse proposed three components of myocardial protection by cardioplegia during cardiac surgery, namely: (a) rapid diastolic cardiac arrest to conserve energy by intracellular Na^+ and Ca^{2+} depletion, extracellular K^+ and Mg^{2+} elevation and infusion of local anesthetic agents or Ca^{2+} antagonists; (b) hypothermia to slow down cellular metabolic demands; and (c) application of substances (i.e. oxygen, energy substrates, etc.) to prevent or reverse ischemia-reperfusion (I/R) injury [34]. These principles now form the foundation for most cardioplegic solutions.

In the 1970s, Bretschneider and his group formulated the Custodiol Histidine-Tryptophan-Ketoglutarate (HTK) solution as an intracellular crystalloid for administration during cardiac surgery [35]. Although the HTK solution was primarily intended for cardiac surgery, it has been used to preserve other organs, such as the liver, pancreas, and kidneys. Currently, after several modifications, it is known as Custodiol and has been widely used as a cardioplegic strategy for myocardial protection [36].

Cold blood cardioplegia (BCP) was proposed by Buckberg [37] to arrest, perfuse, and then reperfuse the myocardium using a hyperkalemic crystalloid cardioplegic solution mixed with blood in a 1:4 ratio. Calafiore et al. [38] later introduced warm BCP containing K^+/Mg^{2+}, which provides myocardial protection when applied continuously. BCP has been shown to have potent myocardial protective effects against I/R injury, as evidenced by improved parameters compared with crystalloid cardioplegia (CCP), including greater oxygen supply and better acid-base balance [39].

In the early 1990s, Pedro Del Nido and colleagues developed a cardioplegic solution that met the specific needs of the pediatric hearts during surgery [40]. It has mainly been used in pediatrics, but after the 2010s began to be actively used in adult cardiac surgery. The Del Nido solution is much more diluted than a traditional blood cardioplegic solution. It has a 1:4 blood-to-crystalloid ratio compared with the traditional 4:1 blood-to-crystalloid ratio [41]. The history of CP described in the text is present in Table 1.2.1 [42].

Table 1.2.1. *Summarized the history of cardioplegic solutions*

Decade	**Milestone**	**Notes**
1950s	Early cardiac surgery begins	Hypothermia only (no solution)
1955s	Melrose solution introduced	CCP; high K^+
1960s	Decline of CP	CCP; high K^+
1970s	Revival of CP	CCP; ST and HTK
1980s	Cold BCP gains more	BCP > warm BCP better
1990s	Retrograde CP explored	Route change, the ST CP is being widely
2000s	DN CP (pediatrics → adults)	DN
2000–2020	HTK solution adopted, DN CP raised	Crystalloid/Single dose
2020s	Focus on minimally invasive & robotic surgery	Modified delivery method/Single dose

CP – cardioplegia, BCP – blood CP, CCP – crystalloid CP, DN – del Nido, ST – St. Thomas, HTK – histidine-tryptophan-ketoglutarate solution (known as Custodiol).

As patient conditions become more serious and complex surgeries become longer, and as advancements in minimally invasive cardiac interventions progress, an increasing number of cardiac surgical centres have adopted the single-dose cardioplegic solutions, such as Del Nido and Custodiol HTK (Fig. 1.2.1) [43, 44].

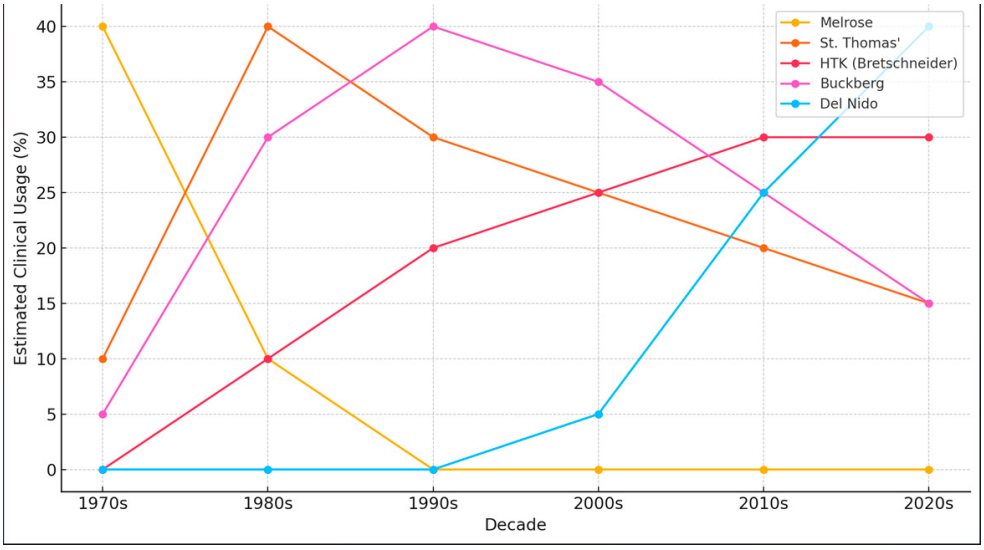


Fig. 1.2.1. *Trends in cardioplegic solution usage over time*

The increasing volume of major cardiac surgery, together with advances in minimally invasive approaches, is hastening the transition from multi-dose cardioplegia to single-dose strategies [43, 44].

1.3. Mechanisms underlying ischemic myocardial injury

The development of cardioplegic solutions is predicated on a comprehensive understanding of how ischemia disrupts normal cellular processes and activates damage-induced pathways. Fundamentally, an optimal cardiac function and aerobic metabolism depend on maintaining proper intracellular pH, high-energy phosphate reserves (ATP), and cell membrane/ionic homeostasis [3]. Irreversible cardiac damage can occur if any of these components deviate significantly from physiological levels. Cellular injury is generally reversible if perfusion is restored within the first 5 to 20 minutes of ischemia. However, tissue edema and cellular necrosis ensue after 20 minutes without intervention [45].

The severity of ischemic injury escalates significantly over time. The key to preventing irreversible damage or cell death is to restore blood flow to ischemic tissue while the cells are still undergoing reversible injury. Hypothermia and chemical agents slow cellular metabolism, extending the reversible state of ischemic injury and allowing full function to return once blood flow is restored. Reducing cellular metabolism slows ATP consumption and pH alterations, both of which contribute to cellular injury when disrupted [46].

The primary mechanisms of ischemic myocardial injury depend on the following factors:

- *Intracellular pH and ionic homeostasis disruption*. Ischemia significantly affects intracellular pH by shifting cellular metabolism from aerobic to anaerobic pathways, resulting in lactate accumulation and hydrogen ion build-up [47]. This acidification inhibits key enzymes in glycolysis and impairs mitochondrial function, exacerbating the ATP depletion.
- *ATP depletion and metabolic failure*. ATP is crucial for maintaining cellular ionic homeostasis and energy metabolism. During ischemia, the cessation of oxidative phosphorylation in mitochondria leads to rapid ATP depletion [48]. This energy deficit impairs the function of ion channels and pumps, causing ionic imbalance, cellular swelling, and subsequent membrane rupture [47, 49]. The inability to restore ATP levels during prolonged ischemia leads to irreversible damage and necrosis.
- *Mitochondrial dysfunction and reactive oxygen species (ROS) production*. Mitochondria are central to energy production and apoptotic signalling. Ischemia impairs mitochondrial oxidative phosphorylation, leading to increased production of ROS during reperfusion [50]. Excess ROS causes oxidative damage to lipids, proteins, and DNA, triggering cell death pathways, including apoptosis and necrosis [47].
- *Tissue edema and cellular necrosis*. The accumulation of intracellular metabolites increases osmotic pressure, resulting in water influx, cellular swelling, and membrane rupture [47]. The release of intracellular contents into the extracellular space promotes inflammatory responses, exacerbating tissue injury and necrosis.
- *Role of hypothermia in reducing ischemic injury*. Hypothermia reduces metabolic demand, slows ATP consumption, and minimizes pH alterations, thereby reducing the extent of ischemic injury [48].

Future clinical implications should focus on understanding the mechanisms of ischemic myocardial injury, which could lead to significant advances in cardioplegic solutions and hypothermic strategies for reducing myocardial damage during cardiac surgery [50].

1.4. Myocardial ischemic/reperfusion (I/R) injury

I/R injury leads to cell death primarily due to the sudden restoration of blood flow and oxygen to previously oxygen-deprived tissues, which

generates excessive ROS and triggers inflammatory responses, ultimately causing significant cellular damage and death [51].

A complex series of mechanisms is involved in myocardial I/R injury pathogenesis, including metabolic changes, mitochondrial dysfunction, inflammation, cytokine responses, autophagy deregulation, and ROS overproduction. Paradoxically, reperfusion causes rapid changes in pH, Ca^{2+} overload, and hyperoxia, leading to altered metabolism. Cell damage induced by prolonged I/R injury may lead to apoptosis, autophagy, necrosis, and necroptosis. This presents clinically as myocardial stunning, arrhythmias, and lethal reperfusion injury, in which salvageable cardiomyocytes in the area at risk undergo necrosis and/or additional forms of regulated cell death [52-55].

Factors that may influence cell damage include oxidative stress and overproduction of ROS. During ischemia, mitochondrial ATP production declines, leading to dysfunction of the electron transport chain (ETC) and resulting in the accumulation of semi-reduced oxygen species. Upon reperfusion, a sudden oxygen influx fuels an overproduction of ROS, exacerbating lipid peroxidation, protein oxidation, and DNA damage [56].

In addition, the inflammatory response and cytokine storms may contribute to cell damage. I/R triggers the activation of nuclear factor kappa-B (NF-κB), leading to upregulation of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6. Neutrophil infiltration and activation further amplify inflammation, causing microvascular obstruction and exacerbating myocardial damage. Neutrophils stimulate the production of reactive oxygen species (ROS), tumor necrosis factor alpha (TNF-α), and local inflammatory mediators, aggravating tissue damage [57].

Two other factors that may cause cell damage include the Na^+/Ca^{2+} exchanger (NCX) and Ca^{2+} overload. The NCX plays a central role in Ca^{2+} overload during ischemia. Under normal physiological conditions, NCX exports intracellular Ca^{2+} in exchange for Na^+. However, during ischemia, anaerobic metabolism and ATP depletion lead to acidosis, triggering increased Na^+ influx via Na^+/H^+ and Na^+/HCO_3^- transporters. This results in NCX reverse mode operation, driving an influx of Ca^{2+}, which accumulates in mitochondria, exacerbating hypercontractility and mitochondrial damage [58-60].

Clinical implications and future directions in myocardial I/R injury remain a critical challenge in cardiovascular medicine. While reperfusion therapy is essential, the associated oxidative stress, calcium overload, inflammation, and metabolic disturbances significantly exacerbate myocardial damage. Current research is focused on targeting mitochondrial dysfunction, oxidative stress, and calcium handling to develop novel cardioprotective therapies.

1.5. Types of cardioplegic solutions

Most cardioplegic solutions can be categorized as either depolarizing or hyperpolarizing solutions. The most prevalent method of myocardial protection during cardiac surgery is depolarized diastolic arrest with a hyperkalemic infusion [61]. During the administration phase, myocardial cells undergo depolarization, which leads to the opening of fast Na^+ channels. This process facilitates the influx of Na^+ ions into the cell, resembling the behaviour observed during a standard action potential. However, due to elevated extracellular K^+ concentration, fast Na^+ channels remain locked in an inactive state. This condition hinders repolarization of cells and effectively maintains them in an unexcitable state [61].

These solutions typically contain 10–25 mEq/L of K^+, which, when administered, elevates the cell membrane potential to approximately −50 mV from a resting membrane potential of −90 mV [46]. Diastolic arrest occurs at −50 mV because fast Na^+ channels, which have a threshold potential between −65 and −70 mV, are inactivated at this potential. The Nernst potential for NCXs is also −50 mV, meaning that at a diastolic arrest of −50 mV, no net flux of Ca^{2+} or Na^+ ions should occur across the cell membrane [46].

Hyperpolarizing solutions are utilized as an alternative to hyperkalemic solutions and function by rendering the membrane potential more negative than the resting potential [46]. This is accomplished by employing solutions devoid of Ca^{2+} and with low Na^+ to induce arrest. This methodology has the theoretical advantage of mitigating the severity of ionic imbalance during the ischemic period. Additionally, a few ion channels and pumps are active, and the metabolic demand of the myocardium is significantly diminished [62].

The potential for influxes and overload of Na^+ and Ca^{2+} during hyperpolarized arrest is attenuated because, at hyperpolarized membrane potentials, Na^+ and Ca^{2+} channels are inactivated. Additionally, the solution itself is primarily low in Na^+ and devoid of Ca^{2+} [46].

These channels have threshold potentials near −40 mV, which is proximate to predicted membrane potentials of cells exposed to depolarizing cardioplegic solutions. Elevating intracellular Ca^{2+} is a concern because it is associated with irreversible muscle contracture and myocyte necrosis [63, 64]. Additionally, placing cells in a prolonged depolarized state augments the consumption of high-energy phosphate reserves when compared with hyperpolarizing solutions [64]. Based on these concerns, some surgical teams prefer the utilization of hyperpolarizing solutions, especially in cases of prolonged myocardial ischemia.

Furthermore, cardioplegic solutions can be categorized into crystalloid CP (CCP) and blood CP (BCP) solutions. The CCP can be subdivided into

intracellular (e.g., Custodiol HTK) and extracellular (e.g., Del Nido, St. Thomas) solutions. The BCP can be classified into warm and cold solutions.

1.6. Advantages and disadvantages of blood and crystalloid cardioplegia

Zeng et al. [65] found that cold BCP was associated with a significantly lower incidence of perioperative myocardial infarction than cold CCP. The oxygen-carrying capacity of BCP is advantageous [66]. Hypothermia during cardiac surgery may counteract the benefits by shifting the oxyhaemoglobin dissociation curve to the left, thereby reducing the oxygen availability to myocardial tissue [65]. At 20 °C, only 50% of the total oxygen is released from BCP, falling to 37% at 10 °C [39]. This is in stark contrast to CCP, which releases all its oxygen at all temperatures [39]. Delivering oxygen to the ischemic myocardial tissue in the presence of BCP may produce oxygen-free radicals, which cause ischemia and reperfusion injury. However, blood also contains endogenous oxygen radical scavengers, which protect against this [65]. It is important to note that hemoglobin has six times higher buffering capacity than plasma proteins [39]. Whole blood also contains physiological oncotic pressure constituents. This minimizes myocardial edema. Some studies have found that CCP causes intracellular edema, depletion of glycogen stores, and higher release of CK-MB [39].

It also causes a higher degree of intra-operative hemodilution, which is associated with increased blood transfusion requirements, significantly greater intensive care requirements, longer hospital stays, higher operative costs, and higher mortality rates and postoperative organ failure, specifically renal failure [67]. Mullen et al. [68] concluded that while CCP is linked to a higher incidence of MI and increased CK-MB release postoperatively, it also results in improved right ventricular systolic function [68].

1.7. Advantages and disadvantages of cold and warm cardioplegia

Electromechanical cardiac arrest reduces myocardial oxygen demand by 90% [69]. Both warm and cold BCP use potassium-induced electromechanical cardiac arrest. Hypothermic CP (4–10 °C), given every 15 to 30 minutes, provides a bloodless operative field and reduces oxygen consumption by an additional 5% to 20%. On the other hand, warm BCP, delivered every 15 minutes at 34–36 °C, reduces I/R injury and improves oxygen delivery to cells [69].

However, the ideal temperature for BCP remains controversial. Buckberg has argued that myocardial temperatures below 20 °C are unnecessary, given

that the oxygen demand of an arrested heart at this temperature is only 0.3 mL/100 g per min [70].

In vitro, studies have shown that, at 20 °C, only 50% of the total oxygen content of BCP is available to the tissue, and this drops an additional 30% when the temperature is lowered to 10 °C [70]. The primary advantages of warm BCP include the reduction of I/R injury and the enhancement of oxygen delivery to myocardial cells, also decreasing intracellular swelling and improving membrane stabilization [71]. However, warm BCP lacks neuroprotective effects, necessitates larger volumes of CP solutions, poses a greater risk of hyperkalemia, and impairs visualization of distal coronary anastomosis. The primary advantages of cold BCP include the reduction of oxygen consumption and the provision of neuroprotective effects. However, it is associated with increased I/R injury, the induction of myocardial edema, and also causes cell membrane instability [71].

1.8. Properties of cardioplegic solutions

1.8.1. St. Thomas CP solution

The original extracellular CP solution developed by Hearse and colleagues in the early 1970s was known as St. Thomas Hospital Solution [72]. Over time, this solution was refined and evolved into Plegisol or St. Thomas's Hospital Solution No. 2 (ST2), which has become the most widely used CCP solution worldwide [73]. One of the main differences between ST and ST2 is the inclusion of procaine hydrochloride in ST, which acts as a membrane stabilizer with known cardioplegic effects [72]. The high concentrations of potassium and magnesium in St. Thomas CP solution induce rapid cardiac arrest [74]. As a result, repeated perfusion is required during ischemia, typically administered every 20–40 minutes [75]. It is important to note that St. Thomas CP also leads to increased cellular edema and can damage endothelial function [72]. It is administered at a cold temperature, usually at 4–10 °C. The solution is administered antegrade or retrograde, at a dose of 10–15 mL/kg.

1.8.2. Del Nido CP solution

During the 1980s and 1990s, CP was universally employed in both adult and pediatric cardiac surgery, with modifications made to delivery flow, volume, and pressure [3]. Since it was discovered that the pediatric myocardium was more tolerant to ischemia and more susceptible to calcium overload, Del Nido CP was developed and has been widely used in pediatric centers since its development in the 1990s [3, 63]. The crystalloid base component

of del Nido CP is 1 liter of Plasma-Lyte A, chosen for its ability to mimic normal extracellular ion concentrations in the body. This base solution and additives are delivered in a 1:4 ratio of patient blood from the bypass circuit to a crystalloid solution [3]. No Ca^{2+} ions are added to the base solution, and the only source of Ca^{2+} is from the patient's whole blood. Del Nido CP achieves electromechanical arrest via depolarization caused by the final K^{+} concentration of 24 mEq/L, which is achieved by adding 13 mL (26 mEq) of K^{+} to the Plasma-Lyte A base.

Mannitol functions as an osmotic diuretic and is conventionally used during cardiopulmonary bypass to increase urine production, reduce cerebral edema, and scavenge oxygen-free radicals [3]. Myocardial edema is another common problem during cardiac surgery and has also been shown to play a large role in post-ischemic cardiac injury [3]. The hyperosmotic nature and free-radical scavenging abilities of mannitol help attenuate both reactive oxygen species accumulation and myocardial edema during ischemia and reperfusion [3].

The addition of magnesium and lidocaine renders Del Nido a modified depolarizing solution, as these two components reduce calcium and sodium influx during the arrest. Magnesium possesses intrinsic calcium channel blocking properties, while lidocaine functions as a sodium channel blocker; both indirectly inhibit NCX and suppress action potential formation [3]. Since myocardial contraction is highly dependent upon intracellular Ca^{2+} levels, reducing Ca^{2+} entry helps prevent diastolic rigidity. The addition of magnesium has been shown to improve cardiac myocyte recovery after ischemic events [76].

The final delivery temperature is usually cold (4–8 °C). The solution is administered antegrade or retrograde, with an initial dose of 20 mL/kg and maintenance doses of 10 mL/kg as needed, and a dose of 30 mL/kg in pediatric patients.

In addition to calcium handling, cardioplegic solutions need to support ATP production and limit acidosis while cells are in an anaerobic state. The Del Nido CP does not contain any substrates because they are available within cardiac cells, but the inclusion of bicarbonate acts as a buffer to mitigate acidic conditions. The incorporation of sodium bicarbonate into Del Nido CP enhances buffering capacity and helps maintain an intracellular pH close to 7.4, allowing glycolysis to be sustained throughout the ischemic period [3]. The addition of 20% whole blood further promotes anaerobic glycolysis due to its endogenous buffering capacity and allows for aerobic metabolism to occur during the delivery period [3]. The inclusion of blood in the solution reduces ischemic stress and reperfusion injury compared with completely crystalloid solutions [7].

Standard dosing for Del Nido CP is a single dose of 20 mL/kg, with a maximum dose of 1 liter for patients weighing more than 50 kg [3]. Redosing is generally performed at the surgeon's discretion. However, Del Nido CP was developed as a single-dose model; therefore, redosing may not occur unless the cross-clamp time exceeds 90 minutes or electrical activity is observed. In summary, Del Nido CP was developed to address the specific requirements of the pediatric and infant heart, with the primary objective being to prevent Ca^{2+} overload during ischemia and reperfusion. This is achieved by adding lidocaine, a fast sodium channel blocker, and magnesium sulfate, a calcium channel competitor. The inclusion of mannitol scavenges oxygen-free radicals during ischemia and reduces the risk of reperfusion injury. Sodium bicarbonate is used to maintain a pH of approximately 7.4, counteracting acidosis and allowing anaerobic glycolysis to be sustained during ischemia. During infusion, the patient's whole blood is mixed into the solution at a 1:4 ratio. This enhances the solution's buffering capacity and aids in coronary perfusion during induction and subsequent doses.

1.8.3. Custodiol HTK CP solution

The Histidine-Tryptophan-Ketoglutarate (HTK) solution was initially introduced in the early 1970s by Hans Jürgen Bretschneider [77]. Hyperpolarizing solutions prevent Ca^{2+} overload because few channels or ion pumps are active at hyperpolarized membrane potentials [78]. Additionally, energy consumption is low, and the metabolic demand of cardiac myocytes is minimal at these highly negative potentials [78]. HTK is classified as an intracellular solution because it contains low Na^{+} (15 mmol/L) and Ca^{2+} concentrations. The reduced Na^{+} level in the extracellular space inhibits the fast inward current and achieves cardiac arrest in diastole, providing up to 3 hours of myocardial protection with a single dose [62, 77, 79].

Histidine acts as a buffer, supporting anaerobic glycolysis and preventing acidosis [62, 77].

Ketoglutarate, an intermediate in the Krebs cycle, enhances ATP production during reperfusion. It also regulates cell membrane function, reduces reperfusion injury, and decreases edema [80]. However, caution should be exercised when using the HTK solution because of its low Na^{+} content, which can affect extracellular Na^{+} levels and may cause hyponatremia [81, 82]

Tryptophan serves to stabilize and protect cell membranes during arrest. Mannitol is added to prevent reperfusion injury by scavenging free radicals and counteract myocardial edema by maintaining a slightly hyperosmotic extracellular osmolarity [83]. It is administered at a cold temperature, usually

4–8 °C. The solution is administered antegrade or retrograde, with a dose of 30 mL/kg in adults and 30–40 mL/kg in pediatric patients.

1.9. Differences in the pediatric and adult myocardium

The hearts of newborns and adults have several morphological, physiological, and histological differences. These include increased dependence on glucose, increased sensitivity to calcium, and less contractile fiber [20]. A good understanding of these differences might affect the choice of cardioplegia and the details of its delivery during surgery. We will examine the most significant differences:

- Morphologically, in the newborn heart, only 30% of the myocardial mass is composed of contractile tissue, whereas 60% of the mature myocardium has contractility.
- Neonatal myocardium has fewer mitochondria and lower oxidative capacity.
- 90% of ATP generation in the adult myocardium is derived from fatty acid metabolism, whereas in the neonatal/pediatric heart, glucose is the main source of energy production [20].
- In pediatric/neonatal and adult hearts, Ca^{2+} plays a key role in cardiac contraction, but how it enters the cell differs between immature and mature hearts. In adult hearts, when voltage-gated L-type Ca^{2+} channels open, the influx of Ca^{2+} ions triggers the opening of Ca^{2+} channels located on the sarcoplasmic reticulum (SR) [84], from which 90% of cytosolic Ca^{2+} arises. In neonates, the density of L-type Ca^{2+} channels is lower than it is in older children or adults. As a result, Ca^{2+} ions influx into immature hearts occurs through the NCX instead of L-type calcium channels [84]. It is essential to maintain proper calcium homeostasis and prevent Ca^{2+} overload during cardiac surgery, particularly in pediatric cardiac surgery. Immature cardiac tissue has a reduced capacity to regulate Ca^{2+}, which can be attributed to various structural differences. In pediatric hearts, the SR is still developing and has a smaller capacity to store calcium [20]. Furthermore, the primary source of Ca^{2+} required for contraction comes from the extracellular space via the NCX and L-type calcium channels, not the SR. The combination of these factors diminishes the ability of pediatric hearts to release and reuptake Ca^{2+} from the SR during an action potential, which is why the inclusion of L-type calcium channel blockers in CP is advantageous during pediatric cardiac surgery [20] These differences also elucidate why Ca^{2+} overload is possible during post-ischemic reperfusion and why pediatric

cardioplegic solutions generally contain lower-than-normal Ca^{2+} levels [20].

- In newborns, cardiac ejection fraction (EF) is more dependent on heart rate and sinus rhythm. Increased afterload causes significant hemodynamic changes.
- The immature myocardium is more tolerant to ischemia compared to the mature myocardium because it possesses higher glycogen reserves and utilizes anaerobic glucose metabolism for a longer duration than the adult heart [85].

1.10. Bioenergetics of the heart

Humans produce and consume roughly their body weight in ATP (about 65 kg) every single day [86]. The heart accounts for only ~0.5% of body weight but is responsible for roughly 8% of ATP consumption. This high energy flux is dynamic: the heart stores only enough energy to support pumping for a few heartbeats, turning over the entire metabolite pool approximately every 10 seconds, even at resting heart rates [87]. As the most metabolically active organ in the body, the heart has the highest mitochondrial content of any tissue. The high concentration of mitochondria in cardiomyocytes is crucial for meeting the significant energy demands of both contraction and relaxation, as these processes are also active. About 90% of cellular ATP is utilized to support the contraction–relaxation cycle within the myocardium [88]. ATP-dependent release of actin from myosin is required for both contraction (as myosin heads the cycle through cross-bridges with actin) and relaxation. Cellular sequestration of Ca^{2+} back into the SR during diastole also requires a tremendous amount of ATP. Cardiac mitochondria must operate at high efficiency to meet the fluctuating energetic demands of contractile units, driven by the body's dynamic need for oxygen-rich blood [88].

Decreased capacity of mitochondria to generate and transfer energy within heart cells results in energy deficits, which influence all cellular processes that require energy, most notably the processes of contraction and relaxation

1.11. Mitochondrial oxidative phosphorylation system and response to ischemia

Mitochondria are the powerhouses of the cell. In all eukaryotes that do not depend on photosynthesis, mitochondria are the main source of adenosine triphosphate (ATP), the energy-rich compound that drives fundamental cell functions.

One of the primary functions of mitochondria is the synthesis of ATP during oxidative phosphorylation. This process is carried out by five enzymatic complexes located in the inner mitochondrial membrane, which together constitute the oxidative phosphorylation system. Four of these complexes, such as nicotinamide adenine dinucleotide (NADH) dehydrogenase (reduced nicotinamide dinucleotide dehydrogenase, Complex I), succinate dehydrogenase (Complex II), coenzyme Q-cytochrome C reductase (Complex III), and cytochrome C oxidase (Complex IV), are components of the electron transport chain.

The substrates of this chain are the reduced coenzymes NADH and flavin adenine dinucleotide ($FADH_2$) in its reduced form, which undergo oxidation in Complexes I and II, respectively. The mobile electron carrier coenzyme Q then transfers electrons from Complexes I and II to Complex III. Subsequently, via another mobile carrier, cytochrome C, electrons are delivered to Complex IV, which catalyzes the reduction of molecular oxygen to water.

During this electron transport process, energy is released and utilized to translocate and accumulate protons in the intermembrane space. ATP synthase, or Complex V, catalyzes the phosphorylation of ADP to ATP using inorganic phosphate. This reaction is driven by the electrochemical proton gradient established by the electron transport chain [89-92].

During ischemia, the disruption of oxygen supply leads to hypoxic conditions within cells, which impairs the function of the mitochondrial electron transport chain and results in a reduced rate of ATP synthesis. The inhibition of oxidative phosphorylation promotes a shift towards anaerobic metabolism, accompanied by increased lactate production, which acidifies the intracellular environment and contributes to the development of metabolic acidosis. As ATP becomes depleted, ATP-dependent ion transport mechanisms, particularly Na^+/K^+-ATPase and Ca^{2+} channels, are compromised. Consequently, intracellular accumulation of Na^+ and Ca^{2+} ions occurs, leading to cellular swelling (oncosis) and disruption of enzyme activity [93].

Under hypoxic conditions, oxygen-dependent enzymes such as prolyl hydroxylases are inhibited, resulting in the stabilization and activation of hypoxia-inducible factor 1-alpha (*HIF-1α*). This transcription factor is crucial for regulating gene expression to help cells adapt to low oxygen levels. Additionally, the production of reactive oxygen species (ROS) is markedly increased, particularly within the mitochondrial electron transport chain [94].

Mitochondria are the main source of cellular ROS. Under physiological conditions, 0.2–2% of the electrons in the ETC do not follow the normal transfer order but instead directly leak out of the ETC and interact with oxygen to produce superoxide or hydrogen peroxide [95, 96].

Ischemia-induced alterations in the ETC can lead to significant bioenergetic and functional impairments. The objective of this study was to evaluate the impact of various cardioplegic solutions on mitochondrial ETC function following different durations of ischemia. We sought to determine which cardioplegic solution is most effective in maintaining mitochondrial integrity and function within the immature myocardium.

Ischemia also triggers a cascade of inflammatory processes, activating the innate immune response, with the release of pro-inflammatory mediators and the recruitment of immune cells to clear necrotic and apoptotic debris. However, if unregulated, this inflammatory and immune activation may exacerbate tissue injury, particularly during the reperfusion phase following ischemia [97].

1.12. Apoptotic cell death during ischemia-reperfusion injury

Tissue injury caused by I/R can occur through either necrosis or apoptosis. Apoptosis, also referred to as programmed cell death, is an energy-dependent mechanism defined by a cascade of gene-regulated events that lead to characteristic cellular morphology, controlled DNA fragmentation, and ultimately cell demise. In recent years, the contribution of apoptosis to I/R injury-associated tissue injury has been extensively studied. Oxidative stress and excessive production of ROS act as strong inducers of apoptosis, a phenomenon that can be clearly observed following cerebral I/R injury. Both renal and myocardial I/R injury are associated with measurable levels of apoptosis in affected tissues. Thus, apoptosis appears to represent a central mechanism of cell damage during I/R injury across multiple organ systems. Inhibition of the apoptotic pathway, particularly through the use of selective blockers targeting pro-apoptotic caspase enzymes, has shown partial effectiveness in experimental models, leading to reductions in tissue injury and infarct size after hepatic and cardiac I/R injury [98].

Cardiac dysfunction resulting from acute lesions (myocardial infarction, I/R) or chronic conditions (ischemia, dilated cardiomyopathy) is closely associated with cardiomyocyte apoptosis, a key driver of cardiac remodelling and a contributor to sudden cardiac death [99, 100]. As apoptosis is central to the pathogenesis of many cardiac diseases, its inhibition or modulation represents a potential therapeutic approach for heart failure [101, 102]. Morphologically, cardiac apoptosis is characterized by cell shrinkage, cytoplasmic and chromatin condensation, and persistent low-grade apoptosis has been implicated in the progression of heart failure. Notably, enhanced apoptosis in hypertrophic cardiomyopathy may accelerate ventricular dilatation and ultimately lead to systolic dysfunction [103].

The expression of molecules implicated in apoptosis, such as *Bcl-2* and caspases, has been proposed as a significant marker of cardiomyocyte viability [104]. Toxin-induced cardiomyopathy arises through diverse pathways, including elevated oxidative stress, lipid peroxidation, DNA injury, apoptosis, and autophagy [105]. *BAX* activation has recently been shown to be a rate-limiting step in doxorubicin-induced cardiomyopathy [106].

1.13. Inflammatory processes and cytokine responses to ischemia/reperfusion

Hypoxia and I/R injury both induce the expression of numerous cytokines, including pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), and anti-inflammatory cytokines such as interleukin-10 (IL-10) and interleukin-4 (IL-4). These cytokines are released systemically and are thus important in the development of systemic inflammatory response syndrome and ultimately multi-system organ failure [107].

1.13.1. Pro-inflammatory cytokines

TNF-α is a pro-inflammatory cytokine produced by activated macrophages, monocytes, Tlymphocytes, natural killer cells, and fibroblasts. It is a potent chemoattractant and an early response cytokine that subsequently induces the expression of IL-1, IL-6, IL-8, and PAF. Serum TNFα levels increased rapidly in an animal model of aortic clamping, thus inducing up-regulation of INOS, which increased NO production in the lungs, leading to more severe lung damage [108]. TNF-α stimulates ROS production and the vascular endothelium's vulnerability to neutrophil-induced injury by enhancing ICAM-1 expression, which facilitates neutrophil adhesion to the activated endothelium.

TNF-α stimulates the expression of other pro-inflammatory cytokines, chemokines, and adhesion molecules by leukocytes and endothelial cells, and regulates extracellular matrix metabolism by decreasing collagen synthesis and activating matrix metalloproteinase (MMP) activity [109]. Targeted TNFα overexpression caused adverse cardiac remodelling provoked by progressive cardiomyocyte apoptosis [110]. Following I/R injury, TNF-α deficiency exhibited attenuated chemokine expression and NF-κB activation in the infarcted heart, resulting in reduced infarct size and improved cardiac function [111]. Additional remote ischemic preconditioning before reperfusion attenuated the inflammatory response, causing decreased levels of TNF-α and IL-1β, accompanied by an improved LV function [112].

Numerous studies in animal models attest to the potential of TNF-α blockade as a therapeutic modality to reduce the severity of I/R injury. In the rat model, an anti-TNF-α antibody prevented microvascular damage and thus protected against I/R-induced pulmonary injury. The development of humanised antibodies, such as etanercept and infliximab, has shown promising results in treating various TNF-α-mediated inflammatory diseases, including some forms of arthritis and inflammatory bowel disease [113].

Pharmacological therapy with morphine after I/R injury was demonstrated to lower circulating TNFα concentrations and diminish infarct size, accompanied by concomitant enhancement of LV performance [114]. Moreover, G protein-coupled receptor kinase 2 (GRK2) deficiency led to reduced TNF-α expression and fibrosis, resulting in smaller infarct size and preserved cardiac function [115]. In experimental MI, TNF-α deficiency safeguarded against myocardial rupture and chronic LV impairment by suppressing excessive inflammation, matrix and collagen degradation, and apoptosis [116]. Conversely, protective properties of TNFα signalling have also been reported, since neutralization of TNFα with adenoviral TNFR1 produced harmful outcomes by facilitating ventricular rupture and worsening ventricular dysfunction and remodelling after MI [117].

IL-6 is a pro-inflammatory protein synthesized by monocytes, fibroblasts, keratinocytes, and endothelial cells in response to IL-1 and TNF-α. IL-6 primes and activates the respiratory burst in neutrophils, promotes endothelial cell expression of ICAM-1, and enhances endothelial permeability. IL-6 is generated in hypo-perfused skeletal muscle in individuals with peripheral arterial disease and is secreted from the intestine into the systemic circulation during reperfusion in aortic aneurysm surgery [118]. In the context of renal transplantation, IL-6 was released in substantial quantities from the repercussed transplanted kidney within the first 30 minutes of reperfusion [119].

Production of IL-6 is rapidly triggered in the I/R myocardium [120], and IL-6 has been associated with acute coronary syndrome [121]. In Mendelian randomization analyses, inhibition of IL-6R resulted in a reduction of cardiovascular events, indicating a potential therapeutic strategy to prevent coronary artery disease [122]. Moreover, administration of tocilizumab, an IL-6R blocker, in patients with non-ST-elevation MI diminished the inflammatory response, lowering concentrations of high-sensitivity C-reactive protein and cTnT [123]. Nevertheless, pre-clinical investigations demonstrated inconsistent findings: IL-6 deficiency did not influence infarct size, LV function, remodelling, or survival after unreperfused MI, which was attributed to compensatory activity of alternative mediators [124]; on the other hand, reduced infarct size following MI-R injury [125]. Concerning

pharmacological therapy, treatment with bisoprolol exerted cardioprotective properties after MI-R injury by inhibiting the secretion of both IL-6 and TNF-α, and by attenuating the unfolded protein response, thereby limiting infarct size and enhancing post-ischemic cardiac performance [126].

1.13.2. Anti-inflammatory cytokines

Suppressive mediators, including IL-10, TGF-β, IL-4, and lipid-derived molecules, may represent the primary pathway for downregulation of chemokine signalling. These substances have diverse effects on modulating the immune response and play a crucial role in coordinating the repair processes after ischemia [127].

IL-10 is primarily secreted by activated T-lymphocytes and stimulated monocytes, demonstrating significant anti-inflammatory properties. Its induction parallels the downregulation of IL-6 synthesis in macrophages and monocytes, and further suppresses the release of IL-1α, IL-1β, TNF-α, and IL-8, thereby attenuating the inflammatory response. In addition, IL-10 contributes to matrix stabilization by reducing metalloproteinase production [128].

IL-10 expression is elevated in reperfused myocardium, and blockade of IL-10 activity was associated with diminished levels of tissue inhibitor of metalloproteinases (TIMP)-1, indicating that IL-10 contributes to matrix stabilization [129]. In the context of MI-R injury, IL-10 deficiency led to augmented fibrosis and apoptosis [130], along with an intensified inflammatory response, which correlated with larger infarct size and higher mortality [131].

Moreover, administration of recombinant IL-10 after MI attenuated the inflammatory response and promoted improved LV function and remodelling by reducing fibrosis and enhancing capillary density [132]. Pharmacological interventions, such as colchicine pretreatment [133] or the use of a selective β2-adrenergic receptor agonist during MI-R injury [134], elevated systemic IL-10 levels, demonstrating cardioprotective effects with smaller infarct size. Nevertheless, evidence regarding IL-10's role in resolving post-MI-R inflammation remains inconsistent, as IL-10 deficiency was also associated with timely downregulation of pro-inflammatory cytokine and chemokine mRNA expression, accompanied by a comparable course of neutrophil clearance. This suggests the contribution of multiple overlapping regulatory mechanisms [135].

1.13.3. Neutrophils

Neutrophils play a crucial role in tissue injury associated with I/R injury. Activated neutrophils are a primary source of ROS, which are produced by the

membrane-bound nicotinamide adenine dinucleotide phosphate (NADPH) oxidase complex. While oxidizing NADPH to $NADP^+$, NADPH oxidase simultaneously reduces molecular oxygen, generating the superoxide anion. Activated neutrophils also release a variety of proteases, including matrix metalloproteinases, that degrade the basement membrane and other structural components of tissues, thereby worsening the extent of tissue damage [136].

Neutrophil depletion during cardiac surgery has been extensively studied as a method to reduce post-operative cardiac dysfunction, though results remain inconsistent. Some investigations have demonstrated a reduction in biochemical markers of myocardial injury, whereas others have failed to establish a clinically meaningful benefit [136].

1.14. Ischemic preconditioning

Ischemic preconditioning involves short, repeated episodes of I/R injury before the onset of prolonged organ ischemia and has proven effective in mitigating tissue injury. This protective phenomenon can also be induced remotely rather than directly in the target organ. Such an approach holds potential in various surgical contexts, including transplantation, coronary artery bypass grafting, and elective major vascular procedures, where the onset of ischemia can be precisely controlled. In these settings, brief limb I/R injury (e.g., 10-15 minutes) induced by a tourniquet before surgery has been extensively studied and demonstrates promise as a strategy to lessen the extent of I/R injury [98].

Animal models across various contexts of I/R injury have been employed to explore the mechanisms underlying ischemic preconditioning; yet, the fundamental molecular basis remains incompletely understood, likely owing to the involvement of multiple signal transduction pathways. It is, however, widely accepted that brief preconditioning episodes trigger a cascade of intracellular kinases and ultimately alter mitochondrial function. In a rat model of lower limb, I/R injury, two brief 10-minute episodes of ischemia before a sustained 60-minute insult effectively attenuated pro-inflammatory neutrophil–endothelium interactions. This protective effect was evident not only in the affected limb but also in distant organs, highlighting the systemic nature of the response [137]. Similarly, in a mouse model of hind limb I/R injury, preconditioning markedly reduced tissue injury in the limb as well as in the lungs and small intestine while also conferring significant protection against post-operative mortality [138].

In a single-center randomized controlled trial of 86 patients undergoing elective mitral valve replacement, participants were assigned to either modified remote ischemic preconditioning (mRIPC) or control. The mRIPC protocol

consisted of three cycles of 5-min ischemia and 5-min reperfusion with a blood pressure cuff, applied 24 h, 12 h, and 1 h before surgery. Compared with controls, mRIPC reduced IL-6 and TNF-α levels while increasing IL-10 and was associated with lower rates of myocardial and pulmonary injury, supporting its potential role in valve surgery [139].

A meta-analysis assessed the efficacy of mRIPC in preventing acute kidney injury (AKI) after cardiac surgery. Pooled data demonstrated a significant reduction in AKI incidence with mRIPC compared to controls. Evidence suggests that the benefit is primarily driven by fewer mild-to-moderate AKI cases not requiring renal replacement therapy [140].

In a randomized clinical trial of 43 patients undergoing coronary artery bypass grafting, participants were assigned to mRIPC (n = 21) or control (n = 22). The control group required more packed cell transfusions in the intensive care unit (ICU). At 24 h after ICU admission, IL-10 levels were significantly higher in the mRIPC group, whereas post-cardiopulmonary bypass (CPB) IL-8 levels were lower than in controls. These findings suggest that RIPC modulates circulating inflammatory cytokines by elevating IL-10 and reducing IL-8, which may contribute to protection against I/R injury [141].

1.15. No reflow phenomenon

No reflow is the failure of microvascular perfusion following restoration of flow to previously ischemic tissue. The cause of this phenomenon has not been fully elucidated [142] but is certainly multifactorial. Cytokines and activated neutrophils act synergistically to produce microvascular barrier dysfunction. The resultant increase in permeability leads to the exudation of fluids and proteins, increasing the interstitial pressure and decreasing the net intravascular pressure. In addition, integrin beta chain 2 (CD18) dependent leukocyte plugging produces partial occlusion of post-capillary venules, further contributing to no-reflow. Neutrophil depletion virtually abolishes the phenomenon in the myocardium, brain, and skeletal muscle, confirming a vital role for neutrophils in no-reflow.

A sudden obstruction of the epicardial coronary artery, whether persistent or transient, induces myocardial ischemia that triggers a cascade of pathophysiological events within minutes, ultimately culminating in cardiomyocyte death. The central mechanism involves endothelial injury and dysfunction, which collectively impair microvascular perfusion. Progression to endothelial necrosis further augments the permeability of adherens and gap junctions. Concomitantly, vascular endothelial growth factor (VEGF) expression is upregulated, enhancing endothelial permeability [143]. This

leads to extravascular accumulation of the blood cells, along with impaired nitric oxide (NO) production by damaged endothelium. Leukocyte infiltration, aggregation of neutrophils, platelets, erythrocytes, vasoconstriction mediated by endothelin-1 and other pro-inflammatory mediators, activation of inflammatory signaling cascades, and cellular edema all contribute to compression of the coronary microvasculature and progressive luminal narrowing. In addition, microvascular spasm secondary to endothelial injury represents an important exacerbating factor. During sustained arterial occlusion, the ischemic process extends to cardiomyocytes, resulting in necrosis and interstitial edema, which further aggravate microvascular obstruction [144].

1.16. Changes in cardiac troponin I level (cTnI) after cardiac surgery

Table 1.16.1 presents studies comparing cTnI levels after different types of CP. In studies comparing BCP and CCP [145, 146], no significant differences were found in postoperatively cTnI release at 4-6 hours, 12 hours, and 24 hours. A meta-analysis [147] that included 10 eligible studies directly comparing BCP to CCP also showed no significant difference between the two groups, except for significantly lower cTnI levels at 4 hours postoperatively in the BCP group [147]. In a study comparing Custodiol HTK CP and cold BCP, it was found that cTnI concentrations were higher in the cold BCP group from postoperative hours 1 to 72 [148]. Another study by Dolcino et al. [149] investigated neonates undergoing arterial switch operation with either Custodiol HTK CP or warm BCP, and it showed that postoperative troponin concentrations were higher in the Custodiol HTK CP [149]. Studies comparing Del Nido CP and blood CP [150, 151] concluded that Del Nido CP provides a lower postoperative troponin I concentration compared to the blood CP group. In the study conducted by Panigrahi et al. [152], although no significant difference was observed regarding cTnI levels between the two groups, a greater amount of cTnI release was noticed at 12 hours in the BCP group. Two studies [153, 154] comparing the Custodiol HTK CP with the Del Nido CP showed that the latter was associated with less release of cTnI.

Table 1.16.1. *P-values between different study CP groups in cardiac troponin I level after cardiopulmonary bypass (CPB)*

Authors	**Study type**	**n**	**Type of CP used**	***p-value* at 4-6 h**	***p-value* at 8 h**	***p-value* at 12 h**	***p-value* at 24 h**	***p-value* at 48 h**	***p-value* at 72 h**
Fang et al. [145]	Meta-analysis	323	BCP *vs* CCP	0.09	-	0.53	0.12	-	-
Romolo et al. [146]	Randomized clinical trial	70	BCP *vs* CCP	-	-	-	-	-	-
Mylonas et al. [147]	Meta-analysis	697	BCP *vs* CCP	0.860	-	0.019	0.000	-	-
Pérez-Andreu et al. [148]	Observational	64	Cold BCP *vs* HTK	0.001	-	0.001	0.001	0.001	0.003
Dolcino et al. [149]	Observational	101	Warm BCP *vs* CHTK	-	-	-	-	0.001	-
Isildak et al. [150]	Randomized clinical trial	80	BCP *vs* DN	0.091	-	-	0.045	0.315	-
Haranal et al. [151]	Randomized clinical trial	100	BCP *vs* BSTH	-	-	-	0.629	-	-
Panigrahi et al. [152]	Randomized clinical trial	60	BCP *vs* DN	0.873	-	0.180	0.780	-	-
Dehaki et al. [153]	Randomized clinical trial	40	CHTK *vs* DN	0.001	-	-	-	-	-
Tunçer et al. [154]	Observational	27	CHTK vs DN	-	0.016	-	-	-	-

n – patient number, CP – cardioplegia, BCP – blood CP, CCP – crystalloid CP, CHTK – Custodiol Histidine-Tryptophan-Ketoglutarate CP, DN – Del Nido CP, BSTH – blood-based St. Thomas CP. The table is taken from [167].

Data regarding myocardial metabolism is limited and is derived from a few studies (Table 1.16.2) which evaluated lactate levels after cardiopulmonary bypass. According to meta-analysis [145], lactate levels after CPB were significantly lower in the BCP group compared to the CCP group. In the study conducted by Gholampour et al. [153], which compared Del Nido with Custodiol HTK CP, lactate levels were significantly higher among patients who received Custodiol HTK CP [153] [153]. The cardioplegic solutions' effects on the myocardial energy marker, the ATP level. In the meta-analysis conducted by Mylonas et al. [147], no significant difference between ATP levels was found between the two groups (BCP vs CCP).

Table 1.16.2. *p-values between different CP groups in lactate levels after CPB*

Authors	Study type	n	Type of CP	*p-value*	Study year
Fang et al. [145]	Meta-analysis	323	BCP vs CCP	0.03	2015
Gholampour et al. [153]	Randomized clinical trial	40	DN vs CHTK	0.001	2018

n – patient number, CP – cardioplegia, BCP – blood CP, CCP – crystalloid CP, CHTK – Custodiol Histidine-Tryptophan-Ketoglutarate CP, DN – Del Nido CP. The table is taken from [167].

1.17. Impact of different cardioplegia solutions on intraoperative results

Several factors influence intraoperative outcomes, including CPB's inotropic status. In a study by Talwar et al. [156], which compared Del Nido and Custodiol HTK CP, Del Nido CP was associated with lower inotropic scores compared to Custodiol HTK CP. The inotropic score was evaluated at the end of the first 24 hours, after 48 hours, and after 72 hours. Three studies [148, 150, 152] compared Del Nido CP with blood CP and concluded that Del Nido CP provides lower inotrope scores at 24 hours and at 48 hours [148].

In one study [157], Custodiol HTK CP was compared with blood CP, and the inotrope score was found to be lower in the HTK group. A study comparing Del Nido and St. Thomas CP in 220 patients found no significant difference in inotropic score [158]. Additionally, one study [159] analyzed the outcomes among the three groups – Custodiol HTK, cold BCP, and St. Thomas CP. Patients who received Custodiol HTK solution had a significantly higher need for inotropic support ($p < 0.05$) [159]. The summarized data showing the significance of inotropic status after CPB are presented in Table 1.17.1.

Table 1.17.1. *The reliability of the detected inotropic status after CPB*

Authors	Study type	n	Type of CP used	*p-value* at 0 h	*p-value* at 24 h	*p-value* at 48 h	*p-value* at 72 h	*p-value* at 96 h	*p-value* at 120 h
Talwar et al. [156]	Randomized clinical trial	100	DN vs CHTK	-	0.021	0.036	0.026	0.008	-
Pérez-Andreu et al. [148]	Observational	64	Cold BCP vs CHTK	0.001	0.006	0.059	0.285	0.658	0.924
Isildak et al. [150]	Randomized clinical trial	80	BCP vs DN	0.058	0.032	0.005	0.136	-	-
Panigrahi et al. [152]	Randomized clinical trial	60	BCP vs DN	0.040	0.030	0.610	0.350	-	-
Bibevski et al. [157]	Observational	132	cold BCP vs CHTK	0.05					

Table 1.17.1 cont.

Authors	Study type	n	Type of CP used	*p-value* at 0 h	*p-value* at 24 h	*p-value* at 48 h	*p-value* at 72 h	*p-value* at 96 h	*p-value* at 120 h
Elassal et al. [158]	Observational	220	DN vs ST	0.591					
Hamed et al. [159]	Randomized clinical trial	60	CHTK vs cold BCP vs ST	0.05					

n – patient number, CP – cardioplegia, BCP – blood CP, CCP – crystalloid CP, CHTK – Custodiol Histidine-Tryptophan-Ketoglutarate CP, DN – Del Nido CP, ST – St. Thomas CP. The table is taken from [167].

Another factor that influences intraoperative outcomes is the total volume of CP. Data come from two studies [150, 160] which showed that using DN was associated with lower total volume of CP ($p < 0.001$) [150] (331.67±188.07 vs. 458.67±226.62, $p = 0.022$) [160].

Additionally, shorter CPB and cross-clamp times may have an impact on intraoperative outcomes. Comparing Del Nido vs blood CP [150, 160], it was shown that cardiac arrest with Del Nido CP was associated with reduced CPB and cross-clamp times ($p = 0.006$ and $p = 0.001$, respectively) [150]. While the other two studies [151, 158] found no significant difference regarding CPB and aortic cross-clamp times comparing the same two cardioplegic solutions ($p = 0.24$). Dolcino et al. [149] in their study showed that single-dose Custodiol HTK may be inadequate for prolonged cross-clamping durations.

1.18. Intensive care unit (ICU) and hospital stay

According to the meta-analysis [145], which included five studies with a total of 323 patients, there was no significant difference in the length of ICU stay between the BCP and Crystalloid CP groups. This finding was also confirmed by Mylonas et al. [147] in their meta-analysis, which found no significant difference in ICU and hospital stay between the BCP and Crystalloid CP groups. In studies comparing Del Nido and Custodiol HTK [153, 156], Del Nido was associated with shorter ICU and hospital stays than Custodiol HTK. However, the last meta-analysis [161] did not find significant differences in ICU or hospital stays among the four types of CP (Del Nido, BCP, Custodiol HTK, and St. Thomas CP), but the meta-analysis was more oriented towards adult patients. Among the 67 studies, 55 were adult trials. Additionally, a pairwise meta-analysis of one trial with 101 patients showed that Custodiol HTK was associated with significantly shorter ICU and hospital

stay compared to St. Thomas [161]. Summarized data on ICU and hospital stay for different cardioplegic solutions can be found in Table 1.18.1.

Table 1.18.1. *The p-values between different study CP groups in the intensive care unit and hospital stay after CPB in pediatric patients*

Authors	Study type	n	Type of CP used	ICU length of stay, *p-value*	Hospital length of stay, *p-value*	Study year
Fang et al. [145]	Meta-analysis	323	BCP vs CCP	0.25	-	2015
Mylonas et al. [147]	Meta-analysis	697	BCP vs ST	0.002	0.060	2017
Talwar et al. [156]	Randomized clinical trial	100	DN vs CHTK	0.05	0.001	2017–2018
Dehaki et al. [153]	Randomized clinical trial	40	DN vs CHTK	0.02	-	2018
Tan et al. [161]	Meta-analysis	101	CHTK vs ST	-	-	2022

n – patient number, CP – cardioplegia, BCP – blood CP, CCP – crystalloid CP, CHTK – Custodiol Histidine-Tryptophan-Ketoglutarate CP, DN – Del Nido CP, ST – St. Thomas CP. The table is taken from [167].

Low cardiac output syndrome (LCOS). In a retrospective single-centre study [162] involving 1129 pediatric patients, BCP was compared to Crystalloid CP. It was shown that BCP has potential advantages in reducing the incidence of LCOS [162]. Another study comparing Del Nido CP vs St. Thomas CP [163] found that Del Nido was associated with a lower occurrence of LCOS compared to patients who received standard myocardial protection using a modified St. Thomas solution. Additionally, Quilsy and colleagues [164] investigated the efficiency of Custodiol HTK CP compared to cold BCP and found that Custodiol HTK was associated with a higher risk of LCOS. Summarized data on LCOS after CPB are presented in Table 1.18.2.

Table 1.18.2. *The p-values between different study CP groups in low cardiac output syndrome (LCOS) after CPB using different cardioplegic solutions*

Authors	Study type	n	Type of CP used	*p-value*	Study year
Sobieraj et al.[162]	Observational	1129	BCP vs CCP	0.0017	2006–2012
Caneo et al. [163]	Observational	500	DN vs ST	0.05	2015–2019
Qulisy et al. [164]	Observational	154	CHTK vs cold BCP	0.14	2013–2014

n – patient number, CP – cardioplegia, BCP – blood CP, CCP – crystalloid CP, CHTK – Custodiol Histidine-Tryptophan-Ketoglutarate CP, DN – Del Nido CP, ST – St. Thomas CP. The table is taken from [167].

Resumption of sinus rhythm and postoperative arrhythmias. In a comparative study [152] between Del Nido CP and BCP, it was found that Del Nido leads to a faster resumption of spontaneous regular cardiac rhythm ($p < 0.0001$). Quilsy and colleagues [164] examined the efficiency of Custodiol HTK CP in comparison with cold BCP. Custodiol HTK was associated with a higher occurrence of arrhythmias (20% vs 17%).

1.19. Postoperative outcomes

The largest mortality data is derived from a meta-analysis [147], which found no difference in 30-day mortality when comparing BCP with Crystalloid CP. In the latest meta-analysis [164] with 1634 children from 12 studies, outcomes after four types of CP (Del Nido, BCP, Custodiol HTK, and St. Thomas CP) were compared, and no significant differences in endpoints were observed among the four types of CP. Floh et al. [165] in a retrospective study involving 1534 patients comparing Del Nido CP to BCP found similar mortality rates in both groups.

Left (LV) and right (RV) ventricular functions may also affect postoperative outcomes. Gholampour et al [153] conducted a study comparing the effects of Del Nido CP and Custodiol HTK CP on perioperative clinical outcomes in children with Tetralogy of Fallot. They found no significant differences in LV ejection fraction (EF) after the surgery. Pérez et al. [148] showed that LV EF was higher in the Custodiol HTK group compared to cold BCP immediately after the operation, at 24 hours, and on the first day without inotropic support. However, an experimental animal study [166] found no difference in LV EF at 24 hours post-operation or at discharge. The pre-operative RV function, as measured by fractional area change, was also similar between BCP and Custodiol HTK. In a single-center [165], retrospective study which included 1534 patients undergoing CPB, a significant rise in RV dysfunction was observed in the Del Nido group compared to conventional St. Thomas CP. Summarized data on LV and RV functions using different cardioplegic solutions are presented in Table 1.19.1.

Table 1.19.1. *The p-values between different study CP-groups in LV and RV function after CPB when different CP solutions were used*

Authors	**Study type**	**n**	**Type of CP used**	**Preoperative *p-value* (LV)**	***p-value* after surgery (LV)**	***p-value* at 24 h (LV)**	***p-value* at discharge (LV)**	**Intraoperative *p-value* (RV)**	***p-value* at discharge (RV)**
Dehaki et al. [153]	Randomized clinical trial	40	DN vs CHTK	0.791	0.750	-	0.906	-	-
Pérez-Andreu et al. [148]	Obser-vational	64	Cold BCP vs CHTK	0.880	0.005	0.001	-	-	-
Floh et al. [165]	Obser-vational	1534	DN vs ST	-	-	-	0.43	0,001	0,001

n – patient number, preop. – preoperative, disch. – discharge, Intraop. – intraoperative, CP – cardioplegia, BCP – blood CP, CCP – crystalloid CP, HTK – Custodiol Histidine-Tryptophan-Ketoglutarate CP, DN – Del Nido CP, ST – St. Thomas CP, LV – left ventricle, RV – right ventricle. The table is taken from [167].

1.20. Literature review summary

In summary, our review identifies differences across clinical studies. Observed discrepancies between cardioplegic solutions likely reflect their distinct compositions, as outlined above; however, these differences were not consistently statistically significant and, when present, were often confined to small-scale studies.

Postoperative survival, one of the most important indicators of the safety and clinical suitability of a cardioplegic solution, did not differ across the solutions reviewed [167]. By contrast, the requirement for inotropic support after cardiopulmonary bypass was significantly lower in the Del Nido CP group. Moreover, single-dose administration of Del Nido CP and Custodiol HTK CP solutions shortened operative interruptions and was associated with reduced CPB and aortic cross-clamp times, thereby decreasing operative duration and cumulative ischemic exposure [167]. Lengths of stay in the intensive care unit and hospital were shorter in some Del Nido CP cohorts, although other studies reported no differences; thus, these outcomes remain inconsistent across the literature [167].

However, the reviewed literature also reveals several important gaps:

- *Limited systematic comparative analyses*. Most available comparative studies focus on clinical outcomes but do not investigate subcellular processes such as mitochondrial respiratory efficiency, oxidative stress regulation, or changes in gene expression during ischemia/reperfusion.
- *Unclear mechanistic relationships*. It remains uncertain whether specific components of solutions (e.g., lidocaine, Mg^{2+}, histidine) directly shape the mitochondrial response and cell survival signaling, or whether these effects are secondary to a more general suppression of cellular metabolism.
- *Clinical insights are not sufficiently based on experimental data*. Many clinical studies rely on indirect markers (e.g., troponin, CK-MB, inotropic support requirements), which do not allow clear identification of the causal mechanisms underlying differences in cardioprotective efficacy.
- *Lack of pediatric experimental studies*. Although Del Nido and Custodiol cardioplegic solutions are widely used in pediatric cardiac surgery, data on their molecular and mitochondrial mechanisms of action in the immature myocardium remain limited. Also, most studies are small, single-center, and mixed in methodological quality. This is noteworthy because immature myocardium differs fundamentally from mature myocardium in calcium handling and metabolism. In addition, robust and ethically acceptable direct myocardial sampling is limited in children.

Considering these gaps, there is a clear need for new experimental studies that can provide an objective assessment of the ability of different cardioplegic solutions to protect the immature heart from I/R injury and elucidate their underlying mechanisms of action. For these reasons, we employed well-controlled animal models of the immature heart to determine the impact of cardioplegic solution composition on mitochondrial function, gene expression, and cytokine detection.

2. MATERIALS AND METHODS

The dissertation work includes experimental studies with an immature animal model. These experiments were designed to evaluate and compare the cardioprotective properties of three clinically used cardioplegic solutions, such as Del Nido, Custadiol HTK, and St. Thomas. The main objective was to assess myocardial perfusion and subcellular responses under I/R conditions; mitochondrial respiratory function and membrane integrity; differential modulation of pro- and anti-inflammatory cytokines; and gene expression related to apoptosis, proliferation, and adaptation to hypoxia. The experiments were designed to ensure clinical relevance and high reproducibility, allowing us to detect their effectiveness and suitability in a clinically relevant context.

All animal procedures complied with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Nos. 49-1883, 49-1884) and the Order of the Director of the State Food and Veterinary Service of the Republic of Lithuania (No. B1-866). The experimental protocol was reviewed and approved by the State Food and Veterinary Service of the Republic of Lithuania (Approval Nr. G2-265).

The number of animals used in the studies was kept as low as possible, and experimental planning adhered to the 3R principle (Replacement, Reduction, Refinement). Efforts were made to minimize animal suffering. Analgesia and anesthesia were used in all procedures. Animals were euthanized humanely under deep anesthesia according to AVMA Guidelines.

Male Wistar rats, aged 1 month and weighing 90 ± 30 g, were obtained from the Lithuanian University of Health Sciences (LUHS) Veterinary Academy. The animals were housed in cages with a maximum of five rats per cage under controlled environmental conditions: a standard 12-hour light / 12-hour dark cycle at a temperature of 22 ± 2 °C, a standard pelleted feed, and free access to drinking water. Group allocation was randomized, and investigators performing laboratory assays were blinded to group identity to minimize bias. The schematic study design is depicted in Fig. 2.1.

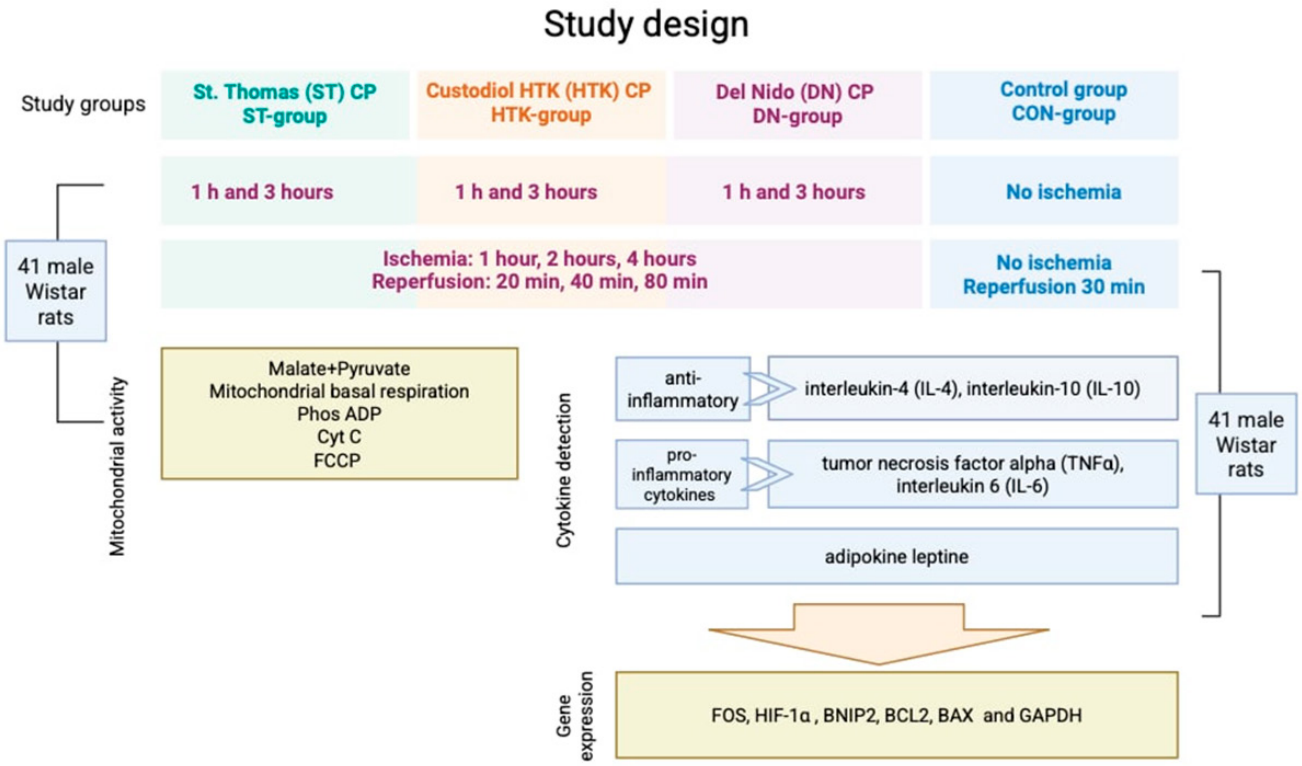


Fig. 2.1. *Workflow scheme*

The schematic illustrates the allocation of test animals to experimental groups. Each experiment was performed in duplicate. CP – cardioplegia, ST-group (incubation/perfusion with St. Thomas CP), HTK-group (incubation/perfusion with Custodiol Histidine-Tryptophan-Ketoglutarate CP), DN-group (incubation/perfusion with Del Nido CP), and CON-group (incubation/perfusion with normal Tyrode solution; without CP).

2.1. *In vivo* induction of cardiac ischemia

The study was performed at the Preclinical Research Laboratory for Medicinal Products, Institute of Cardiology, Medical Academy, LUHS. Forty-one male Wistar albino rats were used and handled in accordance with standard animal-experiment protocols. General anesthesia was induced in a CO_2 chamber. After sterile preparation of the thorax, a median sternotomy was undertaken, the aorta was isolated, and the inferior vena cava was transected.

A cold cardioplegic solution: St. Thomas, Del Nido, or Custodiol HTK, supplemented with heparin (5 IU/g body weight), was infused via the aorta using an antegrade perfusion. Each heart was arrested, and venous effluent was drained through the inferior vena cava. Animals were allocated into four experimental groups: CON-group (n = 5; metabolic control) without CP, ST-group (n = 12) with St. Thomas CP, HTK-group (n = 12) with Custodiol HTK CP, and DN-group (n = 12) with Del Nido CP (n = 12). Cardioplegic solutions were evaluated at two global ischemia durations, 1 h (moderate) and 3 h (prolonged), with six experiments per time point in each group. These intervals were selected to represent clinically relevant cross-clamp durations observed in pediatric cardiac surgery. All experiments were conducted in duplicate to ensure reproducibility. A schematic of the workflow is shown in Fig. 2.1, and the compositions of various CP solutions are shown in Tables 2.1.1–2.1.3.

Table 2.1.1. *Components of St. Thomas cardioplegic solution*

Component	Concentration (mmol/L)	Purpose
Sodium chloride	120	Maintains osmolarity and ionic balance
Potassium chloride	16	Induces diastolic cardiac arrest (membrane depolarization)
Magnesium chloride	16	Stabilizes cell membranes and inhibits calcium influx
Calcium chloride	1.2	Maintains myocardial function and contractility
Sodium bicarbonate	10	pH buffer to maintain physiological pH during ischemia

Table 2.1.2. *Components of Custodiol HTK cardioplegic solution*

Component	Concentration (mmol/L)	Purpose
Histidine	198	Buffering agent to maintain pH
Tryptophan	2	Membrane stabilizer
Ketoglutarate	1	Substrate for ATP generation, supports metabolism during ischemia
Mannitol	30	Osmotic substance reduces cellular edema and acts as a free radical scavenger
Sodium chloride	15	Maintains osmotic balance
Potassium chloride	9	Induces diastolic cardiac arrest
Magnesium chloride	4	Stabilizes the cell membrane, antiarrhythmic
Calcium chloride	0.015	Minimal calcium to avoid calcium overload

Table 2.1.3. *Components of Del Nido cardioplegic solution*

Component	Concentration (mmol/L)	Purpose
Sodium (from Plasma-Lyte A)	132	Maintains osmotic balance
Chloride (from Plasma-Lyte A)	93	Maintains osmotic balance
Potassium (Plasma-Lyte A + added KCl)	29.3	Induces rapid diastolic arrest (depolarizing arrest)
Magnesium (Plasma-Lyte A + added $MgSO_4$)	10.5	Calcium antagonism; membrane stabilization
Acetate (from Plasma-Lyte A)	25.5	Buffer precursor, supporting acid–base balance
Gluconate (from Plasma-Lyte A)	21.7	Maintains osmotic balance
Mannitol	16.9	Osmotic substance reduces cellular edema and acts as a free radical scavenger
Sodium bicarbonate	12.3	Buffering substance to mitigate acidosis during ischemia
Lidocaine	0.45	Sodium channel blockade; reduces excitability / arrhythmias; helps limit Na^+/Ca^{2+} loading

The CON-group did not undergo induced myocardial ischemia; hearts were excised and immediately minced for mitochondrial measurements. For the Del Nido CP formulation, heparinized autologous rat blood obtained from the tail was mixed with the solution at a 1:4 ratio (blood:solution). Cardioplegic solutions served to flush the intracardiac vascular bed, arrest

the heart, and provide myocardial protection, with venous effluent drained through the inferior vena cava.

Cardioplegic solutions (St. Thomas and Custodiol HTK) were sourced from the Department of Cardiac, Thoracic, and Vascular Surgery, LUHS. A list of additives is found in Tables 2.1.1 and 2.1.2. The Del Nido CP solution was compounded at the Department of Drug Chemistry, Faculty of Pharmacy, MA, LUHS. A list of additives is found in Table 2.1.3.

2.2. Isolation of cardiac mitochondria

Following induction of cardiac arrest, hearts were excised and immediately immersed in ice-cold cardioplegic solution (St. Thomas, Custodiol HTK, and Del Nido) maintained at 4 °C. Incubation proceeded for 1-3 hours, according to group assignment, using approximately 10 mL of solution (≈2-3× tissue volume). After 1 h and 3 h intervals of global ischemia, hearts were excised and immediately transferred to pre-cooled 35-mm glass dishes containing 5 mL of solution for mitochondrial isolation. Residual blood was removed by rinsing with homogenization medium (HM). Myocardial tissue was then rapidly minced on ice, and the resulting fragments were transferred into tubes containing HM for subsequent homogenization.

Mitochondrial respiration (MIR) medium and HM were prepared at the Preclinical Research Laboratory for Medicinal Products, Institute of Cardiology, MA, LUHS. A list of additives is found in Table 2.2.1.

Table 2.2.1. *Preparation of buffers and reagents*

Buffer	**Composition**
Mitochondrial respiration (MIR) buffer	Used for Oroboros measurements: $MgCl_2 \cdot 6H_2O$ 3 mmol, KH_2PO_4 10 mmol, EGTA 0.5 mmol, lactobionic acid 60 mmol, taurine 20 mmol, HEPES 20 mmol, D-sucrose 110 mmol, BSA (fatty-acid-free) 1 g/L. pH adjusted to 7.1 at 30 °C.
Homogenization medium (HM)	KCl 160 mmol, NaCl 10 mmol, Tris-HCl (Tris base) 20 mmol, EGTA 2 mmol. pH adjusted to 7.7 at 2 °C.

2.3. Homogenization and protein quantification

Tissue was homogenized directly in tubes, and the crude homogenate was subjected to differential centrifugation: 5 min at 1,000 × *g* followed by 10 min at 6,800 × *g*. These fractionation steps yielded a mitochondrial pellet suitable for assessing respiratory pathways in rat myocardium under the specified cardioplegic conditions. After the second spin, the mitochondrial pellet was washed and resuspended in HM (Table 2.2.1). Aliquots (1.5 mL Eppendorf tubes) were kept on ice until analysis. Protein quantification was performed using the Bradford assay; membrane permeabilization was employed with Triton (TX, 0.1%).

Prepared mitochondrial suspensions were diluted with Triton X-100 (TX, 0.1%) at 1:50. For the assay mix, 490 µL TX and 10 µL mitochondrial suspension were combined and incubated for 5 min at room temperature. From this mix, 10 µL was dispensed into three wells of a microplate: a fourth well received TX alone (blank). Bradford dye was added to each well: 190 µL Bradford to 10 µL sample (or TX blank), yielding a 1:20 ratio. Absorbance was recorded according to the Bradford protocol. Protein concentrations were calculated from a dedicated calibration curve.

2.4. Measurement of mitochondrial respiration (Oroboros Oxygraph-2k)

Mitochondrial oxygen consumption was measured using the Oroboros Oxygraph-2k® high-resolution respirometer (Oroboros Instruments, Austria). Assays were performed at 37 °C in 2.0 mL chambers filled with MIR buffer. The Oxygraph-2k® is a closed, dual-chamber system equipped with barometric pressure sensing for automated oxygen calibration integrated within DatLab, enabling precise, high-resolution respirometry [168, 169]. Mitochondrial bioenergetics were evaluated by sequential titration of substrates, uncouplers, and inhibitors to resolve distinct respiratory states.

The Oroboros machine (Fig. 2.4.1) was started, and 2.1 mL MIR (see Table 2.2.1) was added to each chamber. Complex I–linked substrates were introduced: 20 µL pyruvate (1 mol) and 4 µL malate (1 mol). Mitochondria corresponding to ~2 mg protein were then added, and chambers were closed.

Fig. 2.4.1. *Schematic representation of the sample preparation and measurement using high-resolution respirometry equipment for analysis of homogenized tissue (Oroboros instrument, Innsbruck, Austria)*

Respiratory activity was quantified in freshly isolated mitochondria. The following titration sequence was employed:

- *ADP addition*. 8 µL ADP (1 mol) was injected to elicit oxidative phosphorylation (OXPHOS); the oxygen consumption rate in the phosphorylating state was recorded.
- *Cytochrome c test*. 5 µL cytochrome c (2.8 mmol) was added, and the rate was re-measured. Because the outer mitochondrial membrane is normally impermeable to cytochrome c, an increase in flux indicates compromised outer-membrane integrity.
- *Carboxyatractyloside* (CAT) *addition*. 1 µL CAT (1.5 mmol), an inhibitor of the adenine nucleotide translocase, was added to assess leak respiration and inner-membrane coupling/permeability; the corresponding oxygen consumption rate was recorded.
- *FCCP* (Carbonyl cyanide p-trifluoromethoxyphenylhydrazone) for terminal inhibition. 10 µL FCCP (2 mmol) was titrated to achieve the maximal capacity of the electron-transport system (uncoupled respiration).
- *Sodium azide addition*. 20 µL NaN_3 (1 mol) was applied to inhibit Complex IV and confirm mitochondrial respiratory dependence.

An exemplary scheme for titration of the respiration medium with substrates, inhibitors, and uncouplers is presented in Fig. 2.4.2.

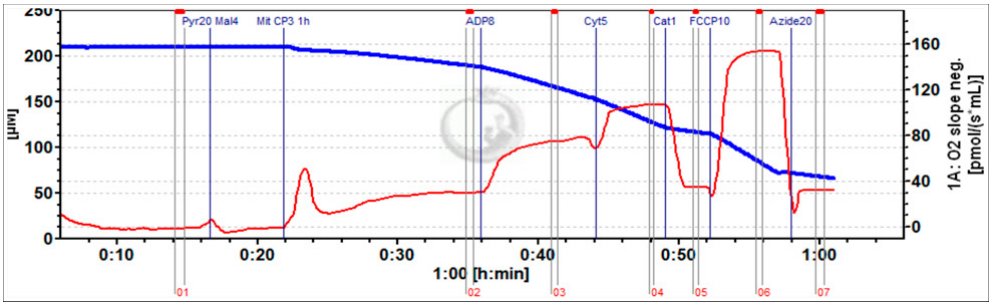


Fig. 2.4.2. *A graphical representation of the respiration rate measurement experiment conducted using DatLab software (Oroboros instruments)*

The bold blue line represents the oxygen concentration in the cuvette (nmol/mL), and the red line represents the oxygen consumption rate (pmol O_2/(s·mL)). The vertical lines represent the additives: Pyr20 Mal4 – complex I substrates pyruvate (20 µL, 1 mol) and malate (4 µL, 1 mol), to measure oxidative phosphorylation respiration, Mit CP3 1h – cardioplegic solution after 1 h of ischemia to detect mitochondrial (MT) basal respiration, ADP8 – ADP (8 µL, 1 mol) to stimulate phosphorylated respiration (state 3), Cyt5 –Cytochrome c (5 µL, 2 mmol) to verify MT outer membrane integrity, Cat1 – carboxyatractyloside (1 µL, 1.5 mmol) to assess leak respiration, FCCP10 – Carbonyl cyanide p-trifluoromethoxyphenylhydrazone (10 µL, 2 mmol) to measure the uncoupled maximal electron transport capacity, Azide20 – sodium azide (20 µL, 1 mol) to inhibit Complex IV.

Data were acquired and analyzed in real time with DatLab (Oroboros Instruments). Derived parameters are presented in Table 2.4.2.

Table 2.4.1. *Derived parameters in freshly isolated mitochondria*

Parameter	**Description**	**Units**
State 3 respiration	ADP-stimulated O_2 consumption	pmol $O_2 \cdot S^{-1} \cdot mg^{-1}$
State 4 (Leak)	Respiration without ADP (non-phosphorylating)	pmol $O_2 \cdot S^{-1} \cdot mg^{-1}$
RCR	Respiratory control ratio (State 3 / state 4)	dimensionless
ETS capacity	Maximal flux after FCCP	pmol $O_2 \cdot S^{-1} \cdot mg^{-1}$
ADP/O ratio	Efficiency of oxidative phosphorylation	mol ADP / mol O_2

FCCP – Carbonyl cyanide p-trifluoromethoxyphenylhydrazone, ETS – electron-transport-system. All respiration rates were normalized to protein content (mg).

In total, 41 experiments were conducted in immature rats using the Oroboros platform. Five experiments were performed without the CP (CON-group). To evaluate moderate and prolonged ischemia, six experiments were

performed per CP group using: St. Thomas CP (ST-group), Custodiol HTK CP (HTK-group), and Del Nido CP (DN-group).

2.5. *Ex vivo* ischemia-reperfusion (I/R) (Langendorff model)

These experiments were carried out at the Laboratory of Membrane Biophysics, Institute of Cardiology, MA, LUHS. Animals were allocated to three CP groups (four rats per group): ST-group, HTK-group, and DN-group. Each group was further subdivided by I/R duration. More detailed information is shown in Fig. 2.1.

A retrograde Langendorff perfusion model was used [170, 171]. The following steps for I/R experiments were performed:

- *Anesthesia and surgical preparation*. Rats were euthanized according to standard animal experimentation protocols. Anesthesia was induced with ketamine (90 mg/kg) and xylazine (9 mg/kg); loss of the pedal withdrawal reflex confirmed surgical depth. Heparin (5 IU/g body weight, s.c.) was administered 10 min before cervical dislocation. After a midline skin incision at the xiphoid sternum, the anterior chest wall was reflected, the thymus excised, the pericardium opened, and the aorta, superior and inferior vena cava were isolated.
- *Heart excision and cannulation*. Immediately after excision, hearts were placed in a chilled perfusion buffer. The aorta was gently cannulated to avoid injury to the aortic valve and secured with double 5-0 silk ties. Hearts were mounted on the system within 5 minutes (Figure 2.5.1) and perfused continuously for 15 minutes, during which global function approached physiological levels.

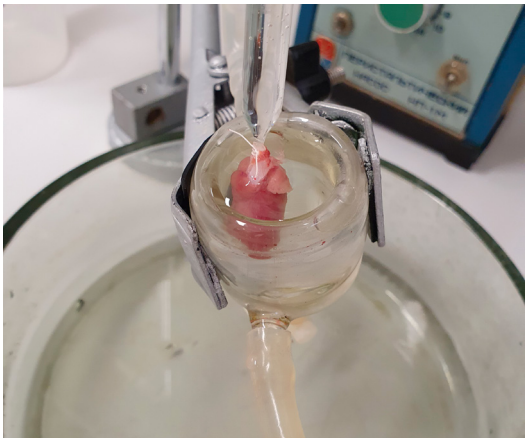


***Fig. 2.5.1**. A schematic view of the heart perfusion in the Langendorff experimental setup*

- *Perfusion conditions and buffer*. Perfusion was delivered in constant-flow mode with meticulous avoidance of air emboli. The modified Krebs–Henseleit buffer (in deionized water) contained (in mmol): NaCl 135.0, $MgCl_2$ 0.9, NaH_2PO_4 0.33, KCl 5.4, $CaCl_2$ 1.0, glucose 10, and HEPES 10. The buffer was oxygenated by bubbling with 95% O_2 and prepared on site at the Laboratory of Membrane Biophysics.
- *Temperature control and flow measurements*. Coronary flow was measured before cardioplegia by collecting effluent volume over 2 min. The water-jacketed reservoir and perfusate were strictly temperature-regulated: perfusion at 37 °C (normothermia) with a heat exchanger, cardioplegic solutions maintained at 4 °C. Following arrest, hearts were held in a bath at 18-22 °C; temperature was monitored every 15 min to ensure stability throughout ischemia.
- *Cardioplegia protocol*. Approximately 10 mL of cold (4 °C) cardioplegic solution (St. Thomas, Del Nido, or Custodiol HTK) was infused via the aorta over 2 min. For DN, heparinized autologous rat blood obtained from the tail was mixed with crystalloid at a 4:1 ratio (solution: blood). Time to asystole was recorded. Re-dosing intervals were group-specific: every 30 min for ST, every 90 min for DN, and a single dose for HTK. At the end of the protocol, the entire LV was harvested and stored at −80 °C pending analysis.

2.6. Gene expression analysis (qPCR)

To delineate molecular differences among cardioplegic solutions, quantitative real-time PCR (qPCR) was used to assess transcripts related to hypoxia, apoptosis, and proliferation in cardiac tissue. *HIF-1α* (*HIF1A*) mediates cellular adaptation to hypoxia highly relevant during cardiac surgery when perfusion is limited. *HIF1A* activates pathways that help cells survive under stress, like promoting oxygen delivery and metabolism adaptations [172]. *FOS*, a component of the AP-1 transcription factor complex, regulates proliferation, differentiation, and stress responses, supporting myocardial adaptation to surgical and ischemic stressors [13]. *BNIP2* participates in apoptotic signaling, and its modulation may limit inappropriate cell death and preserve function during operative I/R [173, 174]. During cardiac surgery, regulating cell death is crucial to preserving heart function, and the modulation of *BNIP2* could help minimize unwanted cell death.

The following steps for gene expression experiments were performed:

- *RNA isolation and reverse transcription*. Heart tissues were pulverized under liquid nitrogen and immediately lysed in TRIzol™ (Invitrogen, Netherlands). Total RNA was isolated using the PureLink™ RNA Mini

Kit (Invitrogen, Netherlands) following the manufacturer's instructions. RNA concentration and purity were determined using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Netherlands). cDNA was synthesized with the High-Capacity RNA-to-cDNA™ Kit (Applied Biosystems, USA).

- *qPCR and analysis*. Gene expression was quantified using TaqMan™ Gene Expression assays (Applied Biosystems, USA) per the manufacturer's protocol. The following probes/primers were employed: *FOS* (Rn02396759_m1), *HIF-1α* (Rn01472831_m1), *BNIP2* (Rn01530716_m1), *BCL2* (Rn99999125_m1), *BAX* (Rn01480161_g1), and *GAPDH* (Rn01775763_g1). Expression levels were normalized to *GAPDH*, and relative quantification was calculated using the 2^-ΔΔCt method [175].

Each biological sample was run in triplicate. Quality controls included no-template and no-RT controls in every run.

2.7. Cytokine quantification by Luminex multiplex assay

We quantified pro- and anti-inflammatory cytokines in immature rat myocardium using a Luminex multiplex platform (Luminex Corporation, Austin, TX, USA). Freshly excised hearts were promptly processed to limit protein degradation. Tissues were pulverized under liquid nitrogen and homogenized; each specimen was weighed and transferred to a 2 mL microcentrifuge tube. Cell Lysis Buffer was added at 500 µL per 100 mg tissue to disrupt cellular architecture and release intracellular constituents. Homogenization was performed with a TissueLyser at 25 Hz for 2 min, an empirically optimized setting yielding uniform lysates without heat build-up. Lysates were clarified by centrifugation at 16,000 × g for 10 min at 4 °C, and the supernatant was collected for analysis. Total protein concentration was determined using the Bio-Rad™ DC Protein Assay Kit.

Cytokine panels included anti-inflammatory interleukins (IL-4, IL-10), the adipokine leptin, and pro-inflammatory mediators (TNFα, IL-6), which were quantified with the Rat Custom ProcartaPlex Mix&Match 5-Plex Kit (Thermo Fisher Scientific, Austria) according to the manufacturer's instructions. All samples were run in duplicate. Data acquisition and concentration calculations were performed in xPONENT software (Luminex), with values derived from cytokine-specific standard curves.

2.8. Statistical analysis

All statistical analyses in mitochondria studies were performed using SPSS version 29 (IBM Corp., Armonk, NY, USA). All values are expressed as median (25–75%) or percentile. Kruskal–Walli's test for independent samples was used to compare median values between groups. A probability value lower than $p < 0.05$ was considered statistically significant.

The sample size for studies using the Langendorff perfusion method was determined by evaluating Cohen's coefficient. The data are expressed as mean ± SD. The normality of the data was evaluated by the Shapiro-Wilk test.

Given the experimental design, including three cardioplegic solutions (St. Thomas, Custodiol HTK, Del Nido) and three ischemia durations (1, 2, and 4 hours), multi-factorial analyses were performed. Specifically, two-way ANOVA was used to assess the main effects of cardioplegic solutions and ischemia duration, as well as their interaction, on gene expression (HIF1α, FOS, BNIP2, BAX/BCL2) and cytokine concentrations (IL-4, IL-6, IL-10, leptin, TNF-α). Where appropriate, post hoc comparisons were conducted to determine differences between groups. The assessment of homogeneity of variance was performed using Levene's test. If variances were unequal, this was noted in the results, and the findings were interpreted with caution.

A *p-value* lower than 0.05 was considered statistically significant. All analyses were performed using SPSS v22.0 and visualized using GraphPad Prism 9 software.

3. RESULTS

3.1. Effect of myocardial ischemia on mitochondrial respiration

3.1.1. Mitochondrial respiration after 1 hour of myocardial ischemia

The initial phase of the study assessed mitochondrial respiration after one hour of myocardial ischemia. This ischemic duration was selected to reflect typical clinical conditions in pediatric cardiac surgery, where the period of myocardial arrest generally does not exceed one hour. By modelling this time frame (see Fig. 2.1.), the study aimed to determine how different CP solutions, such as St. Thomas CP (ST-group), Custodiol HTK CP (HTK-group), and Del Nido CP (DN-group), influence the preservation of mitochondrial function under conditions resembling routine surgical practice.

Compared with other CP solutions, the Del Nido CP (DN-group) demonstrated superior mitochondrial respiratory performance (Figure 3.1.1.1). Basal mitochondrial respiration, representing oxygen consumption in the absence of ADP (non-phosphorylating state) and reflecting the capacity of mitochondria to maintain essential resting activity, was significantly higher in the DN-group than in the ST-group.

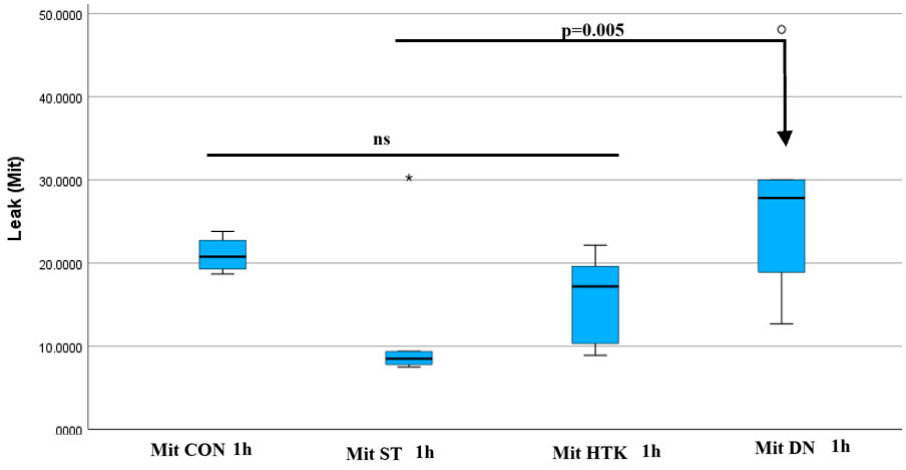


Fig. 3.1.1.1. *The median values of mitochondrial respiration rate without ADP after 1 hour of ischemia among the study groups*

Box and whisker graphs are presented. The box indicates the value from the 25th to the 75th percentile. The line in the middle of the box indicates the median value, with circles – outlier values. The vertical axis – mitochondrial basal respiration (leak), horizontal axis – the study groups, Mit – mitochondrial basal respiration, Mit CON – Mit without cardioplegia (CP), Mit ST – Mit in St. Thomas CP, Mit HTK – Mit in Custodiol HTK CP, Mit DN – Mit in Del Nido CP. A statistically significant difference was found between the ST and DN groups ($p = 0.006$).

The median values of basal respiration in the DN-group were significantly higher than those in the ST-group, with values of 27.8 [17.4; 34.6] for the DN-group compared to 8.5 [7.7; 14.6] for the ST-group (p = 0.006). This finding suggests that the Del Nido CP solution is more effective in sustaining baseline mitochondrial function during short-term ischemic exposure (see Tables 3.1.1.1–3.1.1.3).

Table 3.1.1.1. *Median and percentile values of mitochondrial respiration parameters measured after 1 hour of ischemia in the study groups*

Parameters	CON-group	ST-group	HTK-group	DN-group
Mit	20.76 [18.97–23.26]	8.48 [7.73–14.60]	17.18 [9.98–20.25]	27.81*** [17.34–34.54]
Phos ADP	98.28* [62.67–180.43]	55.51 [25.94–64.17]	60.41** [54.08–68.29]	107.18*** [87.71–130.32]
Cyt C	102.78* [73.59–218.55]	57.67 [38.83–71.36]	90.72** [75.74–95.21]	129.71*** [106.69–167.28]
FCCP	139.78* [67.24–252.70]	71.10 [49.67–84.42]	103.80 [81.25–149.81]	173.35*** [145.96–252.84]
Cyt C/ADP	1.15 [1.03–1.24]	1.19 [1.02–1.42]	1.36 [1.20–1.66]	1.28 [1.04–1.42]
ADP/Mit	4.84 [3.22–7.69]	6.49 [1.61–7.73]	3.58 [3.04–5.99]	4.64 [2.35–7.05]

CON-group – no cardioplegia (CP), ST-group – with St. Thomas CP, HTK-group – with Custodiol HTK CP, DN-group – with Del Nido CP, Mit – mitochondrial basal respiration, FCCP – carbonyl cyanide p-trifluoromethoxyphenylhydrazone for maximal respiration, ADP/Mit – ratio between ATP-production coupled mitochondrial respiration and basal respiration, Cyt C/ADP – assessment of the condition of the outer mitochondrial membrane via the Cyt C effect. In square brackets, the value at the 25th and 75th percentiles. * $p < 0.05$ vs ST, ** $p < 0.05$ vs DN, *** $p < 0.001$ vs ST; n = 6 for each parameter within each CP group.

Table 3.1.1.2. *Calculated p-values for derived median values after 1 hour of ischemia across CP groups*

Parameters	CON vs ST	CON vs HTK	CON vs DN	ST vs HTK	ST vs DN	HTK vs DN
Mit	0.054	0.311	0.591	0.307	**0.006**	0.083
Phos ADP	**0.019**	0.087	0.578	0.477	**0.001**	**0.011**
Cyt C	**0.016**	0.350	0.350	0.100	**0.001**	**0.037**
FCCP	**0.037**	0.462	0.393	0.131	**0.001**	0.075
Cyt C/ADP	0.551	0.067	0.283	0.168	0.594	0.398
ADP/Mit	0.952	0.591	0.858	0.505	0.790	0.689

CON – CON-group (without CP), ST – ST-group with St. Thomas CP, HTK – HTK-group with Custodiol HTK CP, DN – DN-group with Del Nido CP, Mit – mitochondrial basal respiration, FCCP – carbonylcyanide-p-trifluoromethoxyphenylhydrazone for maximal respiration, ADP/Mit – ratio between ATP-production coupled mitochondrial respiration and basal

respiration, Cyt C/ADP – assessment of the condition of the outer mitochondrial membrane via the Cyt C effect. Bolded numbers – detected statistically significant values between the indicated groups.

Table 3.1.1.3. *Determined mean values of mitochondrial respiration parameters after 1 hour of ischemia in the study groups*

Parameters	CON-group	ST-group	HTK-group	DN-group
Mit	21 ± 1.11674	12 ± 3.66738	15 ± 2.16237	27 ± 4.91585
Phos ADP	113 ± 1.89275	47 ± 8.35774	60 ± 3.07206	111 ± 13.57056
Cyt C	131 ± 1.53781	55 ± 8.80473	86 ± 4.94096	140 ± 17.44193
FCCP	153 ± 8.51964	68 ± 7.16078	111 ± 16.62321	188 ± 22.04131
Cyt C/ADP	1.14 ± 0.05457	1.22 ± 0.0878	1.43 ± 0.10612	1.26 ± 0.07982
ADP/Mit	5.2 ± 1.19884	5.2 ± 1.29367	4.2 ± 0.61290	4.8 ± 1.09508

CON-group – no cardioplegia (CP), ST-group – with St. Thomas CP, HTK-group – with Custodiol HTK CP, DN-group – with Del Nido CP, Mit- mitochondrial basal respiration, FCCP – carbonylcyanide-p-trifluoromethoxyphenylhydrazone for maximal respiration, ADP/Mit – ratio between ATP-production coupled mitochondrial respiration and basal respiration, Cyt C/ADP – assessment of the condition of the outer mitochondrial membrane via the Cyt C effect.

A more noticeable difference was found in ADP-stimulated respiration, which reflects ATP-producing mitochondrial activity (Figure 3.1.1.2). The DN-group demonstrated the highest ATP-coupled respiration and reached values comparable to the CON-group, which did not receive CP. In contrast, both the ST-group and HTK-group exhibited significantly lower ADP-stimulated respiration. Statistically significant differences were found between the ST-group and DN-group (55.51 [25.94; 64.17] vs. 107.18 [87.71; 130.32]; p = 0.001) and between the HTK-group and DN-group (60.41 [54.08; 68.29] vs. 107.18 [87.71; 130.32]; p = 0.011). These findings indicate that the Del Nido CP supports mitochondrial oxidative phosphorylation more effectively than the other tested CP solutions during the early phase of ischemia. Additionally, a significant difference was also found between the ST-group and the CON-group (98.28 [62.67; 180.43] vs. 55.51 [25.94; 64.17]; p = 0.019), suggesting that St. Thomas CP provided the weakest mitochondrial protection (see Tables 3.1.1.1–3.1.1.3).

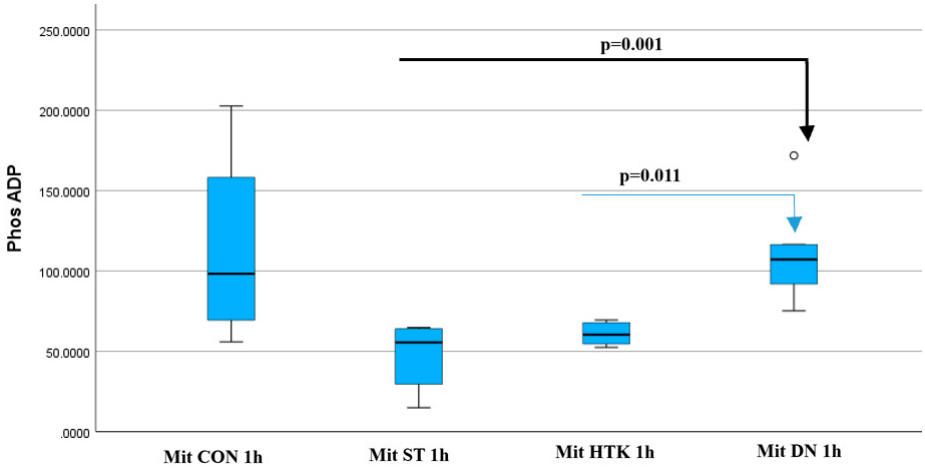


***Fig. 3.1.1.2**. The median values of mitochondrial ADP-stimulated respiration rate after 1 hour of ischemia among the study groups*

Box and whisker graphs are presented. The box indicates the value from the 25th to the 75th percentile. The line in the middle of the box indicates the median value, with circles – outlier values. Vertical axis – ADP-ATP-production coupled mitochondrial respiration (Phos ADP), horizontal axis – the study groups, Mit – mitochondrial basal respiration, Mit CON – Mit without cardioplegia (CP), Mit ST – Mit in St. Thomas CP, Mit HTK – Mit in Custodiol HTK CP, Mit DN – Mit in Del Nido CP. A statistically significant difference was found between ST and DN groups (p = 0.001) and between HTK and DN groups (p = 0.011).

Maximal mitochondrial respiration, assessed by FCCP-induced uncoupling, followed the same trend (Fig. 3.1.1.3). The DN-group demonstrated significantly higher maximal oxygen consumption than the ST-group (173.4 [146.0; 252.9] vs. 71.1 [49.7; 84.4]; p = 0.001). A smaller but statistically significant difference was also observed between the ST-group and the CON-group (p = 0.037) (Table 3.1.1.2). These results suggest that mitochondria in the DN-group retain greater respiratory reserve capacity and, thus, may be better positioned for recovery during reperfusion.

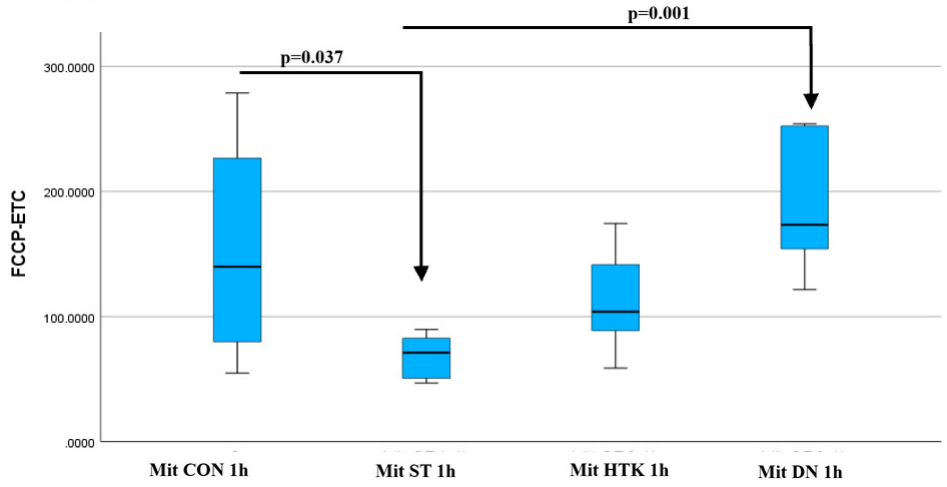


Fig. 3.1.1.3. *The median values of mitochondrial maximal respiration capacity after 1 hour of ischemia among the study groups*

Box and whisker graphs are presented. The box indicates the value from the 25th to the 75th percentile. The line in the middle of the box indicates the median value, with circles – outlier values. The vertical axis – FCCP (carbonyl cyanide p-trifluoromethoxyphenyldrazone) for maximal respiration (FCCP-ETC), horizontal axis – the study groups, Mit – mitochondrial basal respiration, Mit CON – Mit without cardioplegia (CP), Mit ST – Mit in St. Thomas CP, Mit HTK – Mit in Custodiol HTK CP, Mit DN – Mit in Del Nido CP. A statistically significant difference was found between the ST and DN groups (p = 0.001) and between the ST and CON (p = 0.037).

No statistically significant differences were observed among the groups in the ADP/Mit respiration ratio (Figure 3.1.1.4), which reflects mitochondrial coupling efficiency, or in the Cyt C/ADP ratio (Figure 3.1.1.5), which indicates outer mitochondrial membrane permeability. The absence of group differences in these structural parameters suggests that after one hour of ischemia, the degree of mitochondrial membrane injury had not yet progressed substantially. Nonetheless, the functional respiratory data indicate clear differences in mitochondrial performance between the CP groups (Table 3.1.1.1 and Table 3.1.1.2).

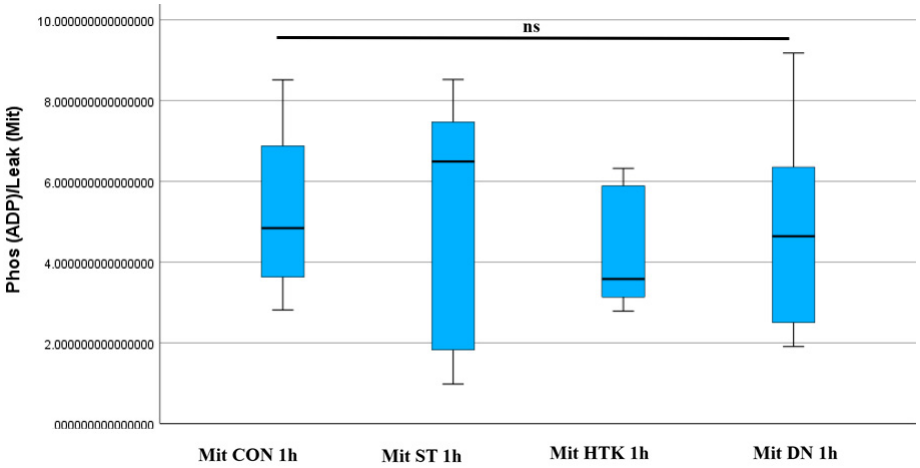


Fig. 3.1.1.4. *The median values of mitochondrial oxidative phosphorylation efficiency after 1 hour of ischemia among the study groups*

Box and whisker graphs are presented. The box indicates the value from the 25th to the 75th percentile. The line in the middle of the box indicates the median value, with circles – outlier values. The vertical axis – mitochondrial respiration ratio Phos (ADP)/Leak (Mit), horizontal axis – the study groups, Mit – mitochondrial basal respiration, Mit CON – Mit without cardioplegia (CP), Mit ST – Mit in St. Thomas CP, Mit HTK – Mit in Custodiol HTK CP, Mit DN – Mit in Del Nido CP. No significant difference was observed between the groups.

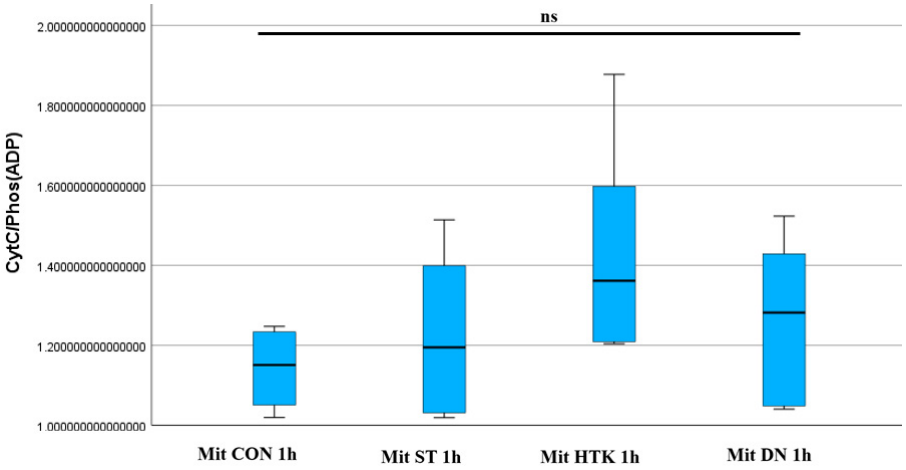


Fig. 3.1.1.5. *The median values of mitochondrial oxidative phosphorylation rate with the addition of cytochrome c after 1 hour of ischemia among the study groups*

Box and whisker graphs are presented. The box indicates the value from the 25th to the 75th percentile. The line in the middle of the box indicates the median value, with circles – outlier values. The vertical axis – mitochondrial respiration Cytochrome C CytC/Phos (ADP), horizontal axis – the study groups, Mit – mitochondrial basal respiration, Mit CON – Mit without cardioplegia (CP), Mit ST – Mit in St. Thomas CP, Mit HTK – Mit in Custodiol HTK CP, Mit DN – Mit in Del Nido CP. No significant difference was observed between the groups.

Figure 3.1.1.6 summarizes all derived parameters measuring mitochondrial respiration among study groups after 1 hour of ischemia.

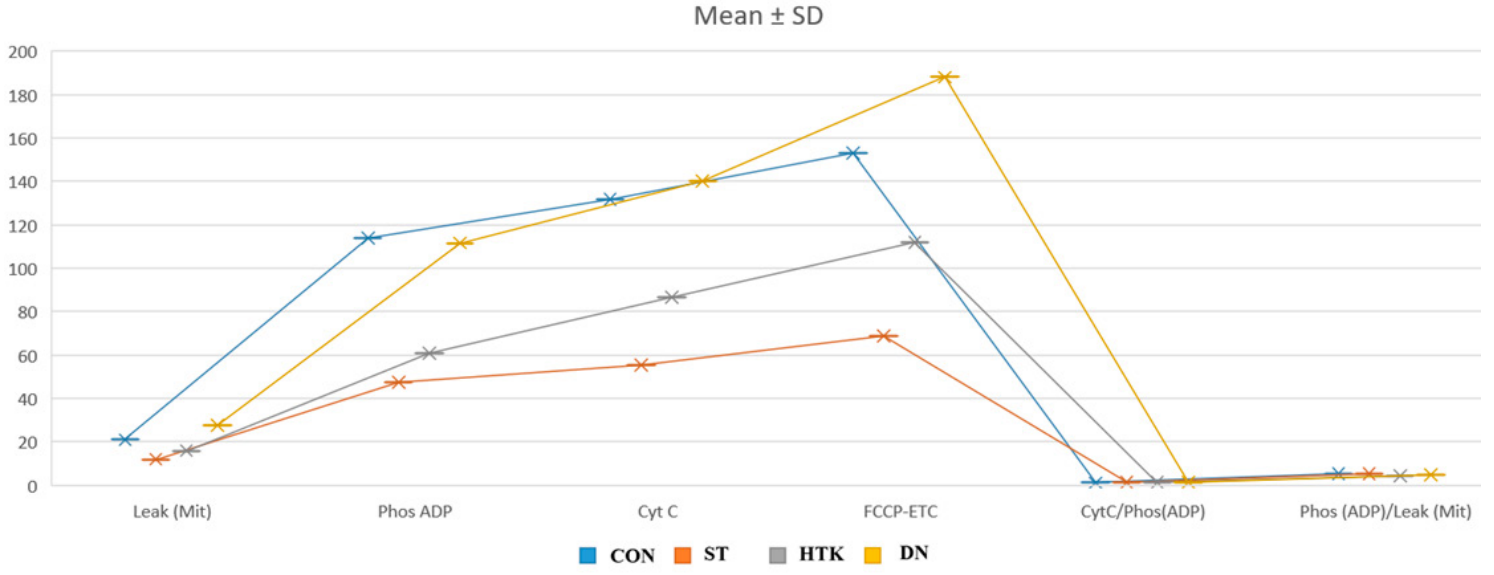


Fig. 3.1.1.6. *Summarized mitochondrial respiration parameters after 1 hour of ischemia in the study groups.*

The vertical axis – normalized values (%), horizontal axis – measured parameters among the study groups, Mit – mitochondrial basal respiration, FCCP – carbonylcyanide-p-trifluoromethoxy phenylhydrazone for maximal respiration, ADP/Mit – ratio between ATP-production coupled mitochondrial respiration and basal respiration, Cyt C/ADP – assessment of the condition of the outer mitochondrial membrane via the Cyt C effect. CON – control in no cardioplegia (CP), ST – St. Thomas CP, HTK – Custodiol HTK CP, DN – Del Nido CP. Data presented as Mean ± SD. n = 6 under each experimental condition.

3.1.2. Mitochondrial respiration after three hours of myocardial ischemia

To reflect more demanding clinical scenarios, the study also examined mitochondrial respiration after three hours of ischemia. Such prolonged ischemic intervals may occur during complex congenital cardiac repairs or in situations involving unanticipated operative challenges. Under these conditions, myocardial tolerance to ischemia is substantially reduced, and cardioplegic protection becomes increasingly important.

After three hours of ischemia, differences in mitochondrial respiration between the CP groups were more pronounced. Basal respiration remained higher in the Custodiol HTK-group and DN-group compared with the ST-group, with significant differences between the ST-group and both the HTK-group (p = 0.003) and the DN-group (p = 0.020). This pattern suggests that St. Thomas CP is less effective at maintaining essential mitochondrial respiration during prolonged ischemia (Figures 3.1.2.1–3.1.2.3, and Tables 3.1.2.1–3.1.2.3). The largest between-group differences were observed in ADP-stimulated respiration. The DN-group preserved significantly higher ATP-coupled respiration (105.7 [86.2; 154.4] nmol/ml) compared to the ST-group (24.2 [11.7; 51.7] nmol/mL; p = 0.008). While the HTK-group exhibited moderately better performance than the ST-group, its values did not reach those of the DN-group. The absence of a statistically significant difference between the HTK-group and DN-group suggests that both Custodiol HTK and Del Nido CP retain mitochondrial ATP-producing capacity during prolonged ischemia, although the numerical values favour the DN-group.

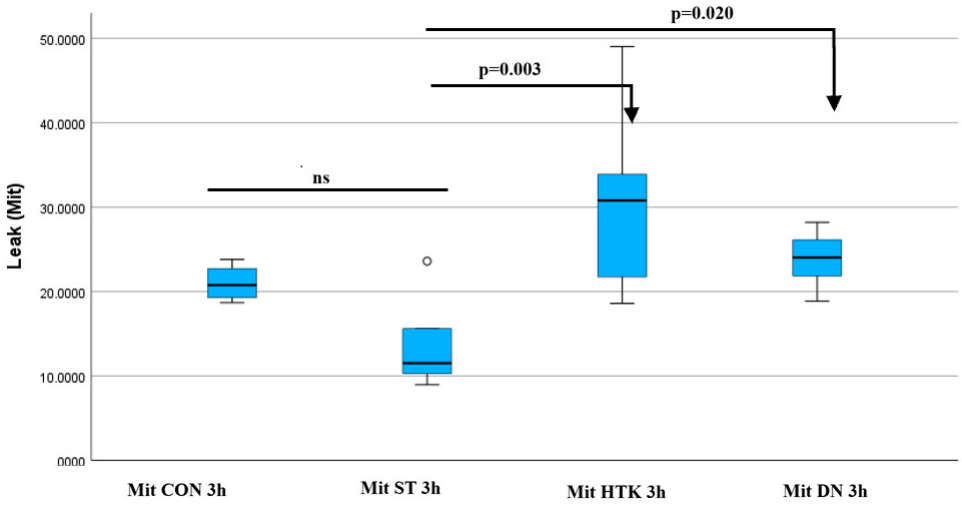


Fig. 3.1.2.1. *The median values of mitochondrial respiration rate without ADP after 3 hours of ischemia among the study groups*

Box and whisker graphs are presented. The box indicates the value from the 25th to the 75th percentile. The line in the middle of the box indicates the median value, with circles – outlier values. The vertical axis – mitochondrial basal respiration (leak), horizontal axis – the study groups, Mit – mitochondrial basal respiration, Mit CON – Mit without cardioplegia (CP), Mit ST – Mit in St. Thomas CP, Mit HTK – Mit in Custodiol HTK CP, Mit DN – Mit in Del Nido CP. A statistically significant difference was found between the ST and DN groups (p = 0.020) and between the ST and HTK (p = 0.003).

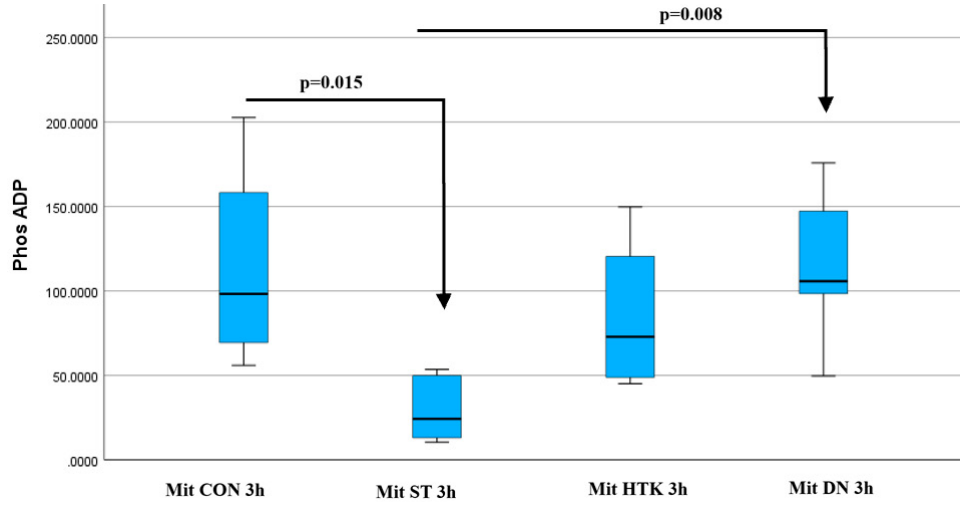


Fig. 3.1.2.2. *The median values of mitochondrial ADP-stimulated respiration rate after 3 hours of ischemia among the study groups*

Box and whisker graphs are presented. The box indicates the value from the 25th to the 75th percentile. The line in the middle of the box indicates the median value, with circles – outlier values. Vertical axis – ADP-ATP-production coupled mitochondrial respiration (Phos ADP), horizontal axis – the study groups, Mit – mitochondrial basal respiration, Mit CON – Mit without cardioplegia (CP), Mit ST – Mit in St. Thomas CP, Mit HTK – Mit in Custodiol HTK CP, Mit DN – Mit in Del Nido CP. A statistically significant difference was found between the ST and DN groups (p = 0.008) and between the ST and CON (p = 0.015).

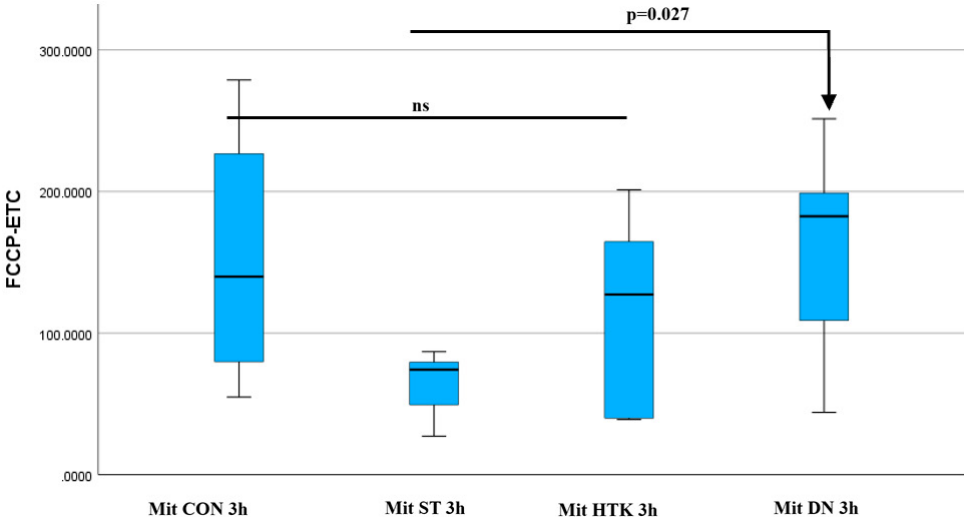


Fig. 3.1.2.3. *The median values of mitochondrial maximal respiration capacity after 3 hours of ischemia among the study groups*

Box and whisker graphs are presented. The box indicates the value from the 25th to the 75th

percentile. The line in the middle of the box indicates the median value, with circles – outlier values. The vertical axis – FCCP (carbonyl cyanide p-trifluoromethoxyphenyldrazone) for maximal respiration (FCCP-ETC), horizontal axis – the study groups, Mit – mitochondrial basal respiration, Mit CON – Mit without cardioplegia (CP), Mit ST – Mit in St. Thomas CP, Mit HTK – Mit in Custodiol HTK CP, Mit DN – Mit in Del Nido CP. A statistically significant difference was found between the ST and DN groups ($p = 0.027$).

Maximal mitochondrial respiration stimulated by FCCP also showed higher values in the DN-group compared with the ST-group ($p = 0.027$). These findings indicate that Del Nido CP helps to preserve mitochondrial respiratory reserve capacity during extended ischemic exposure.

Table 3.1.2.1 *Derived median and percentile values of mitochondrial respiration parameters measured after 3 hours of ischemia in study groups*

Parameters	**CON-group**	**ST-group**	**HTK-group**	**DN-group**
Mit,	20.76 [18.97–23.26]	11.50 [9.62–19.60]	30.79* [20.96–37.68]	24.03* [21.10–26.65]
Phos ADP	98.28* [62.67–180.43]	24.19 [11.71–51.65]	72.84 [47.94–127.77]	105.75* [86.18–154.38]
Cyt C	102.78* [73.59–218.55]	46.50 [23.33–79.65]	109.55* [74.48–160.12]	135.75* [89.46–188.84]
FCCP	139.78 [67.24–252.70]	74.20 [38.25–83.15]	127.16 [39.69–173.65]	182.44* [92.58–212.03]
Cyt C/ADP	1.15* [1.03–1.24]	1.54 [1.48–2.18]	1.37 [1.25–1.57]	1.18* [1.10–1.35]
ADP/Mit	4.84* [3.22–7.69]	1.26 [1.09–3.87]	2.82 [1.46–4.38]	5.22* [3.12–6.32]

CON-group – no cardioplegia (CP), ST-group – with St. Thomas CP, HTK-group – with Custodiol HTK CP, DN-group – with Del Nido CP, Mit – mitochondrial basal respiration, FCCP – carbonylcyanide-p-trifluoromethoxyphenylhydrazone for maximal respiration, ADP/Mit – ratio between ATP-production coupled mitochondrial respiration and basal respiration, Cyt C/ADP – assessment of the condition of the outer mitochondrial membrane via the Cyt C effect. In square brackets, the value at the 25^{th} and 75^{th} percentiles. * $p < 0.05$ vs ST, n = 6 for each parameter within each CP group.

Table 3.1.2.2. *Calculated p-values for derived median values after 3 hours of ischemia across CP groups*

Parameters	CON vs ST	CON vs HTK	CON vs DN	ST vs HTK	ST vs DN	HTK vs DN
Mit	0.244	0.119	0.328	**0.003**	**0.020**	0.515
Phos ADP	**0.015**	0.360	0.967	0.087	**0.008**	0.329
Cyt C	**0.045**	0.917	0.755	**0.035**	**0.011**	0.642
FCCP	0.082	0.466	0.787	0.249	**0.027**	0.264
Cyt C/ADP	**0.003**	**0.061**	0.708	0.201	**0.004**	0.094
ADP/Mit	**0.045**	0.151	0.917	0.489	**0.035**	0.137

CON – CON-group (without CP), ST – ST-group with St. Thomas CP, HTK – HTK-group with Custodiol HTK CP, DN – DN-group with Del Nido CP, Mit – mitochondrial basal respiration, FCCP – carbonylcyanide-p-trifluoromethoxyphenylhydrazone for maximal respiration, ADP/Mit – ratio between ATP-production coupled mitochondrial respiration and basal respiration, Cyt C/ADP – assessment of the condition of the outer mitochondrial membrane via the Cyt C effect. Bolded numbers – detected statistically significant values between the indicated groups.

Table 3.1.2.3. *Determined mean values of mitochondrial respiration parameters after 3 hours of ischemia in four CP groups*

Parameters	CON-group	ST-group	HTK-group	DN-group
Mit	21 ± 1.11674	14 ± 2.64664	30 ± 4.401486	23 ± 1.33585
Phos ADP	113 ± 31.89275	30 ± 9.08557	85 ± 17.72653	113 ± 17.75056
Cyt C	131 ± 41.53781	50 ± 12.90476	115 ± 19.35813	139 ±25.44193
FCCP	153 ± 48.51964	63 ± 11.03386	116 ± 27.25405	161 ± 30.0774
Cyt C/ADP	1.14 ± 0.05457	1.7 ± 0.18722	1.42 ± 0.095271	1.21 ± 0.06016
ADP/Mit	5.2 ± 1.19884	2.24 ± 0.68423	3.11 ± 0.849161	4.8 ± 0.72469

CON-group – no cardioplegia (CP), ST-group – with St. Thomas CP, HTK-group – with Custodiol HTK CP, DN-group – with Del Nido CP, Mit- mitochondrial basal respiration, FCCP – carbonylcyanide-p-trifluoromethoxyphenylhydrazone for maximal respiration, ADP/Mit – ratio between ATP-production coupled mitochondrial respiration and basal respiration, Cyt C/ADP – assessment of the condition of the outer mitochondrial membrane via the Cyt C effect

Markers of mitochondrial membrane integrity provided additional insight into ischemic injury at this time point. The Cyt C/ADP ratio, which increases in response to cytochrome c leakage through a compromised outer mitochondrial membrane, was highest in the ST-group, indicating more pronounced membrane disruption. The DN-group showed significantly lower Cyt C/ADP values (1.2 [1.1; 1.4] vs. 1.6 [1.5; 2.2]; $p = 0.004$), suggesting better preservation of mitochondrial membrane integrity. Such preservation is important because increased membrane permeability facilitates the initiation

of apoptotic pathways during reperfusion. The ADP/Mit ratio was also highest in the DN-group and significantly greater than in the ST-group (5.22 [3.12–6.32] vs. 1.26 [1.09–3.87]; p = 0.035). These findings suggest that mitochondria in the DN-group not only maintain respiratory function but can also effectively couple respiration to ATP synthesis after prolonged ischemia (Figures 3.1.2.4, 3.1.2.5, and 3.1.2.6).

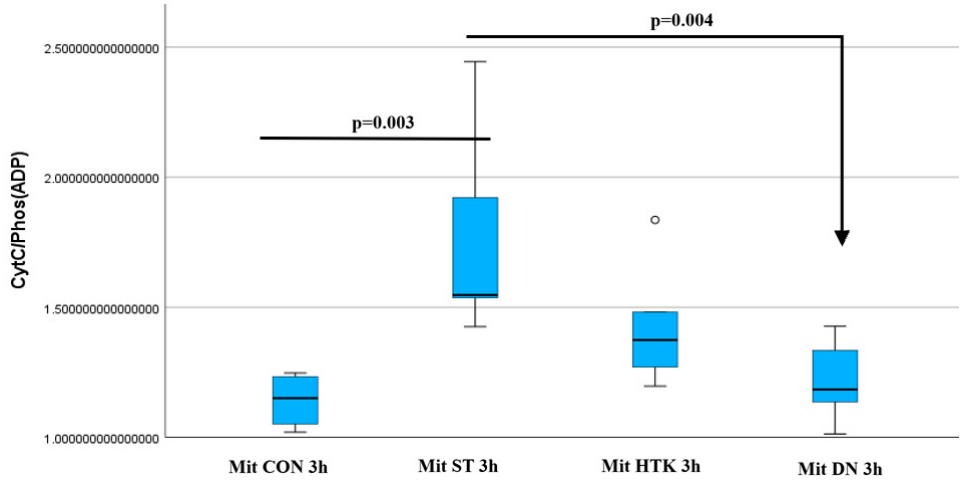


Fig. 3.1.2.4. *The median values of mitochondrial oxidative phosphorylation efficiency after 3 hours of ischemia among the study groups*

Box and whisker graphs are presented. The box indicates the value from the 25th to the 75th percentile. The line in the middle of the box indicates the median value, with circles – outlier values. The vertical axis – mitochondrial respiration Cytochrome C CytC/Phos (ADP), horizontal axis – the study groups: Mit – mitochondrial basal respiration, Mit CON – Mit without cardioplegia (CP), Mit ST – Mit in St. Thomas CP, Mit HTK – Mit in Custodiol HTK CP, Mit DN – Mit in Del Nido CP. A statistically significant difference was found between the CON and ST groups (p = 0.003) and between the DN and ST groups (p = 0.004).

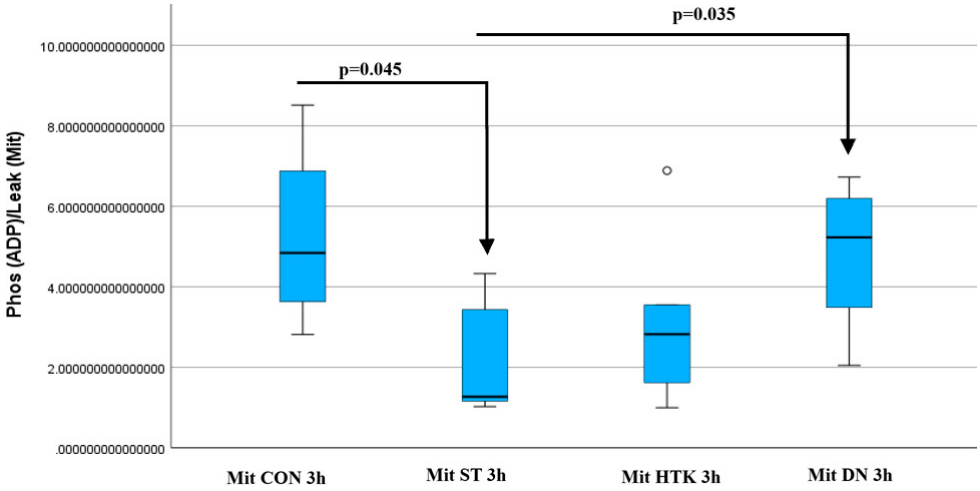


Fig. 3.1.2.5. *The median values of mitochondrial oxidative phosphorylation rate with the addition of cytochrome c after 3 hours of ischemia among the study groups*

Box and whisker graphs are presented. The box indicates the value from the 25th to the 75th percentile. The line in the middle of the box indicates the median value, with circles – outlier values. The vertical axis – mitochondrial respiration ratio Phos (ADP)/Leak (Mit), horizontal axis – the study groups, Mit – mitochondrial basal respiration, Mit CON – Mit without cardioplegia (CP), Mit ST – Mit in St. Thomas CP, Mit HTK – Mit in Custodiol HTK CP, Mit DN – Mit in Del Nido CP. A statistically significant difference was found between the ST and DN groups (p = 0.035) and between the ST and CON groups (p = 0.045).

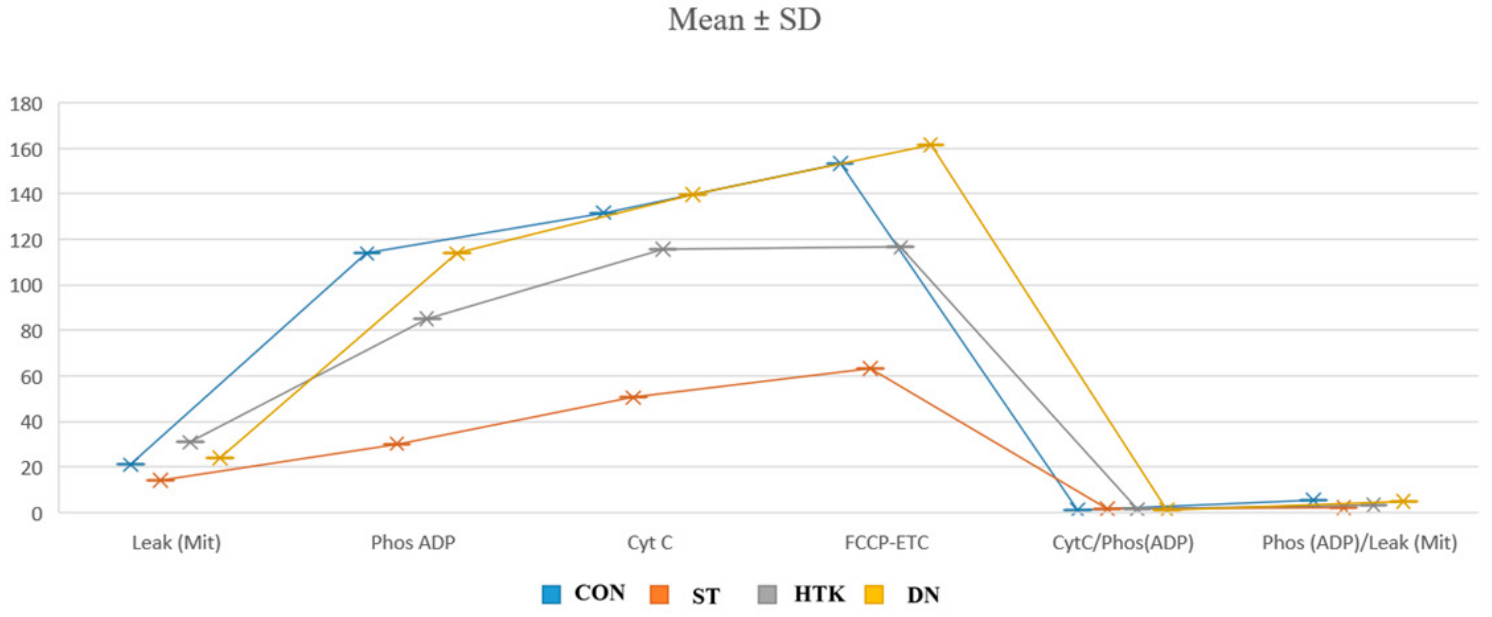


Fig. 3.1.2.6. *Summarized mitochondrial respiration parameters in the study groups after 3 hours of ischemia*

The vertical axis – normalized values (%), horizontal axis – measured parameters among the study groups, Mit – mitochondrial basal respiration, FCCP – carbonylcyanide-p-trifluoromethoxy phenylhydrazone for maximal respiration, ADP/Mit – ratio between ATP-production coupled mitochondrial respiration and basal respiration, Cyt C/ADP – assessment of the condition of the outer mitochondrial membrane via the Cyt C effect. CON – control in no cardioplegia (CP), ST – St. Thomas CP, HTK – Custodiol HTK CP, DN – Del Nido CP. Data presented as Mean ± SD. n = 6 under each experimental condition.

Overall, the results from the prolonged (3 h) ischemia model indicate that Del Nido CP provides the most stable preservation of mitochondrial function, as measured by respiratory activity and membrane stability. The Custodiol HTK CP demonstrates intermediate performance, whereas St. Thomas CP shows the lowest level of protection.

3.2. Baseline characteristics of male Wistar rats in the Langendorff perfusion model

A total of 39 male Wistar albino rats were included in the Langendorff heart perfusion model. The groups did not differ significantly in body weight or heart weight, with mean values of 90.9 ± 12.13 g and 4.7 ± 0.63 g, respectively, confirming that baseline characteristics were well balanced across the experimental conditions (Table 3.2.1).

Table 3.2.1. *Baseline characteristics under the Langendorff perfusion model*

Group	n	Mean body weight, g	Mean heart weight, g	Time to cardiac arrest, sec	Coronary flow before ischemia, mL/min	Time to restoration of spontaneous heart rate, sec	Post-ischemic coronary flow, mL/min
ST	12	90.33 ± 12.55	4.7 ± 0.66	108.08 ± 18.7*	5.1 ± 1.63	151.08 ± 46.5	4.44 ± 1.1
HTK	12	89.67 ± 13.4	4.7 ± 0.7	128.25 ± 24.15**	5.3 ± 1.59	152.91 ± 48.8	4.8 ± 1.2
DN	12	94.58 ± 10.7	4.9 ± 0.56	99.0 ± 8.0***	5.5 ± 0.9	124.17 ± 30.6	5.09 ± 0.81
CON	3	86.40 ± 12.5	4.5 ± 0.5	-	5.3 ± 1.25	-	-
Total	39	90.9 ± 12.13	4.7 ± 0.63	111.78 ± 21.6	5.3 ± 1.36	142.72 ± 43.63	4.7 ± 1.07

$p < 0.05$: * – ST vs. HTK, ** – HTK vs. DN, *** – DN vs. ST

The time to cardiac arrest following administration of the CP differed among the groups, reflecting distinct pharmacodynamic properties of the solutions. The longest arrest time was observed in the HTK-group (128.25 ± 24.15 sec), consistent with its known slow distribution and low sodium content. The ST-group demonstrated intermediate arrest time (108.08 ± 18.7 sec), whereas the DN-group achieved the fastest arrest (99.0 ± 8.0 sec) (Table 3.2.1). These differences likely reflect the specific ionic composition and pharmacodynamic properties of each solution.

Baseline coronary flow before ischemia was similar across the groups (in mL/min: 5.3 ± 1.59 for HTK, 5.1 ± 1.63 for ST, and 5.5 ± 0.9 for DN), indicating that the initial perfusion status of hearts was uniform. After ischemia, all groups had reduced coronary flow, which is expected due to I/R-induced vascular changes. However, the DN-group showed the most favorable postischemic coronary flow (5.09 ± 0.81mL/min), suggesting better preservation of microvascular function (Table 3.2.1).

The time required for restoration of spontaneous heart rhythm after ischemia also differed among the groups. Hearts perfused with Del Nido CP recovered significantly faster (124.17 ± 30.6 sec) compared with those receiving St. Thomas CP (151.08 ± 46.5 sec) or Custodiol HTK CP (152.91 ± 48.8 sec). These functional outcomes are consistent with the mitochondrial respiration findings and further support the conclusion that Del Nido CP provides the most effective overall myocardial protection.

3.3. Gene expression

3.3.1. Impact of CP on cellular susceptibility to apoptotic triggers

Ischemia-reperfusion injury is a major contributor to cardiomyocyte death, arising predominantly from the abrupt restoration of oxygenated blood to ischemic tissue. This process leads to excessive production of ROS, activation of inflammatory cascades, and subsequent initiation of apoptotic and necrotic pathways, resulting in substantial cellular injury and death [176].

To assess cellular susceptibility to apoptotic signalling under different CP solutions, the expression of genes coding *BCL2* apoptosis regulator (*BCL2*) and *BCL2*-associated X apoptosis regulator (*BAX*) was evaluated. The *BAX/BCL2* expression ratio serves as an established molecular indicator of a cell's propensity toward apoptosis: an elevated ratio reflects enhanced vulnerability to apoptotic stimuli, whereas a reduced ratio suggests greater cellular resistance and survival potential [177].

In the present study, myocardial tissue treated with Del Nido CP exhibited an approximately 50% lower *BAX/BCL2* ratio compared with tissue exposed

to St. Thomas and Custodiol HTK solutions. Although these differences did not reach statistical significance (Figure 3.3.1.1), they suggest that Del Nido CP may enhance cellular resistance to apoptosis, thereby offering superior cardioprotective efficacy relative to ST and HTK formulations.

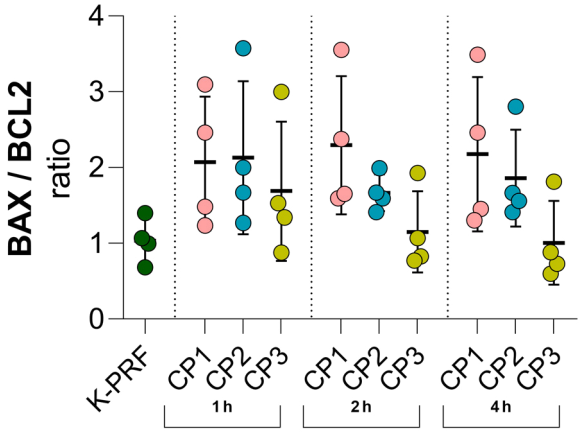


Fig. 3.3.1.1. *The ratio of BAX and BCL2 gene expression in rat heart tissues at different time intervals*

The relative gene expression study of heart tissues treated with cardioplegic solutions showed that DN has the strongest anti-apoptotic effect, which becomes evident at 2 h. Despite quantitative differences, these results were not statistically significant: at the 2 h time point – DN vs. ST ($p = 0.08$) and HTK ($p = 0.12$), at the 4 h time point – DN vs. ST ($p = 0.07$) and HTK ($p = 0.09$), Student's t-test was applied (n = 4 for each).

3.3.2. Differential effects of cardioplegic solutions on cellular processes

To further investigate CP effects on cellular processes in cardiac tissue, expression of genes coding BCL2 interacting protein (*BNIP2*), FOS proto-oncogene (*FOS*), and hypoxia inducible factor 1 subunit alpha (*HIF-1α*) was evaluated (Tables 3.3.2.1–3.3.2.3).

Table 3.3.2.1. *Relative gene expression data at the 1h time point*

1 h		*HIF-1α*			*FOS*			*BNIP2*		
Group	**n**	**Mean ΔCT ± SD**	**Mean fold change ± SD**	***p*- value**	**Mean ΔCT ± SD**	**Mean fold change ± SD**	***p*- value**	**Mean ΔCT ± SD**	**Mean fold change ± SD**	***p*- value**
ST	4	2.36 ± 0.22	0.58 ± 0.09	[a]**0.007** [b]0.195 [c]**0.002**	–4.59 ± 0.22	0.49 ± 0.07	[a]**0.000** [b]0.292 [c]0.287	1.38 ± 0.21	0.68 ± 0.40	[a]**0.000** [b]0.598 [c]0.094
HTK	4	2.18 ± 0.11	0.65 ± 0.05	[a]**0.011** [c]**0.003**	–4.28 ± 0.50	0.41 ± 0.14	[a]**0.002** [c]0.117	1.47 ± 0.24	0.51 ± 0.09	[a]**0.000** [c]0.074
DN	4	1.41 ± 0.32	1.14 ± 0.25	[a]0.532	–5.38 ± 1.33	1.13 ± 1.01	[a]0.699	0.48 ± 0.88	1.07 ± 0.77	[a]0.784

HIF-1α – Hypoxia inducible factor 1 subunit alpha, *BNIP2* – expression of genes coding BCL2 interacting protein, *FOS* – FOS proto-oncogene, ST – St. Thomas, HTK – Custodiol HTK, DN – Del Nido, Student's t-test was applied (n = 4 for each), *p*-values are given – [a] vs. CON, [b] vs. HTK, [c] vs. DN.

Table 3.3.2.2. *Relative gene expression data at the 2h time point*

1 h		*HIF-1α*			*FOS*			*BNIP2*		
Group	**n**	**Mean ΔCT ± SD**	**Mean fold change ± SD**	***p*- value**	**Mean ΔCT ± SD**	**Mean fold change ± SD**	***p*- value**	**Mean ΔCT ± SD**	**Mean fold change ± SD**	***p*- value**
ST	4	2.22 ± 0.22	0.64 ± 0.07	[a]**0.015** [b]**0.002** [c]**0.0008**	–4.60 ± 0.55	0.51 ± 0.2	[a]**0.008** [b]0.935 [c]**0.017**	1.51± 0.16	0.45 ± 0.05	[a]**0.000** [b]**0.013** [c]**0.005**
HTK	4	1.64 ± 0.16	0.96 ± 0.1	[a]0.728 [c]**0.028**	–4.64 ± 0.93	0.56 ± 0.26	[a]**0.024** [c]**0.025**	0.96± 0.27	0.68 ± 0.11	[a]**0.009** [c]0.085
DN	4	1.13 ± 0.31	1.38 ± 0.3	[a]0.102	–6.93 ± 0.86	2.75 ± 1.4	[a]**0.024**	0.37± 0.51	1.03 ± 0.35	[a]0.928

HIF-1α – Hypoxia inducible factor 1 subunit alpha, *BNIP2* – expression of genes coding BCL2 interacting protein, *FOS* – FOS proto-oncogene, ST – St. Thomas, HTK – Custodiol HTK, DN – Del Nido, Student's t-test was applied (n = 4 for each), *p*-values are given – [a] vs. CON, [b] vs. HTK, [c] vs. DN.

Table 3.3.2.3. *Relative gene expression data at the 4h time point*

1 h		***HIF-1α***			***FOS***			***BNIP2***		
Group	**n**	**Mean ΔCT ± SD**	**Mean fold change ± SD**	***p*- value**	**Mean ΔCT ± SD**	**Mean fold change ± SD**	***p*- value**	**Mean ΔCT ± SD**	**Mean fold change ± SD**	***p*- value**
ST	4	2.29 ± 0.31	0.62 ± 0.12	[a]**0.018**[b] 0.092 [c]0.102	-4.80 ± 0.42	0.60± 0.15	[a]**0.007** [b]0.815 [c]**0.032**	1.82 ± 0.54	0.42 ± 0.13	[a]**0.002** [b]0.153 [c]0.108
HTK	4	1.84 ± 0.34	0.85 ± 0.19	[a]0.291 [c]0.456	-4.93 ± 1.03	0.73 ± 0.47	[a]0.215 [c]0.088	1.18 ± 0.56	0.66 ± 0.25	[a]**0.030** [c]0.479
DN	4	1.52 ± 0.74	1.14 ± 0.55	[a]0.904	-6.52± 1.17	2.32± 1.8	[a]0.187	0.75 ± 1.1	0.90 ± 0.54	[a]0.462
CON	3	1.57 ± 0.33	-	-	-5.64 ± 0.05	-	-	0.35 ± 19	-	-

HIF-1α – Hypoxia inducible factor 1 subunit alpha, *BNIP2* – expression of genes coding BCL2 interacting protein, *FOS* – FOS proto-oncogene, ST – St. Thomas, HTK – Custodiol HTK, DN – Del Nido. Student's t-test was applied (n = 3 – 4, as indicated), *p*-values are given: [a] vs. CON, [b] vs. HTK, [c] vs. DN.

It is well established that *BNIP2* plays a crucial role in skeletal muscle differentiation[178] and contributes to myosin II–dependent contractility, where *BNIP2* knockdown has been shown to reduce the recoil velocity of actin filaments [178].

In the present study, treatment with the Del Nido CP preserved *BNIP2* mRNA expression levels, while both St. Thomas CP and Custodiol HTK CP demonstrated significantly diminished effects (Fig. 3.3.2.1).

Specifically, *BNIP2* transcript levels were reduced to approximately 70% of those observed in perfused control tissue across all time points ($p < 0.05$). The stabilizing effect of DN cardioplegia was evident during the initial two hours of ischemia but gradually declined thereafter.

Collectively, these findings suggest that Del Nido CP may confer a protective advantage in maintaining cardiomyocyte contractile function through the preservation of *BNIP2* expression.

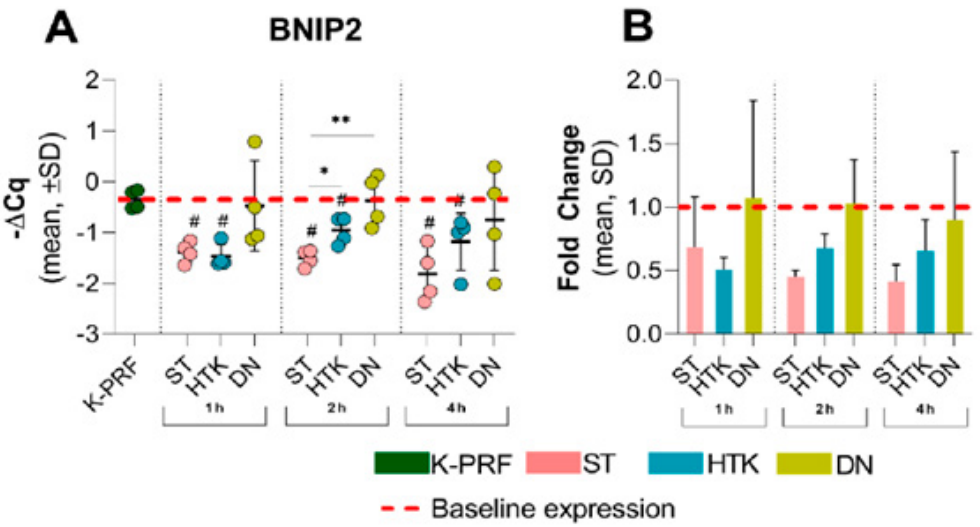


Fig. 3.3.2.1. *Results of the real-time PCR study for BNIP2*

(**A**) – ΔCt values normalized to the reference gene (*GAPDH*). In all groups, the DN CP induced the highest gene expression, comparable to the perfusion procedure. The statistically significant differences were observed after 2 h of incubation. # compared to perfusion $p < 0.05$, * $p < 0.005$, ** $p < 0.01$, *** $p < 0.001$. Student's t-test was used. (**B**) average fold change values normalized to the perfusion group. In all groups, the DN CP treatment resulted in similar or upregulated expressions of *BNIP2* genes compared to perfused heart tissue.

In addition to *BNIP2*, the expression of hypoxia-inducible factor *HIF-1α* mRNA was also evaluated. *HIF-1α* is a pivotal transcriptional regulator mediating cellular adaptation to myocardial ischemia by promoting

angiogenesis, stimulating the formation of collateral vessels, and inhibiting cardiomyocyte apoptosis [179].

Previous studies have demonstrated that during I/R injury, *HIF-1α* mitigates reactive oxygen species (ROS) generation through the upregulation of glycolytic enzymes and pyruvate dehydrogenase kinase 1 (PDK1), thereby restricting mitochondrial substrate influx and limiting oxidative stress [180]. Our findings revealed that treatment with the Del Nido CP solution markedly enhanced *HIF-1α* mRNA expression (Figure 3.3.2.2).

Statistically significant upregulation was detected at the 1-hour and 2-hour ischemic time points, although this effect diminished after 4 hours of ischemia. Compared with the St. Thomas CP and Custodiol HTK CP solutions, Del Nido CP induced approximately 90% higher relative *HIF-1α* expression ($p < 0.01$), providing further evidence of its superior cytoprotective and metabolic adaptive properties under ischemic conditions.

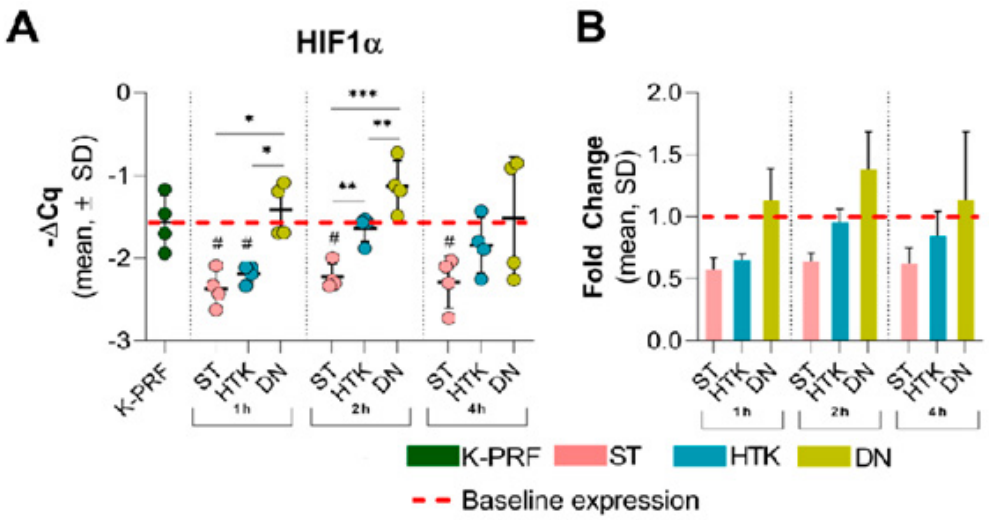


Fig. 3.3.2.2. *Results of the real-time PCR study for HIF1α*

(**A**) – ΔCt values normalized to the reference gene (*GAPDH*). In all groups, the DN CP induced the highest gene expression, comparable to the perfusion procedure. The statistically significant differences were observed after 2 h of incubation. # compared to perfusion $p < 0.05$, * $p < 0.005$, ** $p < 0.01$, *** $p < 0.001$. Student's t-test was used. (**B**) – average fold change values normalized to the perfusion group. In all groups, the DN CP treatment resulted in similar or upregulated expressions of *HIF-1α* genes compared to perfused heart tissue.

FOS is a member of the *FOS* family of transcription factors that dimerize with proteins of the JUN family to form the activator protein-1 (AP-1) complex. This complex regulates a broad spectrum of cellular and biological

processes, including cell cycle progression, proliferation, differentiation, and programmed cell death [181].

Our data demonstrate that relative *c-FOS* mRNA expression remained significantly higher in the DN-group compared with both the ST-group ($p < 0.05$) and HTK-group ($p < 0.05$). Following exposure to Del Nido CP, *FOS* gene expression levels were comparable to those observed in perfused control tissue at the 1- and 4-hour ischemic time points, whereas a significant upregulation ($p < 0.05$) was detected after 2 hours of ischemia. In contrast, myocardial tissue treated with St. Thomas CP and Custodiol HTK CP solutions exhibited a significant reduction in *FOS* mRNA expression relative to perfused tissue ($p < 0.05$) (Figure 3.3.2.3).

Collectively, these findings suggest that Del Nido CP sustains transcriptional activation of the AP-1 pathway, which may contribute to enhanced cellular stress adaptation and survival signalling during ischemic conditions.

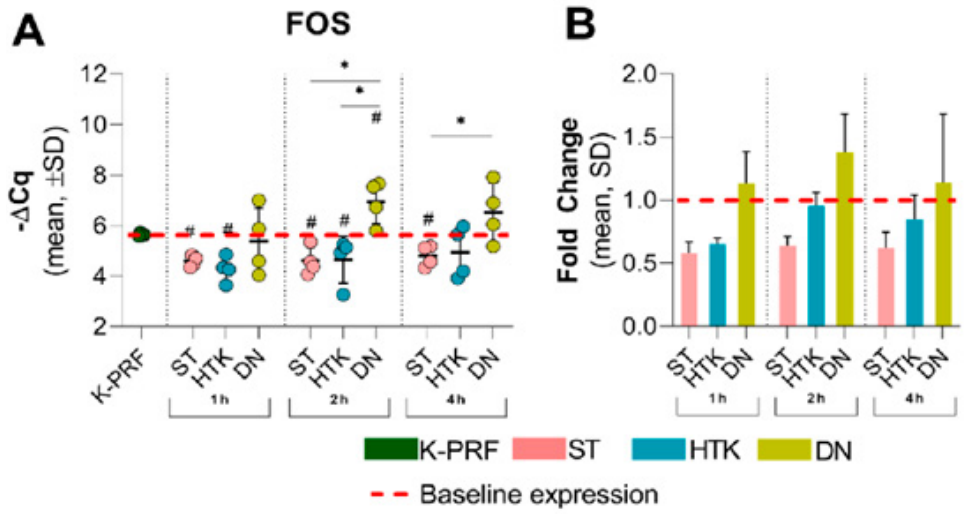


Fig. 3.3.2.3. *Results of the real-time PCR study for FOS*

(**A**) – ΔCt values normalized to the reference gene (*GAPDH*). In all groups, the DN CP induced the highest gene expression, comparable to the perfusion procedure. The statistically significant differences were observed after 2 h of incubation: compared to perfusion $p < 0.05$, * $p < 0.005$, ** $p < 0.01$, *** $p < 0.001$. Student's t-test. (**B**) – average fold change values normalized to the perfusion group. In all groups, the DN CP treatment resulted in similar or upregulated expressions of *FOS* genes compared to perfused heart tissue.

In summary, the gene expression analyses indicate that the Del Nido CP solution exhibits significantly stronger cardioprotective properties compared with both St. Thomas CP and Custodiol HTK CP solutions. Moreover, the expression profiles observed in the DN-treated tissue closely resembled those

of perfused (non-ischemic) myocardium, further supporting the conclusion that Del Nido CP provides superior preservation of cellular integrity and molecular homeostasis under ischemic conditions.

3.3.3. Gene expression response to cardioplegic solutions and ischemia duration

Analysis of myocardial gene expression revealed significant differences between cardioplegic solutions, particularly for markers of hypoxia and cellular stress (Tables 3.3.3.1-3.3.3.4). As shown in Table 3.3.3.1, two-way ANOVA demonstrated a significant main effect of solution on *HIF1α* expression at both 1 hour ($p = 0.007$) and 2 hours ($p = 0.015$), with post hoc analysis indicating that the DN-group exhibited significantly lower ΔCt values (reflecting higher expression) compared to the ST-group and HTK-group. The highest *HIF1α* expression was observed in the DN-group at 2 hours (1.13 ± 0.31), while the highest ΔCt values (indicating the lowest expression) were noted in the ST-group at 1 hour (2.36 ± 0.22). By 4 hours, differences between groups were less pronounced and not statistically significant ($p > 0.05$).

Table 3.3.3.1. *HIF1α ΔCt by solution and ischemia duration*

Solution	Time (h)	Mean *HIF1α* (ΔCt)	± SD
ST	1	2.36	0.22
ST	2	2.22	0.22
ST	4	2.29	0.31
HTK	1	2.18	0.11
HTK	2	1.64	0.16
HTK	4	1.84	0.34
DN	1	1.41	0.32
DN	2	1.13	0.31
DN	4	1.52	0.74

Similarly, *FOS* gene expression (Table 3.3.3.2) differed significantly between groups at 2 hours, with DN showing the lowest ΔCt values (highest expression; –6.93 ± 0.86) compared to ST (–4.60 ± 0.55; $p = 0.008$) and HTK (–4.64 ± 0.93; $p = 0.024$). Although *FOS* expression was also numerically higher in DN at 1 hour and at 4 hours (–6.52 ± 1.17), these differences were not statistically significant at those points.

Table 3.3.3.2. *FOS ΔCt by solution and ischemia duration*

Solution	Time (h)	Mean *FOS* (ΔCt)	± SD
ST	1	–4.59	0.22
ST	2	–4.60	0.55
ST	4	–4.80	0.42
HTK	1	–4.28	0.50
HTK	2	–4.64	0.93
HTK	4	–4.93	1.03
DN	1	–5.38	1.33
DN	2	–6.93	0.86
DN	4	–6.52	1.17

BNIP2 gene expression patterns (Table 3.3.3.3) mirrored these findings, with significantly higher expression in the DN at both 1 hour (ΔCt 0.48 ± 0.88) and 2 hours (0.37 ± 0.51) compared to ST (1 h: 1.38 ± 0.21, $p < 0.001$; 2 h: 1.51 ± 0.16, $p = 0.005$) and HTK (1 h: 1.47 ± 0.24, $p < 0.001$). At 2 hours, the difference between DN and HTK did not reach statistical significance ($p = 0{,}085$), and by 4 hours, no significant group differences were detected.

Table 3.3.3.3. *BNIP2 ΔCt by solution and ischemia duration*

Solution	Time (h)	Mean *BNIP2* (ΔCt)	± SD
ST	1	1.38	0.21
ST	2	1.51	0.16
ST	4	1.82	0.54
HTK	1	1.47	0.24
HTK	2	0.96	0.27
HTK	4	1.18	0.56
DN	1	0.48	0.88
DN	2	0.37	0.51
DN	4	0.75	1.10

Finally, the *BAX/BCL2* ratio (Table 3.3.3.4), a marker of cellular susceptibility to apoptosis, tended to be lower in the DN at 2 hours (0.46 ± 0.10) and 4 hours (0.65 ± 0.17) relative to ST (2 h: 0.80 ± 0.15, 4 h: 0.94 ± 0.12) and HTK (2 h: 0.89 ± 0.17, 4 h: 0.94 ± 0.14); however, these differences did not reach statistical significance (all $p > 0{,}05$).

Table 3.3.3.4. *BAX/BCL2 Ratio by solution and ischemia duration*

Solution	Time (h)	Mean *BAX/BCL2* Ratio	± SD
ST	1	0.79	0.12
ST	2	0.80	0.15
ST	4	0.94	0.12
HTK	1	0.86	0.13
HTK	2	0.89	0.17
HTK	4	0.94	0.14
DN	1	0.56	0.18
DN	2	0.46	0.10
DN	4	0.65	0.17

3.4. Effects of cardioplegic solution on cytokine expression

Inflammatory cytokines represent key biomarkers in experimental models of myocardial injury. To assess the cardioprotective efficacy of different cardioplegic solutions in preserving immature rat myocardium, the pro-inflammatory mediators TNF-α and IL-6 were quantitatively evaluated.

As demonstrated in Table 3.4.1 and Figure 3.4.1, no statistically significant intergroup differences were observed ($p > 0.05$). Nevertheless, during prolonged ischemia, the Custodiol HTK solution demonstrated the most pronounced attenuation of the inflammatory response. Further analysis was performed to evaluate the dynamics of the anti-inflammatory cytokines IL-4 and IL-10. Although intergroup differences did not reach statistical significance (Fig. 3.4.1, Table 3.4.1; $p > 0.05$), a general declining trend in anti-inflammatory cytokine levels was observed over time across all cardioplegic groups. Regarding IL-10, concentrations in the DN group remained comparatively higher after 4 hours of ischemia, whereas the HTK group demonstrated a more stable profile.

Table 3.4.1. *Results of cytokine expression. Despite quantitative differences between groups, these results were not statistically significant ($p > 0.05$)*

group	IL-6	TNF-α	IL-10	IL-4	Leptin	*p*-value
CON	11,38 (8,58;2,70)	26.89 (24,66; 34,75)	84,37 (80,27; 207,77)	4,18 (1,46; 4,89)	156,10 (143,89;198,65)	
ST 1h	17.25 (9.54; 2,01)	41.22 (31.35; 48.93)	120.59 (104.69;225.95)	5.70 (3.50; 7.76)	217,83 (180,17;254,50)	> 0.05
ST 2h	18.14 (9.72; 0.13)	45.12 (28.76;66.78)	169.30 (84.29; 305.21)	6.80 (3.24; 1.35)	218,32 (172,75;279,39)	> 0.05
ST 4h	12.52 (10.32;30.32)	24.68 (21.48;33.22)	81.83 (73.96; 177.43)	3.20 (1.98; 6.30)	145,14 (122,48;177,06)	> 0.05
HTK 1h	14,33 (10.44;19.84)	28.70 (16.71;32.95)	95.61 (57.54; 180.23)	2.59 (0.92;11.66)	174,07 (123,19;193,03)	> 0.05
HTK 2h	12.52 (9.48; 23.85)	34.40 (28.37; 40.52)	93.61 (87.61; 214.97)	3.89 (2.89; 5.15)	176,44 (167,55;215,44)	> 0.05
HTK 4h	13.50 (8.91; 20.79)	28.15 (24.33;32.47)	81.83 (72.52; 212.48)	2.58 (2.02; 5.18)	148,55 (132,43;191,69)	> 0.05
DN 1h	13.68 (9.67; 26.30)	37.33 (27.22;49.53)	165.97 (94.91; 276.25)	5.09 (2.33; 6.88)	198,63 (178,48;266,98)	> 0.05
DN 2h	10.33 (6.51; 31.18)	23.33 (21.20;50.59)	85.26 (82.52; 277.10)	2.85 (1.68; 5.45)	166,83 (128,79;285,99)	> 0.05
DN 4h	8.98 (8.24; 26.27)	28.55 (26.50;39.44)	97.79 (85.63; 226.99)	1.68 (1.21; 5.38)	171,49 (143,48;209,46)	> 0.05

CON – control group without cardioplegia (CP), ST – St. Thomas CP, HTK – Custodiol HTK CP, DN – Del Nido CP. Data are presented as Median. In brackets, the value at the 25th and 75th percentiles.

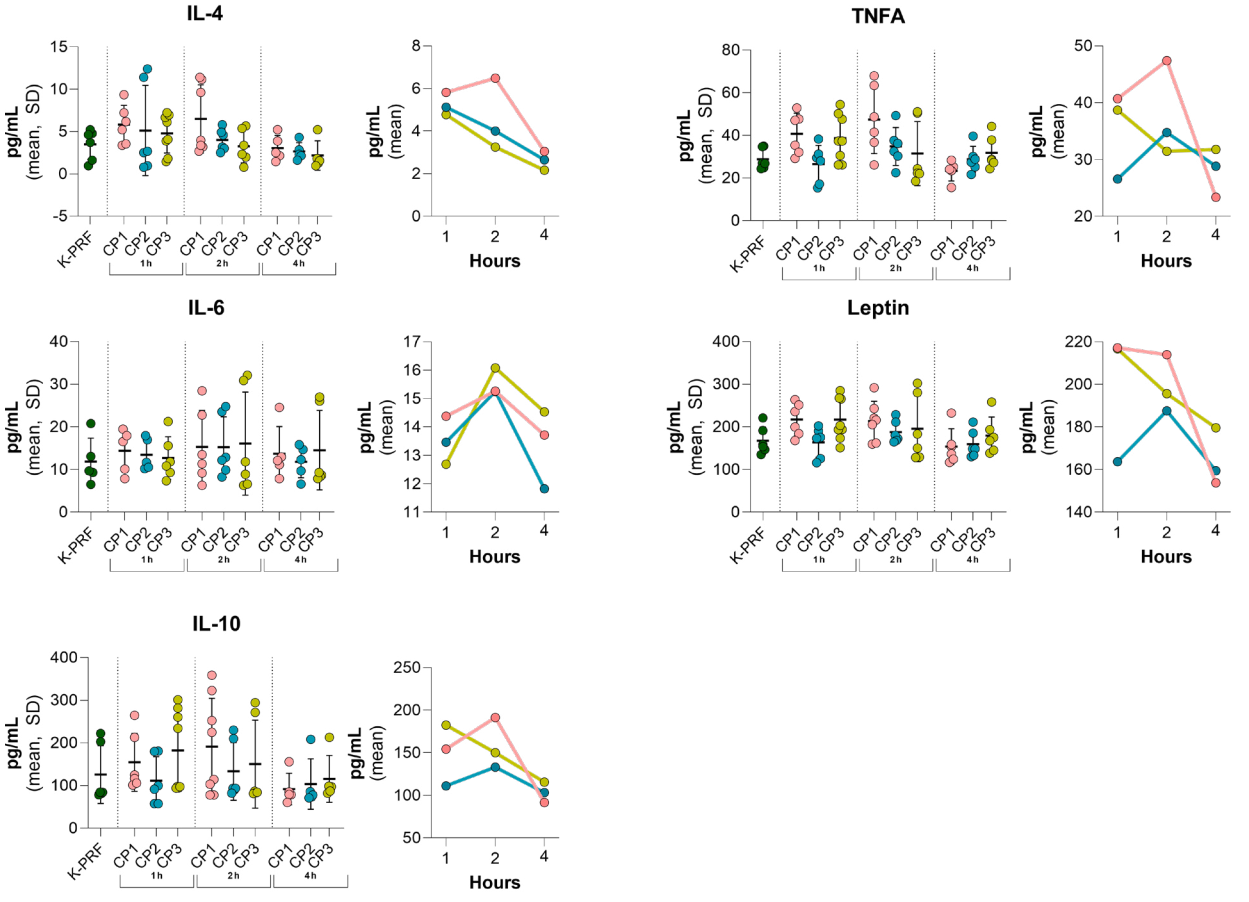


Fig. 3.4.1. *Results of cytokine expression. Despite quantitative differences between groups, these results were not statistically significant*

In contrast, IL-4 expression exhibited similar patterns in the HTK and DN groups, while the ST group showed an early elevation at 2 hours, followed by a marked decline at 4 hours of ischemia.

Additionally, the effects of I/R on leptin expression were examined. Although no statistically significant differences were detected among the cardioplegic solutions ($p > 0.05$), leptin levels uniformly decreased after 4 hours of ischemia across all experimental groups (Fig. 3.4.1, Table 3.4.1).

3.5. Cytokine profile across experimental groups

Analysis of cytokine concentrations across cardioplegic solutions and ischemia durations revealed no significant differences for any measured marker (Tables 3.5.1–3.5.5).

IL-4 levels are summarized in Table 3.5.1. Two-way ANOVA demonstrated no significant main effect of solution or ischemia duration, nor interaction effect, on IL-4 concentration ($F(8.48) = 0.81$, $p = 0.599$, partial $\eta^2 = 0.13$). The highest mean IL-4 was seen in ST at 2 hours (7.36 ± 4.48 pg/mL), and the lowest in DN at 4 hours (2.78 ± 2.16 pg/mL).

Table 3.5.1. *IL-4 levels by solution and ischemia duration*

Solution	Time (h)	Mean IL-4 (pg/mL)	± SD
ST	1	5.82	2.29
ST	2	7.36	4.48
ST	4	4.12	2.96
HTK	1	5.12	5.32
HTK	2	4.01	1.30
HTK	4	3.51	2.28
DN	1	4.77	2.31
DN	2	3.25	1.95
DN	4	2.78	2.16

Similarly, as shown in Table 3.5.2, IL-6 concentrations did not vary significantly by solution or ischemia duration (all $p > 0.05$), with means ranging from 24.09 ± 18.22 pg/mL (ST, 2 h) to 14.53 ± 9.29 pg/mL (DN, 4 h), and Levene's test indicating homogeneity of variances ($p = 0.092$).

Table 3.5.2. *IL-6 levels by solution and ischemia duration*

Solution	Time (h)	Mean IL-6 (pg/mL)	± SD
ST	1	16.93	7.73
ST	2	24.09	18.22
ST	4	19.38	14.98
HTK	1	15.48	5.94
HTK	2	15.25	7.10
HTK	4	15.79	10.29
DN	1	17.11	9.32
DN	2	16.08	12.09
DN	4	14.53	9.29

For IL-10 (Table 3.5.3), two-way ANOVA again showed no significant effects of solution, ischemia duration, or their interaction (all $p > 0.05$), with mean values from 49.45 ± 16.55 pg/mL (ST, 4 h) to 58.41 ± 22.41 pg/mL (DN, 1 h); Levene's test confirmed variance homogeneity ($p = 0.289$).

Table 3.5.3. *IL-10 levels by solution and ischemia duration*

Solution	Time (h)	Mean IL-10 (pg/mL)	± SD
ST	1	52.31	19.22
ST	2	57.34	30.08
ST	4	49.45	16.55
HTK	1	50.32	13.87
HTK	2	54.05	12.71
HTK	4	54.09	19.97
DN	1	58.41	22.41
DN	2	58.25	15.11
DN	4	52.33	13.18

Leptin concentrations (Table 3.5.4.) also did not differ significantly between solutions or durations (all $p > 0.05$), with the highest value in ST at 1 hour (25.20 ± 6.73 pg/mL) and the lowest in DN at 4 hours (17.37 ± 5.33 pg/mL), and Levene's test supporting homogeneity of variance ($p = 0.545$).

Table 3.5.4. *Leptin levels by solution and ischemia duration*

Solution	Time (h)	Mean Leptin (pg/mL)	± SD
ST	1	25.20	6.73
ST	2	22.83	6.28
ST	4	19.45	6.12
HTK	1	22.56	5.36
HTK	2	18.44	6.66
HTK	4	19.80	7.29
DN	1	23.13	7.26
DN	2	20.23	7.33
DN	4	17.37	5.33

TNF-α levels by CP solution and ischemia duration are presented in Table 3.5.5. Two-way ANOVA found no significant main effects of solution or ischemia duration, and no significant interaction, for myocardial TNF-α levels (all $p > 0,05$). Mean TNF-α ranged from 6.46 ± 2.20 pg/mL (ST at 4 h) to 7.84 ± 1.80 pg/mL (HTK at 1 h), with no statistically significant differences detected. Levene's test confirmed homogeneity of variances ($p = 0.877$).

Table 3.5.5. *TNF-α levels by solution and ischemia duration*

Solution	Time (h)	Mean TNF-α (pg/mL)	± SD
ST	1	7.52	2.17
ST	2	7.28	2.30
ST	4	6.46	2.20
HTK	1	7.84	1.80
HTK	2	7.52	2.56
HTK	4	7.58	2.21
DN	1	7.60	2.27
DN	2	7.57	1.90
DN	4	6.93	2.18

Collectively, these findings suggest that, within the examined parameters, neither the type of cardioplegic solution nor the duration of ischemia exerted a statistically significant influence on the myocardial release of IL-4, IL-6, IL-10, leptin, or TNF-α following I/R injury.

4. DISCUSSION

The findings of this study demonstrate that the choice of cardioplegia solution has a measurable influence on myocardial preservation during ischemia and I/R injury in the immature myocardium. By evaluating Del Nido, Custodiol HTK, and St. Thomas CP solutions using functional, molecular, and mitochondrial outcomes, this study provides additional insight into the mechanisms through which these solutions protect, or fail to protect, the developing heart. We demonstrated that Del Nido CP provided the most effective protection of the immature myocardium during ischemia and reperfusion, as evidenced by superior preservation of mitochondrial respiration, maintenance of outer mitochondrial membrane integrity, and upregulation of adaptive gene expression (*HIF1A*, *FOS*, *BNIP2*). Custodiol HTK CP conferred an intermediate level of protection, whereas St. Thomas CP solution was consistently associated with the poorest mitochondrial and molecular outcomes. Although cytokine and leptin responses did not differ significantly among groups, trends indicated that Del Nido and HTK cardioplegia maintained a more balanced inflammatory profile compared to St. Thomas.

These findings align with a recent report that demonstrated that Del Nido CP more effectively preserved the ATP-synthase tetramer-to-monomer ratio during ischemia [182, 206]. In pediatric cohorts, Del Nido has likewise been associated with superior preservation of ventricular function and attenuation of myocardial injury across basic-science investigations [183], retrospective analyses [184], and prospective studies [185].

4.1. Mitochondrial Respiratory Function During Ischemia

It is essential to emphasize the pivotal role of mitochondria in cardiomyocytes. These organelles are the primary generators of cellular ATP, accounting for approximately 90% of the heart's total energy production. Even subtle structural or functional mitochondrial impairments can lead to profound disturbances in cardiomyocyte metabolism, calcium homeostasis, and overall myocardial contractility [186, 187].

One of the key findings of the study is that mitochondrial respiration was better preserved in the del Nido group at both the 1-hour and 3-hour ischemia time points. After one hour of ischemia, del Nido performed better than St. Thomas in maintaining basal, ATP-coupled, and maximal respiration, and was more effective than Custodiol in several parameters. These differences became even more evident when ischemia was extended to three hours. Notably, after prolonged ischemia, mitochondria in the ST-group showed

significant decreases in ATP production capacity and respiratory reserve, as well as increased cytochrome C release, indicating compromised membrane integrity. In contrast, del Nido cardioplegia preserved both mitochondrial respiratory function and structural stability, as indicated by higher ADP/Mit ratios and lower Cyt C/ADP values. These mitochondrial findings align with the known properties of the del Nido CP solution, including its sodium–calcium balance, lidocaine-mediated membrane stabilization, and reduced excitatory ion load, which may collectively help limit mitochondrial depolarization and calcium overload during ischemia.

Custodiol HTK cardioplegia demonstrated an intermediate level of mitochondrial protection. It preserved basal respiration and ATP-coupled respiration more effectively than St. Thomas, particularly under prolonged ischemia, likely reflecting its ability to suppress metabolism and buffer intracellular pH. However, several mitochondrial parameters still fell short of the levels maintained in the DN-group. This suggests that, although HTK can support metabolic quiescence, the immature myocardium may still benefit more from the combined ionic and membrane-stabilizing effects of del Nido cardioplegia.

An interesting observation was made during the 3-hour ischemia period with the use of the Custodiol HTK cardioplegic solution. The observed improvement is most likely attributable to the specific biochemical composition of this solution. Although HTK is characterized by a low calcium concentration, it contains magnesium chloride, which is known to attenuate calcium influx and stabilize cellular membranes. In addition to magnesium chloride, HTK includes tryptophan, another membrane-stabilizing substrate that contributes to the preservation of sarcolemmal integrity. These compositional features distinguish Custodiol from the St. Thomas solution, which has a simpler formulation, is not hypocalcemic, and contains only magnesium chloride as its principal stabilizing component.

Furthermore, Custodiol HTK contains ketoglutarate, a key substrate for ATP generation that supports mitochondrial metabolism under ischemic conditions. The presence of mannitol, an osmotic agent, helps mitigate ischemia-induced cellular edema by promoting osmotic balance and reducing tissue swelling. Considering that ischemic durations exceeding 60 minutes are known to induce significant cellular edema, the inclusion of mannitol is likely to contribute to maintaining structural and metabolic stability during prolonged ischemia.

Importantly, the mitochondrial findings were mirrored in the Langendorff-perfused heart model. Del Nido CP resulted in the shortest time to cardiac arrest and the fastest restoration of spontaneous rhythm, as well as the highest

post-ischemic coronary flow, indicating better preservation of microvascular function. This concordance between preserved mitochondrial respiration and improved global functional recovery strengthens the causal link between mitochondrial competence and post-ischemic contractile performance. Given that even subtle impairments in oxidative phosphorylation can compromise ATP-dependent processes, including SR Ca^{2+} reuptake, cross-bridge cycling, and membrane transport [186,187], the reduced recovery observed in the St. Thomas group may reflect an inability of mitochondria to rapidly meet energetic demands upon reperfusion.

More broadly, mitochondrial dysfunction is increasingly recognized as a central contributor to cardiovascular disease [188]. Reduced oxidative phosphorylation capacity, increased reactive oxygen species (ROS) production, and impaired substrate utilization have been linked to cardiac remodelling and heart failure progression [189-191]. Disruption of mitochondrial bioenergetics shifts substrate use toward glycolysis and is associated with cardiac hypertrophy and progression to heart failure [192–196]. In patients with coronary artery disease, elevated mitochondrial DNA deletions further compromise respiratory chain efficiency [197]. Against this background, strategies that preserve mitochondrial integrity during surgical ischemia may have implications extending beyond the immediate perioperative period.

4.2. Gene expression responses to cardioplegic solutions

Myocardial gene expression profiling in our experimental model extended and mechanistically reinforced the mitochondrial findings. Beyond preserving oxidative phosphorylation, Del Nido cardioplegia induced a transcriptional pattern consistent with adaptive hypoxic signalling and controlled stress activation in immature myocardial tissue.

Among the most notable observations was the upregulation of *HIF-1α*, *FOS*, *and BNIP2* transcripts in the Del Nido group compared with St. Thomas and Custodiol HTK. This coordinated response suggests not merely passive preservation, but active engagement of endogenous cytoprotective pathways.

The induction of *HIF-1α* is particularly relevant in the context of immature myocardium. While neonatal hearts are often described as relatively glycolysis-dependent [18-20], their oxidative reserve remains limited and highly vulnerable to sustained ischemia [21,22]. *HIF-1α* activation promotes glycolytic flux, enhances glucose uptake, and reduces mitochondrial ROS generation, thereby facilitating metabolic adaptation during hypoxic stress [195-197]. Experimental overexpression of *HIF-1α* has been shown to protect neonatal cardiomyocytes from I/R injury [199], whereas pharmacological inhibition reduces glucose utilization and impairs metabolic recovery [200, 201].

In our model, enhanced *HIF-1α* expression in the Del Nido group paralleled superior preservation of mitochondrial coupling and reduced cytochrome c release. This association suggests that transcriptional activation of hypoxia-responsive pathways may have supported the maintenance of mitochondrial integrity, rather than merely reflecting downstream injury. In contrast, the comparatively attenuated *HIF-1α* response observed with St. Thomas CP may indicate insufficient metabolic adaptation under prolonged ischemic stress.

The upregulation of *FOS* further supports the presence of adaptive stress signalling. As an immediate early gene and component of the AP-1 transcriptional complex, *c-FOS* participates in regulating survival, proliferation, and stress responses. Experimental deficiency of *c-FOS* has been associated with increased infarct size and augmented apoptosis following myocardial injury [201]. However, the role of *FOS* is not unidimensional; it has also been implicated in pathways that may exacerbate injury under certain conditions, including regulation of miR-27a and apoptosis-inducing factor translocation during I/R [202].

This apparent duality underscores an important point: the protective value of *FOS* likely depends on context, magnitude, and temporal dynamics. In our study, *FOS* upregulation occurred alongside improved mitochondrial respiration and reduced apoptotic markers in the Del Nido group, suggesting that its activation represented a coordinated adaptive response rather than maladaptive overactivation. Conversely, the lower *FOS* expression observed with St. Thomas CP coincided with greater mitochondrial dysfunction, which may reflect insufficient activation of stress-response pathways in immature myocardium.

BNIP2 modulation adds another layer to this transcriptional profile. Although evidence regarding BNIP2 in cardiac ischemia is less extensive than for *HIF-1α* or c-FOS, available data suggest that maintenance of BNIP family expression contributes to cytoskeletal organization, cell survival, and preservation of contractile phenotype [202,203]. Furthermore, interactions between *HIF-1α* and related BNIP family members (e.g., *BNIP3*) have been shown to regulate autophagy and enhance cardiomyoblast survival during oxygen-glucose deprivation [204]. While *BNIP2* is not identical to *BNIP3*, its upregulation in our study, together with reduced cytochrome c release, suggests a potential role in balancing apoptosis and adaptive autophagic processes.

Importantly, most prior comparisons of cardioplegic solutions have concentrated on biochemical injury markers or early postoperative functional outcomes, frequently in adult cohorts [15-17]. Data on transcriptional responses in immature myocardium remain sparse and methodologically heterogeneous. Moreover, few studies have directly interrogated hypoxia-

responsive and apoptosis-modulating genes in the context of specific cardioplegic formulations. By demonstrating differential modulation of *HIF-1α*, *FOS*, and *BNIP2* in an immature heart model, our study contributes mechanistic insight that complements existing clinical observations.

Notably, the transcriptional profile induced by Del Nido CP was consistent with previously reported increases in heat-shock protein expression in pediatric settings [12,198], recognized markers of cellular stress adaptation. In pediatric populations, blood-based Del Nido cardioplegia has been shown to induce greater *HSP70-1* expression compared with crystalloid regimens such as St. Thomas or Custodiol HTK [198]. Our findings extend this observation by showing that Del Nido not only modulates stress proteins but also activates upstream regulatory pathways linked to hypoxic adaptation and apoptosis control.

Taken together, the gene expression data suggest that Del Nido cardioplegia does more than transiently suppress metabolism. It appears to orchestrate a transcriptional response that enhances metabolic flexibility, stabilizes mitochondrial function, and modulates apoptotic signalling in immature myocardium. In contrast, the weaker induction of these adaptive genes in the St. Thomas group and the intermediate response observed with Custodiol HTK parallels their comparatively inferior mitochondrial performance.

Nevertheless, caution is warranted in interpreting transcriptional changes as direct functional determinants. Gene expression does not always translate proportionally into protein abundance or activity, and temporal dynamics may differ between ischemia and reperfusion phases. Further studies integrating proteomic and post-translational analyses would be required to delineate the precise molecular cascade by which cardioplegic formulations influence these pathways.

Within the broader debate regarding the optimal cardioplegic strategy for pediatric cardiac surgery, our findings suggest that the choice of solution influences not only energetic preservation but also the molecular programming of stress adaptation in immature myocardium. This integrated mitochondrial-transcriptional perspective provides a mechanistic framework that may help explain divergent clinical observations reported in the literature and highlights the importance of age-specific myocardial biology when evaluating cardioprotective strategies.

4.3. Cytokine responses to I/R across CP solutions

Although no statistically significant differences were observed between the groups, the overall trend indicates that both Custodiol HTK CP and del Nido CP most closely approximate the control-group (representing continuous

myocardial perfusion without ischemia.) Interestingly, peak values at the 2-hour time point likely reflect an early surge in the inflammatory response to I/R. These observations align with Gorjipour et al., who, comparing del Nido with a modified St. Thomas solution, reported no significant intergroup differences for TNF-α and IL-6, while documenting significant postoperative increases across cytokines following cardiac surgery [205].

In our dataset, anti-inflammatory cytokines declined over time in all cardioplegia arms, with the most pronounced decrease observed for IL-4. By contrast, IL-10 exhibited a comparatively attenuated decline and remained more closely aligned with CON-group throughout the observation window. Gorjipour et al. likewise noted a more favorable anti-inflammatory profile with the modified St. Thomas solution versus del Nido, including a moderately significant postoperative rise in IL-10; the authors attributed this to shorter dosing intervals that may limit myocardial rewarming, curb metabolic demand, and mitigate hypoxia [205].

Cytokine analysis revealed that, in our case, anti-inflammatory cytokines more accurately reflected the initial changes in the immature myocardium under ischemic conditions. However, there is no doubt that further and broader studies are needed to observe more pronounced differences.

Leptin, an adipocyte-derived adipokine, is a key regulator of energy balance, cellular metabolism, inflammatory/immune signaling, and cardiovascular homeostasis. Leptin resistance, prevalent in obesity and type 2 diabetes mellitus, denotes attenuated tissue responsiveness to leptin [208]. In the cardiovascular system, this resistance negatively affects the heart's stress response, promoting cardiac remodeling through impaired metabolism, increased fibrosis, vascular dysfunction, and heightened inflammation [208].

Elevated circulating leptin levels have been linked to an increased cardiovascular risk in humans. However, recent meta-analyses indicate that some epidemiological studies did not find this association. Studies conducted on mice deficient leptin or leptin receptors often yield contradictory results, showing both protective and harmful effects of leptin [209]. Additionally, mouse models have a significantly different lipoprotein metabolism compared with humans, which limits the extrapolation of these results to human physiology [209].

In our cohort, all cardioplegic regimens were associated with reductions in leptin concentrations following ischemia/reperfusion, with the effect most evident after prolonged ischemic intervals. This decline may reflect time-dependent modulation of cardiomyocyte protein networks during recovery. However, between group differences were not statistically significant, indicating that under the present conditions no single solution demonstrated superior leptin modulation.

It is also worth highlighting two important methodological aspects of our study that may have directly or indirectly influenced the results. The first concerns the use of the well-established Langendorff perfusion model employing a modified Krebs–Henseleit buffer. While this perfusate is widely accepted for ex-vivo cardiac experimentation, it does not fully replicate the full immunological and biochemical complexity of blood. Therefore, a different cytokine response could be expected if autologous blood were used for reperfusion following ischemia. It is plausible that reperfusion with autologous blood would yield more pronounced intergroup differences, particularly in pro-inflammatory markers.

The second aspect relates to the variation in perfusion and ischemia durations. In this study, we aimed to reproduce clinical conditions of cardiac surgery as realistically as possible; consequently, we applied different ischemic intervals in both absolute terms and in an approximate 3:1 ratio relative to reperfusion. It is plausible that maintaining an identical reperfusion time across groups might have attenuated the extent of reperfusion injury. Conversely, a disproportionately short reperfusion period following prolonged ischemia would likely exacerbate myocardial stress, as insufficient time for gradual reoxygenation and thermal recovery would prevent the myocardium from returning to a near-physiological state.

These considerations raise intriguing yet unresolved questions regarding the optimal balance between ischemia and reperfusion durations in experimental and clinical settings. Future studies, both in refined animal models and clinical investigations, will be essential to elucidate these mechanisms and optimize myocardial protection strategies.

4.4. Mechanistic insights into the superiority of Del Nido CP over Custodiol HTK and St. Thomas solutions

The superiority of Del Nido CP observed in our study appears to arise from a convergence of ionic modulation, mitochondrial stabilization, and transcriptional adaptation rather than from a single compositional attribute. When interpreted alongside the mitochondrial, gene expression, and cytokine data, a coherent mechanistic framework emerges. Mechanistically, improved maintenance of the ATP-synthase supramolecular organization with del Nido may relate to its capacity to limit pathological Ca^{2+} influx [182]. Consistent with this, experimental Ca^{2+} loading of mitochondria has been shown to trigger permeability transition pore (PTP) opening and reduce ATP-synthase synthesome levels by ≈20% [210] the PTP, an inner-membrane channel, is a key determinant of mitochondrial Ca^{2+} handling and matrix pH homeostasis [210, 211]. In our study, del Nido and the low-calcium Custodiol HTK regimen

outperformed St. Thomas on the relevant endpoints, although Gulsum Karduz et al. reported inferior functional and cellular protection with Custodiol versus St. Thomas and del Nido in mature-rat models. The discrepancy likely reflects age-dependent differences in Ca^{2+} handling and metabolic demand [212].

In addition to calcium homeostasis, Del Nido cardioplegia may exert its protective effects through modulation of mitochondrial redox balance. The reduced Ca^{2+} overload limits activation of the mitochondrial permeability transition pore (mPTP), thereby preventing cytochrome c release and downstream apoptotic signaling.

The presence of oxygen-carrying erythrocytes in the blood component also facilitates a more gradual reoxygenation profile, attenuating the burst of ROS typically seen at reperfusion a critical determinant of mitochondrial and sarcolemmal integrity.

Notwithstanding the shared single-dose delivery paradigm, several features plausibly account for del Nido's advantage over Custodiol HTK, particularly in immature myocardium, which exhibits heightened Ca^{2+} sensitivity. Custodiol HTK is a low-calcium, low-sodium crystalloid, whereas del Nido incorporates additional Ca^{2+}-modulating elements: magnesium sulfate (a physiological Ca^{2+} antagonist that dampens L-type Ca^{2+} current) and lidocaine, which blocks fast Na^{+} channels and thereby limits reverse-mode Na^{+}/Ca^{2+} exchanger–mediated Ca^{2+} entry [182] Moreover, the crystalloid base of del Nido is Ca^{2+}-free, further constraining trans sarcolemma Ca^{2+} flux during arrest.

Beyond Ca^{2+} control, the blood component of del Nido confers additional benefits germane to pediatric hearts with high metabolic rates: provision of oxygen during ischemia, delivery of metabolic substrates (e.g., glucose) and micronutrients, and superior buffering capacity via hemoglobin and plasma proteins, which stabilizes myocardial pH under low flow/ischemic conditions [150, 207, 213].

Collectively, these compositional and biophysical attributes offer a coherent rationale for the more favorable mitochondrial and functional profiles observed with del Nido in immature myocardium. While our findings demonstrate clear mitochondrial advantages of del Nido in immature myocardium, clinical validation in pediatric cardiac surgery is required to confirm translational relevance.

4.5. Integrative perspective

The present study provides an integrated analysis of mitochondrial respiration, transcriptional adaptation, and inflammatory modulation in immature myocardium exposed to three cardioplegic strategies. Across these

complementary levels of investigation, a consistent pattern emerged: Del Nido CP conferred the most coherent preservation of cellular homeostasis during I/R.

At the bioenergetic level, Del Nido preserved oxidative phosphorylation capacity, respiratory reserve, and mitochondrial coupling efficiency more effectively than St. Thomas and, to a lesser extent, Custodiol HTK. This preservation was accompanied by reduced cytochrome c release, indicating structural stabilization of mitochondrial membranes.

At the molecular level, Del Nido induced a coordinated transcriptional response characterized by upregulation of *HIF-1α*, *FOS*, and *BNIP2*. These changes suggest activation of adaptive hypoxic signalling, modulation of stress responses, and regulation of apoptosis. Importantly, the transcriptional profile did not indicate uncontrolled injury signalling but rather a regulated stress-adaptive program consistent with preserved anti-inflammatory markers and restrained pro-inflammatory activation.

When interpreted alongside mitochondrial preservation, these findings support the concept that limiting primary mitochondrial injury may attenuate secondary inflammatory amplification.

Taken together, our data suggest that mitochondrial stability constitutes the central axis of cardioprotection in immature myocardium. Ionic modulation, metabolic buffering, and adaptive gene activation appear to converge on preservation of mitochondrial membrane integrity and oxidative phosphorylation activity. Rather than acting through isolated mechanisms, effective CP in the immature heart may require coordinated control of calcium flux, redox balance, and stress-responsive transcription.

This integrative perspective is particularly relevant in pediatric cardiac surgery, where myocardial physiology differs fundamentally from that of the adult heart. Given the ongoing debate regarding the optimal cardioplegic strategy and the scarcity of subcellular data in immature myocardium, our findings contribute mechanistic clarity to an area characterized by clinical heterogeneity and methodological variability.

Although extrapolation from an experimental model must be undertaken cautiously, the convergence of mitochondrial, molecular, and inflammatory findings in this study supports the rationale for age-adapted cardioplegic strategies. Future translational investigations incorporating longer reperfusion intervals, proteomic analyses, and clinical correlation will be essential to determine whether these subcellular advantages translate into improved perioperative and long-term outcomes in pediatric patients.

CONCLUSIONS

1. Del Nido cardioplegic solution preserved mitochondrial respiratory function in immature myocardial tissue more effectively than Custodiol HTK and St. Thomas cardioplegic solutions, demonstrating greater mitochondrial stability and resistance to ischemic injury.
2. Del Nido cardioplegic solution elicited more favourable gene expression responses related to hypoxia adaptation, stress regulation, and apoptosis resistance under ischemia/reperfusion conditions compared with the Custodiol HTK and St. Thomas cardioplegic solutions, suggesting enhanced cellular protection at the molecular level.
3. Although cytokine changes were subtle, the patterns observed indicate that cardioplegic solutions differentially influence inflammatory responses in the immature myocardium, with Del Nido CP showing a tendency toward a more balanced inflammatory profile compared with Custodiol HTK and St. Thomas cardioplegic solutions.

SUMMARY

Direct head-to-head comparison of cardioplegic solutions in pediatric surgery is ethically and logistically challenging. Therefore, controlled *ex vivo* models, particularly the Langendorff perfusion system, remain indispensable for dissecting I/R mechanisms under standardized conditions, allowing precise control of temperature, perfusion parameters, and solution composition while simultaneously capturing functional, mitochondrial, and molecular endpoints.

The present study provides an integrated evaluation of mitochondrial respiration, gene expression responses, and inflammatory modulation in immature myocardium exposed to three cardioplegic strategies. Across the complementary levels of analysis, Del Nido cardioplegic solution demonstrated the most consistent preservation of myocardial homeostasis. It maintained oxidative phosphorylation capacity, limited cytochrome c release, promoted adaptive transcriptional responses (including *HIF-1α*, *FOS*, and *BNIP2*), and exhibited trends toward balanced inflammatory signalling.

Custodiol HTK cardioplegic solution provided intermediate protection, particularly through metabolic suppression and buffering capacity, whereas St. Thomas cardioplegic solution showed reduced mitochondrial and molecular resilience, especially during prolonged ischemia.

Collectively, these findings support the concept that mitochondrial stability constitutes the central determinant of effective myocardial protection in the immature heart. The data underscore the importance of age-specific cardioplegic selection and provide mechanistic insight into why Del Nido CP may confer advantages in pediatric cardiac surgery.

CLINICAL IMPLICATIONS

The findings of this study have meaningful translational implications. In pediatric cardiac surgery, where myocardial metabolic vulnerability and calcium handling differ from adult physiology, cardioplegic composition plays a critical role in postoperative recovery.

The demonstrated superiority of Del Nido cardioplegic solution in preserving mitochondrial function and promoting adaptive molecular responses supports its continued and potentially expanded application, particularly in procedures involving prolonged ischemic intervals.

Custodiol HTK cardioplegic solution may remain a viable alternative for prolonged procedures due to its metabolic suppression properties; however, its mitochondrial preservation appears less robust in immature myocardium. St. Thomas solution may be less optimal in pediatric settings, where mitochondrial resilience is paramount.

These findings emphasize the necessity of tailoring cardioplegic strategies to the myocardial developmental stage rather than extrapolating from adult data.

FUTURE DIRECTIONS

Future research should aim to correlate mitochondrial and transcriptional biomarkers with functional recovery in translational clinical pediatric models and clinical cohorts.

Integration of molecular markers, such as *HIF-1α* expression profiles or mitochondrial respiratory indices, into predictive models of myocardial recovery may enable more individualized cardioplegic strategies.

Long-term *in vivo* investigations and large-animal or clinical pediatric studies will be essential to determine whether the subcellular advantages observed experimentally translate into improved postoperative contractile function, reduced complications, and enhanced survival.

SANTRAUKA

ĮVADAS

Svarbiausias kardioplegijos tikslas yra apsaugoti širdį nuo išeminio miokardo pažeidimo. Miokardo audinio išemija yra progresuojantis procesas – ilgėjant išemijos trukmei, ląsteliniai ir molekuliniai pokyčiai darosi vis ryškesni, o laiku neatkūrus kraujotakos, galiausiai įvyksta ląstelių žūtis [1].

Per visą širdies chirurgijos istoriją buvo sukurta įvairių miokardo apsaugos metodų. Du dažniausiai naudojami komponentai yra hipotermija ir elektrocheminių gradientų moduliavimas naudojant kardiopleginį tirpalą. Keičiant elektrocheminius gradientus sukeliamas širdies sustojimas, kuris sukuria nejudrų operacinį lauką ir sumažina širdies raumenų ląstelių energijos sąnaudas. Visų kardiopleginių tirpalų tikslas – sustabdyti širdį ir sumažinti išeminį pažeidimą. Tačiau šiuo metu nėra bendros nuomonės, kuris tirpalas yra geriausias visoms pacientų grupėms, todėl buvo sukurta daugybė skirtingų tirpalų ir technikų [2].

Šie tirpalai gali būti plačiai suskirstyti į dvi kategorijas – depoliarizuojamuosius arba hiperpoliarizuojamuosius, atsižvelgiant į tai, kaip jie veikia miokardo ląstelių membraninį potencialą. Depoliarizuojamosios medžiagos dažniau naudojamos klinikinėje praktikoje, sukelia širdies sustojimą sukeliant hiperkalemiją. Hiperpoliarizuojamosios medžiagos yra hiponatremiškos ir hipokalceminės – jos sukelia širdies sustojimą mažindamos natrio ir kalcio koncentraciją užląstelinėje erdvėje.

Tiesioginis kardiopleginių tirpalų palyginimas vaikų chirurgijoje yra etiškai ir logistiškai neįmanomas. Todėl izoliuotos širdies Langendorff'o perfuzijos modeliai, išlieka auksiniu standartu kontroliuojamai širdies išemiaji bei perfuzijai / reperfuzijai tirti, leidžiantys kontroliuoti tirpalo temperatūrą, slėgį ir sudėtį, tuo pačiu fiksuojant funkcinius ir bioenergetikos rodmenis.

Mūsų tyrimo tikslas buvo imituoti klinikinę situaciją ir įvertinti trijų skirtingų kardiopleginių tirpalų – Del Nido, Custodiol HTK ir Šv. Tomo – poveikį širdies funkcijai nesubrendusių žiurkių išemijos / reperfuzijos (I/R) modelyje, naudojant Langendorff'o perfuzijos metodą.

Klinikinėje praktikoje naudojami kardiopleginiai tirpalai buvo vertinami atsižvelgiant į jų kardioprotekcines savybes. Šiai analizei atlikti buvo tiriama genų, susijusių su ląstelių ciklu, proliferacija, atsparumu apoptozei ir atsaku į hipoksiją, raiška širdies audinyje, taip pat buvo matuojami uždegimą skatinančių ir slopinančių citokinų lygmenys.

HIF-1α genas atlieka pagrindinę funkciją – skatina deguonies tiekimą, metabolinį prisitaikymą ir padeda ląstelėms prisitaikyti prie sumažėjusio de-

guonies kiekio širdies chirurgijos metu [12]. *FOS* genas, esantis AP-1 transkripcijos faktoriaus komplekse, palaiko ląstelių atsakus, pvz., proliferaciją ir išgyvenamumą chirurginio streso sąlygomis [13]. Tačiau *BNIP2* dalyvauja apoptozės reguliacijos procesuose ir jo kontrolė gali būti svarbi siekiant sumažinti širdies ląstelių netekimą bei išsaugoti širdies funkciją išeminių įvykių metu [14].

Taip pat mūsų tyrimo tikslas buvo įvertinti minėtų kardiopleginių tirpalų poveikį nesubrendusio miokardo mitochondrijų funkcijai. Mitochondrijų kvėpavimo indeksas buvo vertintas membranos skaidulose – palaipsniui buvo leidžiami tam tikri substratai, kurie tiekia elektronus per skirtingas elektronų pernašos grandinės dalis.

Mūsų tyrimas atliktas parodo, kaip kiekvienas kardiopleginis tirpalas veikia ląstelės subląstelinius kelius ir mitochondrijų bioenergetiką tiek išemijos sąlygomis, tiek I/R pažeidimo atveju. Šiame tyrime siekėme atskleisti kardioprotekcinius šių tirpalų veikimo mechanizmus nesubrendusiame miokarde ir jų reikšmę gerinant chirurginius rezultatus.

Darbo tikslas

Tyrimo tikslas buvo įvertinti skirtingų kardiopleginių tirpalų (Del Nido, Custodiol HTK, Šv. Tomo) poveikį mitochondrijų kvėpavimo funkcijai, genų ekspresijai ir citokinų atsakui nesubrendusių žiurkių širdies modelyje.

Tyrimo uždaviniai

1. Įvertinti ir palyginti Custodiol HTK, Šv. Tomo ir Del Nido kardiopleginių tirpalų poveikį mitochondrijų kvėpavimo funkcijai nesubrendusio miokardo audinyje.
2. Palyginti genų raiškos atsakus į Del Nido, Custodiol HTK ir Šv. Tomo kardiopleginius tirpalus nesubrendusio miokardo audinyje išemijos / reperfuzijos sąlygomis, taikant Langendorff metodą.
3. Įvertinti skirtumus tarp uždegimą skatinančių ir slopinančių citokinų moduliacijos nesubrendusio miokardo audinyje, reaguojant į Del Nido, Custodiol HTK ir Šv. Tomo kardiopleginius tirpalus.

Tyrimo naujumas ir aktualumas

Idealaus kardiopleginio tirpalo paieškos tęsiasi jau daugiau nei dešimtmetį. Per šį laikotarpį atlikta daugybė mokslinių tyrimų, siekiant išanalizuoti įvairių kardiopleginių tirpalų poveikį miokardui. Tačiau dauguma šių tyrimų daugiausia dėmesio skyrė tradicinės kraujo kardioplegijos ir Custodiol, Del Nido arba kitų kristaloidinių tirpalų palyginimui ir jų poveikiui širdies rau-

mens ląstelėms. Net ir naujausi 2019–2024 m. publikuoti straipsniai nepateikia galutinių išvadų, o kai kuriuose pateikiami netgi prieštaringi rezultatai. Todėl diskusijos dėl optimalaus kardiopleginio tirpalo pasirinkimo išlieka aktualios.

Taip pat svarbu pažymėti, kad vaikų miokardo fiziologija reikšmingai skiriasi nuo suaugusiųjų širdies raumens. Literatūroje pateikiami prieštaringi duomenys apie nesubrendusią širdį: vieni tyrimai rodo didesnį atsparumą išemijai [18–20], o kiti – mažesnę toleranciją [21, 22].

Kardiopleginiai tirpalai yra būtini nesubrendusio miokardo apsaugai širdies operacijų metu, nes jie sukelia laikiną širdies sustojimą, sumažina išemijos ir reperfuzijos pažeidimus ir padeda išlaikyti mitochondrijų bei bendrą širdies funkciją. Miokardo apsauga tampa ypač sudėtinga ilgai trunkančių ir sudėtingų operacijų metu arba, naujagimiams kurių miokardas prieš operaciją jau yra pažeistas [23]. Tokiais atvejais optimalaus kardiopleginio tirpalo pasirinkimas sukelia daugiau klausimų nei pateikia atsakymų. Eksperimentiniais tyrimais nustatyta, kad naujagimių širdžiai tinkamesnė vienos dozės kardioplegija [24], tačiau kitais tyrimais, palyginti su daugkartine kardioplegija, nenustatyta reikšmingų skirtumų [25].

Mūsų tyrimas buvo sutelktas į viduląstelinius nesubrendusių kardiomiocitų pokyčius, nes būtent jie atspindi ankstyviausius pakitimus po kardioplegines intervencijos. Siekiant įvertinti, ar tam tikros kardiopleginės formulės optimaliai apsaugo nesubrendusio miokardo audinį širdies chirurgijos metu, būtina atlikti tikslinį ikiklinikinį tyrimą, kuriame būtų nagrinėjami mitochondrijų kvėpavimo keliai, su kardioprotekcija ir ląstelių stresu susiję genų ekspresijos profiliai bei pro- ir antiuždegiminių citokinų atsakų spektras.

Ši tyrimų kryptis yra itin aktuali, nes tarptautinėje literatūroje vis dar išlieka metodologinis nevienalytiškumas ir prieštaringi rezultatai, todėl egzistuoja reikšmingos žinių spragos, susijusios su nesubrendusia širdimi. Nauja, išsami informacija, gauta tiriant nesubrendusio miokardo audinį naudojant skirtingus kardiopleginius tirpalus, būtų vertinga tiek tarptautiniu mastu, tiek ir Lietuvoje. Nors disertacijoje atliktas tyrimas buvo paremtas gyvūnų modeliu, įgytos naujos žinios galėtų būti pritaikytos ir klinikinėje praktikoje

DARBO METODIKA

Disertaciniame darbe atlikti eksperimentiniai tyrimai, naudojant gyvūnų modelį. Šių eksperimentų tikslas – įvertinti ir palyginti klinikinėje praktikoje taikomus kardiopleginius tirpalus, atsižvelgiant į jų poveikį nesubrendusio miokardo perfuzijai, pokyčius ląsteliniame ir vidųlasteliniame lygyje, mitochondrijų kvėpavimo funkciją išemijos / reperfuzijos sąlygomis bei į pro- ir

antiuždegiminių citokinų atsakų skirtumus, siekiant nustatyti jų efektyvumą ir tinkamumą kliniškai reikšmingame kontekste.

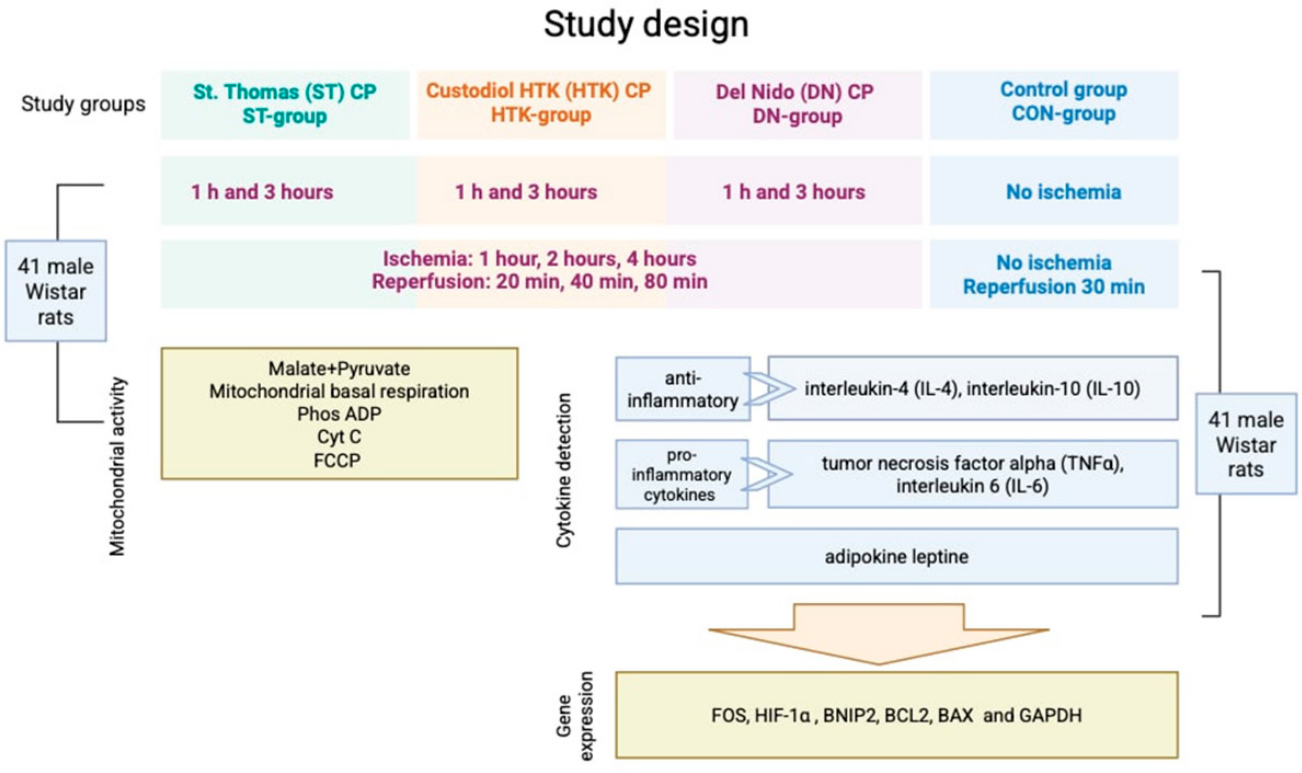
Tyrimai su gyvūnais buvo vykdomi laikantis Europos konvencijos dėl stuburinių gyvūnų apsaugos, naudojamų eksperimentiniais ir kitais moksliniais tikslais (Nr. 49-1883, 49-1884), bei Valstybinės maisto ir veterinarijos tarnybos direktoriaus įsakymo (Nr. B1-866) „Dėl reikalavimų, taikomų gyvūnų laikymui, priežiūrai ir naudojimui mokslo ir mokymo tikslais, patvirtinimo“. Tyrimo protokolą patvirtino Lietuvos Respublikos Valstybinė maisto ir veterinarijos tarnyba (Nr. G2-265).

Tyrimuose naudotų gyvūnų skaičius buvo kiek įmanoma mažesnis, o eksperimentinis planavimas atitiko 3R principus (pakeitimas, mažinimas, tobulinimas). Skausmas ir stresas buvo maksimaliai sumažinti taikant anesteziją ir humaniškus baigiamuosius kriterijus.

Vieno mėnesio Wistar linijos žiurkės patinai, sveriantys 90 ± 30 g, buvo gauti iš Lietuvos sveikatos mokslų universiteto (LSMU) Veterinarijos akademijos (Kaunas, Lietuva). Gyvūnai buvo laikomi narvuose, po ne daugiau kaip penkias žiurkes viename narve, esant standartiniam 12 val. šviesos / 12 val. tamsos režimui ir 22 ± 2 °C temperatūroje. Jie buvo šeriami standartinėmis granulėmis ir turėjo neribotą prieigą prie geriamojo vandens.

Tyrimų struktūra

Kaip pavaizduota 1 pav., šią disertaciją sudaro skirtingos dalys, apjungiančios trijų skirtingų kardiopleginių tirpalų poveikio miokardo mitochondrijų kvėpavimo rodikliams, genų ekspresijai ir uždegiminių citokinų raiškai palyginamuosius tyrimus. Toks integruotas požiūris leidžia viename modelyje ne tik išsamiai įvertinti fiziologinį skirtingų kardiopleginių tirpalų poveikį, bet ir atskleisti molekulinius mechanizmus, lemiančius miokardo apsaugą.



1 pav. *Schematinis suskirstymas į grupes ir darbų eigos schema*

Tyrimuose naudojant jaunos žiurkės išeminio miokardo modelį, pirmame etape nagrinėjome, kaip kardiopleginiai tirpalai (Custodiol HTK, Del Nido arba Šv. Tomo) paveikia mitochondrijų kvėpavimo grandinės veiklos ir oksidacinio fosforilinimo efektyvumą. Antrame etape tyrėme genų, susijusių su hipoksijos atsaku (*HIF1α*), proliferacijos ir apoptozės reguliacijos (*FOS*, *BNIP2*, *BCL2/BAX*) raišką. Trečiame etape siekėme išsiaiškinti pro- ir anti-uždegiminių citokinų (IL-4, IL-6, IL-10, TNF-α, leptino) pokyčius I/R metu.

In vivo širdies išemijos sukėlimas

Tyrimas buvo atliktas LSMU Medicinos akademijos (MA) Kardiologijos instituto Vaistinių medžiagų ikiklinikinių tyrimų laboratorijoje. Buvo panaudota 41 Wistar žiurkė. Žiurkės buvo ruošiamos vadovaujantis standartiniais eksperimentinių gyvūnų protokolais. Anestezija buvo sukeliama naudojant CO_2 kamerą. Krūtinės ląsta buvo steriliai paruošta, atlikta vidurinė sternotomija. Aorta buvo izoliuota, o apatinė tuščioji vena – perpjauta

Į aortą buvo suleista šalto kardiopleginio tirpalo (Custodiol HTK, Del Nido arba Šv. Tomo), turinčio heparino (5 TV/g kūno svorio). Gyvūnai buvo suskirstyti į keturias grupes (1 pav.): CON-grupė – be kardioplegijos (n = 5), ST-grupė – su Šv. Tomo kardioplegija (n = 12), HTK-grupė – su Custodiol HTK kardioplegija (n = 12) ir DN-grupė – su Del Nido kardioplegija (n = 12). Kardioploginiai tirpalai buvo vertinami esant dviem skirtingoms išemijos trukmėms (1 val. ir 3 val.) ir kiekvienoje grupėje atlikta po 6 eksperimentus.

Pažymėtina, kad CON-grupėje miokardo išemija nebuvo taikyta – atvėrus krūtinės ląstą širdis buvo skubiai išimta ir supjaustyta smulkiais gabaliukais, ruošiant mitochondrijų tyrimui. Del Nido tirpalui buvo naudojamas heparinizuotas autologinis žiurkės kraujas, paimtas iš uodegos ir sumaišytas su tirpalu santykiu 4:1 (tirpalas:kraujas).

Kardiopleginiai tirpalai buvo naudojami intrakardialinei kraujagyslių sistemai išplauti, širdžiai sustabdyti ir užtikrinti miokardo apsaugą, o veninis nutekėjimas buvo nukreipiamas per apatinę tuščiąją veną.

Širdies mitochondrijų izoliavimas

Po širdies sustabdymo širdis buvo išimama. Iškart po išėmimo, širdys buvo panardintos į kardiopleginį tirpalą 4 °C temperatūroje. Išemijos trukmė buvo 1–3 val., atsižvelgiant į eksperimentinę grupę, naudojant maždaug 2–3 kartus didesnį kardiopleginio tirpalo tūrį (apie 10 ml). Pasibaigus 1 ar 3 val. inkubacijai, širdys buvo perkeltos į iš anksto atšaldytus 35 mm stiklo indelius (5 ml). Likutinis kraujas buvo pašalintas naudojant homogenizacijos terpę (HT). Vėliau širdys buvo supjaustytos mažais gabaliukais, kurie laikyti ant

ledo, visa procedūra buvo atlikta kiek įmanoma greitai. Maži audinio gabaliukai perkelti į mėgintuvėlį su HT tolesnei homogenizacijai.

Terpės ir reagentai

MIR ir HM tirpalai buvo paruošti LSMU Medicinos akademijos (MA) Kardiologijos instituto Ikiklinikinių vaistų tyrimų laboratorijoje. Pridėtinių medžiagų sąrašas pateiktas lentelėje.

1 lentelė. *Tyrimuose naudotų buferių / terpių sudėtis*

Tirpalas	**Paruošimas**
Mitochondrijų kvėpavimo (MIR) buferis	200 mL; naudojamas Oroboros matavimams: $MgCl_2 \cdot 6H_2O$ – 3 mmol (0,35 g) KH_2PO_4 – 10 mmol (0,2722 g) EGTA – 0,5 mmol (0,038 g) Laktoninė rūgštis – 60 mmol (24 mL atsargų tirpalo) Taurinas – 20 mmol (0,5004 g) HEPES – 20 mmol (0,954 g) D-sacharozė – 110 mmol (7,53 g) BSA (be riebalų rūgščių) – 1 g/L (0,2 g) pH sureguliuotas iki 7,1 esant 30 °C
Homogenizacijos terpė (HM)	100 mL: KCl – 160 mmol (1,22 g) NaCl – 10 mmol (58 mg) Tris–HCl (Tris bazė) – 20 mmol (242 mg) EGTA – 2 mmol (76 mg) pH sureguliuotas iki 7,7 esant 2 °C

Baltymų kiekis buvo nustatomas Bradford metodu, o membranų pralaidumui įvertinti naudotas Triton X-100 (0,1%).

Kardiopleginiai tirpalai (Šv. Tomo ir Custodiol HTK) buvo gauti iš LSMU Širdies, krūtinės ir kraujagyslių chirurgijos klinikos. Del Nido tirpalas buvo paruoštas LSMU MA Farmacijos fakulteto Vaistų chemijos katedroje. Jų sudėtys pateiktos lentelėse žemiau.

2 lentelė. *Tyrimuose naudotų kardiopleginių tirpalų sudėtis*

Tirpalas	Sudėtis (mmol/l)	Savybės
Custodiol HTK	K 9 Na 15 Mg 4 Ca 0,015 Ketogluratas 1 Tryptofanas 2 Histidinas 198 Manitolis 30	Triptofanas naudojamas membranos stabilizacijai. Ketogluratas skirtas padidinti energijos gamybą reperfuzijos metu – ATF pirmtakas. Manitolis ląstelių membranų reguliavimui, osmoliariškumui, turi diuretines savybes. Naudojant manitolį mažėja išemijos-reperfuzijos sukelti miokardo edemos pokyčiai. Histidino buferis, palaikantis anaerobinę glikolizę ir geriau palaikantis pH, pooperacinių elektrolitų ir metabolizmo lygius. Custodiol HTK yra hiperpolarizuojantis tirpalas, tokių būdų apsaugo nuo kalcio perkrovos, nes nedaug kanalų ar jonų siurblių yra aktyvūs esant hiperpoliarizuotam membranos potencialui.
Del Nido	Plasma-Lyte A bazė Manitolis 16,3 Magnio sulfatas 4 Natrio bikarbonatas 13 Kalio chloridas 13 Lidokainas 13	Magnio sulfatas, veikiantis kaip kalcio antagonistas, kalcio kanalo konkurentas, blokuoja kalcio patekimo į kardiomiocitus. Natrio bikarbonatas – naudojamas kaip buferis, padeda artinti Ph rodiklį link 7,4, taip palaikant glikolizę visos išemijos metu. Lidokainas, IB klasės antiaritminis vaistas, veikiantis kaip Na^+ kanalų blokatorius. Del Nido tirpalo kristaloido ir kraujo santykis yra 4: 1. Pats kardioplegijos tirpalas yra ekstraląstelinis tirpalas, sumaišytas su autologiniu krauju, gautu iš dirbtinės kraujo apytakos. Kraujo pridėjimas į tirpalą sumažina išemijos-reperfuzijos pažeidimus, lyginant su visiškai kristaloidinių tirpalu
Šv. Tomo	K 16 NaCl 120 Ca 1,2 Mg 16 $NaHCO_3$ 10	Sukelia greita širdies sustojimą diastolės metu, dėl didelio kalio kiekio. Mažina išemijos poveikį miokardui. Kardioplegiją reikia kartoti kas 30 min.

Homogenizacija

Homogenizacija atlikta mėgintuvėlyje. Gautas homogenatas centrifuguotas du kartus: pirmą kartą – 5 minutes, esant 1000 *g*, antrą kartą – 10 minučių, esant 6800 *g*. Šie etapai leido išsiskirstyti bei tirti mitochondrijų kvėpavimo grandinės kelius, naudojant specifinius kardiopleginius tirpalus žiurkių širdies audinyje. Po antrojo centrifugavimo mitochondrijų nuosėdos buvo išplautos ir suspenduotos homogenizacijos terpėje (HT). Tirpalai buvo surinkti į 1,5 ml Ependorf'o mėgintuvėlius ir laikomi ant ledo.

Eksperimentinė įranga ir Oroboros vertinimo protokolas

Oksidacinio fosforilinimo parametrų matavimai izoliuotose širdies mitochondrijose buvo atlikti naudojant Oxygraph-2k® (*Oroboros Instruments*®, Austrija). Matavimai buvo atliekami 37 °C temperatūroje 2 ml kameroje, pripildytoje mitochondrijų kvėpavimo terpės (MIR). Duomenų surinkimas ir analizė buvo vykdoma naudojant DatLab programinę įrangą (*Oroboros Instruments*®).

Oxygraph-2k® yra didelės raiškos, uždaros dviejų kamerų respirometras, skirtas tiksliai kvėpavimo funkcijai matuoti [168, 169]. Prietaisas turi barometrinio slėgio jutiklį, kuris automatiškai atlieka deguonies kalibravimą ir yra integruotas su DatLab programine įranga. Eksperimento metu mitochondrijų energijos apykaita buvo išsamiai įvertinta realiuoju laiku titruojant įvairius substratus, atjungiklius, inhibitorius ir kitas medžiagas.

Išemijos sukėlimas *ex vivo* širdyje

Eksperimentai buvo atlikti LSMU Medicinos akademijos (MA) Kardiologijos instituto Membranų biofizikos laboratorijoje. Gyvūnai buvo suskirstyti į tris kardioplegijos grupes (po keturias žiurkes kiekvienoje grupėje): Šv. Tomo (ST), Custodiol HTK (HTK) ir Del Nido (DN). Kiekviena grupė buvo papildomai suskirstyta pagal išemijos–reperfuzijos trukmę:

1 pogrupis – 1 val. išemijos + 20 min. reperfuzijos,
2 pogrupis – 2 val. išemijos + 40 min. reperfuzijos,
3 pogrupis – 4 val. išemijos + 80 min. reperfuzijos.

Kontrolinės grupės (K-PRF, n = 5) žiurkėms perfuzija buvo atliekama 30 minučių nesukeliant miokardo išemijos.

Tyrimuose naudotas retrogradinis Langendorf'o perfuzijos širdies modelis [170, 171]. Buvo atlikti sekantys I/R eksperimentų etapai:

- *Anestezija ir chirurginis paruošimas*. Žiurkių eutanazija atlikta pagal standartinius eksperimentinių gyvūnų protokolus. Anestezija buvo sukelta ketaminu (90 mg/kg) ir ksilazinu (9 mg/kg), gilioji anestezija patvirtinta išnykus pėdos atitraukimo refleksui. Po oda buvo suleista heparino (5 TV/g kūno svorio) likus 10 minučių iki kaklo dislokacijos. Oda įpjauta ties kardine atauga. Krūtinės ląsta atverta, pašalinta užkrūčio liauka, atvertas perikardas. Izoliuota aorta, viršutinė ir apatinė tuščiosios venos
- *Širdies išėmimas ir kanuliavimas*. Širdis buvo greitai išpjauta (išimta) ir nedelsiant perkelta į indą su šaltu perfuziniu tirpalu. Aorta buvo atsargiai kaniuliuota, siekiant išvengti aortos vožtuvo pažeidimo, ir užveržta naudojant dvigubą šilko siūlą (Nr. 5-0). Tyrime naudotas Langendorff retrogradinis širdies perfuzijos metodas. Širdis prijungiama prie Lan-

gendorff sistemos per 5 minutes. Vėliau, 15 minučių buvo atliekama širdies perfuzija sistemoje, kad atsistatytų beveik fiziologinis funkcinis lygis.

- *Perfuzijos sąlygos ir tirpalai*. Perfuzija buvo vykdoma pastovaus srauto režimu, stebint, kad į širdį nepatektų oro burbulai ir nesukeltų embolijų. Perfuzijai naudotas modifikuotas Krebs-Henseleit tirpalas (NaCl 135,0 mmolM, $MgCl_2$ 0,9 mmolM, NaH_2PO_4 0,33 mmolM, KCl 5,4 mmolM, $CaCl_2$ 1,0 mmolM, gliukozė 10 mmolM, HEPES 10 mmolM), paruoštas dejonizuotajame vandenyje. Tirpalas prisotintas deguonimi burbuliuojant 95 proc. O_2.
- *Temperatūros kontrolė ir tirpalo pratekėjimo matavimai*. Temperatūros kontrolė ir srauto matavimai.Vainikinė kraujotaka matuota pagal 2 minučių laikotarpiu surinkto perfuzijos skysčio tūrio matavimus. Visos procedūros metu buvo nuosekliai kontroliuojama perfuzijos tirpalo ir rezervuaro, apgaubto vandens apvalkalu, temperatūra. Šilumokaitis palaikė normotermiją (+37 °C), o kardiopleginiai tirpalai buvo palaikomi +4 °C temperatūroje. Po sustabdymo širdis buvo laikoma vonelėje, kurios temperatūra siekė 18–22 °C; temperatūra buvo stebima ir registruojama kas 15 minučių, siekiant užtikrinti stabilumą viso išemijos laikotarpio metu.
- *Kardioplegijos protokolas*. Maždaug 10 ml šalto (+4 °C) kardiopleginio tirpalo (ST, HTK arba DN) suleista į aortą. Kardiopleginio tirpalo infuzija buvo atliekama 2 minutes. Į DN tirpalą buvo pridėta heparinizuoto žiurkės kraujo, paimto iš uodegos, santykiu 4 : 1 (4 dalys kristaloido ir 1 dalis kraujo). Fiksuotas laikas iki asistolijos pradžios. Kardioplegijos papildomos dozės (kartotinė kardioplegija) buvo skiriamos kas 30 min. ST grupėse ir kas 90 min. DN grupėse. HTK grupėse buvo naudojama tik vienkartinė dozė.

Pasibaigus eksperimentui, visas kairysis skilvelis buvo išpjautas ir užšaldytas –80 °C temperatūroje tolesnei analizei.

Realiojo laiko PGR (qPCR)

Genų raiškos analizė realiojo laiko PGR (qPCR) metodu buvo atlikta norint tiksliau įvertinti kardiopleginių tirpalų skirtumus ir įvertinti apoptozės, hipoksijos ir proliferacijos procesus širdies audinio ląstelėse. *HIF1α* genas yra svarbus ląstelių atsakui į hipoksiją – dažną būklę širdies chirurgijos metu, kai sumažėja kraujo tiekimas. *HIF1α* aktyvina kelius, padedančius ląstelėms išgyventi streso metu – skatina deguonies tiekimą ir metabolinį prisitaikymą [172]. *FOS* genas yra AP-1 transkripcijos faktoriaus komplekso dalis,

reguliuojantis ląstelių proliferaciją, diferenciaciją ir išgyvenimą, ypač esant stresui [13]. Jo moduliacija padeda širdies audiniui reaguoti į chirurginį ir išeminį stresą. *BNIP2* genas dalyvauja apoptozės procese [173, 174]. Chirurginės intervencijos metu apoptozės reguliavimas yra esminis siekiant išsaugoti širdies funkciją, o *BNIP2* moduliavimas gali sumažinti nereikalingą ląstelių žūtį.

Širdies audiniai buvo homogenizuoti naudojant skystąjį azotą, kaip aprašyta toliau. Mėginiai nedelsiant buvo paveikti lizės TRIzol™ reagentu (*Invitrogen*, Nyderlandai). Bendroji RNR buvo išskirta naudojant *PureLink*™ RNA *Mini Kit* (*Invitrogen*, Nyderlandai) pagal gamintojo instrukciją. RNR koncentracija ir grynumas buvo įvertinti *NanoDrop* 1000 spektrofotometru (*Thermo Fisher Scientific*, Nyderlandai). Atvirkštinė transkripcija atlikta naudojant *High-Capacity* RNA-to-cDNA™ rinkinį (*Applied Biosystems*, JAV). Realiojo laiko qPCR atlikta naudojant *TaqMan*™ *Gene Expression* metodiką (*Applied Biosystems*, JAV) pagal gamintojo protokolą.

Buvo tiriami: ***FOS*** (Rn02396759_m1), ***HIF-1α*** (Rn01472831_m1), ***BNIP2*** (Rn01530716_m1), ***BCL2*** (Rn99999125_m1), ***BAX*** (Rn01480161_g1) ir ***GAPDH*** (Rn01775763_g1) genai. Genų raiškos duomenys normalizuoti pagal *GAPDH* geną ir apskaičiuoti taikant 2-ΔΔCT metodą.

Citokinų nustatymo analizė

Taikytas *Luminex*® (*Luminex Corporation*, *Austin*, TX, JAV) daugialypės analizės metodas, siekiant kiekybiškai įvertinti prouždegiminius ir antiuždegiminius citokinus nesubrendusioje žiurkių širdies audinio terpėje. Iš pradžių širdies audiniai buvo surinkti ir nedelsiant paruošti, siekiant sumažinti baltymų degradaciją. Audiniai sumalti ir homogenizuoti naudojant skystąjį azotą. Kiekvienas mėginys buvo pasvertas ir perkeltas į 2 ml mikrocentrifuginį mėgintuvėlį. Vėliau į kiekvieną 100 mg audinio buvo pridėta 500 µl ląstelių lizės buferio, siekiant suardyti ląstelines struktūras ir išlaisvinti vidulląstelinius komponentus.

Homogenizacija atlikta naudojant *TissueLyser*, veikiančią 25 Hz dažniu 2 minutes – ši trukmė nustatyta kaip optimali, siekiant gauti vientisą homogenatą neperkaitinant mėginio. Po homogenizacijos mėginiai centrifuguoti 16 000 × *g*, 10 min. esant 4 °C temperatūrai. Skaidrus supernatantas buvo perkeliamas į naują mėgintuvėlį, o bendras baltymų kiekis nustatytas naudojant *Bio-Rad*™ DC *Protein Assay Kit*.

Naudojant *Rat Custom ProcartaPlex Mix&Match 5-Plex Kit* (*Thermo Fisher Scientific*, Austrija) pagal gamintojo protokolą kvantifikuoti (įvertinti kiekybiškai) šie citokinai:

- Antiuždegiminiai: interleukinas-4 (IL-4), interleukinas-10 (IL-10);

- Adipokinas: leptinas;
- Prouždegiminiai: naviko nekrozės faktorius alfa (TNFα), interleukinas-6 (IL-6).

Mėginiai buvo analizuoti po du kartus. Duomenų analizė atlikta naudojant *Luminex* xPONENT programinę įrangą, citokinų koncentracijos nustatytos pagal kiekvienam citokinui sudarytas kalibracines kreives.

Statistinė analizė

Visos statistinės analizės, susijusios su mitochondrijų tyrimu, buvo atliktos naudojant SPSS 29 versiją (IBM *Corp., Armonk*, NY, JAV). Visi duomenys pateikiami kaip mediana arba procentiliai (25–75). Grupėms palyginti taikytas nepriklausomų imčių Kruskal-Wallis testas. Statistiškai reikšminga buvo laikoma p vertė, mažesnė nei $p < 0,05$.

Langendorff'o perfuzijos metodu atlikto tyrimo imties dydis nustatytas įvertinus Cohen'o d efektą. Duomenų normalumas buvo tikrinamas naudojant Shapiro-Wilk testą.

Remiantis tyrimo dizainu buvo naudoti trys kardiopleginiai tirpalai (Šv. Tomo, Custodiol HTK, Del Nido) ir trys išemijos trukmės (1, 2 ir 4 valandos). Buvo atlikta duomenų daugiafaktorinė analizė. Dviejų veiksnių dispersijos analizė (ANOVA) buvo taikoma siekiant įvertinti pagrindinius kardiopleginių tirpalų ir išemijos trukmės poveikius, taip pat jų sąveiką genų ekspresijai (*HIF1α*, *FOS*, *BNIP2*, *BAX/BCL2*) ir citokinų koncentracijoms (IL-4, IL-6, IL-10, leptinas, TNF-α). Buvo atliekamos post hoc palyginimo analizės tarp grupių. Dispersijų vienodumas buvo vertinamas naudojant Levene testą.

Statistiškai reikšminga buvo laikoma p vertė < 0,05. Visos analizės atliktos naudojant SPSS v22.0 programinę įrangą, o rezultatai vizualizuoti naudojant GraphPad Prism 9 programą.

REZULTATAI

Mitochondrijų kvėpavimas

Mitochondrijų kvėpavimas buvo vertinamas praėjus 1 valandai nuo miokardo išemijos.

Rezultatai parodė, kad po 1 val. išemijos reikšmingai didesnis mitochondrijų aktyvumas buvo stebėtas DN grupėje:

1. Lyginant pagrindinį mitochondrijų deguonies suvartojimo greitį tarp skirtingų kardiopleginių grupių, didžiausias mitochondrijų suvartojimo greitis buvo nustatytas DN, o mažiausias – ST grupėje:
   - DN *plg.* ST: 27,8 [17,4; 34,6] *plg.* 8,5 [7,7; 14,6]; $p = 0,006$.

2. Įdėjus ADP substrato, didžiausias mitochondrijų kvėpavimas, susijęs ATP sinteze, buvo nustatytas DN grupėje, panašus į CON kontrolinę grupę, kurioje kardiopleginis tirpalas nebuvo naudotas. ADP vertės buvo reikšmingai didesnės DN grupėje, palyginti su ST ir HTK:
   - ST *plg.* DN: 55,51 [25,94; 64,17] *plg.* 107,18 [87,71; 130,32]; p = 0,001
   - HTK *plg.* DN: 60,41 [54,08; 68,29] *plg.* 107,18 [87,71; 130,32]; p = 0,011
   - CON *plg.* ST: 98,28 [62,67; 180,43] *plg.* 55,51 [25,94; 64,17]; p = 0,019
3. Taip pat buvo lygintas mitochondrijų kvėpavimas tarp grupių, įdėjus mitochondrijų oksidacinio fosforilinimo atjungiklį. Įdėjus FCCP substrato, maksimalus kvėpavimas buvo reikšmingai didesnis DN grupėje lyginant su ST-grupe:
   - DN *plg.* ST: 173,4 [146,0; 252,9] *plg.* 71,1 [49,7; 84,4]; p = 0,001

Tarp grupių reikšmingo skirtumo nenustatyta pagal su ATP sinteze susijusiu ir pagrindiniu kvėpavimu santykio (ADP/Mit). Taip pat nebuvo reikšmingų skirtumų vertinant išorinės mitochondrijų membranos būklę pagal Cyt. C poveikį (Cyt/Phos [ADP]).

4. Mitochondrijų kvėpavimo vertinamas po 3 valandų miokardo išemijos.
   - Ilgalaikės išemijos (3 val.) metu Mit, ADP ir FCCP parametrai išliko statistiškai reikšmingi – rodė DN grupės pranašumą. HTK ir DN grupėse mitochondrijų aktyvumas buvo didesnis nei ST, o DN grupėje ADP vertės buvo reikšmingai didesnės nei ST grupėje:
   - DN *plg.* ST: 105,7 [86,2; 154,4] *plg.* 24,2 [11,7; 51,7]; p = 0,008, nors reikšmingo skirtumo tarp DN ir HTK grupių nenustatyta. Taip pat buvo ištirtas mitochondrijų membranų vientisumas po 3 val. išemijos skirtingose grupėse. Kvėpavimo kreivėse užfiksuotas 5 μM egzogeninio Cyt C poveikis kvėpavimui. Didžiausias CytC/ADP santykis buvo stebėtas ST grupėje: buvo reikšmingai didesnis, palyginti su DN:
   - ST *plg.* DN: 1,6 [1,5; 2,2] *plg.* 1,2 [1,1; 1,4]; p = 0,004, tai rodo išorinės mitochondrijų membranos pažeidimą. DN grupėje taip pat buvo nustatytas didžiausias ADP/Mit santykis, kuris buvo reikšmingai didesnis nei ST grupėje:
   - DN *plg.* ST: 5,22 [3,12–6,32] *plg.* 1,26 [1,09–3,87]; p = 0,035, tai rodo geriausią mitochondrijų kokybės indeksą.

Genų raiška

Kardiopleginių tirpalų poveikis ląstelių atsparumui apoptoziniams dirgikliams vertinimas

Išemijos ir reperfuzijos (I/R) pažeidimas lemia ląstelių žūtį daugiausia dėl staigaus kraujo ir deguonies tiekimo atsikūrimo anksčiau hipoksinėms audinių zonoms. Tai lemia perteklinę reaktyviųjų deguonies formų (ROS) gamybą ir sukelia uždegiminį atsaką, galiausiai – reikšmingą ląstelių pažeidimą ir žūtį [176].

Siekiant įvertinti ląstelių jautrumą apoptoziniams dirgikliams, širdies chirurginių intervencijų metu taikant skirtingus kardiopleginius (CP) tirpalus, buvo tiriama genų, koduojančių *BCL2* (apoptozės reguliatorių) ir *BAX* (su *BCL2* susijusį apoptozės reguliatorių), raiška. *BAX/BCL2* raiškos santykis gali būti naudojamas kaip ląstelės atsako į apoptozinius dirgiklius indikatorius: padidėjęs santykis rodo sumažėjusį atsparumą apoptozei. Šie genai yra glaudžiai susiję ir veikia kaip atitinkamai proapoptoziniai ir antiapoptoziniai reguliatoriai [177].

Rezultatai parodė, kad *BAX/BCL2* santykis audiniuose, gydytuose Del Nido (DN) tirpalu, buvo maždaug 50 proc. mažesnis nei ST ir HTK grupėse, tačiau šie rezultatai nebuvo statistiškai reikšmingi. Galima manyti, kad DN gali skatinti ląstelių atsparumą apoptoziniams dirgikliams – taip suteikti geresnį kardioprotekcinį efektą, palyginti su ST (Šv. Tomo) ir HTK (Custodiol HTK) tirpalais.

Kardiopleginių tirpalų poveikio ląstelių funkcijoms skirtumai

Norint išsamiau įvertinti kardiopleginių tirpalų poveikį širdies audinio ląstelių procesams, buvo tiriama genų, koduojančių *BCL2* sąveikaujantį baltymą *BNIP2*, protoonkogeną *FOS* ir hipoksijos sukeliamąjį veiksnį *HIF1α*, raiška.

Žinoma, kad *BNIP2* yra svarbus griaučių raumenų diferenciacijai [178], taip pat nuo miozino II priklausomam kontraktiliškumui – *BNIP2* geno slopinimas mažina aktino siūlų atsikūrimo greitį [178]. Pagal mūsų duomenis, DN tirpalu paveiktame audinyje išlaikomas santykinis *BNIP2* mRNR kiekis, tuo tarpu ST ir HTK tirpalų poveikis buvo daug silpnesnis. Jų raiška sumažėjo apie 70 proc. ($p < 0,05$) visose laiko grupėse, lyginant su perfuzuotu širdies audiniu. Tačiau šis Del Nido poveikis buvo pastebimas tik per pirmąsias 2 valandas ir vėliau palaipsniui silpnėjo. Šie duomenys leidžia daryti prielaidą, kad Del Nido gali padėti palaikyti kardiomiocitų kontraktilinę funkciją.

Papildomai įvertinta *HIF1α* mRNR genų raiška. *HIF1α* genas atlieka svarbią funkciją prisitaikant prie miokardo išemijos, skatindamas kolateralinių kraujagyslių formavimąsi ir slopindamas kardiomiocitų žūtį [179]. Tyrimai rodo, kad I/R metu *HIF1α* mažina ROS gamybą skatindamas glikolitinių fer-

mentų ir piroviato dehidrogenazės kinazės 1 (PDK1) raišką, taip ribodamas substratų patekimą į mitochondrijas [180]. Mūsų tyrimas parodė, kad DN veiksmingai sukelia *HIF1α* mRNR raišką. Reikšmingi pokyčiai buvo stebimi 1 ir 2 val. grupėse, tačiau po 4 val. šis poveikis išnyko. Palyginti su ST ir HTK grupėmis, DN sukėlė net 90 proc. didesnę *HIF1α* raišką ($p < 0,01$), tai dar kartą pagrindžia kardioprotekcines jo savybes.

FOS priklauso *FOS* baltymų šeimai, kurie dimerizuojasi su JUN baltymais ir formuoja AP-1 transkripcijos faktoriaus kompleksą. AP-1 reguliuoja daugybę ląstelinių ir biologinių procesų, įskaitant ląstelės ciklą, proliferaciją ir programuotą ląstelių žūtį [181]. Mūsų rezultatai rodo, kad c-*FOS* mRNR dydis DN grupėje išliko reikšmingai didesnis, palyginti su ST ($p < 0,05$) ir HTK ($p < 0,05$). *FOS* genų raiška po DN tirpalo poveikio 1 ir 4 val. grupėse buvo panaši į perfuzuoto audinio lygį, o 2 val. grupėje buvo statistiškai reikšmingai didesnė ($p < 0,05$). ST ir HTK grupėse, palyginti su perfuzuotu audiniu, *FOS* raiška jose buvo daug mažesnė ($p < 0,05$).

Apibendrinant, genų raiškos rezultatai rodo, kad Del Nido tirpalas sukelia daug stipresnį kardioprotekcinį poveikį, palyginti su Šv. Tomo ir Custodiol HTK. Be to, visi Del Nido rezultatai buvo panašūs į perfuzuoto audinio, o tai dar labiau patvirtina ankstesnes išvadas.

Kardiopleginių tirpalų poveikis citokinų raiškai

Gyvūnų modeliuose uždegiminiai citokinai yra vieni iš pagrindinių miokardo pažeidimo rodiklių. Siekiant nustatyti, kuris kardiopleginis tirpalas geriau apsaugo nesubrendusį žiurkės miokardą, buvo įvertinta pro-uždegiminių citokinų TNF-α ir IL-6 raiška, visgi statistiškai reikšmingų tarpgrupinių skirtumų nenustatyta ($p > 0,05$). Tačiau ilgalaikės išemijos sąlygomis uždegimo sumažėjimas buvo ryškiausias HTK grupėje.

Papildomai buvo vertinti antiuždegiminiai citokinai IL-4 ir IL-10. Nors tarp grupių statistiškai reikšmingų skirtumų nenustatyta ($p > 0,05$), akivaizdu, kad visose kardioplegijos grupėse antiuždegiminių citokinų skaičius bėgant laikui mažėjo. Vertinant IL-10, DN grupėje jo kiekis išliko didžiausias po 4 val., o HTK grupėje rodikliai buvo stabilesni viso eksperimento metu. IL-4, HTK ir DN grupių rezultatai buvo panašūs, o ST grupėje 2 val. laikotarpiu stebėtas padidėjimas, tačiau po 4 val. nustatytas didesnis mažėjimas. Vertinant leptino koncentraciją po išemijos ir reperfuzijos poveikio, statistiškai reikšmingų skirtumų tarp kardiopleginių tirpalų nenustatyta ($p > 0,05$). Tačiau visose grupėse leptino kiekis sumažėjo 4 val. išemijos metu.

IŠVADOS

1. Del Nido kardiopleginis tirpalas veiksmingiau nei Custodiol HTK ir Šv. Tomo kardiopleginiai tirpalai išsaugojo mitochondrijų kvėpavimo funkciją nesubrendusio miokardo audinyje, parodant didesnį mitochondrijų stabilumą ir atsparumą išeminiams pažaidos procesams.
2. Del Nido kardiopleginis tirpalas sukėlė palankesnius genų raiškos pokyčius, susijusius su hipoksijos prisitaikymu, ląstelių streso reguliacija ir atsparumu apoptozei išemijos–reperfuzijos sąlygomis, palyginti su Custodiol HTK ir Šv. Tomo kardiopleginiais tirpalais.
3. Nors citokinų pokyčiai buvo nedideli, stebėtos tendencijos rodo, kad skirtingi kardiopleginiai tirpalai įvairiai veikia uždegimines reakcijas nesubrendusio miokardo audinyje, o Del Nido kardiopleginis tirpalas pasižymėjo labiau subalansuotu uždegiminio atsako profiliu.

PRAKTINĖS REKOMENDACIJOS

Gauti rezultatai rodo, kad miokardo pažeidimai viduląstelinių lygiu tampa reikšmingai ryškesni, kai išemija trunka ilgiau nei dvi valandas. Todėl išemijos trukmės ribojimas iki mažiau nei dviejų valandų, ypač naujagimiams, turintiems nesubrendusią miokardo struktūrą, gali būti lemiamas veiksnys siekiant sumažinti negrįžtamą ląstelių pažeidimą.

Tarp tirtų kardiopleginių tirpalų Del Nido kardiopleginis tirpalas užtikrino geriausią mitochondrijų kvėpavimo funkcijos ir genų raiškos išsaugojimą nesubrendusiame žiurkių miokarde. Šie duomenys patvirtina, kad Del Nido kardiopleginis tirpalas yra tinkamesnis pasirinkimas vaikų širdies chirurgijoje, kur ypač svarbi veiksminga miokardo apsauga viduląstelinių lygmeniu, siekiant sumažinti išemijos ir reperfuzijos pažeidimą ir išsaugoti ilgalaikę širdies funkciją.

REFERENCES

1. Chambers DJ, Fallouh HB. Cardioplegia and cardiac surgery: Pharmacological arrest and cardioprotection during global ischemia and reperfusion. Pharmacology and Therapeutics 2010; 127: 41–52.
2. Ali JM, Miles LF, Abu-Omar Y, et al. Global Cardioplegia Practices: Results from the Global Cardiopulmonary Bypass Survey. 2018.
3. Matte GS, Del Nido PJ. History and Use of del Nido Cardioplegia Solution at Boston Children's Hospital.
4. Waterford SD, Ad N. Del Nido cardioplegia: Questions and (some) answers. Journal of Thoracic and Cardiovascular Surgery 2023; 165: 1104–1108.
5. Ibrahim MF, Venn GE, Young CP, et al. A clinical comparative study between crystalloid and blood-based St Thomas' hospital cardioplegic solution, https://academic.oup.com/ejcts/article/15/1/75/450314 (1999).
6. Garg SK, Rawat RS, Saad DA, et al. Del Nido cardioplegia in adults: a retrospective observational study in comparison to modified St. Thomas cardioplegia in cardiac surgery. J Cardiothorac Surg; 19. Epub ahead of print 1 December 2024. DOI: 10.1186/s13019-024-02683-1.
7. Caputo M, Modi P, Imura H, et al. Cold Blood Versus Cold Crystalloid Cardioplegia for Repair of Ventricular Septal Defects in Pediatric Heart Surgery: A Randomized Controlled Trial. 2002.
8. Mercan I, Dereli Y, Topcu C, et al. Comparison between the effects of bretschneiders htk solution and cold blood cardioplegia on systemic endothelial functions in patients who undergo coronary artery bypass surgery: A prospective randomized and controlled trial. Braz J Cardiovasc Surg 2020; 35: 634–643.
9. Veres G, Radovits T, Merkely B, et al. Custodiol-N, the novel cardioplegic solution reduces ischemia/reperfusion injury after cardiopulmonary bypass. J Cardiothorac Surg; 10. Epub ahead of print 28 February 2015. DOI: 10.1186/s13019-015-0226-9.
10. Schaefer M, Gebhard MM, Gross W. The effect of melatonin on hearts in ischemia/reperfusion experiments without and with HTK cardioplegia. Bioelectrochemistry 2019; 129: 170–178.
11. Chambers DJ. Mechanisms and Alternative Methods of Achieving Cardiac Arrest Polarized Arrest An alternative to inducing arrest by depolarization (with elevated K concentrations) is to maintain polarization of Presented at the 3rd International Symposium on Myocardial Protection From Surgical Ischemic-Reperfusion Injury, Asheville, NC.
12. Yayla-Tunçer E, Şengelen A, Tan-Recep BZ, et al. Acute Changes in Myocardial Expression of Heat Shock Proteins and Apoptotic Response Following Blood, delNido, or Custodiol Cardioplegia in Infants Undergoing Open-Heart Surgery. Pediatr Cardiol 2022; 43: 567–579.
13. Peng T, Zhang T, Lu X, et al. JNK1/c-fos inhibits cardiomyocyte TNF-α expression via a negative crosstalk with ERK and p38 MAPK in endotoxaemia. Cardiovasc Res 2009; 81: 733–741.
14. Sall A, Zhang HM, Qiu D, et al. Pro-apoptotic activity of mBNIP-21 depends on its BNIP-2 and Cdc42GAP homology (BCH) domain and is enhanced by coxsackievirus B3 infection. Cell Microbiol 2010; 12: 599–614.
15. Chaturvedi RR, Lincoln C, Gothard JWW, et al. Left ventricular dysfunction after open repair of simple congentinal heart defects in infants and children: quantitation with the use of a conductance catheter immediately after bypass. 1998.
16. Bull C, Cooper J, Stark J. Cardioplegic protection of the child's heart. 1984.

17. Kane AE, Howlett SE. Novel cardioprotection strategies for the aged heart: evidence from pre-clinical studies. Clin Exp Pharmacol Physiol 2016; 43: 1251–1260.
18. Lopas Chuk GD, Spafford MA, Marsh Cardiovascular DR. Glycolysis is predominant source of myocardial ATP production immediately after birth, www.physiology.org/journal/ajpheart (1991).
19. Kevin H. Teoh, MD, Donald A. G. Mickle, MD, Richard D. Weisel, MD, Ren-ke Li, MD, Laura C. Tumiati, BSc, John G. Coles, MD, and William G. Williams, MD, Effect of oxygen tension and cardiovascular operations on the myocardial antioxidant enzyme activities in patients with tetralogy of Fallot and aorta-coronary bypass. Toronto, Ontario, Canada J Thorac Cardiovasc Surg 1992;104:159-64
20. Doenst T, Schlensak C, Beyersdorf F. Cardioplegia in pediatric cardiac surgery: Do we believe in magic? Annals of Thoracic Surgery 2003; 75: 1668–1677.
21. C Wittnich, C Peniston, D Ianuzzo, J G Abel, T A Salerno. Relative vulnerability of neonatal and adult hearts to ischemic injury. Circulation 1987 Nov;76(5 Pt 2): V156-60.
22. Parrish MD, Payne A, Fixler DE. Global Myocardial Ischemia in the Newborn, Juvenile, and Adult Isolated Isovolumic Rabbit Heart Age-Related Differences in Systolic Function, Diastolic Stiffness, Coronary Resistance, Myocardial Oxygen Consumption, and Extracellular pH.
23. Turko R. Myocardial Protection in Pediatric Cardiac Surgery. Artificial Organs 2013; 37: 16–20.
24. L J Kohman 1 LJV. Single-dose versus multidose cardioplegia in neonatal hearts. J Thorac Cardiovasc Surg 1994 Jun;107(6):1512-8.
25. Edward L. Bove, M.D., Alfred H. Stammers, B.S., and Kim P. Gallagher, Ph.D., Ann Arbor, Mich., and Syracuse, Protection of the neonatal myocardium during hypothermic ischemia N. Y. J Thorac Cardiovasc Surg 1987;94:115-23
26. Villa AD, Sammut E, Nair A, et al. Coronary artery anomalies overview: The normal and the abnormal. World J Radiol 2016; 8: 537.
27. Shriki JE, Shinbane JS, Rashid MA, Hindoyan A, Withey JG, DeFrance A, Cunningham M, Oliveira GR, Warren BH, Wilcox A. Identifying, characterizing, and classifying congenital anomalies of the coronary arteries. Radiographics 2012; 453–68.
28. Melrose DG, Dreyer B, Bentall HH, Baker JB. Elective cardiac arrest. Lancet 1955 Jul 2;269(6879):21-2.
29. Carvajal C, Goyal A, Tadi P. Cardioplegia. 2023 Jul 24 In: StatPearls [Internet] Treasure Island (FL): StatPearls Publishing; 2025 Jan.
30. Gerdobe F, Melrose D. The use of potassium arrest in open cardiac surgery. Am J Surg Aug;96(2):221-7.
31. Todd GJ, Niebauer IM, Manley NJ, Waldhausen JA. The mechanism of myocardial damage following potassium citrate (Melrose) cardioplegia. Surgery 1975 Jul;78(1):45-53.
32. Jynge P, Hearse DJ, Feuvray D, Mahalu W, Cankovic-Darracott S, O'Brien K, Braimbridge MV. The St. Thomas' hospital cardioplegic solution: a characterization in two species. Scand J Thorac Cardiovasc Surg Suppl 1981; 30:1-28.
33. Ledingham SJ, Braimbridge MV, Hearse DJ. The St. Thomas' Hospital cardioplegic solution. A comparison of the efficacy of two formulations. J Thorac Cardiovasc Surg 1987 Feb;93(2):240-6.
34. Hearse DJ. Cardioplegia. Postgrad Med J 1983;59 Suppl 2:11-24.
35. Bretschneider HJ. Myocardial protection. Thorac Cardiovasc Surg 1980 Oct;28(5):295-302.

36. Reynolds AC, Asopa S, Modi A, et al. HTK versus multidose cardioplegias for myocardial protection in adult cardiac surgery: A meta-analysis. J Card Surg 2021; 36: 1334–1343.
37. Buckberg GD. Strategies and logic of cardioplegic delivery to prevent, avoid, and reverse ischemic and reperfusion damage. J Thorac Cardiovasc Surg 1987 Jan;93(1):127-39.
38. Calafiore AM, Teodori G, Mezzetti A, et al. Intermittent Antegrade Warm Blood Cardioplegia. 1995.
39. Barner HB. Blood cardioplegia: A review and comparison with crystalloid cardioplegia. The Annals of Thoracic Surgery 1991; 52: 1354–1367.
40. Matte GS, Del Nido PJ. History and Use of del Nido Cardioplegia Solution at Boston Children's Hospital.
41. Cayir MC YA. The Use of del Nido Cardioplegia for Myocardial Protection in Isolated Coronary Artery Bypass Surgery. Heart Lung Circ 2020 Feb;29(2):301-307.
42. Bradić J, Andjić M, Novaković J, et al. Cardioplegia in Open Heart Surgery: Age Matters. Journal of Clinical Medicine; 12. Epub ahead of print 1 February 2023. DOI: 10.3390/jcm12041698.
43. Al-Hasan-Al-Saegh S, Takemoto S, Benenati S, et al. Optimizing Myocardial Protection in Minimally Invasive Cardiac Surgeries: A Network Comparison of Del Nido, Histidine-Tryptophan-Ketoglutarate, and Blood Cardioplegia. Journal of Clinical Medicine; 13. Epub ahead of print 1 November 2024. DOI: 10.3390/jcm13226977.
44. Russell S, Butt S, Vohra HA. In search of optimal cardioplegia for minimally invasive valve surgery. Perfusion (United Kingdom) 2022; 37: 668–674.
45. Kalogeris T, Baines CP, Krenz M, et al. Cell Biology of Ischemia/Reperfusion Injury. In: International Review of Cell and Molecular Biology. Elsevier Inc., 2012, pp. 229–317.
46. Chambers DJ, Hearse DJ. Developments in Cardioprotection: 'Polarized' Arrest as an Alternative to 'Depolarized' Arrest Presented at the International Symposium on Myocardial Protection From Surgical Ischemic-Reperfusion Injury. 1999.
47. Ji MJ, Hong JH. A cardioplegic solution with an understanding of a cardiochannelopathy. Antioxidants; 10. Epub ahead of print 1 December 2021. DOI: 10.3390/antiox10121878.
48. Buja LM. Pathobiology of Myocardial Ischemia and Reperfusion Injury: Models, Modes, Molecular Mechanisms, Modulation, and Clinical Applications. Cardiology in Review 2023; 31: 252–264.
49. R. Zucchi1 SG and SE. Biochemical Basis of Ischemic Heart Injury and of Cardioprotective Interventions. Current Medicinal Chemistry, Volume 14, Issue 15, Jun 2007, p 1619 - 1637.
50. Sagris M, Apostolos A, Theofilis P, et al. Myocardial Ischemia–Reperfusion Injury: Unraveling Pathophysiology, Clinical Manifestations, and Emerging Prevention Strategies. Biomedicines; 12. Epub ahead of print 1 April 2024. DOI: 10.3390/biomedicines12040802.
51. Dugbartey GJ. Cellular and molecular mechanisms of cell damage and cell death in ischemia–reperfusion injury in organ transplantation. Mol Biol Rep 51, 473 (2024). Molecular Biology Reports.
52. Ibáñez B, Heusch G, Ovize M, et al. Evolving therapies for myocardial ischemia/reperfusion injury. Journal of the American College of Cardiology 2015; 65: 1454–1471.
53. Hausenloy DJ, Yellon DM. Myocardial ischemia-reperfusion injury: A neglected therapeutic target. Journal of Clinical Investigation 2013; 123: 92–100.
54. Hausenloy DJ, Botker HE, Engstrom T, et al. Targeting reperfusion injury in patients with ST-segment elevation myocardial infarction: Trials and tribulations. European Heart Journal 2017; 38: 935–941d.

55. Liu Y, Li L, Wang Z, et al. Myocardial ischemia-reperfusion injury; Molecular mechanisms and prevention. Microvascular Research; 149. Epub ahead of print 1 September 2023. DOI: 10.1016/j.mvr.2023.104565.
56. Caccioppo A, Franchin L, Grosso A, et al. Ischemia reperfusion injury: Mechanisms of damage/protection and novel strategies for cardiac recovery/regeneration. International Journal of Molecular Sciences; 20. Epub ahead of print 2 October 2019. DOI: 10.3390/ijms20205024.
57. Soares ROS, Losada DM, Jordani MC, et al. Ischemia/reperfusion injury revisited: An overview of the latest pharmacological strategies. International Journal of Molecular Sciences; 20. Epub ahead of print 2 October 2019. DOI: 10.3390/ijms20205034.
58. Wang R, Wang M, He S, et al. Targeting Calcium Homeostasis in Myocardial Ischemia/Reperfusion Injury: An Overview of Regulatory Mechanisms and Therapeutic Reagents. Frontiers in Pharmacology; 11. Epub ahead of print 9 June 2020. DOI: 10.3389/fphar.2020.00872.
59. Metin Avkiran, Garrett Gross, Morris Karmazyn, Hermann Klein, Elizabeth Murphy, Kirsti Ytrehus. Na+/H+ exchange in ischemia, reperfusion and preconditioning. Cardiovasc Res.
60. Murphy E, Cross H, Steenbergen C. Sodium Regulation During Ischemia Versus Reperfusion and Its Role in Injury, http://www.circresaha.org (1999).
61. F. Hensley DM and GG. A Practical Approach to Cardiac Anesthesia. Philadelphia: Lippincott Williams & Wilkins, 2013., 2013.
62. Viana FF, Shi WY, Hayward PA, et al. Custodiol versus blood cardioplegia in complex cardiac operations: An Australian experience. European Journal of Cardio-thoracic Surgery 2013; 43: 526–531.
63. Sorabella RA, Akashi H, Yerebakan H, et al. Myocardial protection using del Nido cardioplegia solution in adult reoperative aortic valve surgery. J Card Surg 2014; 29: 445–449.
64. He GW, Ge ZD, Yim APC, et al. Electrophysiologic and mechanical evidence of superiority of hyperpolarizing versus depolarizing cardioplegia in protection of endothelium-derived hyperpolarizing factor-mediated endothelial function: A study in coronary resistance arteries. Journal of Thoracic and Cardiovascular Surgery 2004; 127: 1773–1780.
65. Zeng J, He W, Qu Z, Tang Y, Zhou Q, Zhang B. Cold blood versus crystalloid cardioplegia for myocardial protection in adult cardiac surgery: a meta-analysis of randomized controlled studies. J Cardiothorac Vasc Anesth 2014 Jun;28(3):674-81 doi: 101053/j. jvca201306005 Epub 2014 Apr 12 PMID: 24721161.
66. Guru V, Omura J, Alghamdi AA, et al. Is blood superior to crystalloid cardioplegia? A meta-analysis of randomized clinical trials. Circulation; 114. Epub ahead of print July 2006. DOI: 10.1161/Circulation aha.105.001644.
67. Günday M, Bingöl H. Is crystalloid cardioplegia a strong predictor of intra-operative hemodilution? J Cardiothorac Surg; 9. Epub ahead of print 27 January 2014. DOI: 10.1186/1749-8090-9-23.
68. Mullen JC, Fremes SE, Weisel RD, et al. Right Ventricular Function: A Comparison Between Blood and Crystalloid Cardioplegia. Annals of Thoracic Surgery 1987; 43: 17–24.
69. Mauney MC, Kron IL. The Physiologic Basis of Warm Cardioplegia. 1995.
70. Caputo M, Ascione R, Angelini GD, et al. The end of the cold era: from intermittent cold to intermittent warm blood cardioplegia, https://academic.oup.com/ejcts/article/14/5/467/431205 (1998).
71. Fan Y, Zhang AM, Xiao Y Bin, et al. Warm versus cold cardioplegia for heart surgery: a meta-analysis. European Journal of Cardio-thoracic Surgery 2010; 37: 912–919.

72. Whittaker A, Aboughdir M, Mahbub S, et al. Myocardial protection in cardiac surgery: how limited are the options? A comprehensive literature review. Perfusion (United Kingdom) 2021; 36: 338–351.
73. Ledingham SJM, Braimbridge M V., Hearse DJ. The St. Thomas' Hospital cardioplegic solution. A comparison of the efficacy of two formulations. Journal of Thoracic and Cardiovascular Surgery 1987; 93: 240–246.
74. Singh SSA, De S Das, Spadaccio C, et al. An overview of different methods of myocardial protection currently employed peri-transplantation. Vessel Plus 2017; 1: 213–229.
75. Lee KC, Chang CY, Chuang YC, et al. Combined St. Thomas and histidine-tryptophan-ketoglutarat solutions for myocardial preservation in heart transplantation patients. In: Transplantation Proceedings. 2012, pp. 886–889.
76. Kronon M, Boiling KS, Allen BS, et al. From the Division of Cardiothoracic Surgery at the University of Illinois at Chicago, Chicago, Ill. Read at the Seventy-seventh Annual Meeting of The American Association for Thoracic Surgery. 1997.
77. Bretschneider HJ, Hübner G, Knoll D, Lohr B, Nordbeck H, Spieckermann PG. Myocardial resistance and tolerance to ischemia: physiological and biochemical basis. J Cardiovasc Surg (Torino) 1975 May-Jun;16(3):241-60 PMID: 239002.
78. He G-W, Yang C-Q. Cardiopulmonary Bypass, Myocardial Management, and Support Techniques Superiority of Hyperpolarizing to Depolarizing Cardioplegia in Protection of Coronary Endothelial Function. 1997.
79. Ghiragosian C, Harpa M, Stoica A, et al. Theoretical and Practical Aspects in the Use of Bretschneider Cardioplegia. Journal of Cardiovascular Development and Disease; 9. Epub ahead of print 1 June 2022. DOI: 10.3390/jcdd9060178.
80. Preusse CJ. Custodiol Cardioplegia: A Single-Dose Hyperpolarizing Solution. 2016.
81. Gankam Kengne F, Decaux G. Hyponatremia and the Brain. Kidney International Reports 2018; 3: 24–35.
82. Lindner G, Zapletal B, Schwarz C, et al. Acute hyponatremia after cardioplegia by histidine-tryptophane-ketoglutarate-a retrospective study, http://www.cardiothoracic surgery.org/content/7/1/52 (2012).
83. Edelman JJB, Seco M, Dunne B, et al. Custodiol for myocardial protection and preservation: a systematic review. Ann Cardiothorac Surg 2013; 2: 717–28.
84. Booker PD. Myocardial stunning in the neonate. 1998.
85. Yano Y, Braimbridge M V., Hearse DJ. Protection of the pediatric myocardium. Differential susceptibility to ischemic injury of the neonatal rat heart. Journal of Thoracic and Cardiovascular Surgery 1987; 94: 887–896.
86. Tö Rnroth-Horsefield S, Neutze R. Opening and closing the metabolite gate, www. pnas.orgcgidoi10.1073pnas.0810654106 (2008).
87. Balaban RS. Cardiac energy metabolism homeostasis: role of cytosolic calcium. J Mol Cell Cardiol 2002 2002; 1259–71.
88. Brown DA, Perry JB, Allen ME, et al. Expert consensus document: Mitochondrial function as a therapeutic target in heart failure. Nat Rev Cardiol 2017; 14: 238–250.
89. Nolfi-Donegan D, Braganza A, Shiva S. Mitochondrial electron transport chain: Oxidative phosphorylation, oxidant production, and methods of measurement. Redox Biology; 37. Epub ahead of print 1 October 2020. DOI: 10.1016/j.redox.2020.101674.
90. Zhao RZ, Jiang S, Zhang L, et al. Mitochondrial electron transport chain, ROS generation and uncoupling (Review). International Journal of Molecular Medicine 2019; 44: 3–15.
91. Kühlbrandt W. Structure and function of mitochondrial membrane protein complexes. BMC Biology; 13. Epub ahead of print 29 October 2015. DOI: 10.1186/s12915-015-0201-x.

92. Tang JX, Thompson K, Taylor RW, et al. Mitochondrial OXPHOS biogenesis: Co-regulation of protein synthesis, import, and assembly pathways. Int J Mol Sci 2020; 21: 1–32.
93. Wu MY, Yiang GT, Liao WT, et al. Current Mechanistic Concepts in Ischemia and Reperfusion Injury. Cellular Physiology and Biochemistry 2018; 46: 1650–1667.
94. Naito H, Nojima T, Fujisaki N, et al. Therapeutic strategies for ischemia reperfusion injury in emergency medicine. Acute Medicine & Surgery; 7. Epub ahead of print January 2020. DOI: 10.1002/ams2.501.
95. Cadenas E DKJ. Mitochondrial free radical generation, oxidative stress, and aging. Free Radic Biol Med 2000; 222–30.
96. Turrens JF. Mitochondrial formation of reactive oxygen species. Journal of Physiology 2003; 552: 335–344.
97. Algoet M, Janssens S, Himmelreich U, Gsell W, Pusovnik M, Van den Eynde J, Oosterlinck W. Myocardial ischemia-reperfusion injury and the influence of inflammation. Trends Cardiovasc Med 2023; 357–366.
98. Prue Cowled and Robert Fitridge. Mechanisms of Vascular Disease: A Reference Book for Vascular Specialists. Discipline of Surgery, The University of Adelaide, The Queen Elizabeth Hospital, Woodville South, South Australia, Australia, 2011.
99. Ho KK, Pinsky JL, Kannel WB, Levy D. The epidemiology of heart failure: the Framingham Study. J Am Coll Cardiol 1993; 6A-13A.
100. Pitt B, L Ho KK, Pinsky JL, et al. Part II: New Insights Pathophysiology of Into the Epidemiology and Heart Failure. The Epidemiology of Heart Failure: The Framingham Study.
101. Kim NH, Kang PM. Apoptosis in cardiovascular diseases: Mechanism and clinical implications. Korean Circulation Journal 2010; 40: 299–305.
102. Wang S, Han HM, Pan ZW, Hang PZ, Sun LH, Jiang YN. Choline inhibits angiotensin II-induced cardiac hypertrophy by intracellular calcium signal and p38 MAPK pathway. Naunyn Schmiedebergs Arch Pharmacol 2012; 823–31.
103. Liu CJ, Cheng YC, Lee KW, Hsu HH, Chu CH, Tsai FJ. Lipopolysaccharide induces cellular hypertrophy through calcineurin/NFAT-3 signaling pathway in H9c2 myocardiac cells. Mol Cell Biochem 2008; 167–78.
104. Das S, Babick AP, Xu YJ, et al. TNF-α-mediated signal transduction pathway is a major determinant of apoptosis in dilated cardiomyopathy. J Cell Mol Med 2010; 14: 1988–1997.
105. Li P-F, Dietz R, Diger Von Harsdorf R. p53 regulates mitochondrial membrane potential through reactive oxygen species and induces cytochrome c-independent apoptosis blocked by Bcl-2. 1999.
106. Shabalala S MCLJJR. Polyphenols, autophagy and doxorubicin-induced cardiotoxicity. Life Sci 2017; 160–170.
107. Lutz J, Thürmel K, Heemann U. Anti-inflammatory treatment strategies for ischemia/reperfusion injury in transplantation. Journal of Inflammation; 7. Epub ahead of print 2010. DOI: 10.1186/1476-9255-7-27.
108. Tassiopoulos AK, Carlin RE, Gao Y, Pedoto A, Finck CM, Landas SK, Tice DG, Marx W, Hakim TS, McGraw DJ. Role of nitric oxide and tumor necrosis factor on lung injury caused by ischemia/reperfusion of the lower extremities. J Vasc Surg 1997; 647–56.
109. Siwik DA, L-F Chang D, Colucci WS. Interleukin-1 and Tumor Necrosis Factor-Decrease Collagen Synthesis and Increase Matrix Metalloproteinase Activity in Cardiac Fibroblasts In Vitro Molecular Medicine, http://www.circresaha.org (2000).

110. Engel D, Peshock R, Armstong RC, et al. Cardiac myocyte apoptosis provokes adverse cardiac remodeling in transgenic mice with targeted TNF overexpression. DOI: 10.1152/ajpheart.00053.2004.-Although.
111. Maekawa N, Wada H, Kanda T, et al. Improved myocardial ischemia/reperfusion injury in mice lacking tumor necrosis factor-α. J Am Coll Cardiol 2002; 39: 1229–1235.
112. Pilz PM et al. Remote ischemic preconditioning attenuates adverse cardiac remodeling and preserves left ventricular function in a rat model of reperfused myocardial infarction. Int J Cardiol 2019; 72–79.
113. Esposito E CS. TNF-alpha as a therapeutic target in inflammatory diseases, ischemia-reperfusion injury and trauma. Curr Med Chem 2009; 3152–67.
114. Sulail Fatima Rajani a AI a, MF a, MI b, GAK c d, ZS b. Post-infarct morphine treatment mitigates left ventricular remodeling and dysfunction in a rat model of ischemia-reperfusion. Eur J Pharmacol 2019; 61–71.
115. Woodall MC, Woodall BP, Gao E, et al. Cardiac Fibroblast GRK2 Deletion Enhances Contractility and Remodeling Following Ischemia/Reperfusion Injury. Circ Res 2016; 119: 1116–1127.
116. Sun M, Dawood F, Wen WH, et al. Excessive tumor necrosis factor activation after infarction contributes to susceptibility of myocardial rupture and left ventricular dysfunction. Circulation 2004; 110: 3221–3228.
117. Yoshiya Monden, Toru Kubota, Takaki Tsutsumi, Takahiro Inoue, Shunichi Kawano, Natsumi Kawamura, Tomomi Ide, Kensuke Egashira, Hiroyuki Tsutsui, Kenji Sunagawa. Soluble TNF receptors prevent apoptosis in infiltrating cells and promote ventricular rupture and remodeling after myocardial infarction. Cardiovasc Res 2007; 794–805.
118. Norwood MG, Bown MJ, Sutton AJ, Nicholson ML, Sayers RD. Interleukin 6 production during abdominal aortic aneurysm repair arises from the gastrointestinal tract and not the legs. Br J Surg 2004; 1153–1156.
119. De Vries DK, Lindeman JHN, Tsikas D, et al. Early renal ischemia-reperfusion injury in humans is dominated by IL-6 release from the allograft. American Journal of Transplantation 2009; 9: 1574–1584.
120. Gwechenberger M, Mendoza LH, Youker KA, et al. Cardiac Myocytes Produce Interleukin-6 in Culture and in Viable Border Zone of Reperfused Infarctions, http://www.circulationaha.org (1999).
121. Empana JP, Jouven X, Canouï-Poitrine F, et al. C-reactive protein, interleukin 6, fibrinogen and risk of sudden death in European middle-aged men: The PRIME study. Arterioscler Thromb Vasc Biol 2010; 30: 2047–2052.
122. Swerdlow DI. Articles The interleukin-6 receptor as a target for prevention of coronary heart disease: a mendelian randomisation analysis. The Lancet 2012; 379: 1214–1224.
123. Ola Kleveland, Gabor Kunszt, Marte Bratlie, Thor Ueland, Kaspar Broch, Espen Holte, Annika E. Michelsen, Bjørn Bend. Effect of a single dose of the interleukin-6 receptor antagonist tocilizumab on inflammation and troponin T release in patients with non-ST-elevation myocardial infarction: a double-blind, randomized, placebo-controlled phase 2 trial. Eur Heart J 2016; 2406–2413.
124. Martin Fuchs, Andres Hilfiker, Karol Kaminski, Denise Hilfiker-Kleiner, Zeynep Guener, Gunnar Klein. Role of interleukin-6 for left ventricular remodeling and survival after experimental myocardial infarction. FASEB Journal, 2003.
125. Jong WMC, ten Cate H, Linnenbank AC, et al. Reduced acute myocardial ischemia–reperfusion injury in IL-6-deficient mice employing a closed-chest model. Inflammation Research 2016; 65: 489–499.

126. Zhang C, He S, Li Y, et al. Bisoprolol protects myocardium cells against ischemia/reperfusion injury by attenuating unfolded protein response in rats. Sci Rep; 7. Epub ahead of print 1 December 2017. DOI: 10.1038/s41598-017-12366-8.
127. Li MO, Flavell RA. Contextual Regulation of Inflammation: A Duet by Transforming Growth Factor-β and Interleukin-10. Immunity 2008; 28: 468–476.
128. Lacraz S, Nicod LP, Chicheportiche R, et al. IL-10 inhibits metalloproteinase and stimulates TIMP-1 production in human mononuclear phagocytes. Journal of Clinical Investigation 1995; 96: 2304–2310.
129. Frangogiannis NG, Mendoza LH, Lindsey ML, Ballantyne CM, Michael LH. IL-10 is induced in the reperfused myocardium and may modulate the reaction to injury. J Immunol 2000; 2798–808.
130. Bagchi AK, Akolkar G, Mandal S, et al. Toll-like receptor 2 dominance over Toll-like receptor 4 in stressful conditions for its detrimental role in the heart. Am J Physiol Heart Circ Physiol 2017; 312: 1238–1247.
131. Yang Z, Zingarelli B, Szabó C. Crucial Role of Endogenous Interleukin-10 Production in Myocardial Ischemia/Reperfusion Injury, http://www.circulationaha.org (2000).
132. Krishnamurthy P, Rajasingh J, Lambers E, et al. IL-10 inhibits inflammation and attenuates left ventricular remodeling after myocardial infarction via activation of STAT3 and suppression of HuR. Circ Res; 104. Epub ahead of print 30 January 2009. DOI: 10.1161/CIRCRESAHA.108.188243.
133. Bakhta O, Blanchard S, Guihot AL, et al. Cardioprotective Role of Colchicine Against Inflammatory Injury in a Rat Model of Acute Myocardial Infarction. J Cardiovasc Pharmacol Ther 2018; 23: 446–455.
134. Tian Y, Miao B, Charles EJ, et al. Stimulation of the Beta2 adrenergic receptor at reperfusion limits myocardial reperfusion injury via an interleukin-10-dependent anti-inflammatory pathway in the spleen. Circulation Journal 2018; 82: 2829–2836.
135. Zymek P, Nah D-Y, Bujak M, et al. Interleukin-10 Is Not a Critical Regulator of Infarct Healing and Left Ventricular Remodeling.
136. Martinez-Mier G, Toledo-Pereyra LH, McDuffie JE, Warner RL, Ward PA. Neutrophil depletion and chemokine response after liver ischemia and reperfusion. J Invest Surg 2001; 99–107.
137. Szabó A, Varga R, Keresztes M, et al. Ischemic limb preconditioning downregulates systemic inflammatory activation. Journal of Orthopaedic Research 2009; 27: 897–902.
138. Eberlin KR, McCormack MC, Nguyen JT, Tatlidede HS, Randolph MA. Ischemic preconditioning of skeletal muscle mitigates remote injury and mortality. J Surg Res 2008; 24–30.
139. Zhang L, Zhou K, Gu T, et al. Cardiopulmonary Protection of Modified Remote Ischemic Preconditioning in Mitral Valve Replacement Surgery: A Randomized Controlled Trial. Cardiovasc Ther; 2024. Epub ahead of print 2024. DOI: 10.1155/2024/9889995.
140. Liu Z, Zhao Y, Lei M, et al. Remote Ischemic Preconditioning to Prevent Acute Kidney Injury After Cardiac Surgery: A Meta-Analysis of Randomized Controlled Trials. Frontiers in Cardiovascular Medicine; 8. Epub ahead of print 18 March 2021. DOI: 10.3389/fcvm.2021.601470.
141. Gorjipour F, Saeedzadeh T, Toloueitabar Y, Kachoueian N, Bahlouli Ghashghaei S. Remote ischemic preconditioning effects on inflammatory markers and myocardial protection in coronary artery bypass graft surgery. Perfusion 2022; 56–61.
142. Niccoli G, Marino M, Spaziani C, Crea F. Prevention and treatment of no-reflow. Acute Card Care 2010; 81–91.
143. Weis SM CDA. Pathophysiological consequences of VEGF-induced vascular permeability. Nature 2005; 497–504.

144. Kaur G, Baghdasaryan P, Natarajan B, et al. Pathophysiology, Diagnosis, and Management of Coronary No-Reflow Phenomenon. International Journal of Angiology 2021; 30: 15–21.
145. Fang Y, Long C, Lou S, et al. Blood versus crystalloid cardioplegia for pediatric cardiac surgery: A meta-analysis. Perfusion (United Kingdom) 2015; 30: 529–536.
146. Romolo H, Hernisa L, Fakhri D, et al. Comparison between blood and non-blood cardioplegia in tetralogy of Fallot. Asian Cardiovasc Thorac Ann 2019; 27: 75–79.
147. Mylonas KS, Tzani A, Metaxas P, et al. Blood Versus Crystalloid Cardioplegia in Pediatric Cardiac Surgery: A Systematic Review and Meta-analysis. Pediatric Cardiology 2017; 38: 1527–1539.
148. Pérez-Andreu J, Fernández-Doblas J, Sao Avilés A, et al. Myocardial protection in the arterial switch operation: Custodiol versus cold blood cardioplegia. Interact Cardiovasc Thorac Surg 2020; 30: 136–143.
149. Dolcino A, Gaudin R, Pontailler M, Raisky O, Vouhé P, Bojan M. Single-Shot Cold Histidine-Tryptophan-Ketoglutarate Cardioplegia for Long Aortic Cross-Clamping Durations in Neonates. J Cardiothorac Vasc Anesth 2020 Apr;34(4):959-965 doi: 101053/j. jvca201908039 Epub 2019 Aug 27 PMID: 31543295.
150. Isildak FU, YY. Comparison of Del Nido and Blood Cardioplegia in Pediatric Patients Undergoing Surgical Repair for Congenital Heart Disease. Pediatr Cardiol 42, 1388–1393 (2021) https://doi.org/101007/s00246-021-02623-z.
151. Haranal M, Chin HC, Sivalingam S, et al. Safety and Effectiveness of Del Nido Cardioplegia in Comparison to Blood-Based St. Thomas Cardioplegia in Congenital Heart Surgeries: A Prospective Randomized Controlled Study. World J Pediatr Congenit Heart Surg 2020; 11: 720–726.
152. Panigrahi D, Roychowdhury S, Guhabiswas R, et al. Myocardial protection following del Nido cardioplegia in pediatric cardiac surgery. Asian Cardiovasc Thorac Ann 2018; 26: 267–272.
153. Gholampour Dehaki M, Gorjipour F, Gorjipour F, et al. The effect of Del Nido versus custodiol cardioplegia on clinical outcomes and troponin-I changes among pediatrics with tetralogy of fallot undergoing cardiopulmonary bypass. Perfusion (United Kingdom) 2024; 39: 317–323.
154. Yayla-Tunçer E, Şengelen A, Tan-Recep BZ, Şavluk ÖF, Yilmaz AA, Ceyran H, Önay-Uçar E. Acute Changes in Myocardial Expression of Heat Shock Proteins and Apoptotic Response Following Blood, delNido, or Custodiol Cardioplegia in Infants Undergoing Open-Heart Surgery. Pediatr Cardiol 2022 Mar;43(3):567-579 doi: 101007/s00246-021-02759-y Epub 2021 Oct 25 PMID: 34694437.
155. Talwar S, Chatterjee S, Sreenivas V, et al. Comparison of del Nido and histidine-tryptophan-ketoglutarate cardioplegia solutions in pediatric patients undergoing open heart surgery: A prospective randomized clinical trial. Journal of Thoracic and Cardiovascular Surgery 2019; 157: 1182-1192.e1.
156. Bibevski S, Mendoza L, Ruzmetov M, et al. Custodiol cardioplegia solution compared to cold blood cardioplegia in pediatric cardiac surgery: a single-institution experience. Perfusion (United Kingdom) 2020; 35: 316–322.
157. Elassal AA, Al-Ebrahim K, Al-Radi O, Zaher ZF, Dohain AM, Abdelmohsen GA, Abdulla AH, Meshak MA, Abdulaziz MA, Eldesouki MS, Hasan MA, Eldib O. Myocardial Protection by Blood-Based Del Nido versus St. Thomas Cardioplegia in Cardiac Surgery for Adults and Children. Heart Surg Forum 2020 Sep 24;23(5):E689-E695 doi: 101532/hsf3099 PMID: 32990578.
158. Elhamamsy MM, Bahi Eldin HH, Mahmoud YM, et al. Comparative Study between Three Solutions for Cardioplegia in Pediatric Cardiac Surgery: Histidine-Tryptophan-Ketoglutarate (HTK) Solution, Blood Cardioplegia and Crystalloid (St. Thomas)

Cardioplegia. J Anesth Clin Res; 09. Epub ahead of print 2018. DOI: 10.4172/2155-6148.1000818.
159. Rushel KZ HAAMIMHKRMSRHMAAB. Comparative Study between the Use of Multidose Standard Cardioplegia and Long-Acting Del Nido Cardioplegia during Intracardiac Repair for Tetralogy of Fallot in Pediatric Patients. Mymensingh Med J 2018 Jul;27(3):610-616 PMID: 30141453.
160. Ji B, Li Y, Ruzmetov M, et al. open access edited by Comparative ects of diferent types of cardioplegia in cardiac surgery: A network meta-analysis, https://www.r-project.org.
161. Sobieraj M, Kilanowska M, Ladzinski P, et al. Type of cardioplegic solution asa factor influencing the clinical outcome of open-heart congenital procedures. Kardiochirurgia i Torakochirurgia Polska 2018; 15: 86–94.
162. Caneo LF, Matte GS, R Turquetto AL, et al. Initial experience with del Nido cardioplegia solution at a Pediatric and Congenital Cardiac Surgery Program in Brazil. Perfusion (United Kingdom) 2022; 37: 684–691.
163. Qulisy EA, Fakiha A, Debis RS, et al. Custodiol versus blood cardioplegia in pediatric cardiac surgery, two-center study. Journal of the Egyptian Society of Cardio-Thoracic Surgery 2016; 24: 38–42.
164. Ji B, Li Y, Ruzmetov M, et al. open access edited by Comparative eeects of diierent types of cardioplegia in cardiac surgery: A network meta-analysis, https://www.r-project.org.
165. Floh AA, Das S, Haranal M, et al. Comparison between Del Nido and conventional blood cardioplegia in pediatric open-heart surgery. Perfusion (United Kingdom) 2023; 38: 337–345.
166. Kohman LJ VLJ. Single-dose versus multidose cardioplegia in neonatal hearts. J Thorac Cardiovasc Surg 1994 Jun;107(6):1512-8 PMID: 8196397.
167. Mamedov A, Rumbinaitė E, Jakuška P, et al. Myocardial protection in pediatric cardiac surgery - deep look to the most often used cardioplegic solutions. Medical Sciences; 12. Epub ahead of print 11 March 2024. DOI: 10.53453/ms.2024.3.3.
168. Pesta D GE. High-resolution respirometry: OXPHOS protocols for human cells and permeabilized fibers from small biopsies of human muscle. Methods Mol Biol 2012; 810:25-58.
169. Gnaiger E. Bioenergetics at low oxygen: dependence of respiration and phosphorylation on oxygen and adenosine diphosphate supply. Respir Physiol 2001 Nov 15;128(3):277-97.
170. Langendorff O. Untersuchungen am überlebenden Säugethierherzen. Pflügers Arch. Epub ahead of print 1895. DOI: https://doi.org/10.1007/BF01812150.
171. Bell RM, Mocanu MM, Yellon DM. Retrograde heart perfusion: The Langendorff technique of isolated heart perfusion. J Mol Cell Cardiol 2011; 50: 940–950.
172. Sato T, Takeda N. The roles of HIF-1α signaling in cardiovascular diseases. Journal of Cardiology 2023; 81: 202–208.
173. Karimi M, Wang LX, Hammel JM, et al. Neonatal vulnerability to ischemia and reperfusion: Cardioplegic arrest causes greater myocardial apoptosis in neonatal lambs than in mature lambs. Journal of Thoracic and Cardiovascular Surgery 2004; 127: 490–497.
174. Feng J, Bianchi C, Sandmeyer JL, et al. Molecular indices of apoptosis after intermittent blood and crystalloid cardioplegia. Circulation; 112. Epub ahead of print 30 August 2005. DOI: 10.1161/CIRCULATIONAHA.104.526160.
175. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCT method. Methods 2001; 25: 402–408.

176. Dugbartey GJ. Cellular and molecular mechanisms of cell damage and cell death in ischemia–reperfusion injury in organ transplantation. Molecular Biology Reports; 51. Epub ahead of print 1 December 2024. DOI: 10.1007/s11033-024-09261-7.
177. Kale J, Osterlund EJ, Andrews DW. BCL-2 family proteins: Changing partners in the dance towards death. Cell Death and Differentiation 2018; 25: 65–80.
178. Wong DCP, Xiao J, Chew TW, et al. BNIP-2 Activation of Cellular Contractility Inactivates YAP for H9c2 Cardiomyoblast Differentiation. Advanced Science; 9. Epub ahead of print 3 November 2022. DOI: 10.1002/advs.202202834.
179. Zhao Y, Xiong W, Li C, et al. Hypoxia-induced signaling in the cardiovascular system: pathogenesis and therapeutic targets. Signal Transduct Target Ther 2023; 8: 431.
180. Kim JW, Tchernyshyov I, Semenza GL, et al. HIF-1-mediated expression of pyruvate dehydrogenase kinase: A metabolic switch required for cellular adaptation to hypoxia. Cell Metab 2006; 3: 177–185.
181. Chambers DJ FHB. Cardioplegia and cardiac surgery: pharmacological arrest and cardioprotection during global ischemia and reperfusion. Pharmacol Ther 2010; 41–52.
182. Murphy E, Ardehali H, Balaban RS, et al. Mitochondrial Function, Biology, and Role in Disease: A Scientific Statement from the American Heart Association. Circ Res 2016; 118: 1960–1991.
183. Lemieux H, Semsroth S, Antretter H, Höfer D, Gnaiger E. Mitochondrial respiratory control and early defects of oxidative phosphorylation in the failing human heart. Int J Biochem Cell Biol 2011 Dec;43(12):1729-38.
184. Poznyak A V., Ivanova EA, Sobenin IA, et al. The role of mitochondria in cardiovascular diseases. Biology 2020; 9: 1–14.
185. Ballinger SW. Mitochondrial dysfunction in cardiovascular disease. Free Radic Biol Med 2005 May 15;38(10):1278-95.
186. Chistiakov DA, Shkurat TP, Melnichenko AA, et al. The role of mitochondrial dysfunction in cardiovascular disease: a brief review. Annals of Medicine 2018; 50: 121–127.
187. Zhang L, Keung W, Samokhvalov V, Wang W, Lopaschuk GD. Role of fatty acid uptake and fatty acid beta-oxidation in mediating insulin resistance in heart and skeletal muscle. Biochim Biophys Acta 2010 Jan;1801(1):1-22.
188. Kolwicz SC, Purohit S, Tian R. Cardiac metabolism and its interactions with contraction, growth, and survival of cardiomyocytes. Circulation Research 2013; 113: 603–616.
189. Tran DH, Wang Z V. Glucose Metabolism in Cardiac Hypertrophy and Heart Failure. Journal of the American Heart Association; 8. Epub ahead of print 2019. DOI: 10.1161/JAHA.119.012673.
190. Neubauer S. The failing heart--an engine out of fuel. N Engl J Med 2007 Mar 15;356(11):1140-51 2007; 1140–51.
191. Casademont J MO. Electron transport chain defects in heart failure. Heart Fail Rev 2002 Apr;7(2):131-9 2002; 131–9.
192. Aubert G, Vega RB, Kelly DP. Perturbations in the gene regulatory pathways controlling mitochondrial energy production in the failing heart. Biochimica et Biophysica Acta - Molecular Cell Research 2013; 1833: 840–847.
193. Corral-Debrinski M, Shoffner JM, Lott MT, Wallace DC. Association of mitochondrial DNA damage with aging and coronary atherosclerotic heart disease. Mutat Res 1992 Sep;275(3-6):169-80 1992; 169–80.
194. Vittorini S, Storti S, Andreani G, et al. Heat shock protein 70-1 gene expression in pediatric heart surgery using blood cardioplegia. Clin Chem Lab Med 2007; 45: 244–248.

195. Date T, Mochizuki S, Belanger AJ, et al. Expression of constitutively stable hybrid hypoxia-inducible factor-1 protects cultured rat cardiomyocytes against simulated ischemia-reperfusion injury. Am J Physiol Cell Physiol 2005; 288: 314–320.
196. Guimarães-Camboa N, Stowe J, Aneas I, et al. HIF1α Represses Cell Stress Pathways to Allow Proliferation of Hypoxic Fetal Cardiomyocytes. Dev Cell 2015; 33: 507–521.
197. Wang F, Liang G-Y, Liu D-X, et al. Effect of Si-RNA-silenced HIF-1α gene on myocardial ischemia-reperfusion-induced insulin resistance. Int J Clin Exp Med 2015; 8: 15514–15520.
198. Wong DCP, Xiao J, Chew TW, et al. BNIP-2 Activation of Cellular Contractility Inactivates YAP for H9c2 Cardiomyoblast Differentiation. Advanced Science; 9. Epub ahead of print 3 November 2022. DOI: 10.1002/advs.202202834.
199. Kang JS, Bae GU, Yi MJ, et al. A Cdo-Bnip-2-Cdc42 signaling pathway regulates p38α/β MAPK activity and myogenic differentiation. Journal of Cell Biology 2008; 182: 497–507.
200. Zhang Y, Liu D, Hu H, et al. HIF-1α/BNIP3 signaling pathway-induced-autophagy plays protective role during myocardial ischemia-reperfusion injury. Biomedicine and Pharmacotherapy; 120. Epub ahead of print 1 December 2019. DOI: 10.1016/j.biopha.2019.109464.
201. Lu X, Lei M, Xiang F, et al. Abstract 162: c-fos Protects the Myocardium from Ischemic Injury and Improves Cardiac Function. Circulation 2007; 116: II_10-II_10.
202. Bao Y, Qiao Y, Yu H, et al. MiRNA-27a Transcription Activated by c-Fos Regulates Myocardial Ischemia-Reperfusion Injury by Targeting ATAD3a. Oxid Med Cell Longev; 2021. Epub ahead of print 2021. DOI: 10.1155/2021/2514947.
203. Poetsch MS, Strano A, Guan K. Role of Leptin in Cardiovascular Diseases. Frontiers in Endocrinology; 11. Epub ahead of print 16 June 2020. DOI: 10.3389/fendo.2020.00354.
204. Genoux A, Bastard J-P, Capeau J, et al. Effects of leptin and adiponectin on the cardiovascular system. Ann Biol Clin (Paris); 2020. DOI: 10.1684/abc.2020.1548ï.
205. Gorjipour F, Dehaki MG, Totonchi Z, et al. Inflammatory cytokine response and cardiac troponin i changes in cardiopulmonary bypass using two cardioplegia solutions; Del Nido and modified St. Thomas': A randomized controlled trial. Perfusion (United Kingdom) 2017; 32: 394–402.
206. Simon B V, Beutner G, Swartz MF, et al. Mitochondrial ATP Synthase Tetramer Disassembly following Blood-Based or del Nido Cardioplegia during Neonatal Cardiac Surgery.
207. Gunaydin S, Akbay E, Orhan, et al. Long-Term Protective Effects of Single-Dose Cardioplegic Solutions in Cell Culture Models. 2020.
208. O'Brien JD, Howlett SE, Burton HJ, O'Blenes SB, Litz DS, Friesen CL. Pediatric cardioplegia strategy results in enhanced calcium metabolism and lower serum troponin T. Ann Thorac Surg 2009 May;87(5):1517-23 2009; 1517–23.
209. Talwar S, Bhoje A, Sreenivas V, Makhija N, Aarav S, Choudhary SK, Airan B. Comparison of del Nido and St Thomas Cardioplegia Solutions in Pediatric Patients: A Prospective Randomized Clinical Trial. Semin Thorac Cardiovasc Surg 2017 Autumn;29(3):366-374 2017; 366–374.
210. Beutner G, Alanzalon RE, Porter GA. Cyclophilin D regulates the dynamic assembly of mitochondrial ATP synthase into synthasomes. Sci Rep; 7. Epub ahead of print 1 December 2017. DOI: 10.1038/s41598-017-14795-x.
211. Hom JR, Quintanilla RA, Hoffman DL, et al. The permeability transition pore controls cardiac mitochondrial maturation and myocyte differentiation. Dev Cell 2011; 21: 469–478.

212. Karduz G, Yaman MO, Altan M, et al. St. Thomas and del Nido cardioplegia are superior to Custodiol cardioplegia in a rat model of donor heart. Scandinavian Cardiovascular Journal 2021; 55: 122–128.
213. Elmahrouk AF, Shihata MS, AL-Radi OO, et al. Custodiol versus blood cardioplegia in pediatric cardiac surgery: a randomized controlled trial. Eur J Med Res; 28. Epub ahead of print 1 December 2023. DOI: 10.1186/s40001-023-01372-4.

LIST OF PUBLICATIONS

Publications related to the results of the dissertation

1. **Mamedov A**, Rumbinaitė E, Romann S, Verikas D, Jakuška P, Aitaliyev S, Benetis R, Stankevičius E. Mitochondrial respiratory pathways in immature rat heart tissue using different cardioplegic solutions. Gen Thorac Cardiovasc Surg. 2024 Nov 5. doi: 10.1007/s11748-024-02097-9. Epub ahead of print. Erratum in: Gen Thorac Cardiovasc Surg. 2025 Jan;73(1):71. doi: 10.1007/s11748-024-02107-w. PMID: 39499491.
2. **Mamedov A,** Rumbinaitė E, Jakuška P, Verikas D, Žūkaitė G, Benetis R, Stankevičius E. Myocardial protection in pediatric cardiac surgery: deep look to the most often used cardioplegic solutions. Journal of Medical Sciences. 11 Mar 2024 - Volume 12 | Issue 2. Electronic - ISSN: 2345-0592 Medical Sciences 2024 Vol. 12 (2), p. 15-30, https://doi.org/10.53453/ms.2024.3.3
3. **Mamedov A**, Gečys D, Jakuška P, Treinys R, Narauskaitė D, Aitaliyev S, Rumbinaitė E, Karčiauskas D, Benetis R, Stankevičius E. How different cardioplegic solutions influence genes expression and cytokine response in an immature rat heart model of ischemia/reperfusion? PLoS One. 2025 Jul 29;20(7):e0329010. doi:10.1371/journal.pone.0329010. PMID: 40729334; PMCID: PMC12306747.

List of presentations at scientific conferences: presentations directly related to the topic of the doctoral dissertation

1. **Mamedov A,** Rumbinaitė E, Romann S, Aitaliyev S, Jakuška P, Benetis R, Stankevičius E. Role of immature rat heart mitochondrial respiration using different cardioplegic solutions for long term myocardial ischemia. International Health Sciences Conference for All (IHSC for All) "Precision Medicine": Abstract book 2024: [March 25-26, 2024, Kaunas] 2024-04-16, p. 449-450
2. **Mamedov A,** Rumbinaitė E, Romann S, Jakuška P, Aitaliyev S, Vaitekūnas P, Schneider W, Benetis R, Stankevičius E**.** Changes of mitochondrial respiratory pathways in immature rat heart tissue using Custodiol HTK, St. Thomas and del Nido cardioplegic solutions. Abstracts of the International Scientific Conference on Medicine organized within the frame of the 82nd International Scientific Conference of the University of Latvia [: 5 April 2024, Riga, Latvia] / 2024-04-05, vol. 60, no. Suppl. 1, p. 154-154

3. **Mamedov A,** Gečys D, Petraitytė V, Treinys R, Stankevičius E. Evaluation of cardioplegic solutions for preserving cardiac cells in isolated rat heart assays. FEBS3+ Baltics: Biochemistry at the Fore Line: Abstract book: Vilnius, Lithuania, April 23-25, 2025, 2025-04-23, p. 164-165
4. **Mamedov A**, Rumbinaitė E, Aitaliyev S, Benetis R, Jakuška P, Karčiauskas D, Stankevičius E, Verikas D, Zhumagaliyev R. Effects of cardioplegic solution on cytokine expression. Proceedings of the Latvian Academy of Sciences. Section B. Natural, Exact, and Applied Sciences: 83rd International Scientific Conference on Medicine and Health Sciences of the University of Latvia: Internal and Cardiovascular Medicine, 2025-04-01, vol. 79, no. 1-2, p. 64-64
5. Petraitytė V, **Mamedov A,** Gečys D, Treinys R, Stankevičius E. Evaluation of Cardioplegic Solutions for Preserving Cardiac Cells in Isolated Rat Heart Assays. International Health Sciences Conference IHSC: Abstract book 2025: [March 13 - 14, 2025, Kaunas] / Edited by Karina Zerr, 2025-03-13, p. 215-216

Other publications

1. **Mamedov A**, Rumbinaitė E, Karčiauskas D, Jakuškaitė G, Veikutienė A, Jakuška P, Benetis R. Surgical coronary angioplasty of both coronary ostia after chest radiotherapy. Is it good alternative to conventional coronary bypass surgery? Perfusion. 2025 Jan;40(1):247-250. doi: 10.1177/02676591231221707. Epub 2023 Dec 8. PMID: 38066688; PMCID: PMC11715061.
2. Tolegenuly A, Ordiene R, Jakuska P, **Mamedov A**, Unikas R, Benetis R. Intraoperative angiography during coronary artery bypass grafting. Perfusion. 2021 Mar 19:2676591211003262. doi: 10.1177/02676591211003262. Epub ahead of print. PMID: 33739157.
3. Rumbinaite E, Karuzas A, Gustienė O, Benetis R, **Mamedov A**, Jankauskiene L. Value of myocardial deformation parameters for detecting significant coronary artery disease. J Cardiovasc Thorac Res, 2022, 14 (3)
4. Tolegenuly A, Ordiene R, **Mamedov A**, Unikas R and Benetis R. 2020. Correlation between preoperative coronary artery stenosis severity measured by instantaneous wave-free ratio and intraoperative transit time flow measurement of attached grafts. *Medicina*, *56*(12), p.714.
5. Tolegenuly A, **Mamedov A**, Benetis R. Impact of Target Coronary Artery Stenosis Severity Measured by Instantaneous Wave-Free Ratio on Bypassed Graft Patency. J Clin Med Kaz. 2021.

6. Rumbinaitė E, Venckus V, **Mamedov A**, Jakuškaitė G, Bučius P, Dobilienė O, Žaliūnas R, Jakuška P, Benetis R. Anterior basal left ventricular pseudoaneurysm in a single vessel disease. Perfusion. **2023** Feb 28:2676591231160269.
7. Rumbinaite E., Karužas A., Verikas D., Jakuškaitė G.,**Mamedov A**.,Sakavičiūtė E., Jakuška P. Žaliūnas R. Vaškelytė J.J "Left atrial strain rate - marker of hemodynamically significant coronary artery stenosis?" FutureMedicine. 1 Jun **2023** https://doi.org/10.2217/fca-2022-0089
8. Rumbinaitė E, Karčiauskas D, Jakuška P, Vajauskas D, **Mamedov A,** Jakuškaitė G, Bolys R, Žūkaitė G, Jankauskienė L, Benetis R, Žaliūnas, R. Case report: ST-elevation myocardial infarction complications. How far will you go? Future Cardiology, 2025-02-26, vol. 21, no. 4, p. 217-221.
9. Raškevičius V, Mickus R, Mačianskienė R, Jurevičius J, **Mamedov A**, Sarapinienė I, Bordel S, Skeberdis VA. Preference of short isoprene-repeat terpenes for Cx43 gap junctions compared with cardiac Na+ and Ca2+ channels: application for cardiac ephaptic transmission research. Pharmacol Res. 2025 Dec;222:108007. doi: 10.1016/j.phrs.2025.108007. Epub 2025 Oct 30. PMID: 41173172.

List of presentations at scientific conferences:

1. Tolegenuly A, **Mamedov A**, Benetis R. Impact of Target Coronary Artery Stenosis Severity Measured by Instantaneous Wave-Free Ratio on Bypassed Graft Patency. 3rd International Conference on Applied Research in Education (ARECONF): 04-06 June 2021, Barcelona, Spain. Barcelona: GKS, 2021., 2021-06-04, p. 1-1.
2. Verikas D, Rumbinaitė E, Jakuškaitė G, **Mamedov A,** Sakavičiūtė E, Jakuška P, Žaliūnas R, Vaškelytė J. Stress longitudinal left atrium strain rate is a marker of significant coronary artery disease. European Journal of Heart Failure: Abstracts of the Heart Failure 2022 and the World Congress on Acute Heart Failure: 21-24 May 2022, Madrid, Spain / European Society of Cardiology. Chichester: Wiley, 2022, vol. 24, suppl. 2., 2022-05-21, p. 200-200: lent.
3. Verikas D, Žukaitė G, **Mamedov A**, Bolys R, Rumbinaitė E. Is noninvasive measured pulmonary pressure a marker of postoperative long-standing instability after heartmate 3 implantation? European Journal of Heart Failure: Abstracts of the Heart Failure 2023 and the World Congress on Acute Heart Failure: May 20-23, 2023, Prague, Czech Republic / Heart Failure Association (HFA), European Society of Cardiology (ESC)., 2023-07-06, vol. 25, no. S2, p. 215-21.

CURRICULUM VITAE

Name, Surname:	**Arslan Mamedov**
Phone:	+37060948264
E-mail:	ars.mamedovas@gmail.com

Work experience

2019-08-01 – Current	Department of Cardiac, Thoracic and Vascular Surgery (now Heart Centre), Kaunas Clinics Eiveniu str. 2, LT-50009, Kaunas, Lithuania, Cardiac surgeon

Education

September 1, 2020, to August 31, 2025	PhD student, Lithuanian University of Health Sciences
2018.01-2018.05	5 months traineeship at the University of Salzburg Hospital (Austria), Department of Cardiac Surgery
2014–2019	Cardiac surgery Residency, Lithuanian University of Health Sciences, Department of Cardiac, Thoracic and Vascular Surgery. Acquisition of cardiac surgery doctor licence in 2019
2014.01–2014.05	Internship at Klaipeda University Hospital
2008–2014	Studies at Lithuanian University of Health Sciences

Participation in scientific projects:

2025 - Present	Junior researcher in the project „Changes in perivascular, epicardial, and paracardial adipose tissue in acute and chronic coronary artery syndromes “(project number: S-MIP-25-70)
2024- Present	Junior researcher in the project „Endothelial Transglutaminase-2“supported by Research Council of Lithuania (project number: S-MIP-23-94)

PADĖKA

Ši disertacija nėra vien tik mano sunkaus darbo rezultatas. Nuo pat pirmosios doktorantūros dienos mane supo žmonės, kurie mokė, kėlė iššūkius, palaikė ir drąsino nepasiduoti. Visi šio darbo eksperimentai, publikacijos ir pasiekimai yra ne tik mano, bet ir šių žmonių pastangų, pagalbos bei įtakos vaisius.

Pirmiausia norėčiau padėkoti prof. dr. R. Benečiui už mokslinę idėją ir suteiktą galimybę pradėti doktorantūros studijas.

Taip pat nuoširdžiai dėkoju prof. E. Stankevičiui, kuris tapo mano doktorantūros vadovu ir lydėjo mane didžiąją šio kelio dalį. Kartu dirbome penkerius metus: ieškojome sprendimų, atlikome eksperimentus, įveikėme sunkumus, ir visą tą laiką profesorius buvo šalia. Jo dėka susipažinau su Vaistinių medžiagų ikiklinikinių tyrimų laboratorija, todėl esu labai dėkingas ir visam šios laboratorijos personalui už suteiktą pagalbą bei bendradarbiavimą.

Kita mano mokslinio darbo dalis vyko Kardiologijos institute, Molekulinės kardiologijos laboratorijoje. Už didelę pagalbą ir bendradarbiavimą nuoširdžiai dėkoju Rimantui Trainiui ir Dovydui Gečiui.

Taip pat norėčiau padėkoti visiems širdies chirurgams, kurie įvairiais būdais prisidėjo prie bendrų darbo rezultatų. Ypatingą padėką skiriu skyriaus vadovui prof. dr. P. Jakuškai už konsultacijas ir suteiktą galimybę darbo metu atlikti sudėtingus eksperimentus su gyvūnais.

Nuoširdžiai dėkoju ir savo recenzentams. Doc. A. Budrikiui dėkoju už disertacijos chirurginės dalies korekcijas ir vertingas diskusijas. Taip pat labai dėkoju dr. Reginai Mačianskienei už nuodugnią disertacijos analizę ir gilias mokslines įžvalgas bei korekcijas.

Ypatingą padėką norėčiau išreikšti Širdies, kraujagyslių ir krūtinės chirurgijos klinikos slaugytojoms, kurios visos doktorantūros metu palaikė mane ir neleido pasiduoti. Didelis ačiū Liucijai už pagalbą sprendžiant kompiuterinius klausimus ir už nuoširdų moralinį palaikymą.

Taip pat dėkoju prof. dr. Š. Kinduriui ir prof. dr. E. Širvinskui už padrąsinimus ir pagalbą jauno mokslininko kelyje.

Atskirai norėčiau labai nuoširdžiai padėkoti prof. dr. E. Rumbinaitei, kuri visą doktorantūros laikotarpį man labai padėjo, beveik kiekviename žingsnyje konsultavo, koregavo ir drąsino eiti pirmyn.

Galiausiai nuoširdžiai dėkoju doc. dr. T. Lenkučiui už ypatingą profesinę pagalbą bei vertingas ir įdomias pastabas.