Protecting The Aged Heart During Cardiac Surgery: Use Of Del Nido Cardioplegia Provides Superior Functional Recovery In Isolated Hearts

by

Arun Govindapillai

Submitted in partial fulfilment of the requirements
for the degree of Master of Science

at

Dalhousie University
Halifax, Nova Scotia
August 2013

TABLE OF CONTENTS

LIST OF TABLES v
LIST OF FIGURES vi
ABSTRACT ix
LIST OF ABBREVIATIONS AND SYMBOLS USED x
ACKNOWLEDGEMENTS xiii
CHAPTER 1: INTRODUCTION 1
1. Overview 1
2. Pathophysiology of Ischemia-Reperfusion Injury 4
3. Aged and Immature Myocardium are Susceptible to Ischemia-Reperfusion Injury 7
4. Cardioplegia Solutions Protect Against Ischemia-Reperfusion Injury 9
5. The Potential Benefits of del Nido Cardioplegia 11
6. Delivery of del Nido Cardioplegia 14
7. The Isolated Perfused Standard "Langendorff" Rat Preparation 17
7.1 Overview 17
7.2 Modes of Retrograde Perfusion 19
8. The Isolated "Working" Heart Rat Model 20
8.1 Overview 20
9. Objectives and Hypotheses 22
CHAPTER 2: METHODS 25
1. Experimental Animals and Anaesthesia 25
2. The Isolated Working Rat Heart 25
2.1 Setup 25
3. Isolated Heart Preparation 30
4. Perfusion Protocol 32
5. Measurements 34
5.1 Calculated Hemodynamic Variables 34
5.2 Spontaneous Activity during Cardioplegic Arrest 35
5.3 Return of Rhythm during Reperfusion 35
5.4 Troponin Release into Coronary Effluent 35

5.5 Myocardial Edema 36
5.6 Tachyarrhythmias during Reperfusion 36
6. Cardioplegia Preparation and Delivery 36
7. Experimental Groups 38
8. Statistical Analysis 38
CHAPTER 3: RESULTS 39
1. Aged Hearts Experiment 39
1.1 Eligibility of Hearts 39
1.2 Cardiac Temperature 39
1.3 Spontaneous Activity during Cardioplegic Arrest 43
1.4 Return of Rhythm at Reperfusion 45
1.5 Hemodynamic Measurements 48
1.5.1 Heart Rate 50
1.5.2 Rate Pressure Product 51
1.5.3 Left Ventricular Developed Pressure 52
1.5.4 Coronary Flow 54
1.5.5 Cardiac Output 55
1.5.6 Stroke Volume 57
1.5.7 Stroke Work 58
1.5.8 Coronary Vascular Resistance during Reperfusion 59
1.6 Troponin Release into Coronary Effluent 61
1.7 Myocardial Edema 62
2. Young Adult Hearts Experiment 63
2.1 Eligibility of Hearts 63
2.2 Cardiac Temperature 63
2.3 Spontaneous Activity during Cardioplegic Arrest 67
2.4 Return of Rhythm at Reperfusion 69
2.5 Hemodynamic Measurements 72
2.5.1 Heart Rate 74
2.5.2 Rate Pressure Product 75
2.5.3 Left Ventricular Developed Pressure 76
2.5.4 Coronary Flow 77
2.5.5 Cardiac Output 78

2.5.6 Stroke Volume 79
2.5.7 Stroke Work 80
2.5.8 Coronary Vascular Resistance during Reperfusion 81
2.6 Troponin Release into Coronary Effluent 83
2.7 Myocardial Edema 84
CHAPTER 4: DISCUSSION 85
1. Overview 85
2. Interpretation of Results 86
2.1 Spontaneous Activity during Cardioplegic Arrest 86
2.2 Return of Rhythm during Reperfusion 89
2.3 Reperfusion Arrhythmias 94
2.4 Troponin Release into Coronary Effluent 95
2.5 Hemodynamics and Functional Recovery in Isolated Hearts 95
2.6 Coronary Vascular Resistance during Reperfusion 99
2.7 Dosing of del Nido Cardioplegia 104
2.8 Impact of Lidocaine on Na^+ Influx during Cardioplegic Arrest 106
3. Limitations 110
4. Conclusion 111
References 112

LIST OF TABLES

Table 1 Composition of cardioplegia solutions: del Nido vs. standard cardioplegia 16

Table 2 Hemodynamic parameters measured during working heart mode: aged hearts 49

Table 3 Hemodynamic parameters measured during working heart mode: young adult hearts 73

LIST OF FIGURES

Figure 1 Model of pathophysiology of ischemia-reperfusion injury in the cardiomyocyte 6

Figure 2 Retrograde reperfusion in standard Langendorff mode 18

Figure 3 "Working" heart mode 21

Figure 4 Schematic diagram of the isolated working heart system 27

Figure 5 Diagram demonstrating cannulation of the isolated heart 31

Figure 6 Isolated working heart protocol 33

Figure 7 Cardiac temperature following delivery of induction dose of standard and del Nido cardioplegia in aged hearts 40

Figure 8 Cardiac temperature prior to the start of reperfusion in hearts arrested with standard and del Nido cardioplegia in aged hearts 41

Figure 9 Change in cardiac temperature over the cardioplegic arrest period for hearts arrested with standard and del Nido cardioplegia in aged hearts 42

Figure 10 Analysis of spontaneous activity during cardioplegic arrest in aged hearts 44

Figure 11 Analysis of the return of first heart beat (rhythm) following the start of reperfusion in aged hearts 46

Figure 12 Percent of aged hearts that had short runs of sustained tachyarrhythmia during retrograde reperfusion, that were resolved and were self-limiting 47

Figure 13 Heart rate in standard and del Nido cardioplegia groups in aged hearts 50

Figure 14 Rate-Pressure Product in standard and del Nido cardioplegia groups in aged hearts 51

Figure 15 Analysis of left ventricular developed pressure before cardioplegic arrest and after reperfusion in aged hearts 53

Figure 16 Coronary flow in standard and del Nido cardioplegia groups in aged hearts 54

Figure 17 Analysis of cardiac output before cardioplegic arrest and after reperfusion in aged hearts 56

Figure 18 Stroke volume in standard and del Nido cardioplegia groups in aged hearts 57

Figure 19 Stroke work in standard and del Nido cardioplegia groups in aged hearts 58

Figure 20 Analysis of coronary vascular resistance before cardioplegic arrest and during reperfusion in aged hearts 60

Figure 21 Troponin-I release measured from coronary effluent during reperfusion in aged hearts 61

Figure 22 Measurement of myocardial edema in aged hearts 62

Figure 23 Cardiac temperature following delivery of induction dose of standard and del Nido cardioplegia in young adult hearts 64

Figure 24 Cardiac temperature prior to the start of reperfusion in hearts arrested with standard and del Nido cardioplegia in young adult hearts 65

Figure 25 Change in cardiac temperature over the cardioplegic arrest period for hearts arrested with standard and del Nido cardioplegia in young adult hearts 66

Figure 26 Analysis of spontaneous activity during cardioplegic arrest in young adult hearts 68

Figure 27 Analysis of the return of the first heart beat (rhythm) following the start of reperfusion in young adult hearts 70

Figure 28 Percent of young adult hearts that had short runs of sustained tachyarrhythmia during retrograde reperfusion, that were resolved and were self limiting 71

Figure 29 Heart rate in standard and del Nido cardioplegia groups in young adult hearts 74

Figure 30 Rate-Pressure Product in standard and del Nido cardioplegia groups in young adult hearts 75

Figure 31 Left ventricular developed pressure before and after arrest and reperfusion in young adult hearts....76

Figure 32 Coronary flow in standard and del Nido cardioplegia groups in young adult hearts....77

Figure 33 Cardiac output in standard and del Nido cardioplegia groups in young adult hearts....78

Figure 34 Stroke volume in standard and del Nido cardioplegia groups in young adult hearts....79

Figure 35 Stroke work in standard and del Nido cardioplegia groups in young adult hearts....80

Figure 36 Analysis of coronary vascular resistance before cardioplegic arrest and after reperfusion in young adult hearts....82

Figure 37 Troponin-I release measured from coronary effluent during reperfusion in young adult hearts....83

Figure 38 Measurement of myocardial edema in young adult hearts....84

Figure 39 Average baseline cardiac output in young adult and aged heart experiments....99

ABSTRACT

The purpose of this study was to determine if del Nido cardioplegia provides superior protection for aged and young adult hearts. We used our isolated working heart model of cardioplegic arrest and reperfusion to compare functional recovery in both senescent and young adult rat hearts, with delivery of del Nido or our standard cardioplegia. In the aged hearts, use of del Nido cardioplegia prevented spontaneous contractions during arrest, reduced troponin release, and provided superior functional recovery during working heart. In contrast, in the young adult hearts, although stroke work was higher in the del Nido group, there were no significant differences in spontaneous activity, troponin release, and cardiac output between del Nido and standard cardioplegia, suggesting that del Nido cardioplegia did not provide superior functional recovery in the young adult heart. Del Nido cardioplegia has the potential to provide superior myocardial protection for elderly patients undergoing cardiac surgery.

LIST OF ABBREVIATIONS AND SYMBOLS USED

H_20	water
Ca^{2+}	calcium ion
Na^{+}	sodium ion
NHE	sodium hydrogen exchanger
H^{+}	hydrogen ion
HCO_3^{-}	bicarbonate ion
NCX	sodium calcium exchanger
ATP	adenosine triphosphate
SR	sarcoplasmic reticulum
min	minutes
CABG	coronary artery bypass grafting
NO	nitric oxide
VT	ventricular tachycardia
VF	ventricular fibrillation
SERCA	sarcoplasmic reticulum calcium ATPase
LV	left ventricle
LVDP	left ventricular developed pressure
K^{+}	potassium ion
TTC	tetrazolium chloride
NHEI	sodium hydrogen exchange inhibitor
MI	myocardial infarction
°C	degrees Celsius

Tn-I	troponin-I
mL	milliliter
kg	kilogram
mmHg	millimeter of mercury
Mg^{2+}	magnesium ion
mOsm	milliosmole
IP	intraperitoneal injection
mm	millimeter
KHB	Krebs-Henseleit buffer
O_2	oxygen
CO_2	carbon dioxide
bpm	beats per minute
RPP	rate-pressure product
HR	heart rate
SP	systolic pressure
LVEDP	left ventricular end-diastolic pressure
CO	cardiac output
AF	aortic flow
CF	coronary flow
SV	stroke volume
SW	stroke work
CVR	coronary vascular resistance
MAP	mean aortic pressure

ECG	electrocardiogram
wt	weight
ANOVA	Analysis of Variance
I_{Na}	sodium current
TTX	tetrodotoxin

ACKNOWLEDGEMENTS

First, I would like to acknowledge my supervisor, Dr. Stacy O'Blenes, for his advice, support, encouragement and guidance throughout this project. Thank you for providing me with the opportunity to develop as a scientist and to gain important life skills. It has been a privilege to learn from such a highly motivated and hard-working scientist and clinician.

I would also like to thank Richard Livingston and Audrey Li for your excellent training, technical support and advice, and helpful troubleshooting throughout this project. Thank you for your willingness to assist me with questions that I encountered throughout my graduate research. Additionally, I would also like to thank my colleague Jeanne Egar for her ongoing support in and out of the lab.

Finally, I would like to thank the staff of the Department of Physiology & Biophysics, Jennifer Graves, Alice Smith, Nikolas Morgunov, and Elizabeth Cowley for always assisting me with administrative tasks and helping to answer all of my questions. Your assistance has been invaluable over the past two years.

CHAPTER 1: INTRODUCTION

1. Overview

Elderly patients undergoing cardiac surgery have a higher risk of significant cardiac dysfunction leading to post operative complications and reduced survival when compared to younger adult patients (Hirose et al., 2000; Shahian et al., 2009; O'Brien et al., 2009). This is because aged hearts are particularly vulnerable to ischemia-reperfusion injury that occurs during cardiac surgery (McCully et al., 2006; Willems et al., 2005; Boucher et al., 1998; Ataka et al., 1992). Cardioplegia is an integral and essential method of myocardial protection for patients of all ages requiring cardiac surgery in which the heart must be stopped (Buckberg et al., 1977). Cardioplegia solutions aim to reduce the metabolic activity of the myocardium so that during periods of ischemia, the development of intracellular acidosis leading to Ca^{2+} accumulation is slowed, thereby attenuating Ca^{2+}-mediated myocardial damage upon reperfusion (Buckberg et al., 1977). Cardioplegia solutions also aim to abolish the electromechanical activity of the myocardium in order to reduce the metabolic demands of the heart when under cardiopulmonary bypass. Cardioplegia solutions are delivered into the coronary arteries once clamping the aorta has disrupted blood flow (Gay, 1975).

A specialized cardioplegia solution, del Nido cardioplegia, designed to protect pediatric myocardium, may also be beneficial for elderly patients, due to the similarities that immature and senescent hearts share with respect to their increased susceptibility to

myocyte injury during ischemia and reperfusion. Researchers at the University of Pittsburgh developed a novel formulation for myocardial protection in the early 1990s. This team, led by Dr. Pedro del Nido, Hung Cao-Danh, K. Eric Sommers, and Akihiko Ohkado, eventually patented this solution**,** known as del Nido cardioplegia. Cardioplegia for infant and pediatric patients was originally the same as that used for adults and was simply adjusted for volume, flow and pressure (Allen, 2004). St. Thomas' Hospital cardioplegia solution was widely used in the 1980s and 1990s in this manner. However, researchers at the University of Pittsburgh recognized the need for a cardioplegia solution that more specifically addressed the needs and differences of the immature heart.

The immature heart has been described as being both more tolerant to ischemia (Hiramatsu et al., 1995; Bove et al., 1986; Baker et al., 1988) and less so (Wittnich et al., 1987). A study in 1989 by Kempsford and Hearse may have explained this contradiction by suggesting that the efficacy of cardioplegia in the immature myocardium may be more related to the cardioplegia solution itself than the underlying physiology of the neonatal heart. The neonatal heart has been shown experimentally to recover better following ischemia-reperfusion with single-dose cardioplegia, leading to the development of del Nido cardioplegia, which is administered as a single induction dose at the beginning of the cardioplegic arrest period (Kohman et al., 1994; Sawa et al., 1989).

The elderly, like the young, have myocardium that is more susceptible to Ca^{2+} mediated ischemia-reperfusion injury. Both immature and aged myocardium are poorly equipped to deal with post-ischemic Ca^{2+} overload (McCully et al., 2006; Willems et al., 2005; Boucher et al., 1998; Ataka et al., 1992, Faulk et al., 1995; Tsukube et al., 1997; Ladilov et al., 2003; Piper et al., 2003; Tsukube et al., 1996; Wittnich et al., 1987; Parrish et al., 1987; Bolling et al., 1996). Given that immature and aged hearts are both susceptible to myocardial injury during ischemia and reperfusion, it is possible that giving del Nido cardioplegia to the aged heart may result in improved functional recovery compared to our "standard" cardioplegia, which is currently being used to treat all adult patients at the Queen Elizabeth II Health Sciences Center Hospital in Halifax, Nova Scotia.

Our lab has previously shown that in isolated aged cardiomyocytes, arrest with del Nido cardioplegia resulted in lower spontaneous and inducible activity during ischemia and lower diastolic Ca^{2+} during ischemia-reperfusion when compared with our "standard" cardioplegia (O'Blenes et al., 2011). This led us to investigate how whole isolated hearts would respond to del Nido cardioplegia.

The overall aim of this study was to determine if the use of del Nido cardioplegia could result in superior functional recovery in both whole, isolated aged and young adult hearts compared to our standard cardioplegia. The isolated working heart Langendorff model of cardioplegic arrest was used to compare the effects of del Nido cardioplegia to that of our standard cardioplegia, on cardiac function, from senescent and young adult rat

hearts. While various cardioplegia formulations have been proposed and evaluated previously, we have chosen to focus on del Nido cardioplegia as our starting point for developing a tailored strategy for myocardial protection for the aged heart, because it has already been used extensively in the clinical setting for pediatric and young adult patients, and we anticipate that this study will facilitate its incorporation into an eventual clinical trial for elderly patients. We also sought to determine whether use of del Nido cardioplegia could provide better cardioprotection than standard cardioplegia to the whole, isolated young adult heart, since there has been a lack of evidence in the literature, in basic science and clinical research, to support the use of del Nido cardioplegia on the young adult population.

The following introduction will discuss the pathophysiology of ischemia reperfusion injury in the heart, unique characteristics of aged and immature myocardium, the cardioprotective role of cardioplegia solutions during cardiac surgery, and potential strategies to reduce post-operative myocardial dysfunction in elderly patients undergoing cardiac surgery.

2. Pathophysiology of Ischemia-Reperfusion Injury

During cardiac surgery, a clamp is typically applied across the ascending aorta to interrupt blood flow to the myocardium, while the patient's circulation is supported on cardiopulmonary bypass (Kinoshita et al., 2012). This allows for surgeons to have a clear visualization of the heart and vasculature in order to perform cardiac procedures.

However, the process of restoring blood flow to the ischemic myocardium can induce injury by ischemic necrosis, cellular damage that occurs when blood flow is restored – known as ischemia-reperfusion injury.

The development of elevated intracellular Ca^{2+} and cardiomyocyte hypercontracture primarily mediate ischemia-reperfusion injury (Ladilov et al., 2003; Kinoshite et al., 2012; Steenbergen et al., 1990). During ischemia, oxygen delivery to the myocardium is impaired, cardiomyocyte energy stores are rapidly depleted, anaerobic metabolism occurs, and intracellular acidosis develops (Steenbergen et al., 1990, Piper et al., 1999). Intracellular acidosis leads to an increase in intracellular Ca^{2+} levels during ischemia by promoting Na^+ influx through the Na^+/H^+ exchanger (NHE) and the Na^+/HCO_3^- symporter (Piper et al., 1999; Allen et al., 1993; Anderson et al., 1990; Avkiran et al., 2001). The increased Na^+ influx causes Ca^{2+} influx by reverse mode action of the Na^+/Ca^{2+} exchanger (NCX; Piper et al., 1999). Upon reperfusion of the myocardium, oxygen supply is restored to the tissue, resulting in myocardial damage due to the elevated intracellular Ca^{2+} levels within the cardiomyocyte. Excessive force generation occurs as a result of the restoration of function of the contractile apparatus in the presence of elevated intracellular Ca^{2+} levels, resulting in hypercontracture during early reperfusion, which can lead to cell injury or death (Ladilov et al., 2003; Piper et al., 2003). **See Figure 1 for an illustrative diagram depicting cellular mechanisms of ischemia reperfusion injury in the cardiomyocyte.**

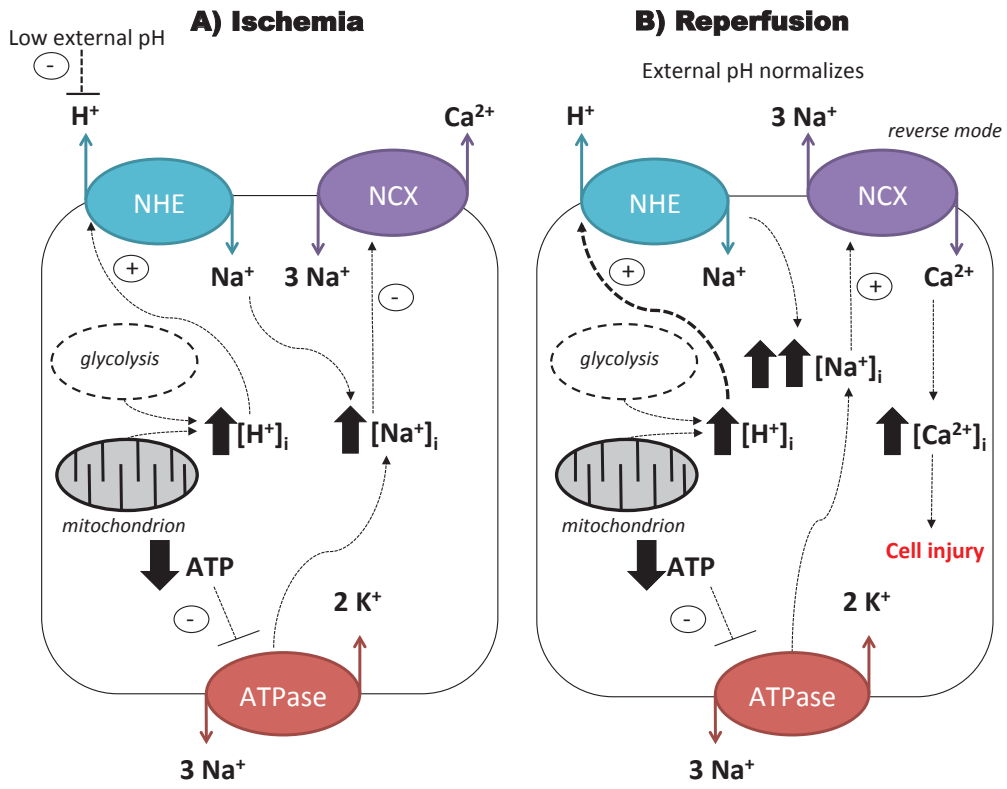

Figure 1. Model of pathophysiology of ischemia-reperfusion injury in the cardiomyocyte. Ischemia leads to intracellular acidosis by anaerobic glycolysis and mitochondrial dysfunction. Na^+/H^+ exchange is driven by the intracellular/extracellular H^+ gradient and is allosterically activated by intracellular protons (dashed curved arrows). Acidification of the interstitium (low extracellular pH) partially suppresses the gradient-driven Na^+/H^+ exchange. At the same time, cellular adenosine triphosphate (ATP) depletion leads to Na^+/K^+-ATPase dysfunction. The net result is intracellular Na^+ loading. Rising intracellular Na^+ slows down the NCX, impairing Ca^{2+} extrusion from the cell. **B:** During reperfusion, extracellular pH normalizes, allowing Na^+/H^+ exchange to be fully activated. With cellular ATP and Na^+/K^+-ATPase activity still insufficient, intracellular Na^+ levels continue to rise. At some point in this process, increased $[Na^+]_i$ leads to reverse mode operation of NCX. NCX extrudes Na^+ at the cost of raising intracellular Ca^{2+} concentration, which reaches deleterious levels, leading to cell injury and apoptosis.

3. Aged and Immature Myocardium are Susceptible to Ischemia Reperfusion Injury

Immature myocardium is less tolerant to ischemia than mature (young adult) myocardium (Wittnich et al., 1987; Parrish et al., 1987). Immature cardiomyocytes are particularly susceptible to Ca^{2+} induced injury during ischemia and reperfusion because in the pediatric heart, the sarcoplasmic reticulum (SR) is underdeveloped and the Ca^{2+}-ATPase responsible for shifting Ca^{2+} into the sarcoplasmic reticulum has lower activity (Boland et al., 1974; Gombosova et al., 1998). Pediatric hearts rely more on trafficking of Ca^{2+} in and out of the extracellular space for excitation-contraction coupling rather than the SR, and therefore, are poorly equipped to deal with the post-ischemic Ca^{2+} overload (Rebeyka et al., 1990). Additionally, it should not be overlooked that there is quantitatively less SR in newborns than in adults (Hoerter et al., 1981). The increased vulnerability to ischemia-reperfusion injury that is experienced by the pediatric heart has been suggested to be characterized by an early and rapid increase in diastolic stiffness during ischemia and severe loss of systolic performance during reperfusion (Parrish et al., 1987). Several studies have argued that while ATP depletion in the tissue is an important mechanism of myocardial injury during ischemia-reperfusion, and although accumulation of rigor contracture and consequential myocardial contracture has been strongly correlated with tissue ATP levels (Hoerter et al., 1981; Hearse et al., 1977; Sink et al., 1980), they have shown that ATP is relatively preserved during ischemia in the immature heart (Nishioka et al., 1982). A third mechanism that may contribute to age-related differences during ischemia is differences in tissue (intracellular) pH. Parrish et al. (1987)

found that pediatric myocardium has a higher tissue pH during the early part of ischemia, than the adult rabbit heart. Intracellular acidosis is an important mechanism for the deactivation of adenosine triphosphatase activity of cardiac myofilaments (Tsien, 1976). Acidosis appears to reduce the affinity of the myofibrillar troponin C for calcium, thus inhibiting myofilament contraction (Blanchard et al., 1984). The increased intracellular hydrogen ion concentration found in adult rabbit hearts may protect the adult heart from ischemic contracture, by inhibiting contractile activity and inhibiting the formation of rigor contractures. Another important mechanism of ischemic cellular damage is free radical injury. Oxygen radicals are important mediators of ischemic injury, particularly during reperfusion (Gaudel et al., 1984; Hess et al., 1984). Immature tissue, including heart and lung, are relatively deficient in the antioxidant enzymes needed to detoxify free radicals (Tanswell et al., 1984; Gerdin et al., 1985; Otani et al., 1985). Furthermore, immature sarcolemma has a particularly high content of polyunsaturated fatty acids, which may increase the susceptibility of the membrane to free radical injury (Awad et al., 1982). Lastly, several groups have reported a rapid increase in coronary vascular resistance during reperfusion in the neonatal rabbit heart, suggesting that perhaps the neonatal heart is more susceptible to ischemia-reperfusion injury due to age-related differences in coronary microcirculation (Parish et al., 1987).

Aged hearts are also vulnerable to the ischemia-reperfusion injury that occurs during cardiac surgery (McCully et al., 2006; Willems et al., 2005; Boucher et al., 1998; Ataka et al., 1992, Faulk et al., 1995; Tsukube et al., 1997; Ladilov et al., 2003;

Piper et al., 2003). The mechanism for this intolerance to ischemia in aged myocardium is thought to be related to changes in Ca^{2+} homeostasis that result in higher intracellular Ca^{2+} levels during ischemia (Ataka et al., 1992; Faulk et al., 1995; Tsukube et al., 1997), which mediate damaging hypercontracture during early reperfusion (Nishioka et al., 1982), and due to impaired Ca^{2+} uptake by the SR, leading to an accumulation of intracellular Ca^{2+}. An age-related decline in basal coronary flow has been documented previously in mouse and rat heart (Willems et al., 2005). The genesis of reduced flow is unknown, but may involve both structural changes in the vasculature, for example, vascular stiffening, together with alterations in vascular control mechanisms (Folkow et al., 1993; Dohi et al., 1995). Other potential causes for the age-related intolerance to ischemia-reperfusion include altered and abnormal mitochondrial function and anti-oxidant defenses (Willems et al., 2005).

Compared to the young adult, differences in the cellular response to cardioplegia in the elderly due to increased ischemic intolerance may help to explain why elderly patients undergoing cardiac surgery have impaired recovery of ventricular function and lower survival than the young adult. It has been shown that strategies to limit accumulation of intracellular Ca^{2+} in aged hearts do improve the recovery of ventricular function after ischemia (Faulk et al., 1995; Tsukube et al., 1997).

4. Cardioplegia Solutions Protect Against Ischemia-Reperfusion Injury

In order to minimize post-operative cardiac dysfunction following cardiac surgery, strategies have been developed to prevent ischemia reperfusion injury and protect myocardium. These strategies aim to reduce the metabolic activity of the myocardium, such that during ischemic periods, the development of intracellular acidosis leading to Ca^{2+} accumulation is slowed, thereby attenuating Ca^{2+} mediated myocardial damage upon reperfusion. Myocardial protection strategies typically aim to abolish the electro-mechanical activity, which accounts for most of the cardiomyocyte's metabolic demands (Buckberg et al., 1977). This is achieved by delivering a cardioplegia solution into the coronary arteries once clamping the aorta has disrupted coronary blood flow. The cardioplegia is formulated with additives that will cause the desired electro-mechanical arrest. The most widely used additive is K^+, which causes an increase in the extracellular K^+ concentration thereby reducing the trans-membrane K^+ gradient leading to membrane depolarization (Gay, 1975). Depolarization of the cell membrane largely inactivates Na^+ channels responsible for the generation of action potentials. In the absence of action potentials, Ca^{2+} influx from the extracellular space and sarcoplasmic reticulum is inhibited, thereby preventing contraction (Fallouh et al., 2009).

Cardioplegia solutions are either mixed with blood or delivered as a clear "crystalloid" solution. Cardioplegia is most commonly delivered hypothermically (≈10°C)**,** which minimizes the remaining metabolic activity, thereby allowing the flow of cardioplegia to be safely interrupted for a period of time, typically 20-30 minutes between doses (Bove et al., 1986). In order to avoid potentially detrimental effects of hypothermia on cardiomyocyte metabolism (Fremes et al., 1985; Weisel et al., 1989),

delivering cardioplegia at normothermia (≈37°C) was proposed (The Warm Heart Investigators, 1994). However, the so-called "warm cardioplegia" has to be delivered on a continual basis to ensure adequate myocardial protection, which complicates the surgical procedure. Tepid cardioplegia (≈24°C), is an intermediate strategy that appears to allow safe intermittent delivery while avoiding potentially damaging profound hypothermia (Chocron et al., 2000).

5. The Potential Benefits of del Nido Cardioplegia

Age related changes in Ca^{2+} homeostasis may alter the action of cardioplegia solutions on aged hearts, since it has been shown in the literature that aged hearts are not as well protected by some cardioplegia solutions (Caldarone et al., 1995). Since there is an increasing number of elderly patients requiring cardiac surgery over the past years (Statistics Canada, 2011), and cardiac surgery is riskier in elderly patients than in young adult patients due to a higher chance of significant myocardial dysfunction after surgery (Hirose et al., 2000; Shahian et al., 2009; O'Brien et al., 2009), it is important to develop cardioplegic strategies that specifically tailor toward benefitting elderly patients.

A specialized cardioplegia solution, del Nido cardioplegia, developed by Dr. Del Nido from the Boston Children's Hospital, was designed to protect pediatric myocardium, and may also be beneficial for elderly patients, due to the similarities that immature and aged hearts share with respect to their susceptibility to myocyte injury (McCully et al., 2006; Willems et al., 2005; Boucher et al., 1998; Ataka et al., 1992,

Faulk et al., 1995; Tsukube et al., 1997; Ladilov et al., 2003; Piper et al., 2003; Tsukube et al., 1996; Wittnich et al., 1987; Parrish et al., 1987; Bolling et al., 1996). Our lab previously reported that compared to our standard cardioplegia, del Nido cardioplegia resulted in significantly lower post-operative serum troponin levels, released from injured cardiomyocytes, in children undergoing cardiac surgery (O'Brien et al., 2009). Our lab has previously shown that in aged isolated cardiomyocytes, arrest with del Nido cardioplegia results in lower spontaneous and inducible activity during ischemia and lower diastolic Ca^{2+} during ischemia and reperfusion when compared with standard cardioplegia (O'Blenes et al., 2011).

There are three main features that distinguish del Nido from our standard cardioplegia. Firstly del Nido cardioplegia is mixed with only a small amount of blood (1:4 blood:crystalloid), which differs from our standard cardioplegia (4:1 blood:crystalloid; **see Table 1 for composition of cardioplegia solutions).** This results in a Ca^{2+} concentration in del Nido cardioplegia that is significantly lower than in standard cardioplegia, thus potentially attenuating hypercontraction-induced ischemia-reperfusion injury. Second, del Nido cardioplegia has a higher K^+ concentration than our standard cardioplegia, hence it may be able to achieve more extensive and prolonged membrane depolarization, thereby optimizing inactivation of Na^+ channels and preventing the generation of action potentials. In the absence of action potentials, hypercontraction is prevented (Fallouh et al., 2009). Lastly, del Nido cardioplegia contains lidocaine which blocks fast Na^+ channels and prevents Na^+ influx, and may potentially reduce the inward Na^+ "window current" that is active at membrane potentials

achieved with K^+ induced depolarization (Chambers et al., 1999; Attwell et al., 1979). Strategies to limit Na^+ influx should indirectly limit potentially injurious Ca^{2+} influx, since increased intracellular Na^+ influx drives Ca^{2+} overload through the reverse mode action of the NCX (**Figure 1**).

Normal contractile function in its most basic form relies on high-energy phosphates, maintenance of intracellular pH, and ionic and cell membrane homeostasis, all contributing to aerobic metabolism (Matte et al., 2012) A disruption of any of these may lead to irreversible damage after myocardial ischemia (Charette et al., 2012). Furthermore, the promotion of anaerobic glycolysis, scavenging of ROS, and prevention of intracellular Ca^{2+} accumulation are thought to be desirable in preserving function during the arrest period (Jennings et al., 1981; Werns et al., 1986). Cardioplegia solutions generally rely on metabolic arrest coupled with hypothermia to address these concerns (Ohkado et al., 1994). The most commonly used method to achieve contractile arrest is by providing a high concentration of K^+ ions into the extracellular space (hyperkalemia); del Nido cardioplegia is hyperkalemic (Matte et al., 2012). Although the advantage of this strategy is its simplicity and the rapid onset of arrest, washout of the potassium-containing solution and the fact that potassium causes cardiomyocyte depolarization are the main drawbacks of this approach. It is well known that hyperpolarizing cells during ischemia slow down the rate of energy consumption and intracellular ion accumulation of the detrimental calcium ion. For this purpose, polarizing agents such as lidocaine, along with calcium-competing ions such as magnesium, were added to the formulations of cardioplegia solutions, as found in del Nido cardioplegia (Matte et al., 2012). Although the solution was developed with the immature myocardium in mind, it is important to

note that its use in adult patients with acquired cardiovascular disease has been reported (New York Presbyterian Hospital and Cleveland Clinic Foundation). Del Nido cardioplegia is used on all patients from neonates to adults with congenital heart disease at the Boston Children's Hospital (Matte et al., 2012).

6. Delivery of del Nido Cardioplegia

Del Nido cardioplegia is used in several centers for myocardial protection during pediatric cardiac surgery (O'Brien et al., 2009; Matte et al., 2012; Charette et al., 2012). Compared to the 'standard' 4:1 blood cardioplegia we use in our adult practice, del Nido cardioplegia is more dilute (1:4 blood:crystalloid), has ≈75% less Ca^{2+}, and contains lidocaine (**Table 1**). Del Nido cardioplegia is usually given as a single dose (O'Brien et al., 2009; Matte et al., 2012; Charette et al., 2012), whereas our standard cardioplegia is given as an induction dose followed by maintenance doses every ≈20 minutes. In the clinic, del Nido cardioplegia is typically given as a single 20 mL/kg dose (Matte et al., 2012). The maximum arresting dose is usually limited to 1 L for patients larger than 50 kg. Additional cardioplegia may be given for hypertrophied hearts, those with aortic insufficiency, or those with known coronary disease based on the effectiveness of the initial dose and surgeon preference. A smaller arresting dose of 10 mL/kg may be used for procedures requiring a cross-clamp time of less than 30 minutes. Subsequent doses are not normally given except for the rare occurrence of electrical activity or for exceptionally long cross-clamp times (greater than 3 hours) at the surgeon's discretion. In the clinical setting, the 20 mL/kg cardioplegia dose is generally given over 1-2 minutes

with a system pressure of 100-200 mmHg. Aortic root pressure is not monitored, although the surgeon monitors aortic root distention closely during delivery to prevent capillary damage from high shear forces with too rapid a delivery. This method results in a cardioplegia delivery flow rate of 10-20 mL/kg/min in infants and toddlers (Matte et al., 2012).

	Standard Cardioplegia	del Nido Cardioplegia
Base solution (1 L)	D5 0.225% NaCl	Plasmalyte A
Components (mmol/L)		
Na	38.8	140
Cl	38.8	98
K	-	5
Mg	-	1.5
Acetate	-	27
Gluconate	-	23
Glucose	278	
Additives (mL/L base solution)		
KCl 2 meq/mL	44	13
$NaHCO_3$ 1 meq/mL	100	13
$MgSO_4$ 0.2g/mL	15	10
Lidocaine 1%	-	13
Mannitol 25%	-	13
Dilution (blood:cardioplegia)	4:1	1:4
Estimated Final Composition (mmol/L)		
Na	136	143
K	18	24
Mg	5	7
Ca	1.0	0.24
Glucose	51	1
Lidocaine	-	0.36
Estimated final Osmolarity (mOsm/L)	396	375
Estimated final Hematocrit (%)	≈25	≈6

Table 1. Composition of Cardioplegia Solutions – del Nido cardioplegia versus "standard" cardioplegia. Del Nido cardioplegia contains lidocaine, less blood (1:4 ratio of blood to crystalloid) and therefore 75% less calcium.

7. The Isolated Perfused Standard "Langendorff" Rat Heart Preparation

7.1 Overview

The isolated Langendorff rat heart preparation was implemented in this study to assess functional recovery of hearts following delivery of either del Nido or our standard cardioplegia. The isolated perfused mammalian heart preparation was established by the German physiologist Oskar Langendorff in 1895 as a tool for studying heart biology. Since then it has been one of the most widely adopted models of mammalian cardiophysiology for basic and pre-clinical drug research (Bell et al., 2011). The isolated perfused "Langendorff" heart system allows for examination of cardiac inotropic, chronotropic, and vascular effects without the complications of an intact animal model. In the Langendorff model, the heart is excised from the animal and cannulated via the aorta (Bell et al., 2011). The basic design of the Langendorff model is such that the perfusate is allowed to flow retrogradely through the ascending aorta, perfusing the coronary arteries (**Figure 2;** Radnoti Working Heart Manual 2013). With the perfusate flowing retrogradely down the aorta, opposite to normal physiological flow, the aortic valve is forced shut under pressure, directing the perfusion fluid into the coronary ostia, thereby perfusing the entire ventricular mass of the heart and draining into the right atrium via the coronary sinus (Bell et al., 2011). With free drainage of the right atrium, the preparation can therefore be maintained without any fluid filling the ventricular chambers. Once coronary flow is established, the perfused heart can undergo various analyses over a

period of time including: contractile function, heart rate, coronary vascular tone, and cardiac metabolism (Bell et al., 2011). **See Materials and Methods section for setup of the standard Langendorff system.**

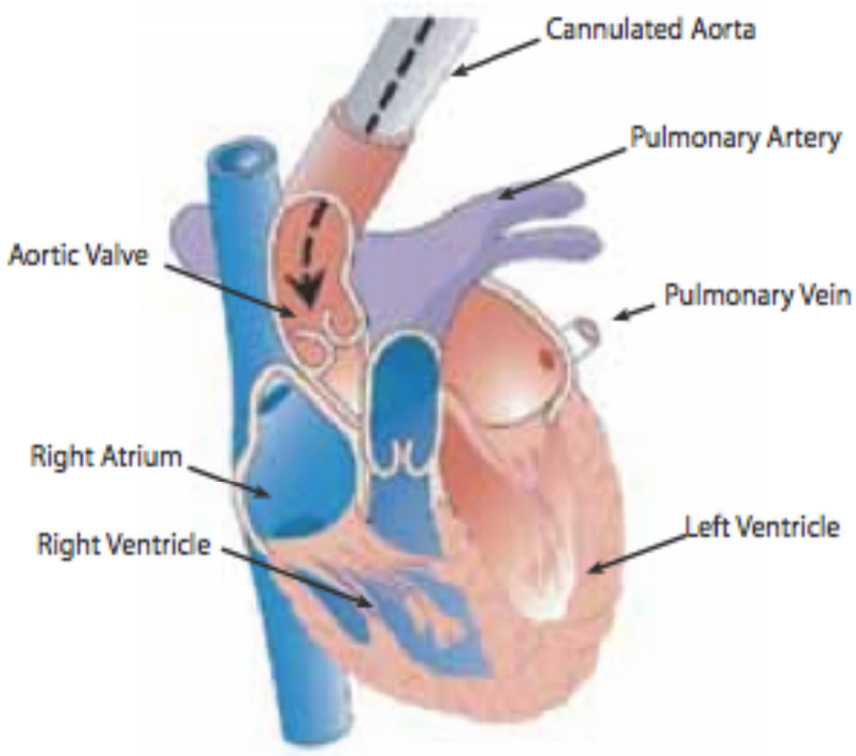

Figure 2. Retrograde reperfusion in standard Langendorff mode. Perfusate flows retrogradely through the aorta (dotted arrow indicates direction of flow) to perfuse the coronary arteries.

The greatest advantages of the standard Langendorff preparation are the reasons for the method's longevity: simplicity of the preparation, low cost, reproducibility, and the ability to study an organ in isolation of other organ systems and exocrine control that may confound physiological measurement (Bell et al., 2011). There are, however,

limitations to consider: the isolation from the whole animal moves any study further away from clinical relevance. Furthermore, the Langendorff preparation, which may be viable over several hours, must nonetheless be considered a dying preparation (Bell et al., 2011). Many groups have reported a 5-10% per hour deterioration in contractile and chronotropic function (Sutherland et al., 2000).

7.2 Modes of Retrograde Perfusion for the Standard Langendorff Preparation: Constant Pressure and Constant Flow

The standard Langendorff system operates in either a constant pressure or constant flow mode, both of which are used in this study. Constant flow mode, where coronary flow is administered by the peristaltic roller-pump, is particularly useful when studying coronary vascular tone and endothelial function (Bell et al., 2011). While in constant flow mode, measurement of perfusion pressure can be achieved by using a pressure transducer, allowing one to calculate coronary vascular resistance during constant flow mode – an advantage of the constant flow mode (Bell et al., 2011). A pressure control circuit is maintained by using a peristaltic roller pump**.** This pump can switch from constant flow to constant pressure seamlessly. Constant pressure can be achieved easily by maintaining a constant hydrostatic pressure through a set height column of fluid, such as positioning a reservoir and its fluid meniscus a known distance above the tip of the perfusion cannula in the heart preparation's aorta (Bell et al., 2011). Constant pressure mode perfusion is ideal for models of ischemia-reperfusion injury, where using constant flow mode instead would administer far more coronary perfusate

per unit volume of available myocardium than before coronary ligation, with the potential risk of coronary artery damage resulting through shear stress (Bell et al., 2011). During this study, before switching to working heart mode, both at baseline and at post-reperfusion, hearts are first retrogradely perfused in constant flow mode, then switched to constant pressure mode and finally to working heart mode, to minimize potential damage to the coronary arteries due to flow of the perfusate causing potential shear stress.

8. The Isolated "Working" Rat Heart Model

8.1 Overview

The isolated working rat heart model was also implemented in this study. During a normal cardiac contraction in a mammalian heart, the blood stored in the LV is ejected at a pressure of about 80-100 mmHg into the aorta (Bell et al., 2011). At the base of the aorta is an ostium which feeds blood under this pressure into the coronary arteries. The standard Langendorff system maintains the isolated heart through the use of a roller pump that is connected via a tube to the aortic cannula (Neely et al., 1967). When the reservoir is opened, the perfusate is forced through the ostia into the coronary bed. This is termed "retrograde perfusion", in the sense that the perfusate flows down into the aorta rather than out the LV through the aorta, as blood does *in situ* (Neely et al., 1967). One of the major disadvantages of the standard Langendorff system is that it does not permit the heart to generate pressure-volume work, because the perfusate does not flow via the normal systemic circulatory pathway (Neely et al., 1967). A modern modification which

permits the heart to pump fluid through the systemic circulatory pathway is the "working" heart model developed by Neely et al**.** (1973), in which perfusate enters the cannulated left atria, passes through the left ventricle, and is ejected out of the aorta (**Figure 3**). We utilized both the standard retrograde Langendorff and working heart modes in our isolated rat heart model. **See Materials and Methods section for setup of the isolated working heart model.**

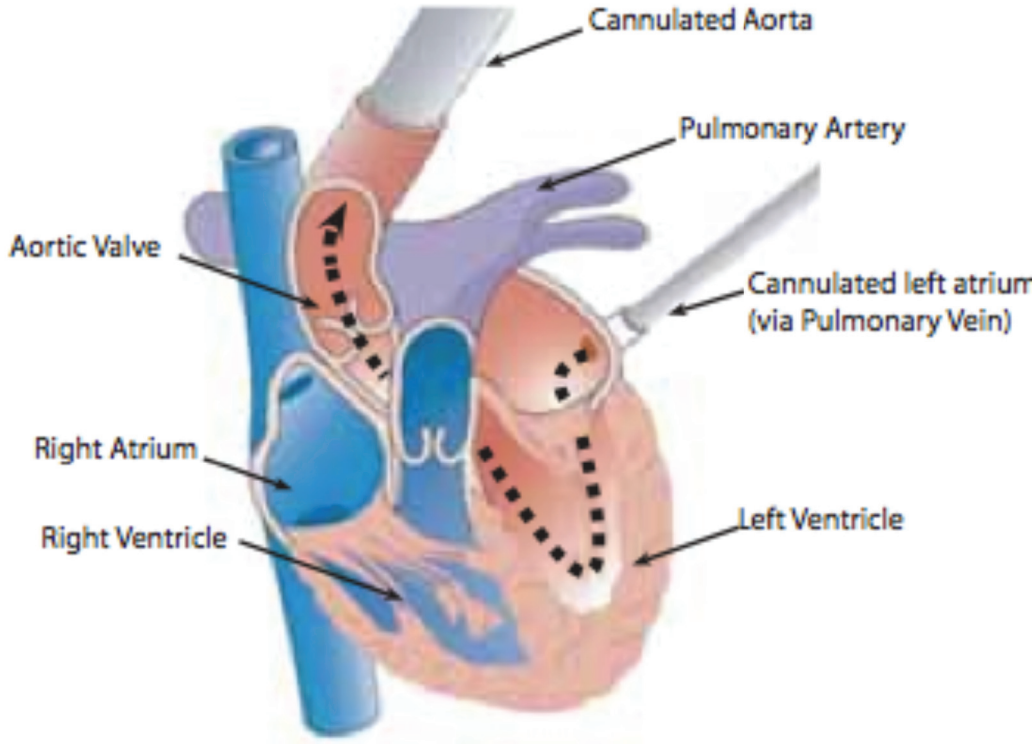

Figure 3. "Working" heart mode. The heart is perfused through the left atria (dotted line indicates direction of flow). Perfusate enters the left ventricle and is ejected out of the aorta.

9. Objectives and Hypotheses

The overall aim of this study was to determine if the use of del Nido cardioplegia could result in superior functional recovery in both whole, isolated aged and young adult hearts compared to our standard cardioplegia. We utilized the isolated working heart Langendorff model of cardioplegic arrest and reperfusion to compare the effects of del Nido cardioplegia to that of our standard cardioplegia on cardiac function in senescent and young adult rat hearts. Since elderly myocardium is similar to immature myocardium in that both are particularly susceptible to reperfusion injury related to Ca^{2+} overload, we hypothesized that a del Nido cardioplegia strategy may also be beneficial in the elderly.

It was hypothesized that since our lab has previously shown that: in isolated cardiomyocytes arrest with del Nido cardioplegia results in lower spontaneous and inducible activity during ischemia, lower diastolic Ca^{2+} during ischemia-reperfusion, and avoidance of hypercontraction during early reperfusion in aged cardiomyocytes when compared with standard cardioplegia (O'Blenes et al., 2011); del Nido cardioplegia has been shown to be clinically beneficial for pediatric patients in reducing post-operative troponin-I levels compared to our standard cardioplegia (O'Brien et al., 2009); and the pediatric and aged heart share similarities as they are both particularly vulnerable to ischemia reperfusion injury, then del Nido cardioplegia would provide superior myocardial protection by improving post-ischemic functional recovery of aged rat hearts versus our standard cardioplegia.

We also sought to determine whether del Nido cardioplegia provides additional benefits in the young adult heart compared to our standard cardioplegia. Since the young adult heart is more tolerant to ischemia-reperfusion injury than the aged heart (Ataka et al., 1992), it was hypothesized that delivery of del Nido cardioplegia to the young adult heart would not exert the same level of cardioprotection than in the aged heart and would not provide any significant cardioprotective advantages over our standard cardioplegia. Although del Nido cardioplegia has been given to young adult patients in some clinics, there is a lack of basic science and clinical evidence to support its superiority in providing cardioprotection to the young adult heart. This second objective will help to determine if del Nido cardioplegia is only an effective cardioplegic strategy for added protection of aged hearts, or whether it should also be implemented as a strategy for protecting younger adult hearts.

The specific objectives of this study, in the form of research questions, are summarized below.

1) **Do the benefits of del Nido cardioplegia seen in isolated cardiomyocytes from aged animals translate into improved function in the whole heart?**

This first objective will be approached by utilizing an isolated working rat heart model of ischemia-reperfusion injury to measure hemodynamics and functional recovery of aged hearts that have been given either del Nido or our standard cardioplegia.

2) **Does del Nido cardioplegia provide improved cardioprotection over our standard cardioplegia in the young adult heart?**

This second objective will be approached by utilizing the same isolated working rat heart model of ischemia-reperfusion injury to examine the impact of each type of cardioplegia on young adult rat hearts.

CHAPTER 2: METHODS

1. Experimental Animals and Anaesthesia

Experiments were performed according to guidelines published by the Canadian Council on Animal Care (CCAC; Ottawa, Ontario: Vol 1, 2nd edition, 1993; Vol 2, 1984). Male Fisher 344 rats (young adult hearts group: 3-4 months old; aged hearts group: 22-24 months old) were fed *ad libitum* and housed in a 12-hour light/dark cycle. Rats were obtained from Charles River Laboratories (Saint-Constant, Canada) and were heparinized to prevent coagulation (IP, 3000 U/kg; Pharmaceutical Partners of Canada, Richmond, ON), and anesthetized with sodium pentobarbital (IP, 160 mg/kg; CDMV; Saint-Hyacinthe, QC).

2. The Isolated "Working" Rat Heart

2.1 Setup

In addition to the use of the standard Langendorff system, the isolated working rat heart model was also used for this study. In the working heart model, which is a slight modification of the standard Langendorff heart system, the heart is no longer perfused retrogradely through the aorta, but is perfused via the left atrium due to the insertion of a second cannula into the pulmonary vein (**Figure 3).** The working heart pumps the perfusate from the LV out of the aorta under experimentally controlled preload (atrial

pressure) and afterload (aortic resistance) conditions (Neely et al., 1967). The preload of the preparation is determined by the height of the overflow from the atrial perfusion bubble trap above the heart. The afterload is determined by the height of the compliance (afterload) reservoir above the aortic cannula (Radnoti Working Heart Manual 2013). The compliance bubble trap contains a 2mm diameter air bubble to mimic normal vascular elasticity. **Figure 4 illustrates the setup of the isolated working heart model.**

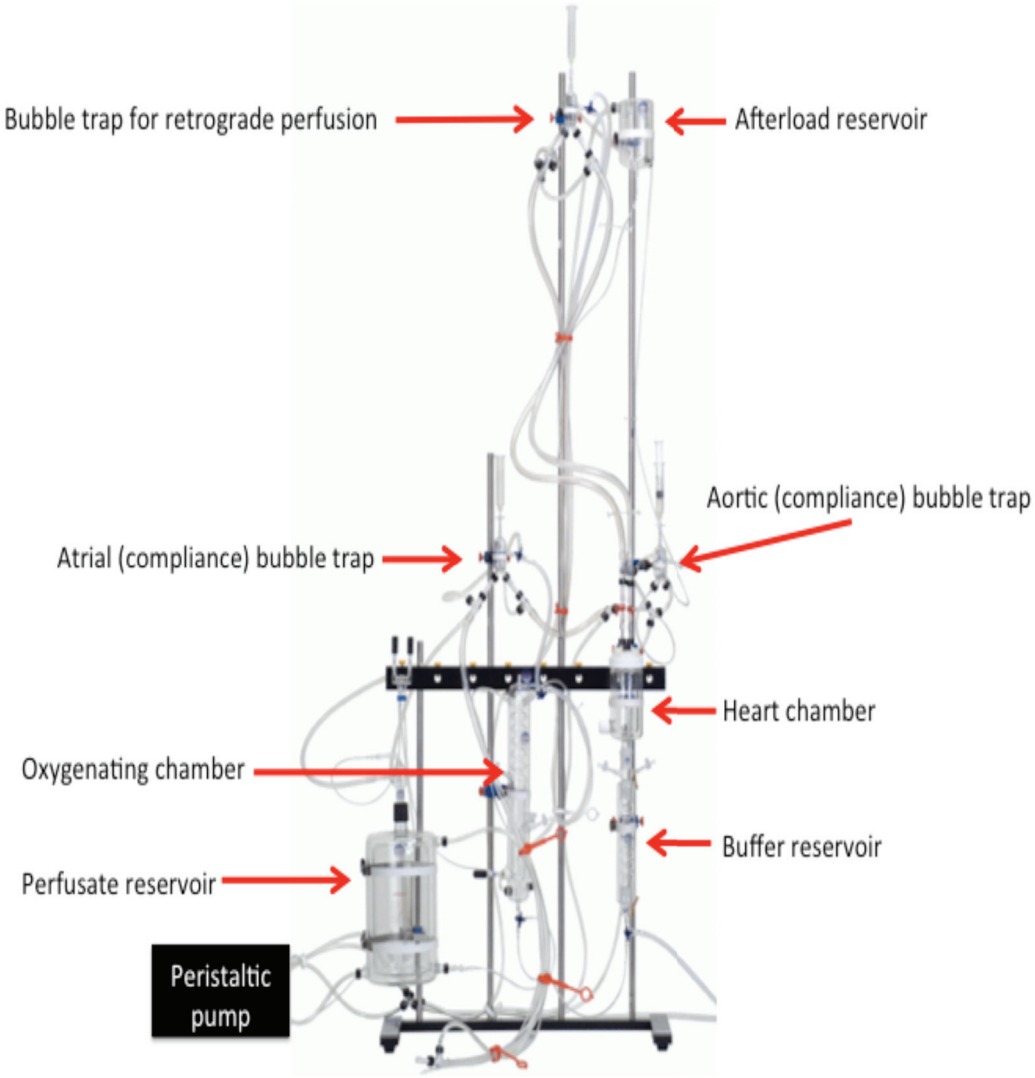

Figure 4. Schematic diagram of the isolated working heart system. The peristaltic pump is used to transport the perfusate from the reservoir to the heart. The oxygenating chamber allows for redirected perfusate to be re-gassed and returned to the heart. After the perfusate has been drawn from the reservoir, it is routed through the atrial bubble trap and diverted up to the aortic bubble trap (for retrograde reperfusion) at the atrial cannula. After the heart has been stabilized in retrograde Langendorff mode, a 3-way valve at the atrial cannula is changed to switch from retrograde to working heart mode by directing perfusate into the left atria under hydrostatic pressure. The perfusate then enters the left ventricle and is pumped by the heart against an afterload pressure.

In our lab setup of the working heart system, perfusate is drawn from the water jacketed reservoir, routed through the atrial bubble trap and diverted up to the aortic bubble trap at the atrial cannula. After the heart has been stabilized, a 3-way valve at the atrial cannula is changed to switch from retrograde to working heart mode by directing perfusate into the left atria under hydrostatic pressure. The perfusate then enters the LV and is pumped by the heart against an adjustable pressure head. Afterload can be adjusted on the aortic line to vary resistance by incorporating the compliance loop on the aortic side during working heart mode (Radnoti Working Heart Manual 2013).

The peristaltic pump is used to transport the perfusate from the reservoir through the system and to the heart. A thermal circulating pump is used to warm and maintain temperature of the system by warming the water and circulating throughout the water jacket of the system. The thermal circulator must have sufficient pump strength to move the water through the system and overcome the hydrostatic pressure head created by the elevated components of the system. In addition, the tank volume must be of sufficient size to minimize the effect of the returning fluids' temperature variation. The combination of these two features ensures an accurate and stable temperature control throughout the system (Radnoti Working Heart Manual 2013).

Afterload Pressure

Having moved from the left atrial cannula into the left atrium, the perfusate is ejected via the mitral valve into the LV, from where it is ejected through the aortic

cannula against a hydrostatic pressure via the compliance bubble trap chamber. The afterload is determined by the height of the compliance (afterload) loop reservoir relative to the position of the aortic cannula. In the compliance loop, the bubble trap compliance chamber is pre-filled with perfusate to the point where it contains approximately a 2 mm diameter bubble of air for the working heart. The trapped air bubble mimics normal vascular elasticity. The height of the fixed hydrostatic pressure head reservoir relative to the aortic cannula determines the afterload, and this is fixed at a specific height during working heart mode (along with the preload; Randoti Working Heart Manual 2013).

Perfusion of the Heart

In the course of LV ejection, a portion of the perfusate is forced into the coronary ostia, thereby perfusing the coronary vessels of the heart (Neely et al., 1973). This coronary effluent can be sampled for assay or collected over time for measurement of coronary flow. The isolated working heart model allows for the measurement and calculation of additional hemodynamic parameters under the conditions of work: cardiac output, stroke volume, stroke work, etc. (see hemodynamic measurements below). Flow in the atrial perfusion inflow cannula and aortic outflow cannula are measured with Transonic inline flow transducers to calculate cardiac output. Coronary flow may be derived from timed collection of coronary effluent.

Once the aortic and left atrial cannulation are accomplished, the aortic cannula stopcock is switched from a closed position during the standard Langendorff setup which

allowed for retrograde perfusion through the aorta, to the opened position, allowing for the perfusate to now be ejected through the aortic cannula against a hydrostatic pressure set via the compliance loop. Perfusion is initiated by the left atrium while simultaneously opening up the aortic outflow line. In this way, oxygenated perfusate from a constant pressure head left atrial reservoir (which is continuously filled by the peristaltic roller-pump) flows under gravity into the left atrial cannula while perfusate also exits anterograde through the aorta.

3. Isolated Heart Preparation

Rats were operated on by thoracotomy to expose the chest. A longitudinal skin and muscle incision was made to open the abdomen from the diaphragm to the throat. The diaphragm was then cut free from the ribs. The thorax was then opened following the bone-cartilage border on the left and right sides parallel to the sternum from the diaphragm cranially to the first rib. The entire anterior thoracic wall was turned upwards over the head to expose the heart. Pericardium was then removed. The ascending aorta was identified and separated from connective tissue and the pulmonary artery using blunt dissection, cutting just beneath the bifurcation of the aorta. The entire heart-lung mass was removed from the chest cavity and placed immediately into an ice-cold petri-dish bath containing Krebs-Henseleit buffer (KHB; NaCl 118 mM, KCl 4.71 mM, $NaHCO_3$ 25.0 mM, KH_2PO_4 1.20 mM, $CaCl_2$ 2.50 mM, $MgSO_4$ 1.20 mM, glucose 11 mM, EDTA 0.5 mM, equilibrated with 95% O_2/5% CO_2, pH 7.4; chemicals obtained from Sigma-Aldrich Co. LLC.). The heart was isolated from the lungs, then inserted onto an aortic

cannula on a standard Langendorff apparatus (Radnoti Inc, Monrovia, CA). Care was taken to ensure that the cannula had already been primed to remove air bubbles prior to cannulation and that the cannula was not inserted too far into the aorta, which could disrupt coronary arterial perfusion during experimentation. Hearts were then cannulated through the pulmonary vein of the left atrium via a second cannula **(Figure 5).**

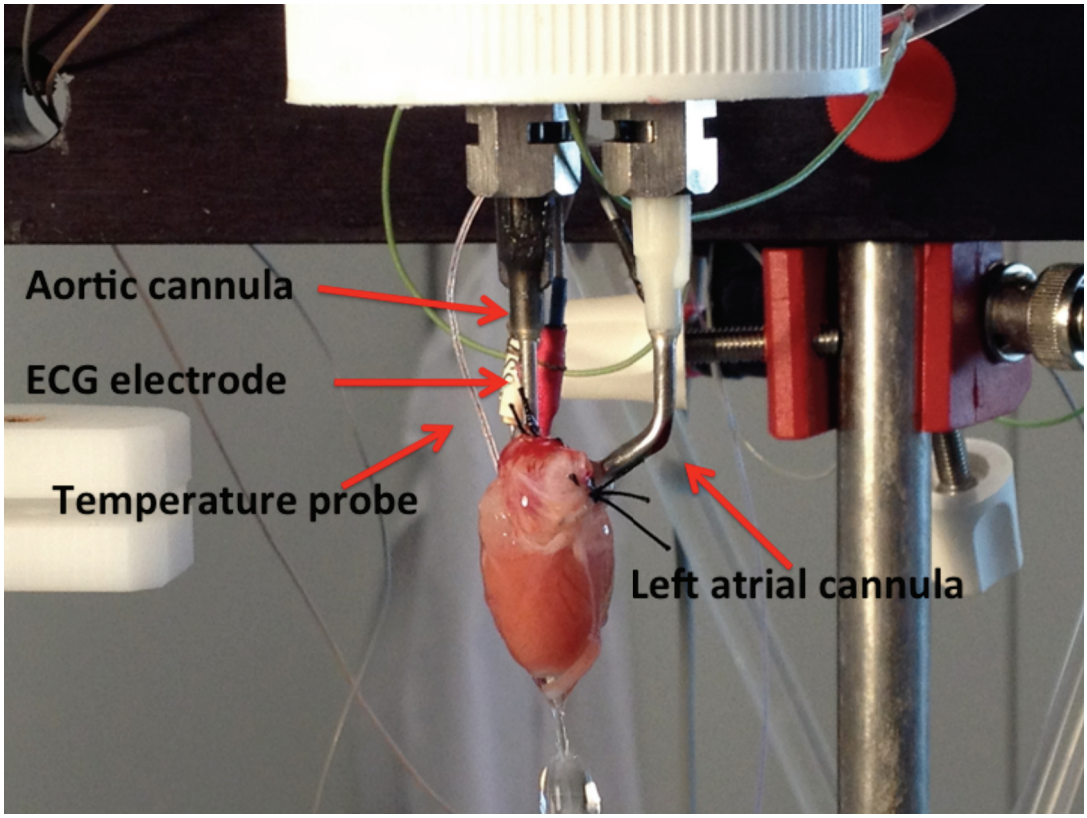

Figure 5. Diagram demonstrating cannulation of the isolated heart. The heart is cannulated through the aorta and left atrium with two cannulae. During retrograde Langendorff mode, perfusion flows retrogradely through the aortic cannula, into the aorta and the coronary arteries. During working heart mode, perfusate flows through the left atrial cannula, then the LV, and lastly the aorta to be ejected against the afterload pressure.

4. Perfusion Protocol

Before working heart mode can be initiated, the heart must first be stabilized into standard Langendorff mode. Following cannulation of the left atrium, hearts were perfused retrograde with KHB at 10 mL/min at constant flow mode of the standard Langendorff setup, such that KHB perfusate flowed retrogradely through the aorta and to the coronary arteries (5 minutes; 37°C, gassed with 95% O_2/5% CO_2). After a 5 minute stabilization period, hearts were switched to constant pressure mode (100 cm H_2O, 37°C, gassed with 95% O_2/5% CO_2) for 5 minutes. The perfusion of the heart is maintained at a constant pressure, thus, changes in resistance of the heart will result in fluctuations in the flow rate that are measured with a Transonic inline flow transducer (Transonic systems Inc., Ithica, NY).

Hearts are then switched into working heart mode for 5 minutes (preload 20 cm H_2O, afterload 100 cm H_2O). Only hearts which met the following pre-determined baseline criteria at the end of working heart mode were included in the study: heart rate > 200 bpm, regular rhythm, cardiac output > 25 mL/min, and coronary flow > 10mL/min (Rudd et al., 2009). After the baseline working heart period, hearts were arrested with cardioplegia as described below, and then exposed to ambient room temperature (22-23°C) for a 60-minute ischemic period. Reperfusion started with retrograde perfusion at 4 mL/min and increased in 0.5 mL/min increments every 30 seconds to 10 mL/min. Coronary effluent was then collected and frozen (-80°C) for later determination of troponin concentration (Life Diagnostics, Inc., 2010-2-HS, Rat Serum Cardiac Troponin-

I ELISA kit). After a total of 15 minutes, hearts were switched to constant pressure mode for an additional 5 minutes, and then into working heart mode for 60 minutes. At the end of the protocol, the ventricles were blotted dry, weighed, then desiccated at 80°C for 24 hours and reweighed. **See Figure 6 for diagram of perfusion protocol.**

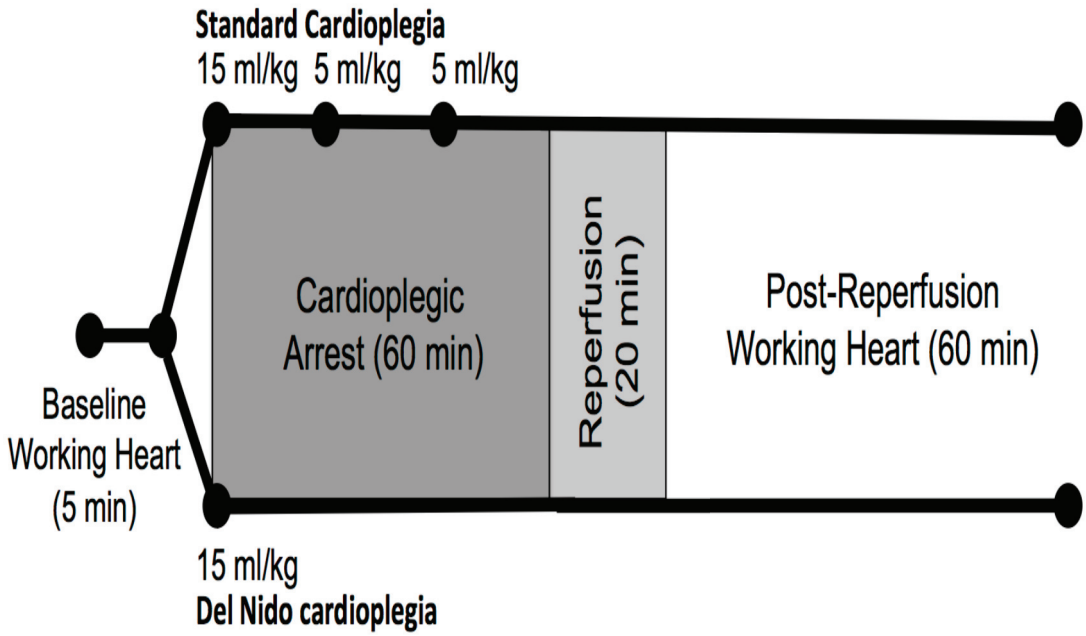

Figure 6. **Isolated working heart protocol.** Room temperature KHB solution was used as the perfusate. Hemodynamic parameter measurements were taken during the baseline working heart period, after which hearts were given either our standard or del Nido cold blood cardioplegia, as they are delivered clinically, at the start of the 60-minute cardioplegic arrest period (standard cardioplegia: 15 mL/kg initial dose followed by subsequent 5 mL/kg doses every 20 minutes; del Nido cardioplegia: 15 mL/kg induction dose only). Hearts were returned to retrograde reperfusion. Once coronary flow rate reached 10 mL/min, coronary effluent was collected for 1 minute for troponin-I analysis. Retrograde reperfusion continued for a total of 15 minutes, then the hearts were switched to constant pressure mode (total of 20 minutes of reperfusion). Then hearts were switched to working heart mode for 60 minutes, during which time hemodynamic parameters were recorded at 5, 10, 15, 30, 45, and 60 minutes into the working heart period.

5. Measurements

A temperature probe and ECG electrode were placed through the pulmonary artery into the right ventricular outflow tract to measure cardiac temperature and heart rate respectively. The values of all hemodynamic variables were averaged over 10 consecutive heart beats. All flow values were indexed to the dry weights of hearts.

5.1 Calculated Hemodynamic Variables

Rate-Pressure Product (RPP) is a measure of the stress put on the cardiac muscle based on the number of times it needs to beat per minute (heart rate; HR) and the aortic blood pressure it is pumping against (max systolic pressure; SP) and was calculated by the following equation: RPP = HR * SP. LVDP is an important measure of contractile function and was calculated as follows: LVDP = SP – LVEDP (LV end diastolic pressure). Left atrial pressure measured at the end of diastole was used as LVEDP. Cardiac output (CO) is an important determinant of cardiac function and was calculated by summation of aortic flow (AF) and coronary flow (CF): CO = AF+ CF. Stroke volume (SV) was determined in order to account for variability in HRs of the rat hearts: SV = CO/HR. Stroke Work (SW) refers to the work done by the ventricles to eject a volume of blood (stroke volume) into the aorta and was calculated as follows: SW = SV*SP. Coronary vascular resistance (CVR) is an important determinant of blood flow through the heart and overall functional recovery and was calculated by: CVR = MAP/CF, where

MAP = mean aortic pressure.

5.2 Spontaneous Activity during Cardioplegic Arrest

Spontaneous activity during cardioplegic arrest was determined by observing changes in ECG and corresponding aortic pressure tracings that indicated the occurrence of a heartbeat. Wide complex electromechanical activity seen on the ECG tracing coupled with a fluctuation in aortic pressure indicated the occurrence of a spontaneous contraction during cardioplegic arrest.

5.3 Return of Rhythm during Reperfusion

Measuring the length of time taken for rhythm to return following the start of reperfusion was done by examining the time scale corresponding to the ECG tracing of each group (standard or del Nido cardioplegia) from the start of constant flow reperfusion until the first heartbeat occurred.

5.4 Troponin Release into Coronary Effluent

During reperfusion, retrograde perfusion was gradually increased from 4 mL/min to 10 mL/min by increments of 0.5 mL/min every 30 seconds. Once retrograde reperfusion reached the flow rate of 10 mL/min, coronary effluent (10 mL) was collected with a tube over a 1-minute period. The collected coronary effluent was then frozen in a -

80°C freezer to be used on a later date to measure the myocardial troponin release. After working heart Langendorff experiments were completed for all hearts in the aged and young adult groups, troponin-I release was analyzed. The coronary sinus effluent was examined using a high sensitivity ELISA for determination of cardiac troponin-I (Life Diagnostics, Inc., 2010-2-HS, Rat Serum Cardiac Troponin-I ELISA kit). Frozen samples were collected from the freezer and thawed. All samples were analyzed with the ELISA assay at the same time. The procedures outlined in the ELISA kit were followed.

5.5 Myocardial Edema

Myocardial edema was determined by calculating the total myocardial water content, by subtracting dry weight of the heart from the wet weight. This value was then indexed to the dry weight: Indexed mass of myocardial water = wet-dry wt/ dry wt.

5.6 Tachyarrhythmias during Reperfusion

ECG tracings were examined throughout the entire reperfusion period for each heart and occurrences of arrhythmias were noted. Arrhythmias were defined as runs of tachycardia that were identified as bursts of rhythm that were faster than the underlying rate.

6. Cardioplegia Preparation and Delivery

Autologous blood was collected from the chest cavity of rats using a syringe containing heparin (200 U) as the hearts were being harvested. Cardioplegia solutions were prepared according to our clinical protocols (**Table 1**), cooled in an ice bath, and oxygenated. Our standard cardioplegia was prepared by obtaining the base solution from the QEII hospital (Halifax, Canada), and then adding sodium bicarbonate and magnesium sulfate to the base solution. After mixing the base solution with the collected blood from the rat, the cardioplegia solution was oxygenated (gently bubbled with O_2) and placed in an ice bath (≈1-5°C). The approximate final ionic concentration of our standard cardioplegia is listed in **Table 1**. Del Nido cardioplegia was prepared by obtaining the base solution from the QEII hospital, and then adding lidocaine, sodium bicarbonate and mannitol to the base solution. Del Nido cardioplegia was similarly oxygenated and cooled in an ice bath (≈1-5°C). Prior to delivery, cardioplegia was filtered using a 20 μm pore size vacuum filter (EMD Millipore, Billerica, MD). The cardioplegia temperature was 1.9±0.4°C. The working heart setup was switched back to nonworking mode, in constant flow mode, prior to delivery of the cardioplegia solutions.

The cardioplegia strategies used in this study were modeled after those we use in the clinic (O'Brien et al., 2009). Standard cardioplegia was delivered as an induction dose (15 mL/kg) followed by additional doses (5 mL/kg) every 20 minutes. Del Nido cardioplegia was delivered as a single induction dose (15 mL/kg) with no additional doses during the 60-minute ischemic period. The infusion rate of the cardioplegic solutions, delivered by hand injection, was pressure controlled with the aortic pressure maintained at ≤ 50 mmHg.

7. Experimental Groups

In the aged hearts experiment, hearts were arrested with either our standard (n=8) or del Nido (n=8) cardioplegia. In the young adults experiment, hearts were arrested with either our standard (n=6) or del Nido (n=6) cardioplegia.

8. Statistical Analysis

Data are presented as mean±SEM. Tests for statistical significance included unpaired t-test (cardiac temperature, myocardial edema, troponin-I, return of rhythm during reperfusion, coronary vascular resistance), Fisher's exact test (spontaneous activity during cardioplegic arrest, incidence of arrhythmias during reperfusion), and mixed linear model analysis followed by Tukey-Kramer test (aged hearts group: all hemodynamic variables for the 60 minutes of post-reperfusion working heart mode). Repeated measures ANOVA was conducted for all hemodynamic variables for the 60 minutes of post-reperfusion working heart mode in the young adults heart group.

CHAPTER 3: RESULTS

1. Aged Hearts Experiment

1.1 Eligibility of Hearts

To determine the relative ability of del Nido cardioplegia to protect aged hearts, we studied senescent rats (male Fisher rats; 22-24 months old) using our working heart model of arrest with blood cardioplegia. Sixteen of 20 hearts (80%) met the pre-determined functional criteria for inclusion into the study (**see Materials and Methods section**). Of the hearts that met baseline criteria, 2/16 (13%) hearts stopped during the protocol (specifically during the 60-minute post-reperfusion working heart mode) due to technical problems (air bubbles entering heart or afterload chamber emptying). However, data for these two hearts have been included up until the time that the hearts stopped beating. Eight hearts were arrested with standard cardioplegia (n=8), and 8 with del Nido cardioplegia (n=8).

1.2 Cardiac Temperature

Cardiac temperature was measured by insertion of a temperature probe through the pulmonary artery into the right ventricular outflow tract and was recorded throughout the experimental protocol. The temperature of the heart can affect its resistance to ischemia-reperfusion injury. Cardiac temperature was 21±1°C in each group after induction (p=NS) and was essentially unchanged immediately prior to reperfusion

(20±1°C in each group; p=NS). Thus, the change in cardiac temperature from the start to the end of the arrest period was also not significantly different between the two cardioplegia solutions (-1.0±0.9°C vs. -1.2±0.7°C, del Nido vs. standard cardioplegia, p=NS; **Figures 7-9**).

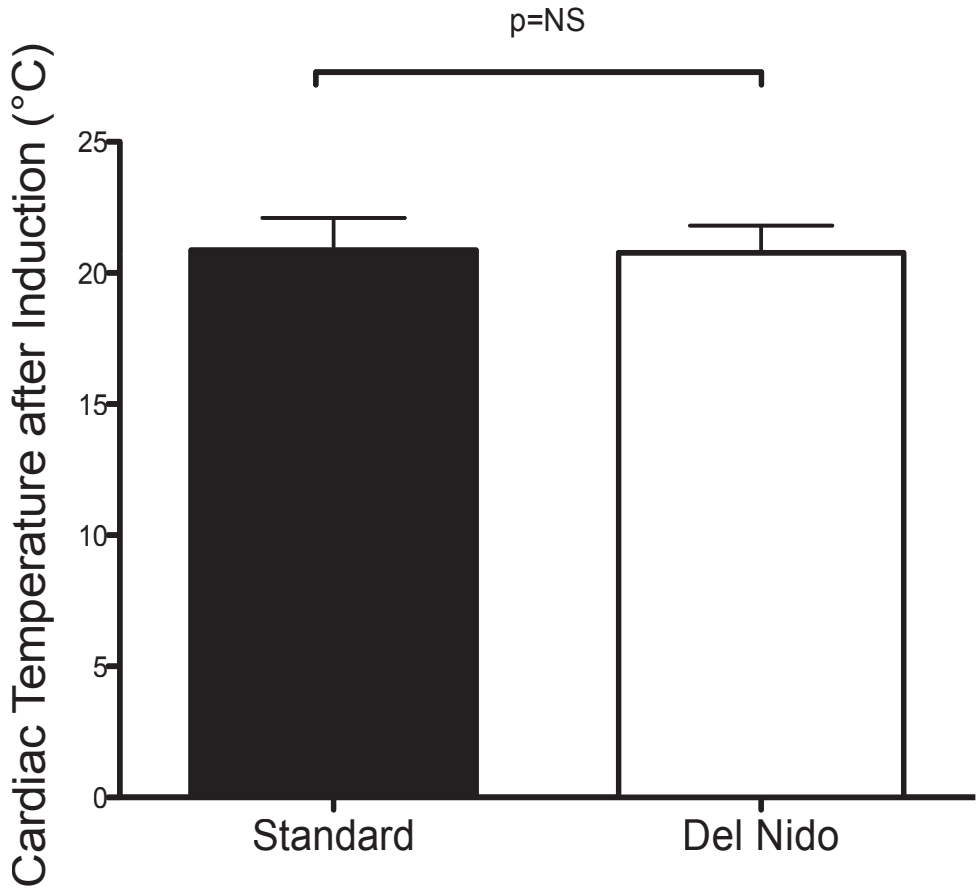

Figure 7. Cardiac temperature following delivery of induction dose of standard and del Nido cardioplegia. Bars represent mean±SEM, n=8 per group.

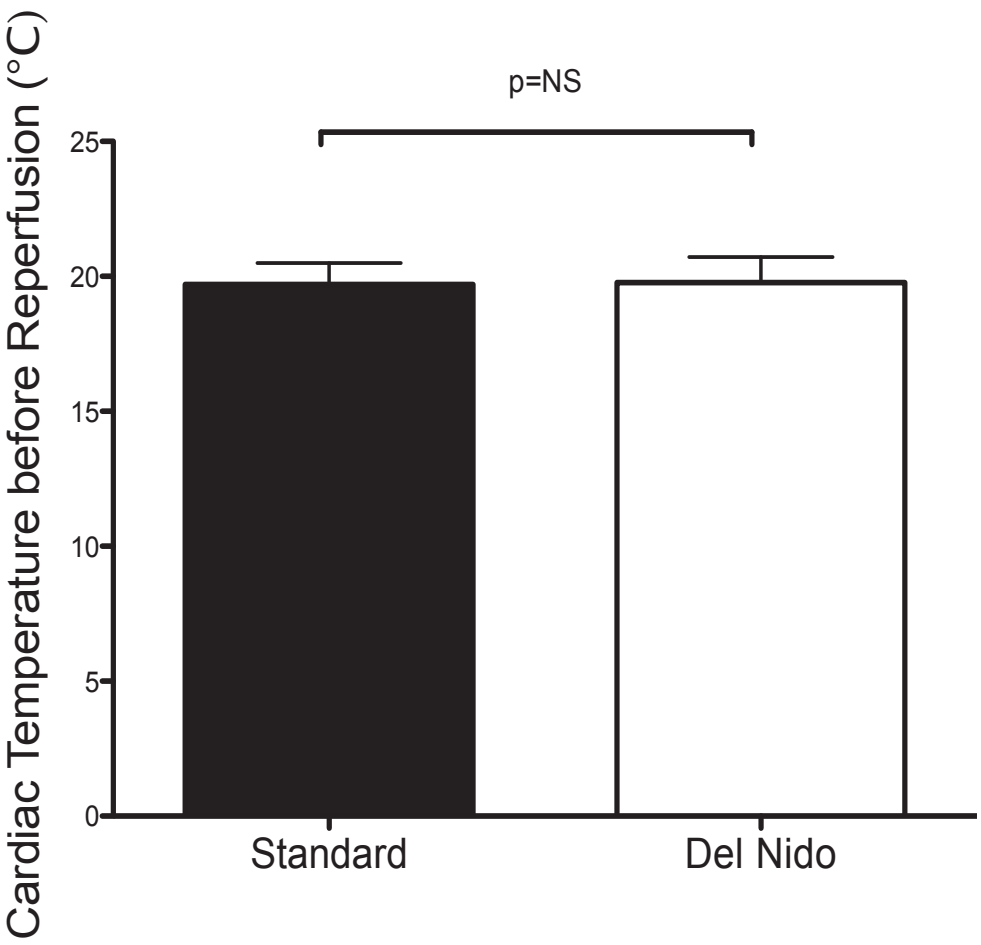

Figure 8. Cardiac temperature prior to the start of reperfusion in hearts arrested with standard and del Nido cardioplegia. Bars represent mean±SEM, n=8 per group.

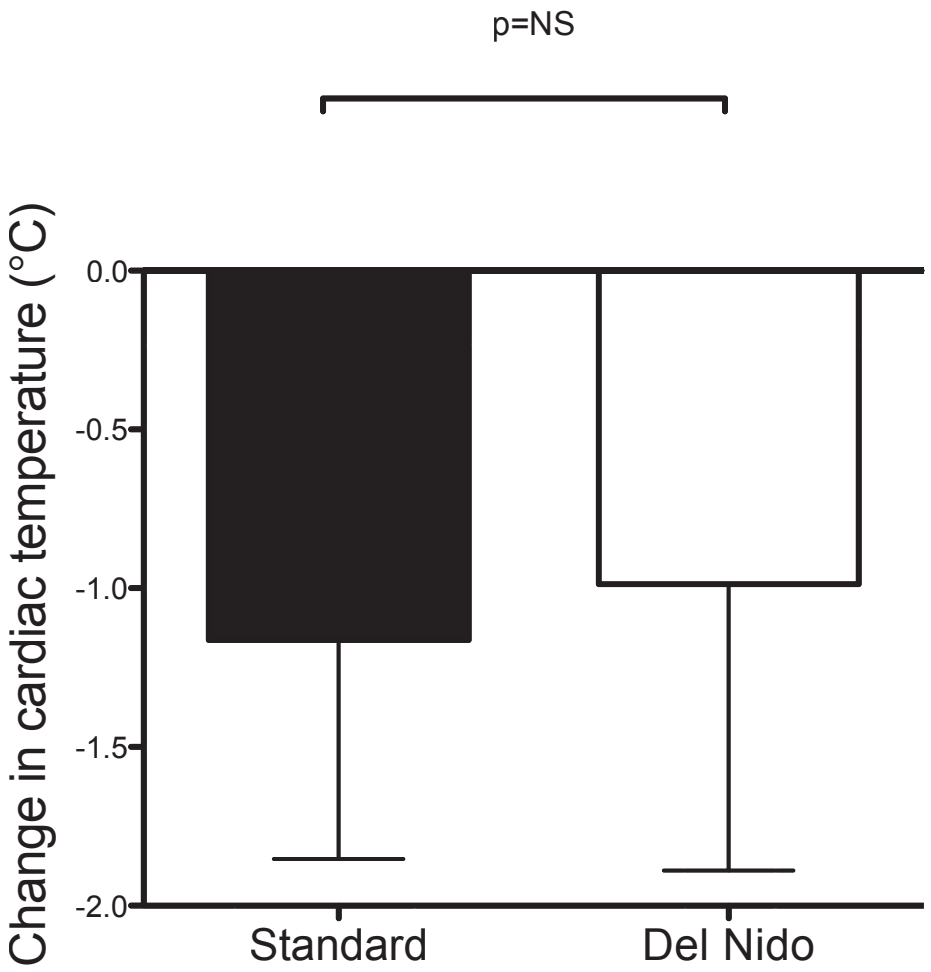

Figure 9. Change in cardiac temperature over the cardioplegic arrest period for hearts arrested with standard and del Nido cardioplegia. Bars represent mean±SEM, n=8 per group.

1.3 Spontaneous Activity during Cardioplegic Arrest

To determine the relative ability of our standard and del Nido cardioplegia to maintain arrest in aged hearts, spontaneous electromechanical activity was examined by observing the electrocardiogram for changes in electrical activity coupled to fluctuations in aortic pressure during the arrest period. Spontaneous electromechanical activity was observed during the arrest period in 7/8 (88%) hearts in the standard cardioplegia group (**Figure 10**). This was in the form of occasional wide complex beats seen on the electrocardiogram (**Figure 10A**) with accompanying mechanical activity. In contrast, spontaneous activity was only seen in 1/8 (13%) hearts arrested with del Nido cardioplegia (**Figure 10B**, p=0.01).

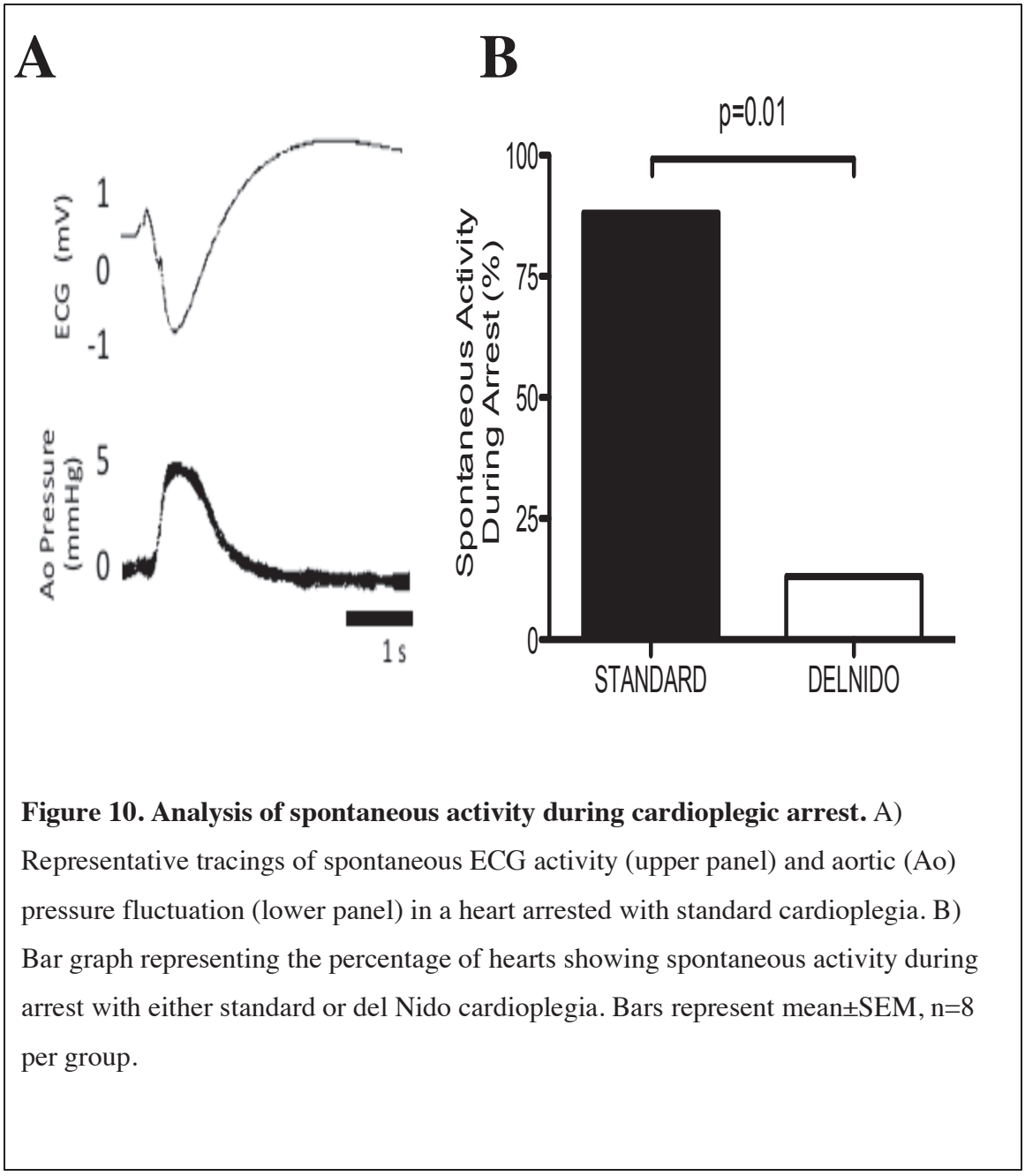

Figure 10. Analysis of spontaneous activity during cardioplegic arrest. A) Representative tracings of spontaneous ECG activity (upper panel) and aortic (Ao) pressure fluctuation (lower panel) in a heart arrested with standard cardioplegia. B) Bar graph representing the percentage of hearts showing spontaneous activity during arrest with either standard or del Nido cardioplegia. Bars represent mean±SEM, n=8 per group.

1.4 Return of Rhythm at Reperfusion

After the start of reperfusion, all hearts had spontaneous return of rhythm. However, the time to return of the first heartbeat was twice as long in the del Nido group when compared to the standard cardioplegia group (**Figure 11 A and B,** 67±6 vs. 32±5 s, p=0.0007). All hearts had occasional extra systoles or short runs of bigeminy during the reperfusion period. Four hearts in the standard cardioplegia group and 1 in the del Nido group (p=NS) had short runs of sustained tachyarrhythmia that resolved spontaneously (**Figure 12**). One heart in the del Nido group had a short period of asystole, which was not seen in any of the hearts protected with standard cardioplegia.

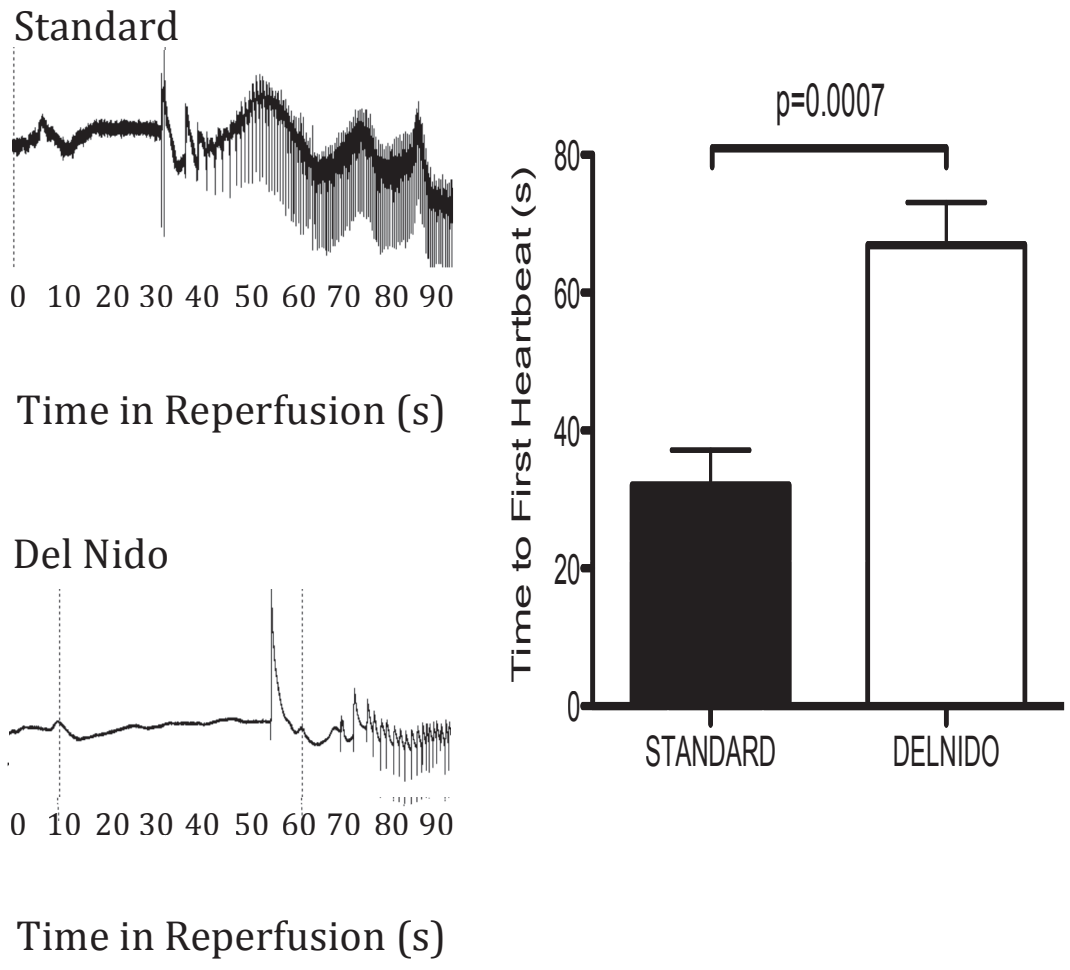

Figure 11. Analysis of return of first heart beat (rhythm) following the start of reperfusion. A) Representative ECG tracings from the start of reperfusion (t=0) in hearts arrested with standard (upper panel) and del Nido cardioplegia (lower panel). B) Bar graph representing average time to return of first heart beat after the start of reperfusion in hearts arrested with standard and del Nido cardioplegia. Bars represent mean±SEM, n=8 per group.

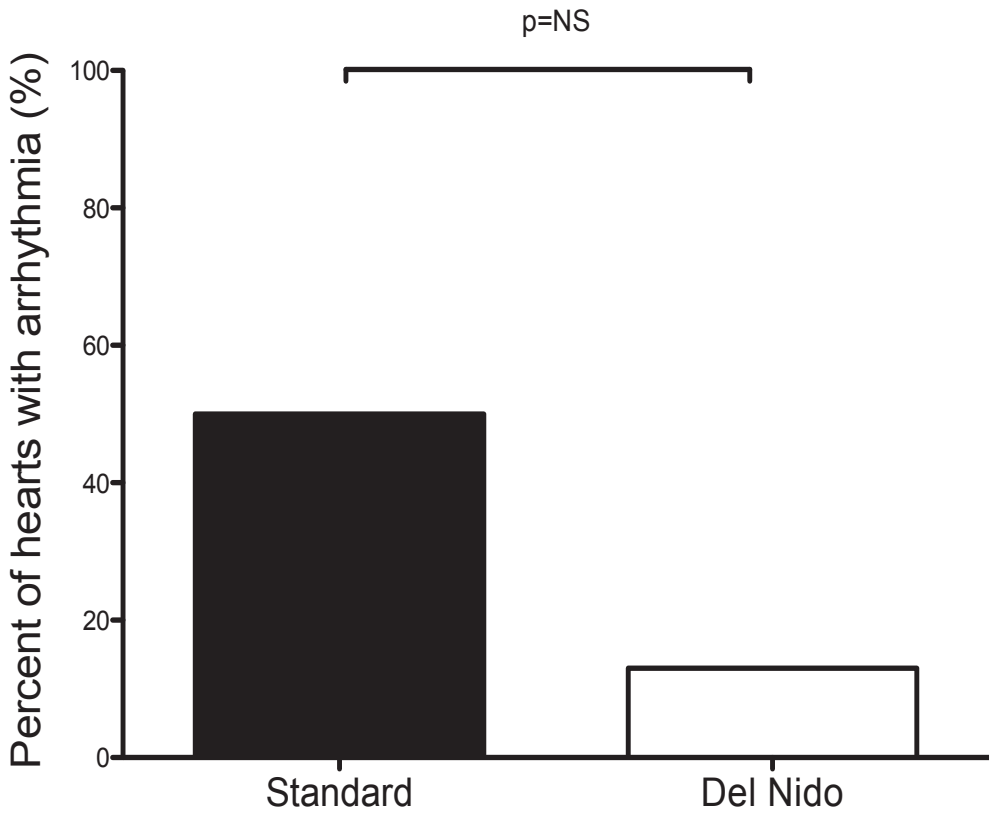

Figure 12. Percent of hearts that had short runs of sustained tachyarrhythmia during retrograde reperfusion, that were resolved and were self-limiting. Bar graph representing percent of hearts that had tachyarrhythmia during reperfusion in hearts arrested with standard and del Nido cardioplegia. Bars represent mean±SEM, n=8 per group.

1.5 Hemodynamic Measurements

To evaluate the impact of del Nido cardioplegia on functional recovery, the aged hearts were switched into working heart mode for 60 minutes after the 20-minute reperfusion period, and hemodynamic parameters were examined at a fixed preload (20 cm H_2O, **Table 2**). All hearts completed the entire protocol with the exception of one in each group, in which air was entrained near the end of the studies and therefore do not contribute data for the last two time points. Hemodynamic measurements were recorded before ischemic arrest (baseline working heart) and repeated again after reperfusion (60-minute working heart mode, post-reperfusion). Baseline measurements were recorded at the end of the initial 5-minute working heart mode before switching off the perfusate flow to deliver the cardioplegia. Post-reperfusion measurements were made during the 60-minute working heart mode period at the time points of 0, 5, 10, 15, 30, 45, and 60 minutes into the working heart mode. A summary of the hemodynamic parameters measured during working heart mode is shown in **Table 2**.

	Baseline	Post Reperfusion					
		5	*10*	*15*	*30*	*45*	*60 min*
Heart Rate (BPM)							
Standard	224±6	197±16	200±16	200±16	204±19	198±21	198±22
Del Nido	219±6	228±8	231±9	235±6	233±9	233±11	229±12
p	NS	NS	NS	NS	NS	NS	NS
Rate Pressure Product ($x10^3$ mmHg*min^{-1})							
Standard	30±1	20±2	20±2	20±2	19±3	20±3	20±3
Del Nido	30±1	29±1	29±1	30±1	29±1	29±1	28±1
p	0.8	0.0005	0.0006	0.0002	0.0002	0.0006	0.0017
Systolic Pressure (mmHg)							
Standard	133±5	99±6	99±6	99±7	93±9	98±9	97±8
Del Nido	138±4	128±4	127±5	128±4	127±4	125±5	123±4
p	0.5	0.0006	0.0008	0.0006	0.0001	0.0011	0.0018
LVDP (mmHg)							
Standard	120±12	85±18	85±18	85±19	79±25	82±23	82±22
Del Nido	125±10	116±8	114±11	115±10	113±10	111±12	110±8
p	0.5	0.0002	0.0004	0.0003	0.0001	0.0005	0.001
Coronary Flow (ml*min^{-1}*g^{-1})							
Standard	97±5	61±9	60±9	61±7	60±11	65±11	61±12
Del Nido	103±7	101±6	101±6	101±6	101±6	99±6	101±9
p	0.6	0.0009	0.0008	0.001	0.0007	0.0014	0.0003
Cardiac Output (ml*min^{-1}*g^{-1})							
Standard	181±18	85±23	87±23	88±24	86±24	92±23	86±22
Del Nido	196±9	177±14	180±16	181±15	177±14	174±13	173±14
p	0.6	0.0007	0.0006	0.0007	0.0009	0.0017	0.0008
Stroke Volume ($x10^{-2}$ ml*g^{-1})							
Standard	81±7	40±10	41±10	41±10	40±10	43±10	40±10
Del Nido	90±4	78±6	78±6	77±5	76±5	75±5	76±4
p	0.4	0.0009	0.0009	0.0013	0.0016	0.0026	0.0008
Stroke Work (ml*mmHg*g^{-1})							
Standard	108±28	43±30	44±30	44±30	42±34	45±30	41±28
Del Nido	123±20	101±23	100±25	98±20	95±19	93±18	93±16
p	0.3	0.0001	0.0001	0.0001	0.0001	0.0005	0.0002

Table 2. Hemodynamic parameters measured during working heart mode: aged hearts.

n = 8 per group up to 30 minute time point and n=7 for 45 and 60 minute time point

NS = Mixed linear model analysis was not significant so post-hoc comparisons not performed

1.5.1 Heart Rate

Heart rate was not significantly different during baseline working heart mode in hearts arrested with either standard or del Nido cardioplegia (219±6 vs. 224±6 bpm, p=NS). Additionally, after reperfusion, heart rates were also not significantly different between del Nido and standard cardioplegia (**t=5:** 228±8 vs. 197±16, **t=10**: 231±9 vs. 200±16, **t=15:** 235±6 vs. 200±16, **t=30:** 233±9 vs. 204±19, **t=45**: 233±11 vs. 198±21, **t=60**: 229±12 vs. 198±22 bpm, del Nido vs. standard cardioplegia, p=NS, **Table 2, Figure 13**).

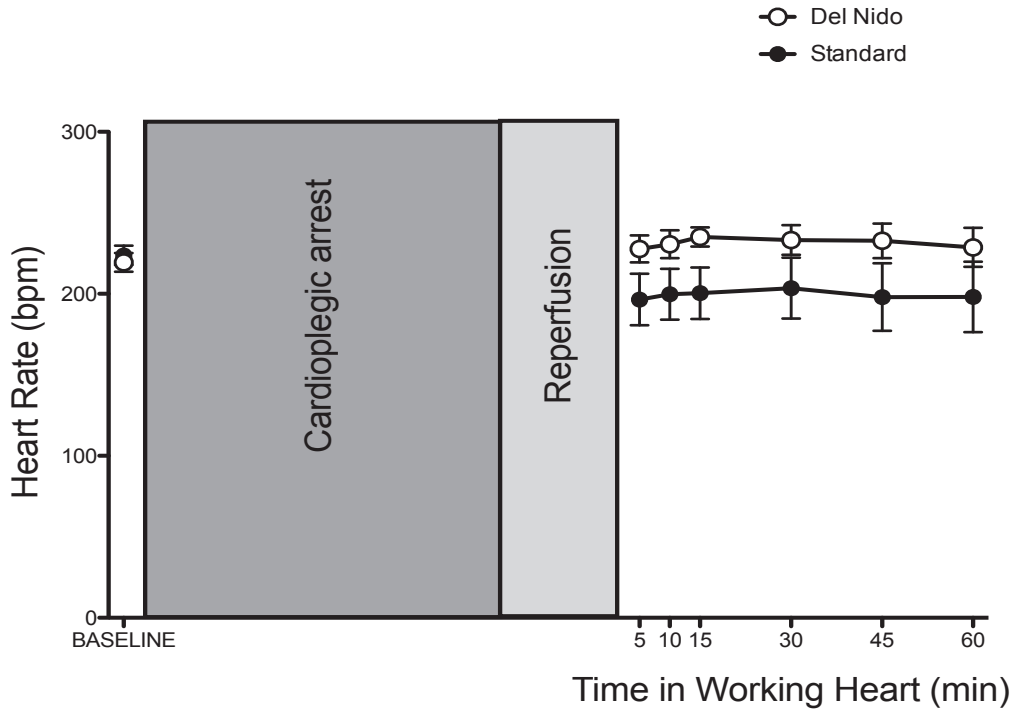

Figure 13. Heart rate in standard and del Nido cardioplegia groups. Graph showing heart rate measured during working heart mode before (baseline) and at several time points after 60 minutes of cardioplegic arrest and 20 minutes of reperfusion for the standard and del Nido cardioplegia groups. Data points represent mean±SEM, n=8 per group up to 30 minutes and 7 per group for 45 and 60 minute time points, *=p≤0.001.

1.5.2 Rate-Pressure Product

RPP was similar between the two cardioplegia groups at baseline for both groups (30 ± 1 x10^3 mmHg*min^{-1} for both groups, **Table 2, Figure 14**). However, during post-reperfusion working heart mode, RPP was significantly greater in hearts arrested with del Nido cardioplegia than standard cardioplegia across the entire time period (**t=5**: 29 ± 1 vs. 20 ± 2, **t=10**: 29 ± 1 vs. 20 ± 2, **t=15**: 30 ± 1 vs. 20 ± 2, **t=30**: 29 ± 1 vs. 19 ± 3, **t=45**: 29 ± 1 vs. 20 ± 3, **t=60**: 28 ± 1 vs. 20 ± 3 x10^3 mmHg*min^{-1}, del Nido vs. standard cardioplegia, $p\leq0.001$; **Table 2, Figure 14**).

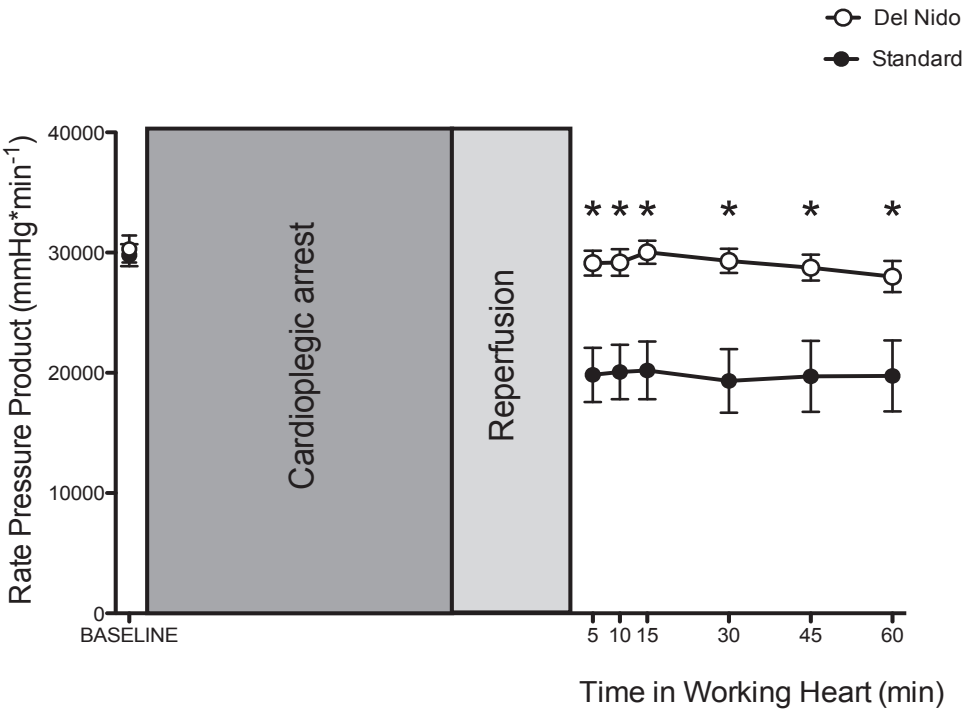

Figure 14. Rate-Pressure Product in standard and del Nido cardioplegia groups. Graph showing Rate-Pressure Product calculated during working heart mode before (baseline) and at several time points after 60 minutes of cardioplegic arrest and 20 minutes of reperfusion for the standard and del Nido cardioplegia groups. Data points represent mean±SEM, n=8 per group up to 30 minutes and 7 per group for 45 and 60 minute time points, *=$p\leq0.001$.

1.5.3 Left Ventricular Developed Pressure

LVDP between the standard and del Nido cardioplegia groups was not significantly different at baseline (125±10 vs. 120±12 mmHg, del Nido versus standard cardioplegia, p=NS). However, LVDP was higher in hearts arrested with del Nido cardioplegia than with standard cardioplegia across the entire working heart period (**t=5**: 116±8 vs. 85±18, **t=10:** 114±11 vs. 85±18, **t=15:** 115±10 vs. 85±19, **t=30:** 113±10 vs. 79±25, **t=45:** 111±12 vs. 82±23, **t=60:** 110±8 vs. 82±22 mmHg, del Nido vs. standard cardioplegia, $p \leq 0.001$, **Table 2, Figure 15B)**. Representative tracings of aortic pressure recorded in working heart mode, for hearts arrested with standard and del Nido cardioplegia are shown in **Figure 15A**. There was a noticeable decline in peak systolic pressure from baseline to post-reperfusion for hearts arrested with standard cardioplegia, not seen to the same extent in hearts arrested with del Nido cardioplegia (**Figure 15A)**.

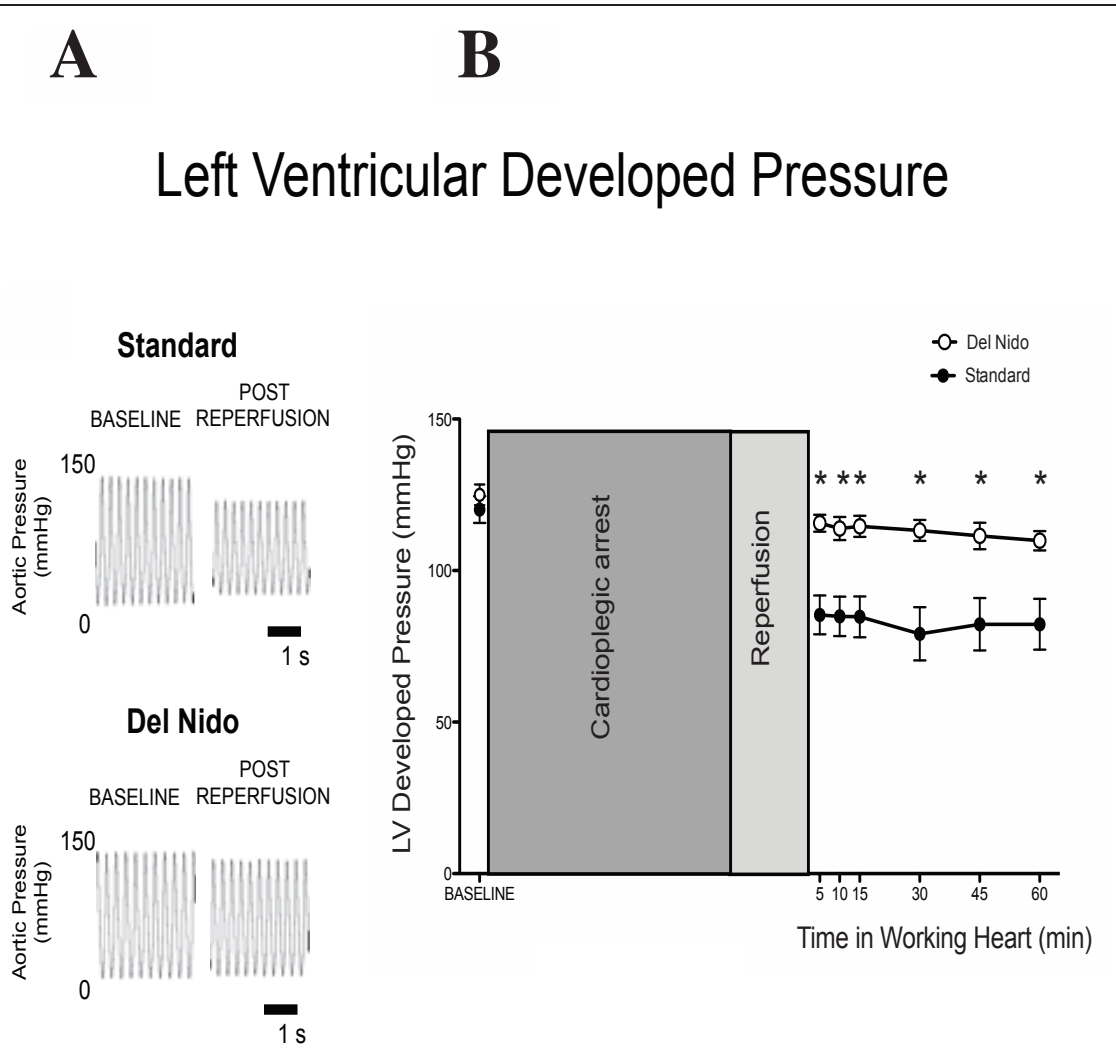

Figure 15. Analysis of LVDP before and after arrest and reperfusion. A) Representative tracings of aortic pressure recorded in working heart mode. Baseline and post-reperfusion tracing are shown from hearts arrested with standard (upper panels) and del Nido cardioplegia (lower panels). B) Graph showing LVDP measured during working heart mode before (baseline) and at several time points after 60 minutes of cardioplegic arrest and 20 minutes of reperfusion for the standard and del Nido cardioplegia groups. Data points represent mean±SEM, n=8 per group up to 30 minutes and 7 per group for 45 and 60 minute time points, *=p≤0.001.

1.5.4 Coronary Flow

Although there were no significant differences in basal coronary flow rates between standard and del Nido cardioplegia (103±7 vs. 97±5 ml*min^{-1}*g^{-1}, del Nido vs. standard cardioplegia, p=NS) during post-reperfusion working heart mode, coronary flow was significantly elevated in hearts arrested with del Nido cardioplegia than in standard cardioplegia, across the entire time period (**t=5:** 101±6 vs. 61±9, **t=10:** 101±6 vs. 60±9, **t=15:** 101±6 vs. 61±7, **t=30:** 101±6 vs. 60±11, **t=45:** 99±6 vs. 65±11, **t=60:** 101±9 vs. 61±12 ml*min^{-1}*g^{-1}, del Nido vs. standard cardioplegia, p≤0.001, **Table 2, Figure 16**).

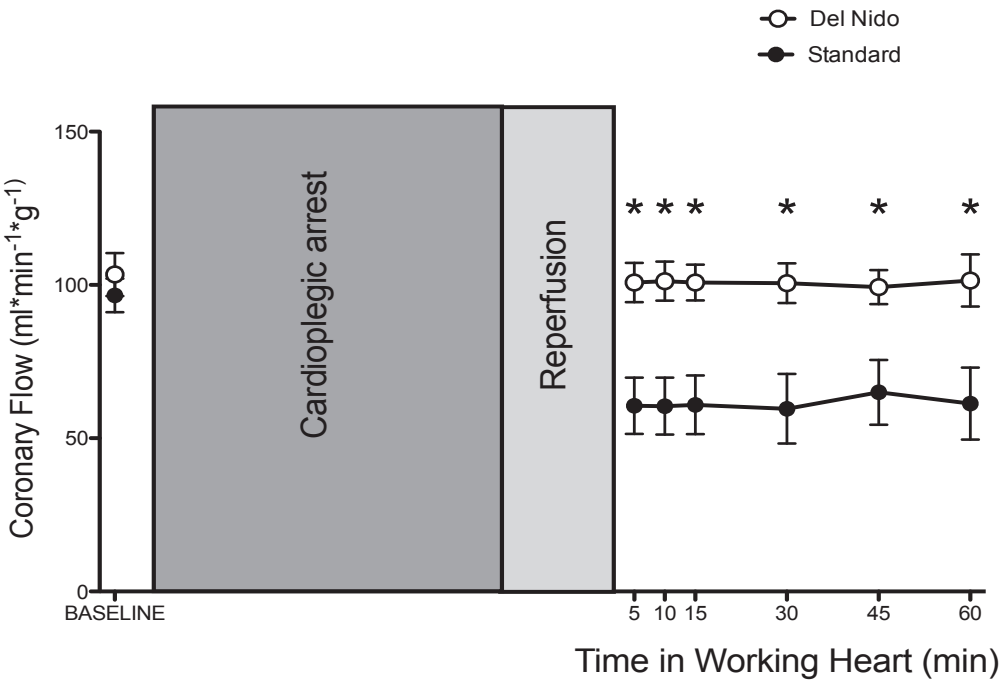

Figure 16. Coronary flow in standard and del Nido cardioplegia groups. Graph showing coronary flow measured during working heart mode before (baseline) and at several time points after 60 minutes of cardioplegic arrest and 20 minutes of reperfusion for the standard and del Nido cardioplegia groups. Data points represent mean±SEM, n=8 per group up to 30 minutes and 7 per group for 45 and 60 minute time points, *=p≤0.001.

1.5.5 Cardiac Output

Cardiac output at baseline was not different between the two cardioplegia groups (196±9 vs. 181±18 ml*min^{-1}*g^{-1} , del Nido vs. standard cardioplegia, p=NS). However, following reperfusion, during working heart mode, CO was significantly higher and recovery was better in hearts arrested with del Nido cardioplegia than with standard cardioplegia, across the entire working heart period (**t=5:** 177±14 vs. 85±23, **t=10:** 180±16 vs. 87±23, **t=15**: 181±15 vs. 88±24, **t=30**: 177±14 vs. 86±24, **t=45:** 174±13 vs. 92±23, **t=60:** 173±14 vs. 86±22 181±18 ml*min^{-1}*g^{-1}, del Nido vs. standard cardioplegia, p≤0.001, **Table 2, Figure 17 A and B).** Representative tracings of aortic flow for each type of cardioplegia, during baseline and post-reperfusion working heart, are shown in **Figure 17 A.**

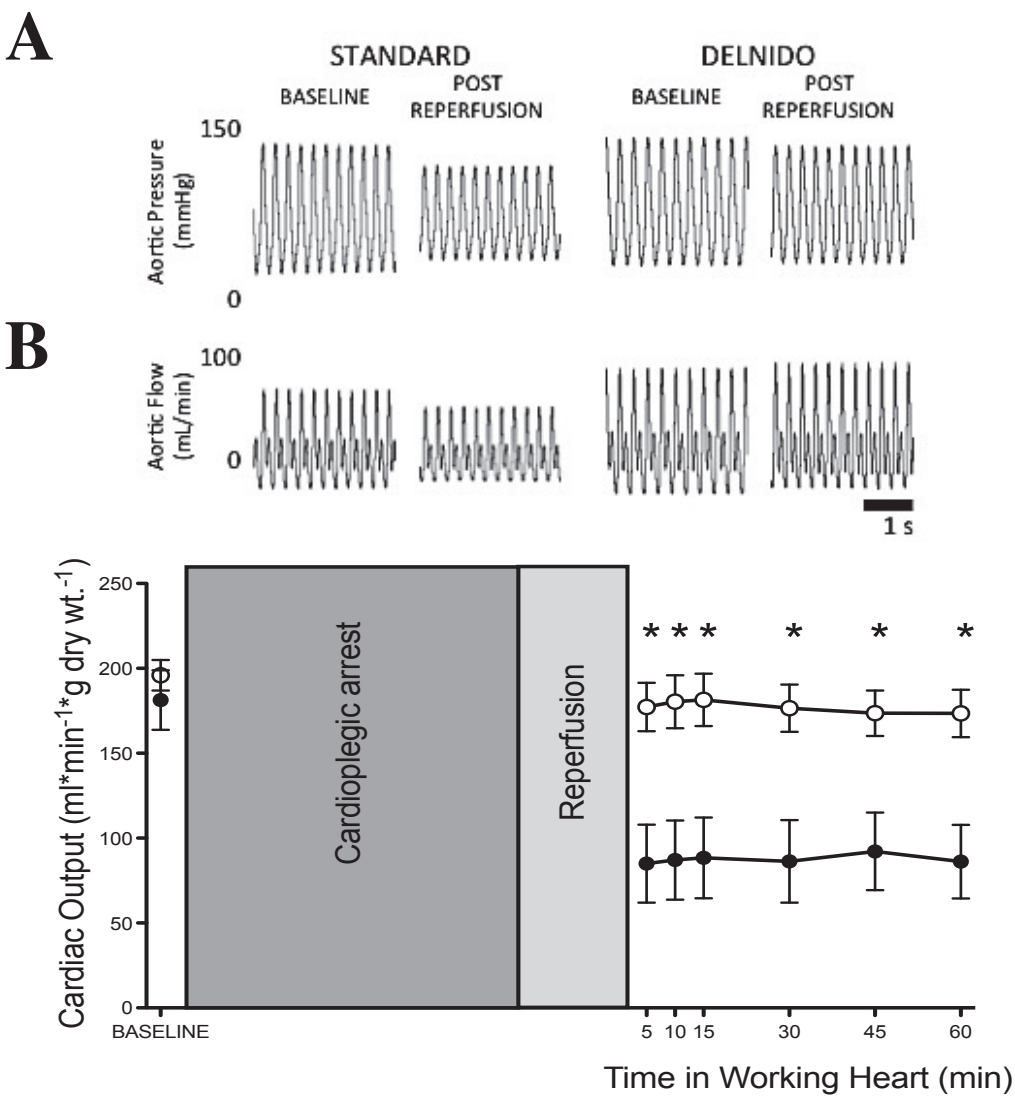

Figure 17. Cardiac output in standard and del Nido cardioplegia groups. A) Representative tracings of aortic flow recorded in working heart mode. Baseline and post-reperfusion tracing are shown from hearts arrested with standard cardioplegia (left panels) and del Nido cardioplegia (right panels). B) Graph showing cardiac output measured during working heart mode before (baseline) and at several time points after 60 minutes of cardioplegic arrest and 20 minutes of reperfusion for the standard and del Nido cardioplegia groups. Data points represent mean±SEM, n=8 per group up to 30 minutes and 7 per group for 45 and 60 minute time points, *=p≤0.001.

1.5.6 Stroke Volume

There were no significant differences in stroke volume values at baseline between standard and del Nido cardioplegia groups (90±4 vs. 81±7 $x10^{-2}$ $ml*g^{-1}$, del Nido vs. standard cardioplegia, p=NS). However, SV was significantly greater in hearts arrested with del Nido cardioplegia than standard cardioplegia throughout the entire working heart period following reperfusion (**t=5:** 78±6 vs. 40±10, **t=10:** 78±6 vs. 41±10, **t=15:** 77±5 vs. 41±10, **t=30**: 76±5 vs. 40±10, **t=45:** 75±5 vs. 43±10, **t=60:** 76±4 vs. 40±10 $x10^{-2}$ $ml*g^{-1}$, del Nido vs. standard cardioplegia, p≤0.001, **Table 2, Figure 18).**

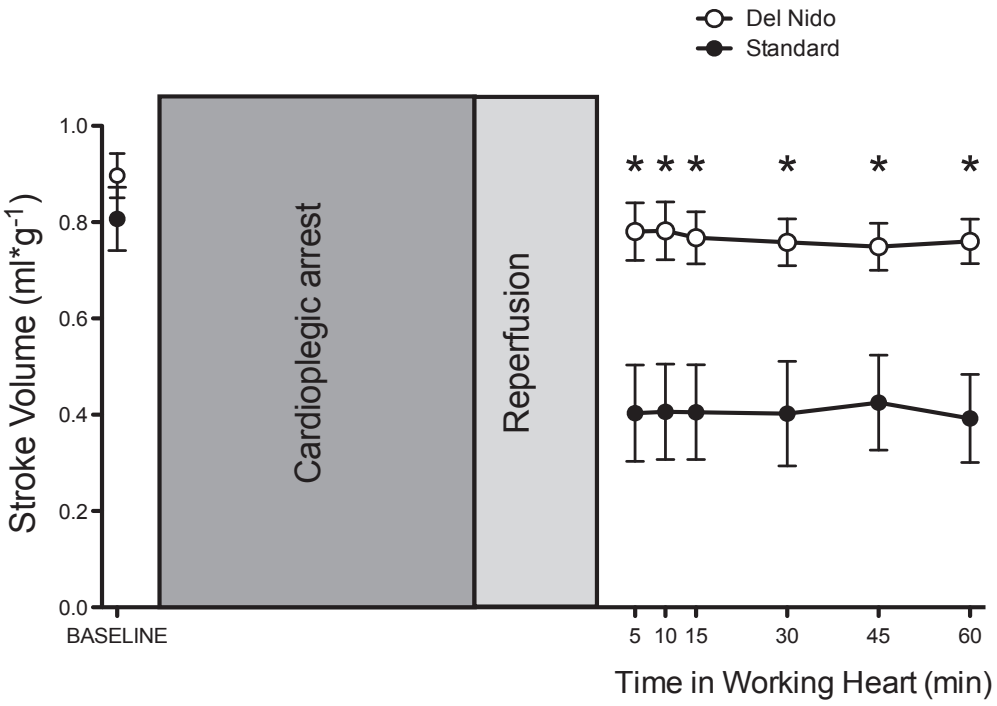

Figure 18. Stroke volume in standard and del Nido cardioplegia groups. Graph showing stroke volume calculated during working heart mode before (baseline) and at several time points after 60 minutes of cardioplegic arrest and 20 minutes of reperfusion for the standard and del Nido cardioplegia groups. Data points represent mean±SEM, n=8 per group up to 30 minutes and 7 per group for 45 and 60 minute time points, *=p≤0.001.

1.5.7 Stroke Work

At baseline, there were no differences in stroke work between standard and del Nido cardioplegia (123±20 vs. 108±28 ml*mmHg*g^{-1}, del Nido vs. standard cardioplegia, p=NS). However, throughout the entire working heart period, SW was higher in hearts arrested with del Nido cardioplegia (**t=5:** 101±23 vs. 43±30, **t=10:** 100±25 vs. 44±30, **t=15:** 98±20 vs. 44±30 vs. **t=30**: 95±19 vs. 42±34, **t=45:** 93±18 vs. 45±30, **t=60:** 93±16 vs. 41±28 ml*mmHg*g^{-1} **,** del Nido vs. standard cardioplegia, p≤0.001, **Table 2, Figure 19**).

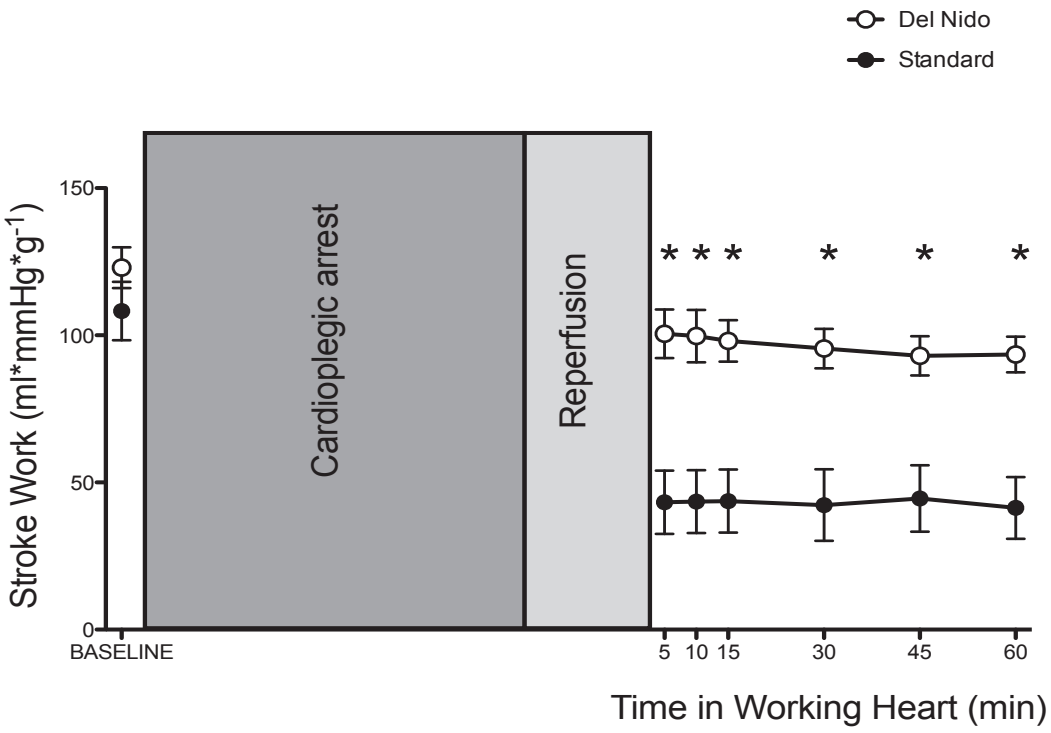

Figure 19. Stroke work in standard and del Nido cardioplegia groups. Graph showing stroke work calculated during working heart mode before (baseline) and at several time points after 60 minutes of cardioplegic arrest and 20 minutes of reperfusion for the standard and del Nido cardioplegia groups. Data points represent mean±SEM, n=8 per group up to 30 minutes and 7 per group for 45 and 60 minute time points, *=p≤0.001.

1.5.8 Coronary Vascular Resistance

Coronary vascular resistance during the retrograde perfusion phase of reperfusion was ≈50% less in hearts arrested with del Nido cardioplegia compared to our standard cardioplegia (0.75±0.05 vs. 1.43±0.31 mmHg*min*ml^{-1}*g ^{1}, del Nido vs. standard cardioplegia, p=0.0497, **Figure 20A**). This was due to an increase in resistance over baseline in the standard cardioplegia group that was not seen in the del Nido cardioplegia group (ΔCVR: 0.68±0.22 vs. -0.05±0.04 mmHg*min*ml^{-1}*g^{-1}, p=0.007, **Figure 20B**).

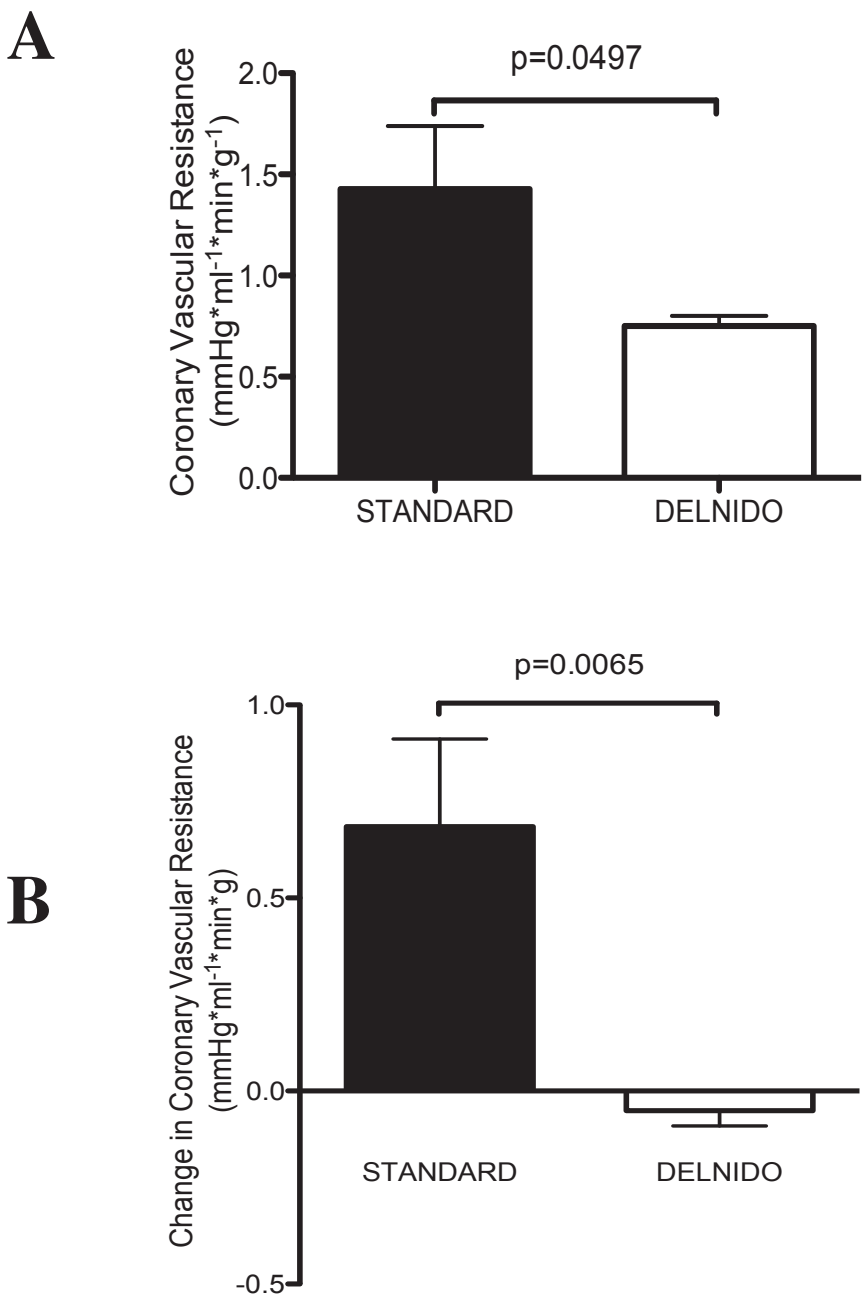

Figure 20. Coronary Vascular Resistance in standard and del Nido cardioplegia groups. A) Bar graph of CVR calculated at the end of the retrograde perfusion phase of reperfusion. B) Bar graph showing the change in CVR (ΔCVR) over baseline (end of reperfusion minus baseline CVR). Bars represent mean±SEM, n=8 per group.

1.6 Troponin Release into Coronary Effluent

To assess the impact of del Nido cardioplegia on cardiomyocyte damage after arrest and reperfusion, we assayed troponin I from the collected coronary effluent. Troponin levels were ≈70% less in hearts protected with del Nido cardioplegia when compared to standard cardioplegia (0.24±0.05 vs. 0.89±0.23 ng/ml, p=0.02, **Figure 21**).

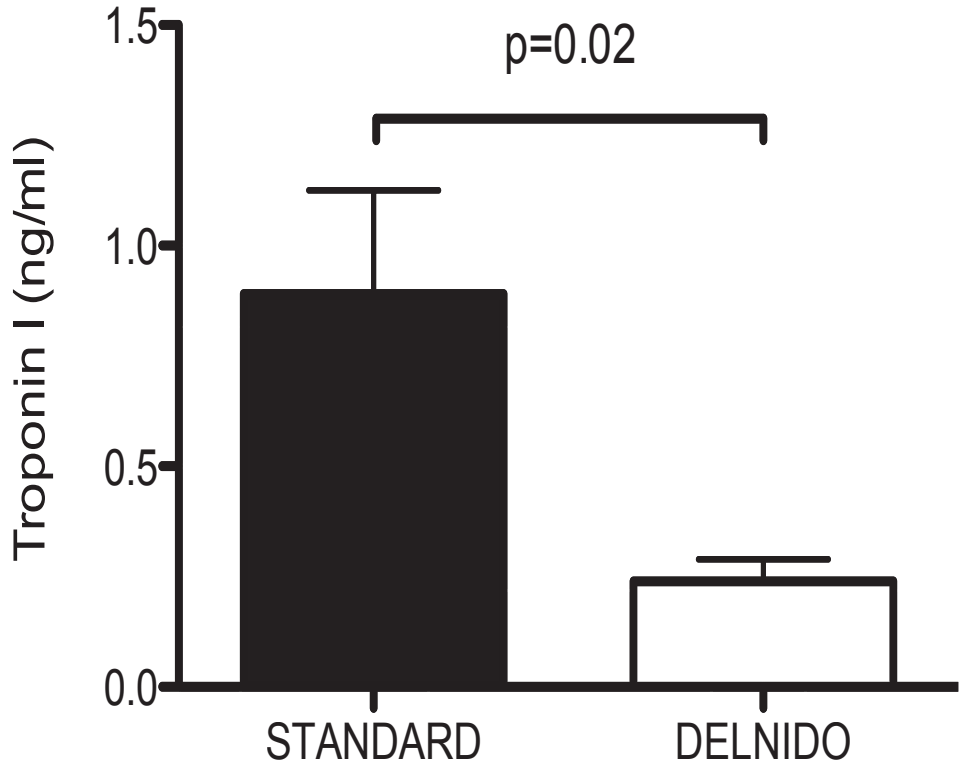

Figure 21. Troponin-I release from aged hearts during reperfusion. Bar graph showing the amount of Tn-I released in hearts arrested with standard and del Nido cardioplegia. Bars represent mean±SEM, n=8 per group.

1.7 Myocardial Edema

To assess the potential impact of cardioplegia strategy on the development of myocardial edema, we examined water content in the ventricular myocardium at the end of each study (**Figure 22**). The amount of myocardial water was similar in each group (0.94±0.05 vs. 0.92±0.02 g for standard and del Nido respectively, p=NS). Similarly, the ratio of water to dry weight of the ventricular myocardium was not different (5.1±0.1 vs. 4.9±0.1 for standard and del Nido respectively, p=NS).

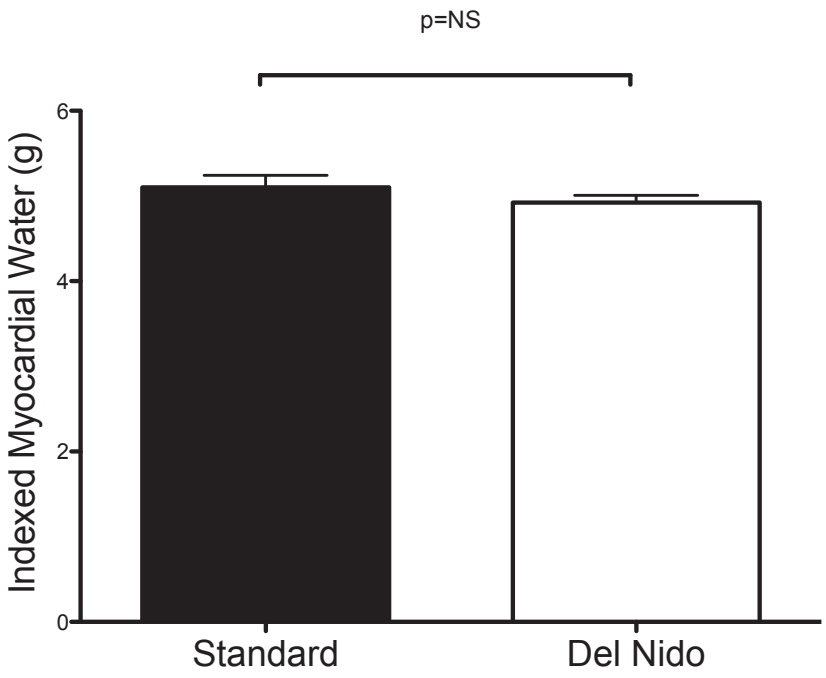

Figure 22. Measurement of myocardial edema in standard and del Nido cardioplegia groups. Bars represent mean±SEM, n=8 per group.

2. Young Adult Hearts Experiment

2.1 Eligibility of Hearts

To determine if del Nido cardioplegia could provide cardioprotection to the young adult heart, we studied young adult rats (male Fischer rats, 3-4 months old) using the same working heart model of arrest with blood cardioplegia. Thirteen of 14 hearts (93%) met the pre-determined functional criteria for inclusion into the study (**see Materials and Methods section**). Of the hearts that met baseline criteria, 2/14 (14%) hearts stopped during the protocol and data was not included. In these two hearts, both of which were given standard cardioplegia, the resistance in the syringe was extremely high during cardioplegic arrest, preventing delivery of the doses of cardioplegia within our predetermined pressure limits and were therefore excluded from the study. This did not occur in any hearts arrested with del Nido cardioplegia, or with the aged hearts experiment. Six hearts were arrested with standard cardioplegia (n=6), and six hearts were arrested with del Nido cardioplegia (n=6).

2.2 Cardiac Temperature

Cardiac temperature was measured in the young adult rat hearts similarly as with the aged hearts group. Cardiac temperatures were similar in both groups following induction (17±1 and 19±1°C, del Nido vs. standard cardioplegia, p=NS) and were also similar prior to reperfusion (17±1 and 18±1°C, del Nido vs. standard cardioplegia,

p=NS). Thus, the change in cardiac temperature from the start to the end of the arrest period was also not significantly different between the two cardioplegia solutions (-0.3±0.5°C vs. -1.1±1.4°C, del Nido vs. standard cardioplegia, p=NS; **Figures 23-25**)

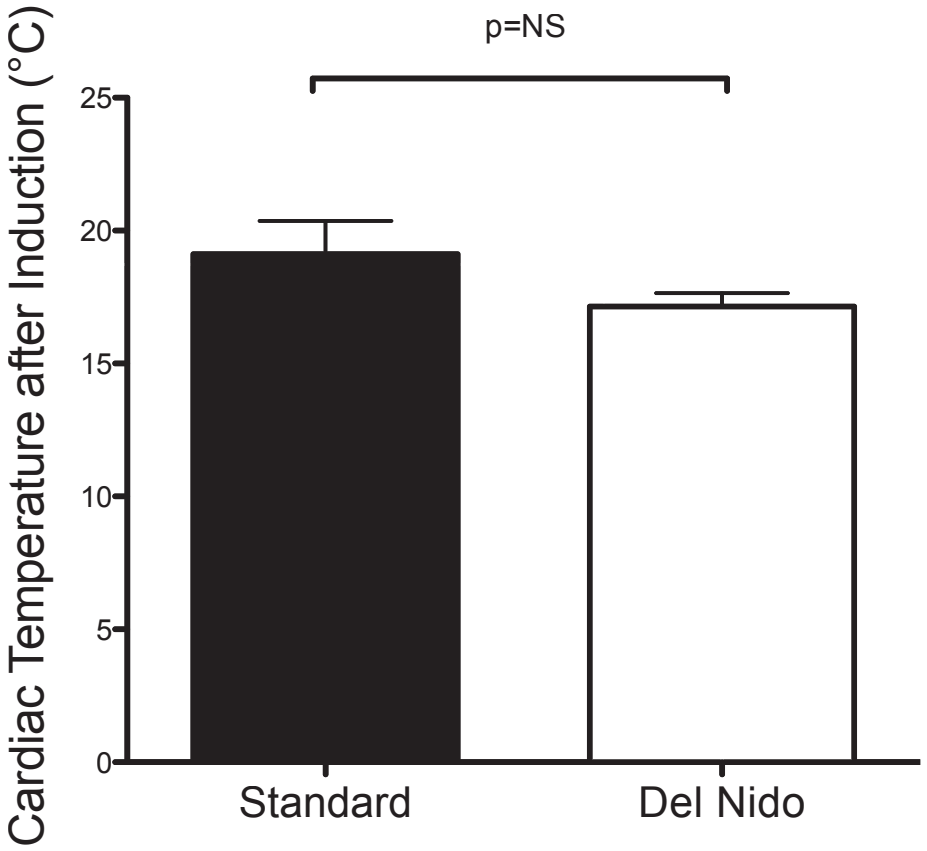

Figure 23. Cardiac temperature following delivery of induction dose of standard and del Nido cardioplegia. Bars represent mean±SEM, n=6 per group.

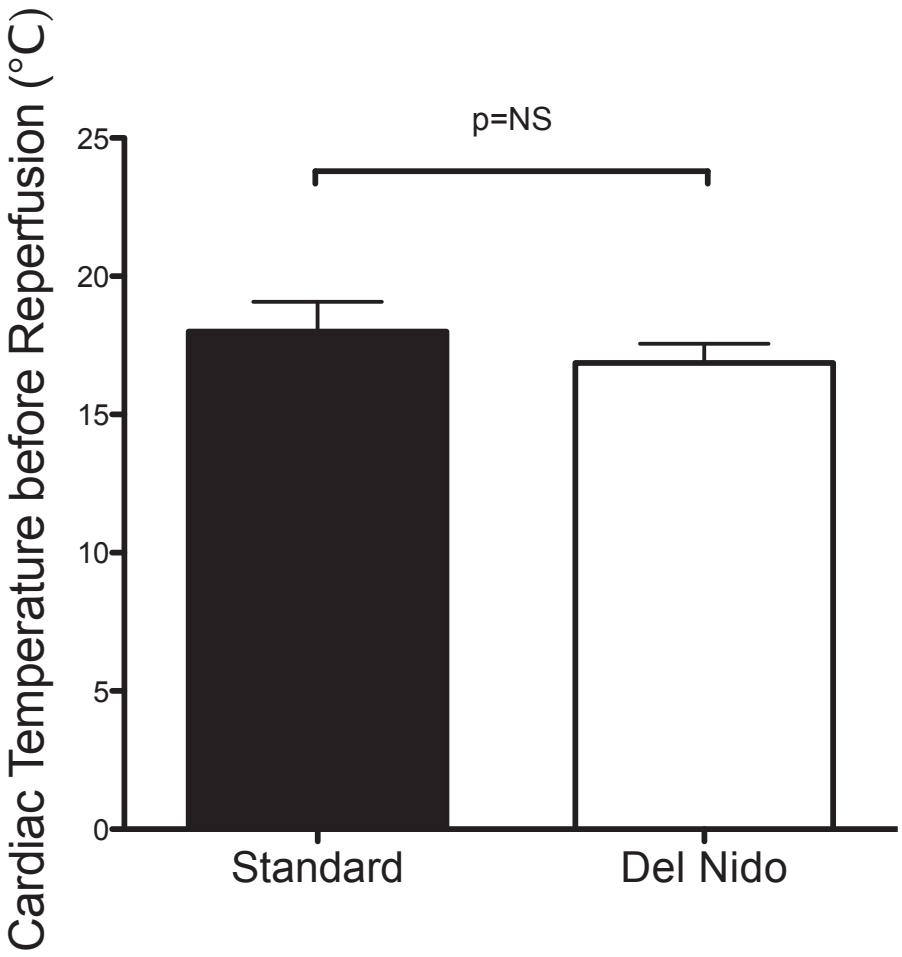

Figure 24. Cardiac temperature prior to the start of reperfusion in hearts arrested with standard and del Nido cardioplegia. Bars represent mean±SEM, n=6 per group.

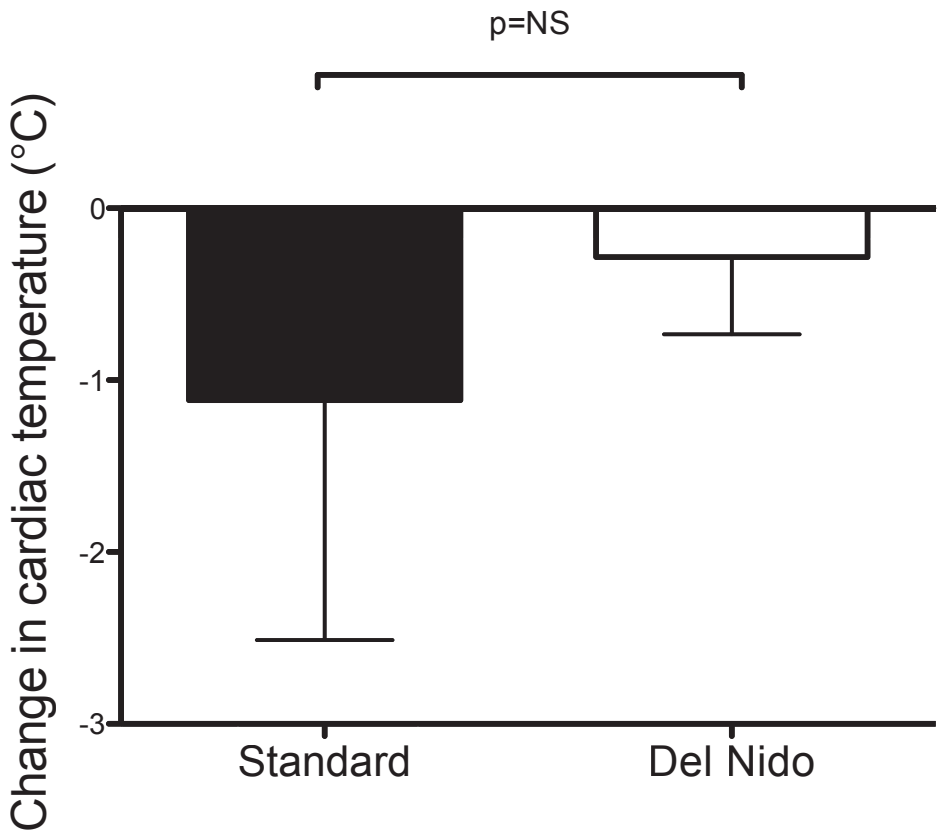

Figure 25. Change in cardiac temperature over the cardioplegic arrest period for hearts arrested with standard and del Nido cardioplegia (p=NS). Bars represent mean±SEM, n=6 per group.

2.3 Spontaneous Activity during Cardioplegic Arrest

Spontaneous electromechanical activity was examined during the cardioplegic arrest period to determine if standard and del Nido cardioplegia could maintain arrest in young adult hearts. Spontaneous activity was observed in 2/6 (33%) hearts in the standard cardioplegia group, and none of the hearts (0/6, 0%) in the del Nido group showed any spontaneous activity. However, there were no significant differences with respect to spontaneous activity between the two groups (p=NS, **Figure 26**). In the two hearts that showed spontaneous activity from the standard cardioplegia group, occasional wide complex beats were also seen on the electrocardiogram with accompanying mechanical activity, similar to **Figure 10A.**

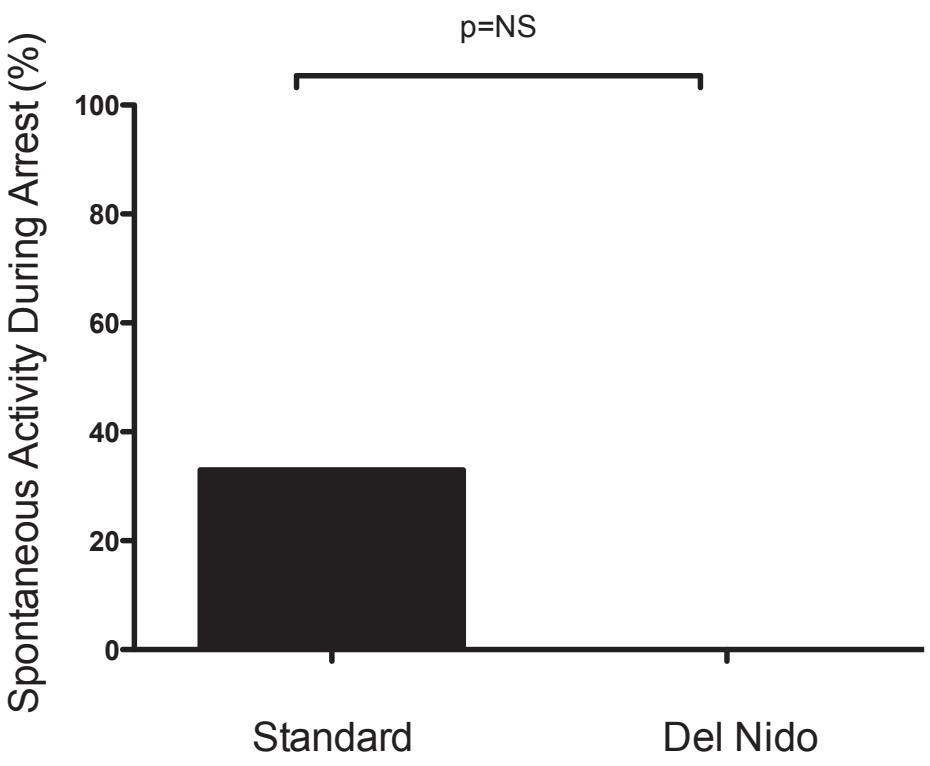

Figure 26. Analysis of spontaneous activity during cardioplegic arrest. Bar graph representing the percentage of hearts showing spontaneous activity during arrest with either standard or del Nido cardioplegia. Bars represent mean±SEM, n=6 per group.

2.4 Return of Rhythm at Reperfusion

In the young adult hearts experiment, it took the hearts arrested with del Nido cardioplegia approximately 1.5x longer for their heartbeat to return following the end of the cardioplegic arrest period and the onset of reperfusion (56±6 vs. 38±5 s, del Nido vs. standard cardioplegia, p=0.0284, **Figure 27**). All hearts had occasional extra systoles or short runs of bigeminy during reperfusion. Although one heart (1/6; 17%) in the standard cardioplegia group had short runs of sustained tachyarrhythmia that resolved spontaneously and were self-limiting, whereas none of the hearts (0/6; 0%) in the del Nido group had runs of tachyarrhythmia, these differences were not significant (p=NS, **Figure 28**).

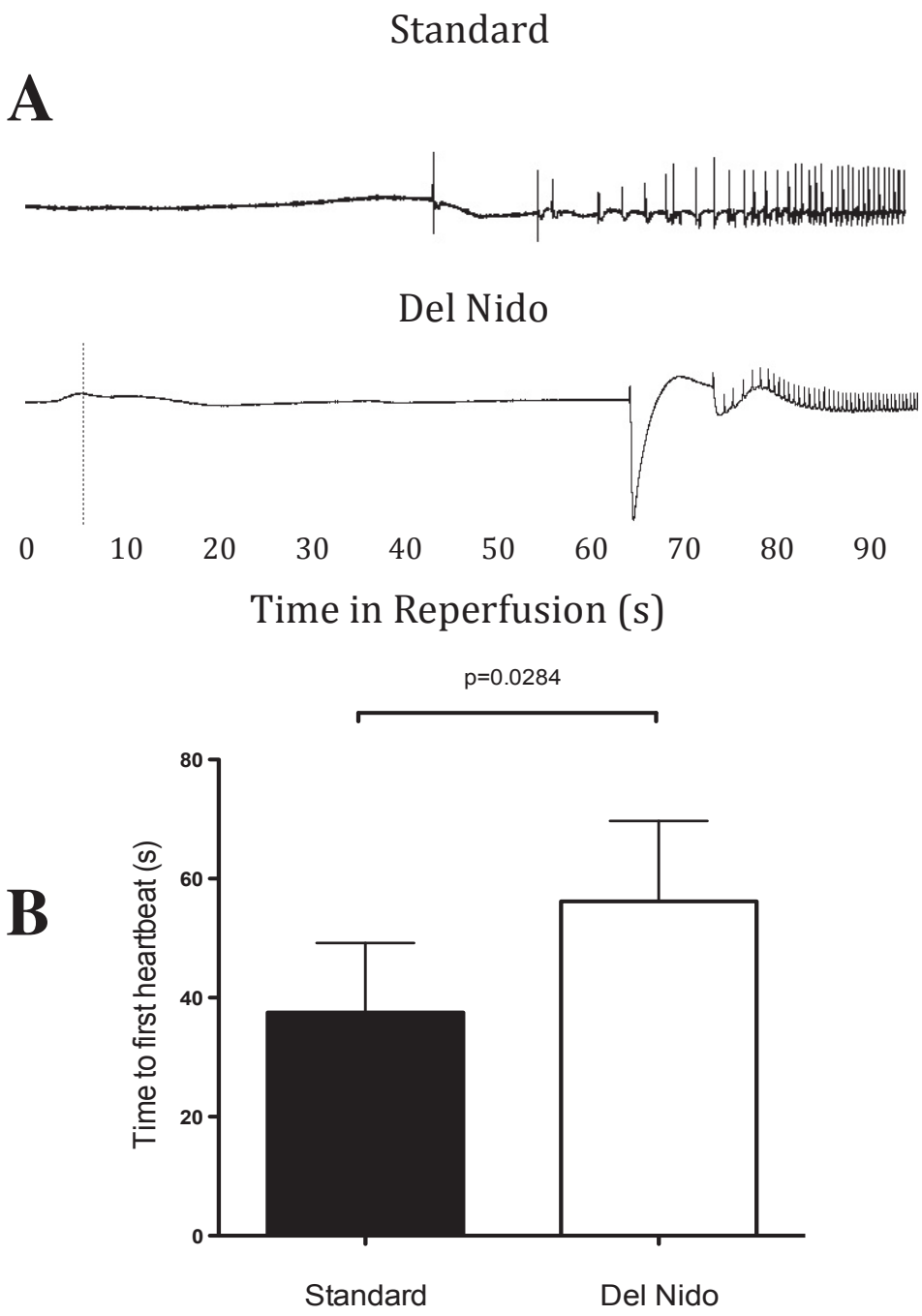

Figure 27. Analysis of return of first heart beat (rhythm) following the start of reperfusion. A) Representative ECG tracings from the start of reperfusion (t=0) in hearts arrested with standard (upper panel) and del Nido cardioplegia (lower panel). B) Bar graph representing average time to return of first heart beat after the start of reperfusion in hearts arrested with standard and del Nido cardioplegia. Bars represent mean±SEM, n=6 per group.

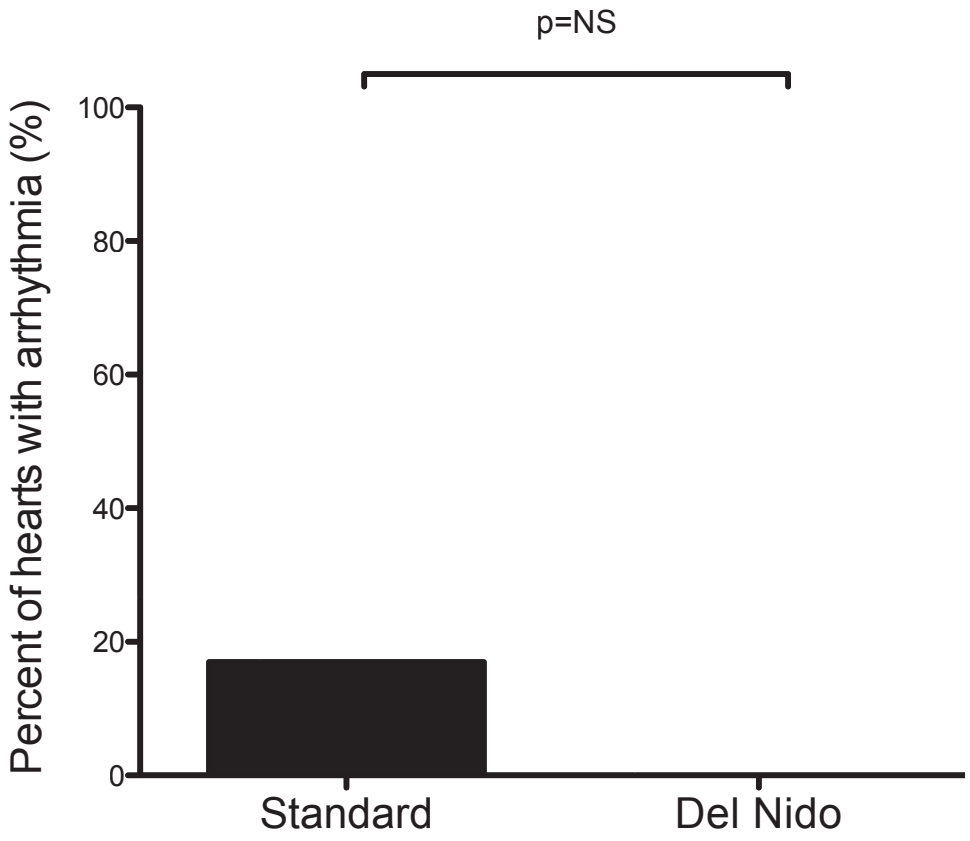

Figure 28. Percent of hearts that had short runs of sustained tachyarrhythmia during retrograde reperfusion, that were resolved and were self-limiting (p=NS). Bar graph representing percent of hearts that had incidences of tachyarrhythmia during reperfusion in hearts arrested with standard and del Nido cardioplegia. Bars represent mean±SEM, n=6 per group.

2.5 Hemodynamic Measurements

Hemodynamic measurements for the young adult hearts experiment were recorded in the same manner as in the aged hearts experiment, following the same perfusion protocol (**Figure 6**). A summary of the hemodynamic parameters measured during working heart mode for the young adult hearts is shown in **Table 3**.

	Baseline	Post Reperfusion					
		5	*10*	*15*	*30*	*45*	*60 min*
Heart Rate (BPM)							
Standard	268±12	257±17	254±19	256±20	258±16	265±13	243±25
Del Nido	248±13	246±14	239±14	242±11	243±10	240±13	235±17
p	NS	NS	NS	NS	NS	NS	NS
Rate Pressure Product ($x10^3$ mmHg*min^{-1})							
Standard	33±1	26±2	25±2	25±2	24±2	25±1	22±2
Del Nido	31±2	28±1	28±1	29±1	28±1	26±2	25±2
p	NS	NS	NS	NS	NS	NS	NS
Systolic Pressure (mmHg)							
Standard	116±3	100±4	99±4	100±5	93±3	93±3	90±2
Del Nido	125±6	116±6	120±7	119±6	114±5	110±4	107±4
p	NS	NS	<0.05	NS	p<0.05	NS	NS
LVDP (mmHg)							
Standard	101±4	85±4	83±4	84±5	77±3	77±3	74±2
Del Nido	111±6	102±6	105±7	103±7	98±5	95±4	91±4
p	NS	NS	p<0.05	NS	NS	NS	NS
Coronary Flow (ml*min^{-1}*g^{-1})							
Standard	127±6	75±7	76±6	77±7	81±7	84±7	80±7
Del Nido	114±8	100±7	100±8	104±8	105±8	103±8	99±9
p	NS	NS	NS	NS	NS	NS	NS
Cardiac Output (ml*min^{-1}*g^{-1})							
Standard	313±9	159±23	150±20	150±19	137±19	129±18	110±18
Del Nido	274±26	229±23	220±24	224±26	204±25	189±27	172±28
p	NS	NS	NS	NS	NS	NS	NS
Stroke Volume ($x10^{-2}$ ml*g^{-1})							
Standard	109±7	62±8	59±7	59±7	54±8	49±7	46±6
Del Nido	109±6	92±5	91±7	91±7	83±8	77±8	71±8
p	NS	NS	NS	NS	NS	NS	NS
Stroke Work (ml*mmHg*g^{-1})							
Standard	127±12	64±9	60±8	60±8	51±9	46±7	42±6
Del Nido	135±6	106±6	108±6	108±9	93±9	84±10	75±9
p	NS	p<0.05	p<0.01	p<0.01	p<0.05	NS	NS

Table 3. **Hemodynamic parameters measured during working heart mode: young adult hearts**; n=6 per group.

2.5.1 Heart Rate

Heart rate was measured throughout the experimental protocol as in the aged hearts experiment. Heart rate was not significantly different during baseline between standard and del Nido cardioplegia groups (248±13 vs. 268±12 bpm, del Nido vs. standard cardioplegia, p=NS, **Table 3, Figure 29**) and was also not significantly different during post-reperfusion working heart (p=NS).

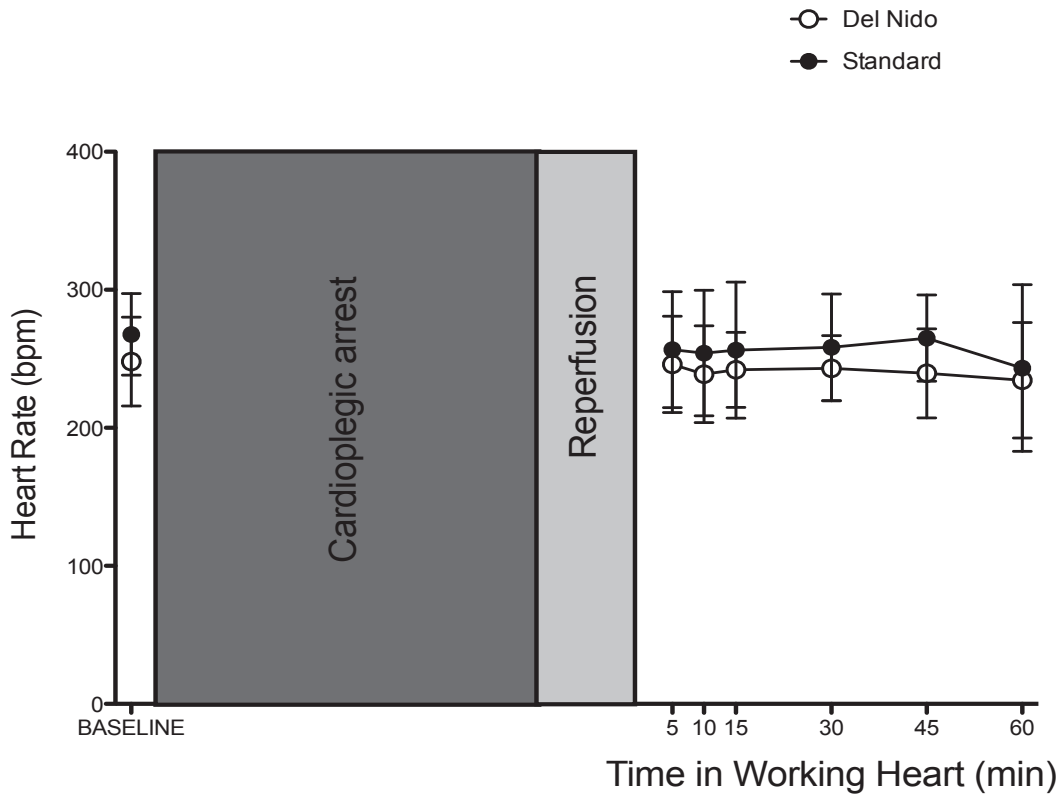

Figure 29. Heart rate in standard and del Nido cardioplegia groups. Graph showing heart rate measured during working heart mode before (baseline) and at several time points after 60 minutes of cardioplegic arrest and 20 minutes of reperfusion for the standard and del Nido cardioplegia groups. Data points represent mean±SEM, n=6 per group.

2.5.2 Rate-Pressure Product

Rate-Pressure Product (RPP) was calculated during baseline and post-reperfusion similarly as with the aged hearts. RPP was similar between the two cardioplegia groups at baseline (31±2 vs. 33±1 x10^3 mmHg*min^{-1}, del Nido vs. standard cardioplegia, p=NS). Following reperfusion, there were also no differences in RPP between the two cardioplegia groups during the 60-minute working heart period (p=NS).

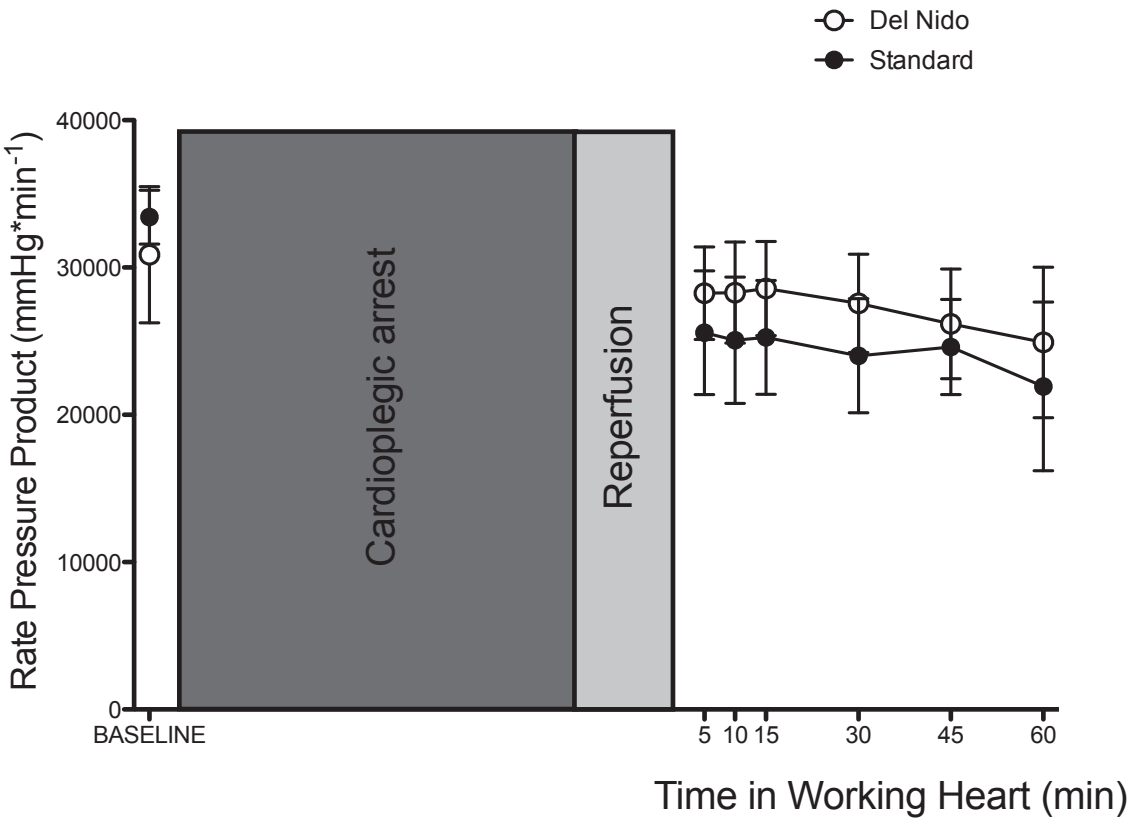

Figure 30. Rate-Pressure Product in standard and del Nido cardioplegia groups. Graph showing Rate-Pressure Product calculated during working heart mode before (baseline) and at several time points after 60 minutes of cardioplegic arrest and 20 minutes of reperfusion for the standard and del Nido cardioplegia groups. Data points represent mean±SEM, n=6 per group.

2.5.3 Left Ventricular Developed Pressure

LVDP was calculated for the young adult rat hearts with both types of cardioplegia. There were no significant differences in LVDP between standard and del Nido cardioplegia at baseline (111±6 vs. 101±4 mmHg, del Nido vs. standard cardioplegia, p=NS). Following reperfusion, during working heart, LVDP was significantly higher in the del Nido group only at **t=10** (105±7 vs. 83±4, del Nido vs. standard cardioplegia, p<0.05, **Table 3, Figure 31**). At all other time points in working heart mode, the increase in LVDP in the del Nido group was not significant (p=NS).

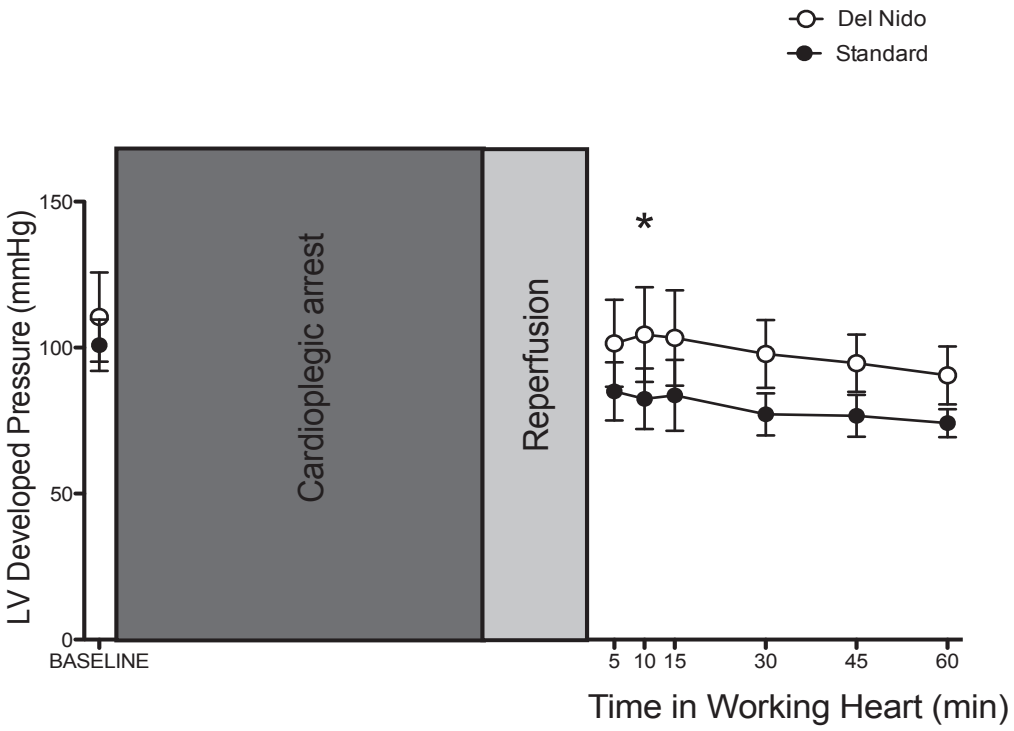

Figure 31. Left ventricular developed pressure before and after arrest and reperfusion. Graph showing LVDP measured during working heart mode before (baseline) and at several time points after 60 minutes of cardioplegic arrest and 20 minutes of reperfusion for the standard and del Nido cardioplegia groups. Data points represent mean±SEM, n=6 per group, *=p<0.05.

2.5.4 Coronary Flow

Coronary flow was measured at baseline and post-reperfusion working heart modes for the young adult hearts. There were no significant differences in coronary flow between the standard cardioplegia and del Nido cardioplegia groups at baseline (127±6 vs. 114±8 ml*min^{-1}*g^{-1}, del Nido vs. standard cardioplegia, p=NS) and during post-reperfusion working heart mode (p=NS).

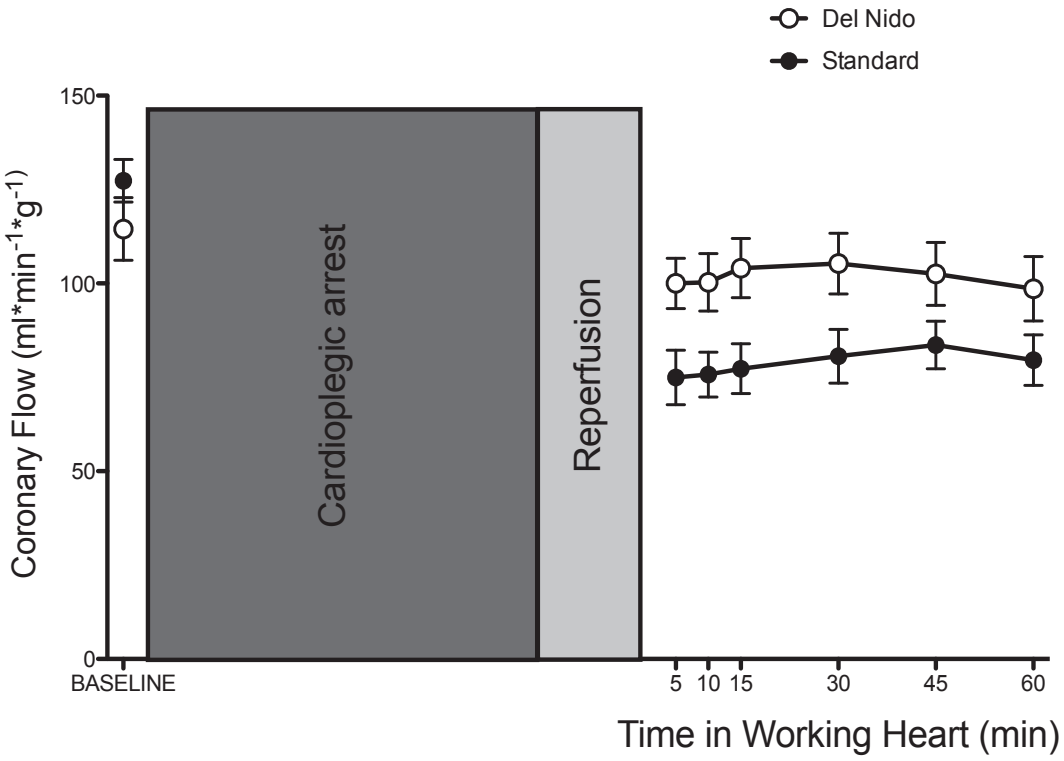

Figure 32. Coronary flow in standard and del Nido cardioplegia groups. Graph showing coronary flow measured during working heart mode before (baseline) and at several time points after 60 minutes of cardioplegic arrest and 20 minutes of reperfusion for the standard and del Nido cardioplegia groups. Data points represent mean±SEM, n=6 per group.

2.5.5 Cardiac Output

Cardiac output was calculated for the young adult hearts. There were no significant differences in cardiac output between standard and del Nido cardioplegia at baseline (274±26 vs. 313±9 ml*min^{-1}*g^{-1}, del Nido vs. standard cardioplegia, p=NS) and following reperfusion, in working heart mode (**Table 3, Figure 33**). Cardiac output values were not significantly different during post-reperfusion working heart mode (p=NS).

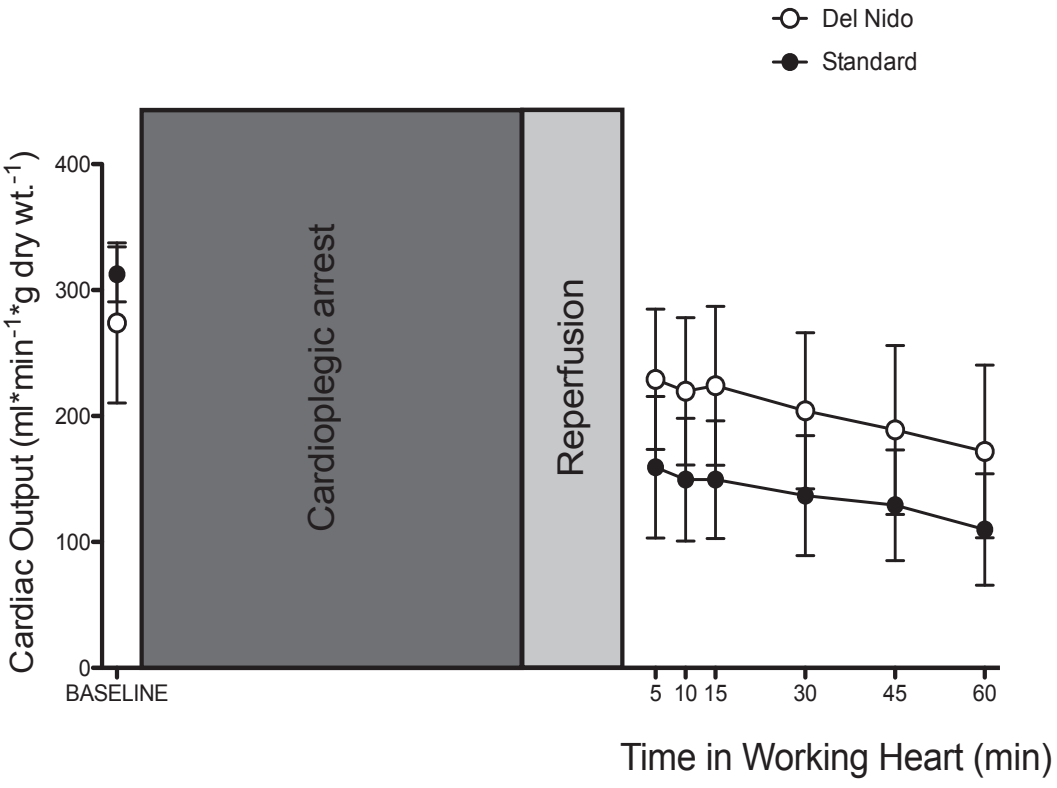

Figure 33. Cardiac output in standard and del Nido cardioplegia groups. Graph showing cardiac output measured during working heart mode before (baseline) and at several time points after 60 minutes of cardioplegic arrest and 20 minutes of reperfusion for the standard and del Nido cardioplegia groups. Data points represent mean±SEM, n=6 per group.

2.5.6 Stroke Volume

Stroke volume was calculated in hearts arrested with standard and del Nido cardioplegia in the young adult similarly as with the aged hearts experiment. There were no significant differences between the two cardioplegia groups at baseline (109±6 vs. 109±7 x10^{-2} ml*g^{-1}, del Nido vs. standard cardioplegia, p=NS). During post-reperfusion working heart, there were no significant differences in stroke volume between del Nido and standard cardioplegia (p=NS).

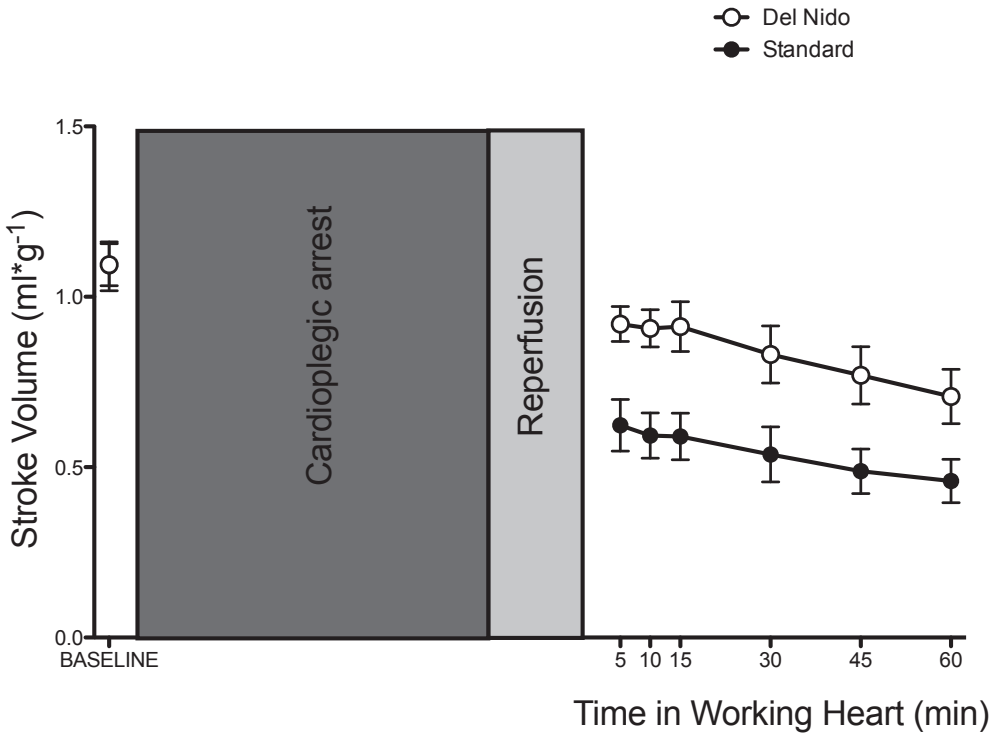

Figure 34. Stroke volume in standard and del Nido cardioplegia groups. Graph showing stroke volume calculated during working heart mode before (baseline) and at several time points after 60 minutes of cardioplegic arrest and 20 minutes of reperfusion for the standard and del Nido cardioplegia groups. Data points represent mean±SEM, n=6 per group.

2.5.7 Stroke Work

Stroke work was calculated for the young adult hearts at baseline and post-reperfusion. At baseline, there were no differences in stroke work between standard and del Nido cardioplegia (135±6 vs. 127±12 ml*mmHg*g^{-1}, del Nido vs. standard cardioplegia, p=NS). However, during the first 30 minutes of post-reperfusion working heart, stroke work was greater in the del Nido group than in the standard group (**t=5:** 106±6 vs. 64±9, **t=10**: 108±6 vs. 60±8; **t=15**: 108±9 vs. 60±8, **t=30**: 93±9 vs. 51±9 ml*mmHg*g^{-1} **,** del Nido vs. standard cardioplegia, p<0.05, **Table 3, Figure 35**) but was not significantly different between the two cardioplegia groups for the next 30 minutes of the working heart period.

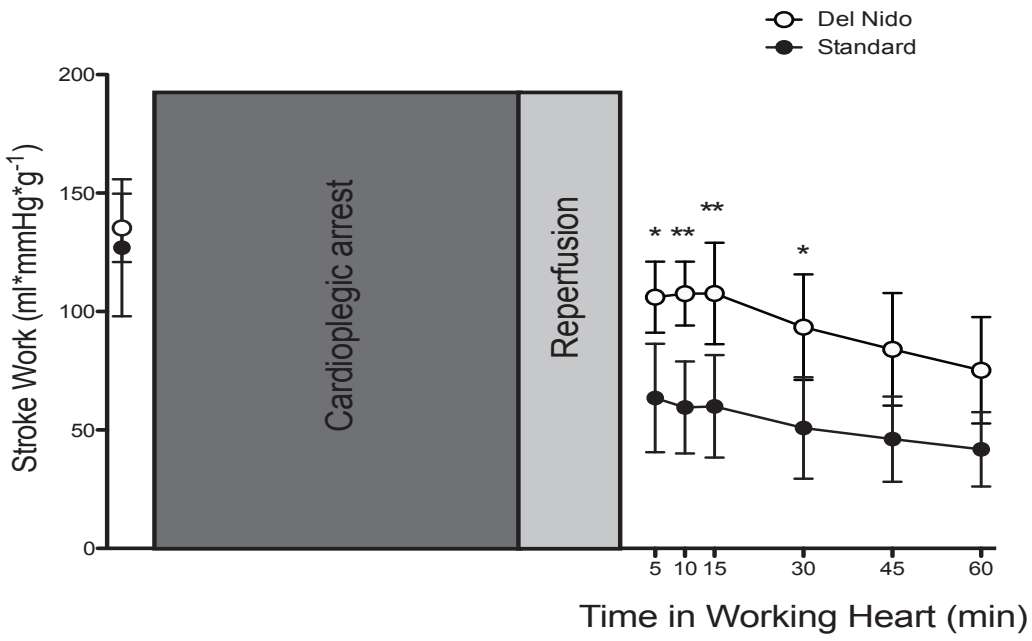

Figure 35. Stroke work in standard and del Nido cardioplegia groups. Graph showing stroke work calculated during working heart mode before (baseline) and at several time points after 60 minutes of cardioplegic arrest and 20 minutes of reperfusion for the standard and del Nido cardioplegia groups. Data points represent mean±SEM, n=6 per group, *=p<0.05; **=p≤0.001.

2.5.8 Coronary Vascular Resistance

Coronary vascular resistance was determined at the end of retrograde reperfusion, before switching to pressure mode, in the same manner as in the aged hearts experiment. During the retrograde perfusion phase of reperfusion, coronary vascular resistance was not different between the two types of cardioplegia solutions (0.79±0.02 vs. 1.00±0.11 mmHg*min*ml^{-1}*g ^{1}, del Nido vs. standard cardioplegia, p=NS, **Figure 36A**). Additionally, the change in CVR from baseline to retrograde reperfusion (ΔCVR) was also not significantly different between the standard and del Nido cardioplegia groups (-0.06±0.14 vs. 0.27±0.16 mmHg*min*ml^{-1}*g^{-1}, p=NS, **Figure 36B).**

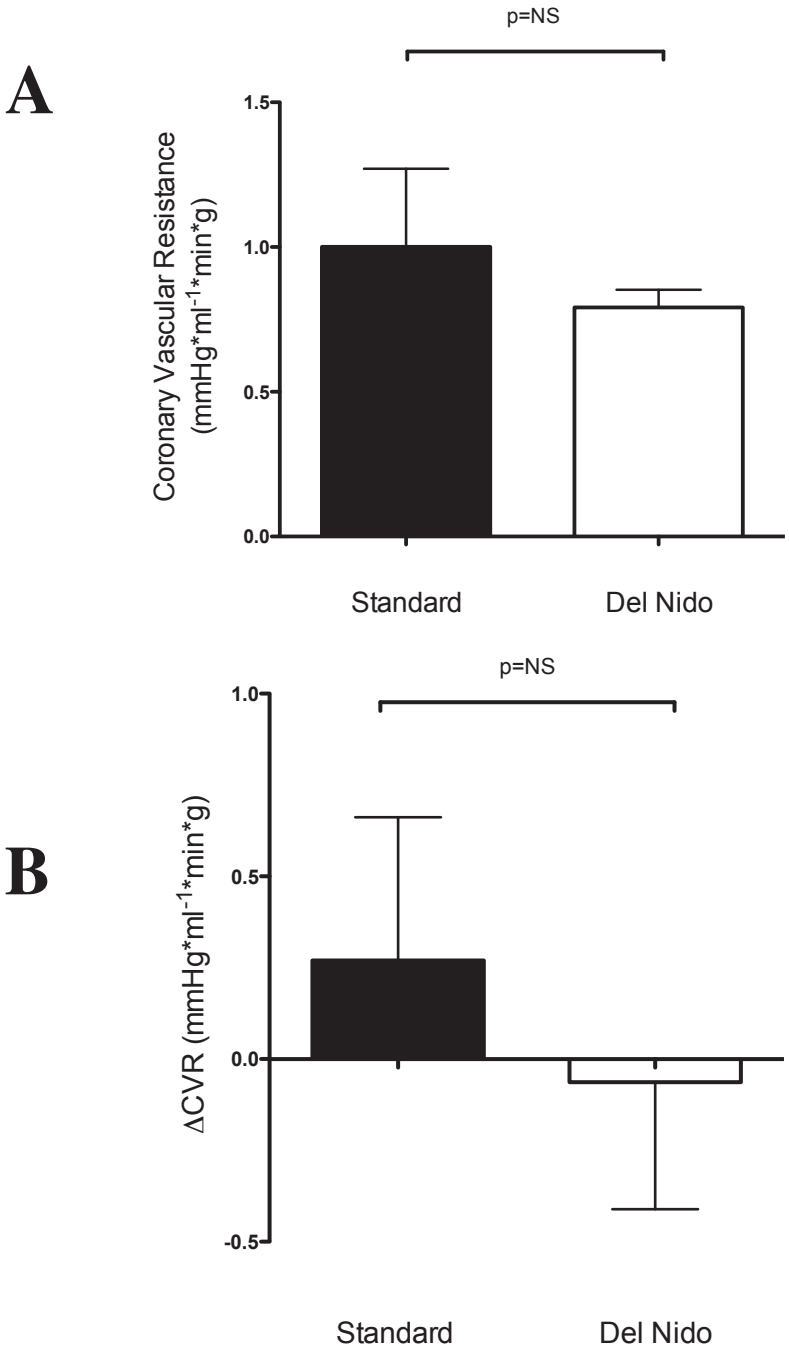

Figure 36. Coronary Vascular Resistance in standard and del Nido cardioplegia groups. A) Bar graph of CVR calculated at the end of the retrograde perfusion phase of reperfusion. B) Bar graph showing the change in CVR (ΔCVR) over baseline (end of reperfusion minus baseline CVR). Bars represent mean±SEM, n=6 per group.

2.6 Troponin Release into Coronary Effluent

To assess the impact of del Nido cardioplegia on cardiomyocyte damage after arrest and reperfusion, we assayed troponin-I from the collected coronary effluent, for the young adult hearts. Between the two cardioplegia groups, there were no significant differences in troponin-I levels (0.67±0.24 vs. 1.44±0.38 ng/ml, del Nido vs. standard cardioplegia, p=NS, **Figure 37**).

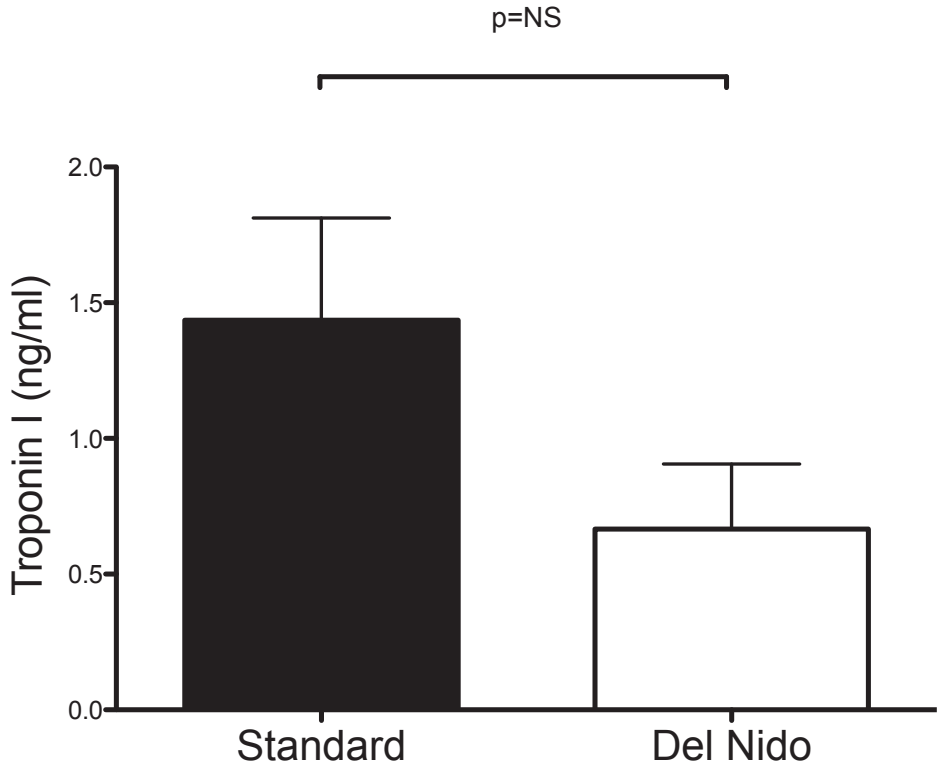

Figure 37. Troponin-I release from young adult hearts during reperfusion. Bar graph showing the amount of Tn-I released in hearts arrested with standard and del Nido cardioplegia. Bars represent mean±SEM, n=6 per group.

2.7 Myocardial Edema

Myocardial edema was quantified in the young adult hearts as in the aged hearts experiment (**Figure 38**). The amount of myocardial water was similar in each group (0.68±0.01 for both groups, p=NS). The ratio of water to dry weight of the ventricular myocardium was also not different (4.6±0.1 vs. 4.4±0.1 for standard and del Nido respectively, p=NS, **Figure 38**).

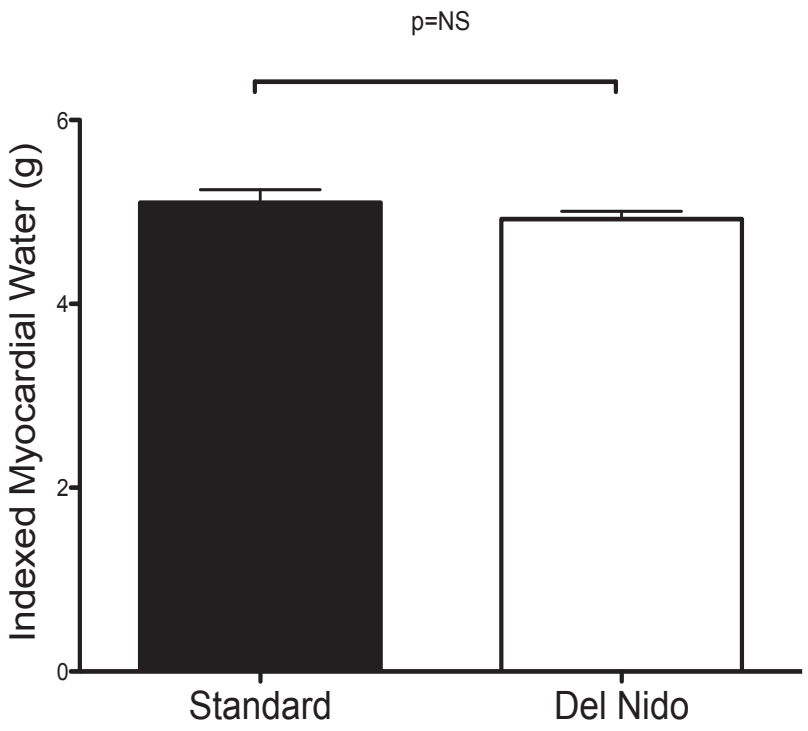

Figure 38. Measurement of myocardial edema in standard and del Nido cardioplegia groups (p=NS)**.** Bars represent mean±SEM, n=6 per group.

CHAPTER 4: DISCUSSION

1. Overview

The overall aim of this study was to determine if the use of del Nido cardioplegia could result in superior functional recovery in both whole, isolated aged and young adult hearts compared to our standard cardioplegia.

The first objective of the study was to determine if the benefits of del Nido cardioplegia, seen in isolated cardiomyocytes from aged animals, translates into improved function in the whole aged heart. It was hypothesized that since our lab has previously shown that in isolated cardiomyocytes, arrest with del Nido cardioplegia resulted in lower spontaneous and inducible activity during ischemia, lower diastolic Ca^{2+} during ischemia-reperfusion, and avoidance of hypercontraction during early reperfusion in aged cardiomyocytes when compared with standard cardioplegia (O'Blenes et al., 2011), that del Nido cardioplegia has been shown to be clinically beneficial for pediatric patients in reducing post-operative troponin-I levels compared to our standard cardioplegia (O'Brien et al., 2009), and that the pediatric and aged heart share similarities because they are both particularly vulnerable to ischemia reperfusion injury, then del Nido cardioplegia could provide superior myocardial protection by improving post-ischemic functional recovery of aged rat hearts versus our standard cardioplegia.

The second objective of the study was to determine if del Nido cardioplegia provides better cardioprotection over our standard cardioplegia in the young adult heart. Although del Nido cardioplegia has been given to young adult patients in the clinic (Matte et al., 2012) there is a lack of basic science and clinical evidence to support the superiority of del Nido cardioplegia over other cardioplegia formulations that are similar in composition compared to our standard cardioplegia. It was hypothesized that since the young adult heart is more tolerant to ischemia-reperfusion injury than the aged heart (Ataka et al., 1992), use of del Nido cardioplegia in the young adult heart would not exert the same level of cardioprotection than in the aged heart, and would not provide significant advantages over our standard cardioplegia. Del Nido and standard cardioplegia could be similar in effectiveness when delivered to the young adult heart.

2. Interpretation of Results

2.1 Spontaneous Activity during Cardioplegic Arrest

Similar to the observations in our lab's previous *in vitro* isolated cardiomyocyte study (O'Blenes et al., 2011), we found a reduction in spontaneous activity in aged hearts arrested with del Nido cardioplegia. This may be related to Na^+ channel blockade with lidocaine, but del Nido cardioplegia also contains slightly more K^+ and Mg^{2+} than our standard cardioplegia resulting in more pronounced membrane depolarization that may also contribute to more effective arrest (O'Brien et al., 2009). We have recently demonstrated that the lidocaine concentration used in del Nido cardioplegia decreases the

potential for Na^+ influx by minimizing the so-called Na^+ "window" current during cardioplegic arrest. This "window" current is a tonally active, inward sodium current that has the potential to provide sodium influx during the cardioplegic arrest period. Myocardial function is closely related to intracellular Ca^{2+} concentration. The normal Ca^{2+} flux in the myocardium increases intracellular Ca^{2+} for contraction and decreases it for relaxation. If Ca^{2+} is allowed to accumulate in the myocardium, relaxation may be interrupted and diastolic stiffness with poor recovery may result (Larach et al., 1995). Magnesium has been shown to be a natural Ca^{2+} channel blocker (Iseri et al., 1984). This effect is likely how Mg^{2+} has been shown to improve ventricular recovery in hypothermic cardioplegia solutions when coupled with a low calcium level, and may also explain why spontaneous activity was reduced in aged hearts arrested with del Nido cardioplegia (Brown et al., 1995). The role of magnesium in blocking Ca^{2+} channels in del Nido cardioplegia would have to be studied further in a cardiomyocyte model of cardioplegic arrest and reperfusion similar to the model utilized by O'Blenes et al. (2011), whereby calcium transients and cell length shortening can be measured, and in a set of isolated working heart experiments where del Nido cardioplegia is delivered during arrest with varying concentrations (or none) of Mg^{2+} in solution, and functional recovery parameters are recorded.

Since all of the Ca^{2+} in the two cardioplegia solutions comes from the blood, and that standard cardioplegia contains more blood and therefore 75% more Ca^{2+} (**Table 1**), the lower amount of Ca^{2+} in del Nido cardioplegia may help to prevent Ca^{2+}-induced hypercontracture to a greater degree than standard cardioplegia. Reduced spontaneous

activity during the ischemic period should limit the development of intracellular acidosis, which drives the Na^+, and subsequently Ca^{2+} influx that contributes to ischemia-reperfusion injury (O'Blenes et al., 2011). In the aged heart, it is possible that more effective elimination of spontaneous activity as seen with del Nido cardioplegia could translate into reduced ischemia-reperfusion injury and improved heart function and therefore, better outcomes after cardiac surgery in elderly patients (O'Blenes et al., 2011).

In contrast, for the young adult hearts, there was no significant difference in the incidence of spontaneous activity during cardioplegic arrest between del Nido and standard cardioplegia. This suggests that del Nido cardioplegia may not be more advantageous than standard cardioplegia in providing an effective arrest for the young adult heart. A possible explanation for this finding is that since young adult hearts are more tolerant to ischemia-reperfusion injury than aged hearts (McCully et al., 2006; Willems et al., 2005; Boucher et al., 1998; Ataka et al., 1992; Faulk et al., 1995; Tsukube et al., 1997; Ladilov et al., 2003; Piper et al., 2003) due to changes in Ca^{2+} homeostasis resulting in lower calcium overload during ischemia (Ataka et al., 1992; Faulk et al., 1995; Tsukube et al. 1997), it is possible that standard cardioplegia may be just as effective as del Nido cardioplegia in providing a depolarized arrest. Since the degree of calcium overload is lessened in the young adult heart (and thus the potential for contractions during the arrest period), the unique additives in del Nido cardioplegia may be exerting a similar level of electromechanical arrest as the standard cardioplegia, and thus no differences were noted. It is possible that neither type of cardioplegia is better than the other at inducing the desired electromechanical arrest in the young adult heart.

2.2 Return of Rhythm during Reperfusion

Del Nido cardioplegia delayed the return of rhythm in both aged and young adult hearts. The time to return of first heartbeat was twice as long with del Nido cardioplegia in the aged hearts study than standard cardioplegia. This corresponds with what cardiac surgeons at the IWK Children's Hospital anecdotally observed in their clinical practice when they switched from standard to del Nido cardioplegia for their pediatric patients (O'Brien et al., 2009; O'Blenes et al., 2011). Similarly, del Nido cardioplegia also delayed the return of rhythm in the young adult hearts. In the young adults hearts, the time to return of the first heartbeat was one and a half times as long with del Nido cardioplegia than standard cardioplegia. This may represent a residual effect of lidocaine but it is not clear if the delayed resumption of rhythm plays any role in the benefit seen with del Nido cardioplegia. It is possible that a period of persistent inactivity during early reperfusion may improve myocardial recovery in a manner similar to that seen with the use of terminal warm blood cardioplegia (Allen et al., 1993).

Warm terminal blood cardioplegia creates a period of asystolic reperfusion during which energy produced is channeled to myocardial reparative processes rather than mechanical work (Hattori et al., 2000). In a study published by Teoh et al. (1986) delivery of a "hot shot" of cold blood followed by warm blood cardioplegia was given to patients undergoing elective coronary bypass grafting. The hot shot removed excess lactate from the arrested heart, providing early evidence that prolonging the onset of reperfusion may

be beneficial in improving metabolic repair of hearts before contractions eventually resume during reperfusion (Teoh et al., 1986).

The major metabolic deficit caused by ischemic myocardial damage is a limited capacity to utilize delivered oxygen (Kawasuji et al., 1998). Kane et al. (1975) found that mitochondrial oxygen uptake remained reduced after reperfusion, and identified a defect in electron transport in the respiratory chain. Kawasuji et al. (1998) measured myocardial tissue oxygen saturation during reperfusion in dogs, using near-infrared spectroscopy. Dogs' hearts that were given normal warm blood reperfusion showed a significant decrease in myocardial tissue oxygen saturation approximately 2.5 minutes after the start of reperfusion. They postulated that this decrease in myocardial tissue oxygen saturation may be attributed to an increase in metabolic requirements as post-ischemic electromechanical activity resumes. Previous studies have shown that myocardial oxygen consumption increases as a result of electromechanical activity (Holman et al., 1994). It is possible that del Nido cardioplegia, in delaying the return of rhythm (and electromechanical activity) following the onset of reperfusion, may also slow down the rate of myocardial oxygen consumption, thereby improving cardiac metabolism vs. standard cardioplegia in both the aged and young hearts. Future studies can measure the rate of oxygen consumption upon reperfusion in isolated hearts arrested with del Nido vs. standard cardioplegia.

A period of prolonged inactivity during the early part of reperfusion may allow for normalization of the intracellular ion concentrations before mechanical activity can resume, thereby potentially reducing the risk of damaging hypercontracture. Future

studies can address this by utilizing a cardiomyocyte model of cardioplegic arrest and reperfusion to study the effects of length of delay in time of reperfusion on the amplitude of calcium transients. Del Nido cardioplegia may exert better cardioprotection than standard cardioplegia by allowing time for some of the calcium overload to be attenuated via blocking of sodium influx (lidocaine), therefore potentially reducing the extent of hypercontracture during reperfusion. During the earliest phase of reperfusion, development of cardiomyocyte hypercontracture has been shown to be the primary cause of cardiomyocyte necrosis. It has been demonstrated that control of hypercontracture during reperfusion reduces the extent of tissue injury (Piper et al., 2004). Literature has shown that reperfusion-induced hypercontracture may originate from Ca^{2+} overload, when energy recovery is rapid due to the resupply of oxygen but cytosolic Ca^{2} load is high (Piper et al., 2004).

This so-called "Ca^{2+} overload-induced hypercontracture" may be attenuated in hearts arrested with del Nido cardioplegia. It is the resupply of energy to the myofibrillar elements in the presence of an increase of cytosolic Ca^{2+} concentration which may be deleterious for the reoxygenated cell (Piper et al., 1998). This is because during the initial phase of reoxygenation, the cytosolic Ca^{2+} is still largely elevated and myofibrillar activation therefore leads to uncontrolled, excessive force generation. This sustained force generation causes hypercontraction (Piper et al., 1998). The hypercontracting cardiomyocyte becomes severely injured in its cytoskeletal structures as the deformation of cytoskeletal elements beyond the degree found under normal contractile shortening is no longer readily reversible. The resulting state of irreversible cell shortening is called

"hypercontracture". In tissue, hypercontraction of adjacent cells may lead to mutual cell disruptions and necrosis (Piper et al., 1998).

Under conditions of energy depletion such as during ischemia-reperfusion injury, the cytosol of the cardiomyocyte becomes loaded with Na^+ and Ca^{2+} (Piper et al., 1998). Recovery of energy production upon resupply with oxygen and metabolic substrates rapidly reactivates two major cation pumps: the Ca^{2+}-ATPase of the SR and the Na^+/K^+-ATPase of the sarcolemma (Piper et al., 1998). Activation of the Ca^{2+}-ATPase of the SR leads to a temporary sequestration of excess Ca^{2+} within the SR (Siegmund et al., 1992; Siegmund et al., 1994). If the capacity of the SR is too small for the amount of Ca^{2+} accumulated in the cytosol, a cycle of continuous release and reuptake of Ca^{2+} from and into the SR is initiated (Piper et al., 1998, Siegmund et al., 1992; Siegmund et al., 1994). These spontaneous oscillations reach an end only if the major mechanism for Ca^{2+} extrusion from the cytosol is sufficiently activated – through activation of the forward mode of the sarcolemmal NCX (Siegmund et al., 1994). Restoration of a sufficiently large Na^+ gradient is the prerequisite for extrusion of Ca^{2+} from reoxygenated cardiomyocytes. It is essential that the Na^+/K^+ ATPase of the sarcolemma is rapidly activated to remove excess Na^+ from the interior of the cell (Piper et al., 1998, Siegmund et al., 1992; Siegmund et al., 1994).

Ischemic cardiomyocytes become energy depleted and subsequently develop Ca^{2+} overload of the cytosol due to an initial accumulation of Na^+ and subsequent uptake of Ca^{2+} through a reverse-mode operation of the sarcolemmal NCX (Piper et al., 2004). At the end of ischemia, this leaves the ischemic cardiomyocytes in a state of cytosolic Ca^{2+}

overload. If the ability of mitochondria to resume ATP synthesis is not critically impaired during the ischemic period, reoxygenation brought on by reperfusion leads to a rapid recovery of oxidative energy production (Piper et al., 2004). Resynthesis of ATP can enable cardiomyocytes to recover from the loss of cytosolic cation balance, but it also reactivates the contractile machinery. Contractile activation is normally faster than Ca^{2+} recovery, and this leads to an uncontrolled Ca^{2+}-dependent contraction (Piper et al., 2004). Experimental evidence has shown that cyclic uptake and release of Ca^{2+} by the sarcoplasmic reticulum (SR) in the reoxygenated cardiomyocytes immediately triggers a Ca^{2+} overload-induced hypercontracture (Schafer et al., 2001). These oscillatory Ca^{2+} shifts lead to high cytosolic peak Ca^{2+} concentrations. The frequency of these Ca^{2+} peaks is influenced by an ongoing Ca^{2+} influx across the sarcolemma during the early phase of reoxygenation. During this period, the transsarcolemmal Na^{+} gradient is still reduced and the NCX still operates in the reverse mode (Schafer et al., 2001).

To summarize, after ischemia, cardiomyocytes contain an excessive cytosolic Ca^{2+}overload (Piper et al., 2004). In the early phase of reoxygenation, this may still be aggravated by a reverse-mode action of the NCX. Reoxygenation causes a re-energizing of the SR due to the return of oxygen inducing oxidative phosphorylation to yield more ATP than during ischemia (Piper et al., 1998). This causes the SR to accumulate Ca^{2+}, and once full, the SR releases Ca^{2+}. These Ca^{2+} movements lead to oscillatory cytosolic intracellular Ca^{2+} elevations that provoke an uncontrolled myofibrillar activation, also fuelled by the resupply of ATP (Piper et al., 2004; Piper et al., 1998). Since our lab has previously shown that diastolic calcium levels in aged cardiomyocytes were decreased

during ischemia with del Nido cardioplegia compared to standard cardioplegia, it is highly possible (although not yet tested) that in the whole isolated heart, both aged and young adult, del Nido cardioplegia could be attenuating calcium overload (possibly through blocking sodium influx via lidocaine), thereby preventing to some extent, the uncontrolled myofibrillar activation leading to hypercontracture. Future studies can address this by utilizing an isolated cardiomyocyte model of cardioplegic arrest and reperfusion, comparing the effects of del Nido and standard cardioplegia on calcium transients, cell length shortening, and force of contractions, to provide an idea of the extent to which del Nido cardioplegia could attenuate hypercontracture-induced injury in the cell.

2.3 Reperfusion Arrhythmias

During ischemia and reperfusion, the late or persistent inward Na^+ current is increased (Belardinelli et al., 2004; Belardinelli et al., 2006), which can predispose hearts to early after-depolarisation-like activity and arrhythmia (Belardinelli et al., 2004). This late Na^+ current can be reduced by Na^+ channel blockers including lidocaine (Belardinelli et al., 2006). Reperfusion ventricular fibrillation and arrhythmias are indicators of reperfusion injury as they impose extra metabolic demand on the ischemic heart while decreasing the oxygen supply due to their deleterious effects on myocardial blood flow, thus aggravating post-ischemic reperfusion injury (Hattori et al., 2000). The low incidence of reperfusion ventricular fibrillation is thought to be related to hyperkalemia (Hattori et al., 2000). However, in our study we saw no episodes of ventricular fibrillation

and only a few runs of tachycardia that were short and self-limiting. We did not see a significant reduction of reperfusion tachyarrhythmias with del Nido cardioplegia, in both the aged and young adult heart studies, even though del Nido cardioplegia contains higher K^+ than standard cardioplegia, but we cannot exclude the possibility that this might become apparent in a larger study.

2.4 Troponin Release into Coronary Effluent

To detect myocardial infarction, troponin-I is highly preferred as a biomarker (Klug et al., 2011). When damage occurs to the myocyte, troponin is released and can be detected in the serum (Xiong et al., 2010). In the aged hearts, use of del Nido cardioplegia resulted in less myocardial damage as reflected by lower troponin-I release during reperfusion, compared to standard cardioplegia. In contrast, in the young adult hearts, there were no significant differences in troponin-I release between del Nido and standard cardioplegia, providing evidence to further support the first hypothesis that del Nido cardioplegia provides superior myocardial protection to the aged heart than standard cardioplegia, and the second hypothesis, that del Nido and standard cardioplegia offer similar levels of protection in the young adult heart, with none being better than the other.

2.5 Hemodynamics and Functional Recovery in Isolated Hearts

Aged myocardium behaves differently than mature myocardium during ischemia and is not as well protected by some cardioplegia solutions (Tsukube et al., 1996;

Caldarone et al., 1995). This may be one reason why older patients undergoing cardiac surgery have impaired recovery of ventricular function and lower survival when compared to younger adult patients (Hirose et al., 2000; Shahian et al., 2009). The mechanism responsible for the intolerance to ischemia appears to be related to accelerated accumulation of intracellular Ca^{2+} (Ataka et al., 1992; O'Brien et al., 2009; Faulk et al., 1995; Tsukube et al., 1997). Strategies to limit the accumulation of intracellular Ca^{2+} in aged hearts have been shown to improve recovery of ventricular function after ischemia (Faulk et al., 1995; Tsukube et al., 1997). In previous studies (O'Blenes et al., 2011), it was observed that del Nido cardioplegia, developed for the protection of immature myocardium, has potentially beneficial effects in aged cardiomyocytes. The results of our current study comparing a del Nido cardioplegia strategy with a 'standard' multi-dose 4:1 blood cardioplegia strategy suggest that those benefits translate into reduced myocardial damage and improved functional recovery in the whole aged heart.

It is well known that cardiac temperature directly affects basal metabolism of the cardiac muscle and resistance to ischemia-reperfusion injury (Loiselle, 1985). We measured cardiac temperature by insertion of a temperature probe into the right ventricular outflow tract, recording the cardiac temperature after induction and before the start of reperfusion (the start and end of the arrest period). This was done to ensure that the cardiac temperatures during arrest did not factor into how the groups recovered. In both aged and young adult groups, cardiac temperatures were not significantly different. Since cardioplegia solutions were delivered hypothermically at the same temperature in

all experiments as well (1-5°C), this allowed us to make a valid comparison between del Nido and standard cardioplegia in terms of hemodynamic parameters.

Hemodynamic parameters were recorded at baseline in the working heart, prior to arrest, and during a 60-minute working heart period following reperfusion. Although there were no significant differences at baseline, the use of del Nido cardioplegia in the aged heart resulted in significantly elevated RPP, SP, LVDP, CF, CO, SV, and SW, throughout the entire 60-minute working heart period, providing evidence that del Nido cardioplegia provides superior myocardial protection to the aged heart than our standard cardioplegia.

In contrast, in the young adult group, only SW was significantly elevated post-reperfusion for the del Nido group, for the first 30 minutes of the 60-minute working heart period. Systolic pressure, LVDP, CF, CO and SV were not significantly different at baseline and during post-reperfusion working heart mode. There was a time point in the recovery of LVDP (t=10) where LVDP in the del Nido group was higher than in the standard group, however, this may be a random point of significance, or may indicate that the study was too underpowered to detect significant differences. Since no other points were significant, this singular point of significance alone is not enough to make a convincing argument that del Nido cardioplegia could provide superior protection to young adult hearts vs. standard cardioplegia. The hemodynamic data suggests that use of del Nido cardioplegia in the aged heart results in superior functional recovery vs. standard cardioplegia; however, use of del Nido cardioplegia in the young adult heart

does not provide the same level of cardioprotection. It is possible that del Nido cardioplegia may exert additional benefits over standard cardioplegia in the young adult group, as evidenced by the increased stroke work during the first 30 minutes of working heart, however further metabolic tests must be performed in order to provide more conclusive evidence. One possible explanation for the lack of significant differences in hemodynamics observed in the young adult group may be that perhaps the young adult hearts are already healthier and robust enough to begin with than the aged heart. This is supported by the fact that on average, the baseline cardiac output of the young adult hearts was much greater than that of the aged hearts (**Figure 39**). Del Nido cardioplegia may impact the aged heart more than the young adult vs. standard cardioplegia because the aged hearts are less robust than the young adult hearts. It is possible that del Nido and standard cardioplegia provide similar benefits in the young adult heart, and that potentially standard cardioplegia may be adequate enough to provide cardioprotection to these young adult hearts.

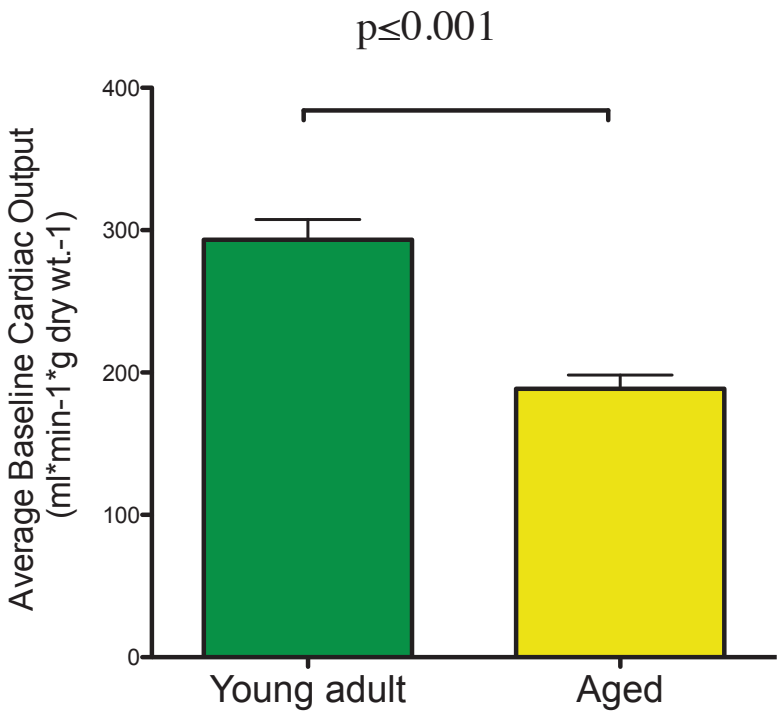

Figure 39. Average baseline cardiac output in young adult and aged groups. Bars represent mean±SEM, n=12 for young adult hearts, n=16 for aged hearts.

2.6 Coronary Vascular Resistance during Reperfusion

Coronary vascular resistance during reperfusion was increased in the standard cardioplegia group but not in the del Nido cardioplegia group for the aged hearts. This was due to an increase in CVR over baseline (prior to arrest) that was not seen in hearts arrested with del Nido cardioplegia. This may also be related to the presence of lidocaine in del Nido cardioplegia, which can promote coronary arteriolar vasodilation (Perlmutter

et al., 1990). However, there are alternative explanations including the possibility of increased microvascular obstruction related to hypothermia induced sludging (Sakai et al., 1988) with the higher hematocrit in the 4:1 (blood:crystalloid) standard cardioplegia. Decreasing the myocardial metabolic rate with hypothermia is a common practice for cardioplegia delivery. Hypothermia decreases oxygen and high-energy phosphate consumption while providing its own additional cardioplegic effect at lower temperatures (Larach et al., 1995; Iseri et al., 1984).

In contrast, interestingly, there were no significant differences in CVR during reperfusion or the change in CVR from baseline retrograde constant flow perfusion to post-ischemic retrograde reperfusion (ΔCVR) in the young adult group. Studies have shown that there is age-related reduction in the sensitivity of endothelial cells to respond to vasodilation. For example, some studies have shown that the ability of endothelial cells to respond to the release of nitric oxide (a vasodilator) declines with age (Homeister et al., 1990). Thus, del Nido cardioplegia may offer better cardioprotection in the aged heart through the use of lidocaine behaving as a coronary vasodilator, to improve the age-related deterioriation in vascular function (while lidocaine is absent in standard cardioplegia, therefore this cardioplegia does not have the same potential to promote vasodilation). However, in the young adult heart, since the vasodilatory responses are more preserved, the additives of del Nido cardioplegia may not have a significant impact on coronary vasodilation compared to standard cardioplegia (Homeister et al., 1990). Furthermore, lidocaine has been shown to reduce neutrophil adherence in the vasculature to correct the "no-reflow phenomenon" – which refers to the reduced blood flow in ischemic tissue during reperfusion. The no-reflow phenomenon is thought to occur due to

the accumulation/buildup of neutrophils in the vasculature that have resulted from ischemia-reperfusion injury (Homeister et al., 1990). Experimental evidence has suggested that the aged heart receives greater post-ischemic endothelial injury during ischemia-reperfusion. It has been suggested that neutrophil adherence in the vasculature contributes toward this endothelial injury, therefore, it is possible that in the aged heart, there may be a greater increase in neutrophil accumulation and adherence in the vasculature compared to the young adult heart, although this must be tested (Homeister et al., 1990). It is highly possible that the presence of lidocaine in del Nido cardioplegia may therefore improve coronary microcirculation by attenuating neutrophil adherence, thereby decreasing coronary vascular resistance and improving functional recovery of the aged hearts. However, in the young adult heart, it is possible that there is a reduced accumulation of neutrophils in the vasculature, therefore the presence of lidocaine in del Nido cardioplegia may not improve coronary microcirculation to the same degree as in the aged heart, which could account for the lack of significant differences in CVR in the young adult hearts, however, this must be addressed in future studies to confirm. Further studies would have to be conducted to confirm if lidocaine, at its current concentration found in del Nido cardioplegia, is adequate enough to improve coronary microcirculation by attenuating neutrophil adherence vs. standard cardioplegia, which does not contain any lidocaine (Homeister et al., 1990).

In the adult, blood cardioplegia appears to offer superior myocardial protection when compared to crystalloid cardioplegia (Fremes et al., 1984; Ibrahim et al., 1999). Blood cardioplegia has been shown to preserve myocardial metabolism and function (Catinella et al., 1984; Guru et al., 2006; Caputo et al., 2002; Amark et al., 2005; Amark

et al., 2006; Gray et al., 2001). A comparison of St. Thomas' I crystalloid solution with blood cardioplegia was performed in 40 patients with ventricular septal defect (Caputo et al., 2002). The need for inotropic support was more frequent and prolonged in the crystalloid group (no blood). The mean total troponin-I release and mean total lactate levels were significantly lower in the blood cardioplegia group. Myocardial biopsies demonstrated a significant decrease in ATP concentration in the crystalloid cardioplegia without blood, but not in the blood cardioplegia group. Therefore, less metabolic ischemic stress and reperfusion injury resulted with use of the blood cardioplegia versus an asanguineous cardioplegia. Another study compared St. Thomas cardioplegia with blood cardioplegia in 30 infants with atrioventricular septal defects (Amark et al., 2005). Coronary sinus blood lactate concentration was significantly higher in the crystalloid cardioplegia group than the blood cardioplegia group, whereas left ventricular function was better and the cardiac index higher in the blood cardioplegia group (Amark et al., 2005; Amark et al., 2006). The mechanism underlying the protective effects of blood cardioplegia may be explained by the upregulation of heat shock proteins in response to stress stimuli. Protective effects of these proteins against ischemia have been suggested in literature (Gray et al., 2001; Vittorini et al., 2007). Heat shock protein 70-1 gene expression was assessed on right atrium biopsies in 59 pediatric patients during blood cardioplegic arrest. An upregulation of this protein correlated with aortic cross-clamping time was observed during blood cardioplegia arrest (Vittorini et al., 2007).

Del Nido cardioplegia is delivered with 20% by volume fully oxygenated patient blood, which supports aerobic metabolism for a finite period of time and provides

buffering properties to promote anaerobic glycolysis as well. Blood in cardioplegia has also been shown to improve coronary perfusion during cardioplegia delivery (Amark et al., 2005; Suaudeau et al., 1982). Furthermore, studies have shown that blood cardioplegia preserves myocardial metabolism and function and results in less metabolic ischemic stress and reperfusion injury when compared with asanguineous cardioplegia in a varied population of patients undergoing congenital heart surgery (Caputo et al., 2002).

However, the optimal dilution of the cardioplegia solution is the subject of ongoing debate. While the benefits of blood cardioplegia are apparent with minimal hematocrit (Ibrahim et al., 1999), it has been suggested that concentrated blood cardioplegia improves recovery by limiting the development of myocardial edema. We did not see any difference in myocardial edema in this study comparing del Nido (1:4 blood:crystalloid) and standard cardioplegia (4:1). Myocardial edema has been implicated in post-ischemic myocardial impairment. Mannitol is incorporated into del Nido cardioplegia (but not standard cardioplegia), with the purpose of attenuating myocardial edema. Mannitol also acts as a scavenger of free radicals. Myocardial injury during cardioplegic arrest and subsequent reperfusion may be in part due to ROS including superoxide anion, hydrogen peroxide, and hydroxyl. These radicals are normally countered enzymatically within the cell, but this is inhibited during myocardial arrest (Braunwald et al., 1985; Powell et al., 1976). Hyperosmotic mannitol has been shown to scavenge free radicals and reduce myocardial cell swelling (Larach et al., 1995). However, despite the inclusion of mannitol in del Nido cardioplegia, there was still no significant improvement in myocardial edema in both the aged and young adult heart groups.

Potential advantages of dilute cardioplegia include reduced viscosity that may enhance cardioplegia delivery and reduce coronary vascular resistance (O'Neill et al., 1981), and reduced potential for sludging and microvascular obstruction with hypothermia (Sakai et al., 1988). Furthermore, all the Ca^{2+} in these cardioplegia solutions comes from the blood component, so del Nido has a lower Ca^{2+} concentration which may be beneficial, particularly in elderly hearts in which strategies to limit Ca^{2+} influx can reduce ischemia-reperfusion injury (Faulk et al., 1995; Tsukube et al., 1997).

In both aged and young adult heart groups, there were no significant differences in CVR during post-reperfusion working heart mode. In the aged group, this finding suggests that although CVR was elevated in the standard cardioplegia group during reperfusion, when the hearts continued to be reperfused in working heart mode, the additional time may have allowed for CVR to recover.

2.7 Dosing of del Nido Cardioplegia

This study is a comparison of two cardioplegia strategies that are currently used clinically (O'Brien et al., 2009; Matte et al., 2012; Charette et al., 2012), therefore del Nido cardioplegia was administered as a single dose and standard cardioplegia was delivered in multiple doses. Evidence suggests that in adults, multi-dose 4:1 blood cardioplegia offers benefits over a single dose (Buckberg et al., 1995), and is the strategy that cardiac surgeons at the QEII and IWK hospitals currently use clinically. In contrast,

del Nido cardioplegia, which is typically used as a single dose, or re-dosed at long intervals (O'Brien et al., 2009; Matte et al., 2012; Charette et al., 2012), compares favorably with multi-dose 4:1 cardioplegia (O'Brien et al., 2009). However, this is primarily in pediatric patients in which there is some evidence that re-dosing of cardioplegia is detrimental (Magovern et al., 1988). Therefore, the efficacy of single dose del Nido cardioplegia in children could in part be related to the patient population rather than the cardioplegia solution itself. While some centers are using del Nido cardioplegia in adults with single dosing or long intervals between doses, it remains to be determined if multiple dosing with del Nido cardioplegia could provide additional benefits in mature or aged hearts. It should be kept in mind that the administration of large volumes of del Nido cardioplegia could result in elevated systemic lidocaine levels that may be a safety issue, particularly in patients with impaired renal function (Yamaguchi et al., 2007).

The neonatal rabbit heart has been shown experimentally to prefer single dose cardioplegia than multidose cardioplegia (Magovern et al., 1988; Kohman et al., 1994; Sawa et al., 1989). One study sought to determine whether multidose St. Thomas' cardioplegia solution would be effective for preservation of the immature myocardium during ischemia as it is for mature myocardium. The percent recovery of pre-ischemic aortic flow was lower in the immature than the mature hearts after 90 minutes (60.3% ± 7.4% versus 101.8% ± 4.3%) and after 120 minutes (57.4% ± 10.6% versus 91.1% ± 13.6%, $p<0.05$; Magovern et al., 1988). The study concluded that multidose st. Thomas cardioplegia did not provide adequate preservation of hemodynamic function in the immature rabbit heart. Another study compared single dose to multidose cardioplegia in

neonatal rabbit hearts and found that neonatal rabbits exhibited better recovery with single dose. The neonatal rabbit hearts showed increased creatine kinase release with multidose cardioplegia versus the adult rabbit hearts (Kohman et al., 1994). A third study compared the effects of single dose, multidose with dosage every 40 min ("M-1") and multidose with dosage every 20 min ("M-2") in neonatal rabbit hearts and found that the M-2 group had significantly lower heart rate, increased creatine kinase release, elevated percent water content, and intracellular edema versus control (no cardioplegia) and the single dose group (Sawa et al., 1989). Intracellular edema was significantly higher in the M-1 group than control, indicating that the single dose method of administering crystalloid cardioplegia may provide better myocardial protection than the multiple dose method in the neonate. Since immature and aged myocardium both handle ischemia-induced calcium overload poorly, it would be reasonable to postulate that the multidose cardioplegia could likewise impair recovery of ventricular function in aged hearts versus the adult heart, although this remains to be tested.

2.8 Impact of Lidocaine on Na^+ Influx during Cardioplegic Arrest

The superior functional recovery of aged hearts shown by use of del Nido cardioplegia may be attributed to the presence of lidocaine. Lidocaine is classified as a Na^+ channel blocker and is used frequently as an anti-arrhythmic drug. Sodium channel blockade increases the refractory period of the cardiac myocyte (Larach et al., 1995). When cardioplegia is given in an ideal environment without washout, this action is

prolonged because the lidocaine remains in adequate concentrations to continually affect the myocardium. Additionally, Na^+ channel blockade helps counteract the negative effects of a hyperkalemic depolarized arrest by polarizing the cell membrane to some degree and preventing Na^+ and Ca^{2+} accumulation within the cell. Depolarized arrest can allow for Na^+ and Ca^{2+} accumulation through exchange mechanisms, discussed earlier (**Figure 1**), and blocking Na^+ channels helps prevent this (Dobson et al., 2004). A 2009 study by Brian et al. showed that del Nido cardioplegia reduced Ca^{2+} accumulation during ischemia in a setting of depolarized arrest. Previous studies have demonstrated that hyperpolarizing cardioplegic solutions supplemented with lidocaine provide superior protection compared with traditional hyperkalemic depolarizing cardioplegic solutions (O'Brien et al., 2009).

Sodium influx is thought to be the main cause of intracellular Ca^{2+} accumulation during ischemia by driving reverse mode Na^+/Ca^{2+} exchange (O'Blenes et al., 2011). During hyperkalemic cardioplegic arrest, membrane depolarization opens a proportion of the voltage gated Na^+ channels spanning the cell membrane. Most of these channels are rapidly inactivated thereby preventing action potential generation and propagation, but a small fraction remain tonically available and allow Na^+ to flow into the cell via a 'window current' (Attwell et al., 1979). Lidocaine used as an additive in del Nido cardioplegia may limit Na^+ influx by blocking the window current and may be part of the mechanisms of benefit with this solution, however this has not been well described. The fact that the young adult hearts did not show superior functional recovery with use of del Nido cardioplegia may be due to the fact that since adult hearts have been shown to be

more tolerant to ischemic injury than the aged heart, and that intracellular Ca^{2+} levels are significantly lower in the adult heart vs. the aged heart, the degree of Ca^{2+} overload in the adult heart is lessened such that del Nido cardioplegia may not exert the same benefits in the young adult heart as it does in the aged heart, or perhaps that standard cardioplegia is adequate enough to provide protection to the young adult heart (Ataka et al., 1992).

An increasing body of evidence over the years suggests that inhibiting the late, persistent sodium current (late I_{Na}) in the heart is beneficial to reducing electrical and mechanical dysfunction during ischemia. Recent literature has shown that an enhanced late I_{Na} is likely to contribute to the sodium overload observed in ischemia/hypoxia and heart failure, and consequently may play a role in the abnormalities of ventricular contractility and repolarization. The increased, late I_{Na} results in a prolonged action potential with the potential for the induction of early after-depolarizations, abnormal relaxation including aftercontractions, and contractions with both phasic and tonic components (Belardinelli et al., 2004; Belardinelli et al., 2006). Both tissue hypoxia and reperfusion of ischemic myocardium are reported to generate metabolites and ROS that act to increase late I_{Na} in ventricular myocytes (Imahashi et al., 1999). Late I_{Na} flows into myocytes through Na^+ channels that fail to inactivate properly. Transient openings of many Na^+ channels create the inward current responsible for the upstroke of the cellular action potential. When a small fraction (as few as two per cell) of these channels either fails to inactivate or reopens during the plateau of the cardiac action potential when Na^+ channels are normally closed, the resultant late Na^+ current slows repolarization of the action potential and increases action potential duration, the heterogeneity of

repolarization and the formation of early after-depolarizations (EADs), and cellular Na^+ loading (Kiyosue et al., 1989). The effect of increased late I_{Na} to slow repolarization (and increase duration) of the action potential is not uniform across the wall of the left ventricle. For example, late I_{Na} is normally larger in M cells than in endo- or epicardial cells of the dog, and enhancement of late I_{Na} may increase the transmural heterogeneity of ventricular repolarization by preferentially prolonging the duration of the M cell action potential. An enhancement of late I_{Na} may therefore prolong the QT interval and delay the relaxation of LV contraction (Zygmunt et al., 2001). The influx of Na^+ contributes at least in part to the $[Na^+]_i$ load in ischemia. This effect is likely to be linked to the fact that the late persistent current is enhanced by hypoxia. The increase in persistent current by hypoxia is blocked by low concentrations of lidocaine and TTX (Saint, 2006). This is consistent with the hypothesis that it is the increase in the persistent current during hypoxia or ischemia that leads to the increase in $[Na+]_i$, since lidocaine (and TTX) also blocks the increases in $[Na+]_i$ during cardiac ischemia. Hence, following this reasoning, agents which block late I_{Na} should be protective in ischemia, and this has been validated with previous studies, including lidocaine (Belardinelli et al., 2006; Haigney et al., 1994).

The use of Na^+ channel blockade has had minimal clinical uptake despite the fact that lidocaine is used clinically as a local anesthetic and an anti-arrhythmic agent at lower doses, and has well identified side-effect and safety profiles. One problem is that lidocaine clearance is dependent on hepatic and renal activity (which is reduced for varying periods as a result of low perfusion during cardiopulmonary bypass), so doses of lidocaine sufficient to induce arrest may lead to accumulation of the drug and its

metabolites in the body after reperfusion and cause potential arrhythmic and neurological toxicity (Matte et al., 2012).

3. Limitations

The isolated heart model used in this study has several limitations that must be considered when interpreting our results. The isolated working heart preparation is viable for only a limited time. While it is possible to examine cardiac function in the short term with this system, it is not feasible to study hearts at more clinically relevant time points (12 or 24 hours post reperfusion). Furthermore, the isolated heart is not subject to non-coronary collateral flow during the ischemic period, which might alter the efficacy of the myocardial protection strategy by washing out the cardioplegia and/or rewarming the myocardium. The volume of autologous blood that we can collect from each rat is limited, and collecting blood from the young adult rat is even more challenging than in the aged rat due to the decreased blood volume. With 4:1 cardioplegia we are able to prepare the induction dose plus two additional doses, which allows a maximum 60-minute ischemic period if re-dosing occurs every 20 minutes. If longer ischemic periods were possible, benefits with del Nido cardioplegia might become less apparent if the single dose strategy becomes inadequate with prolonged cross-clamp times. While ischemia-reperfusion injury is primarily initiated by Ca^{2+} overload, other processes affect the development of myocardial injury and evolution of functional recovery. For example, the inflammatory system plays a role in the myocardial damage that occurs early after reperfusion. When treating elderly patients in the clinical setting,

cardiac surgeons mostly deal with myocardium that is ischemic or perhaps has been subjected to long-term pressure volume overload. The myocardium of the aged rats used in this study can be considered to be relatively "healthy", and thus, the clinical relevance of studies of this design is questioned. Inclusion of diseased myocardium models (example, heart failure models in the rat) would be necessary in the design of future studies evaluating the use of cardioplegia solutions. Most of these limitations will require future studies in an intact animal to address.

4. Conclusion

There have been many undertakings in basic science and in the clinic to develop strategies for myocardial protection, such as pharmacological inhibition of NHEs. A pediatric cardioplegia strategy to protect myocardium, the use of del Nido cardioplegia, may also be tailored toward protecting the myocardium of the elderly. We have demonstrated that in aged hearts, a del Nido cardioplegia strategy is associated with less spontaneous activity during arrest, reduced myocardial injury, and improved functional recovery when compared to a 'standard' multi-dose 4:1 blood cardioplegia strategy, however, use of del Nido cardioplegia in the young adult heart does not provide superior myocardial protection over our standard cardioplegia. Additional studies in a whole animal will help to determine if these results persist in a more clinically relevant model, and justify clinical studies in elderly patients undergoing cardiac surgery.

REFERENCES

Abete, P., Della Morte, D.,Mazzella, F., D'Ambrosio, D., Galizia, G., Testa, G., Gargiulo, G., Cacciatore, F., Rengo, F. Lifestyle and prevention of cardiovascular disease in the elderly: an Italian perspective. Am. J. Geriatr. Cardiol. 2006:15; 28–34.

Abete, P., Ferrara, N., Cioppa, A., Ferrara, P., Bianco, S., Calabrese, C., Cacciatore, F., Longobardi, G., Rengo, F. Preconditioning does not prevent post-ischemic dysfunction in aging heart. J. Am. Coll. Cardiol. 1996: 27;1777–1786.

Allen BS. Pediatric myocardial protection: Where do we stand? J Thorac Cardiovasc Surg. 2004;128:11–3.

Allen DG, Cairns SP, Turvey SE, Lee JA. Intracellular calcium and myocardial function during ischemia. Adv Exp Med Biol 1993;346:19-29.

Amark K, Berggren H, Björk K, et al. Blood cardioplegia provides superior protection in infant cardiac surgery. Ann Thorac Surg 2005; 80:989–994.

Amark K, Berggren H, Björk K, et al. Myocardial metabolism is better preserved after blood cardioplegia in infants. Ann Thorac Surg 2006; 82:172–178.

Anderson SE, Murphy E, Steenbergen C, London RE, Cala PM. Na-H exchange in myocardium: effects of hypoxia and acidification on Na and Ca. Am J Physiol 1990 Dec;259(6 Pt 1):C940-C948.

Ataka K, Chen D, Levitsky S, Jimenez E, Feinberg H. Effect of aging on intracellular Ca2+, pHi, and contractility during ischemia and reperfusion. Circulation 1992 Nov;86(5 Suppl):II371-II376.

Attwell D, Cohen I, Eisner D, Ohba M, Ojeda C. The steady state TTX-sensitive ("window") sodium current in cardiac Purkinje fibres. Pflugers Arch 1979 Mar 16;379(2):137-42

Avkiran M, Gross G, Karmazyn M, Klein H, Murphy E, Ytrehus K. Na+/H+ exchange in ischemia, reperfusion and preconditioning. Cardiovasc Res 2001 Apr;50(1):162-6.

Awad AB, Clay SW. Age-dependent alterations in lipids and function of rat heart sarcolemma. Mech Ageing Dev 1982;19:333-342.

Azhar G, Gao W, Liu L, Wei J. Ischemia-reperfusion in the adultmouse heart influence of age. Exp Gerontol 1999; 34:699-714.

Baker JE, Boerboom LE, Olinger GN. Age-related changes in the ability of hypothermia and cardioplegia to protect ischemic rabbit myocardium. J Thorac Cardiovasc Surg. 1988;96:717– 24.

Barja G. Mitochondrial Free Radical Production And Aging In Mammals And Birds Ann N. Y. Acad. Sci. 1998: 854; 224-238.

Bartling, B., Friedrich, I., Silber, R.E., Simm, A. IP is not cardioprotective in senescent human myocardium. Ann. Thorac. Surg. 2003:76;105–111.

Belardinelli L, Antzelevitch C., Fraser H. Inhibition of late (sustained/persistent) sodium current: a potential drug target to reduce intracellular sodium-dependent calcium overload and its detrimental effects on cardiomyocyte function. European Heart Journal Supplements 2004:6;13-17.

Belardinelli L, Shryock JC, Fraser H. Inhibition of the late sodium current as a potential cardioprotective principle: effects of the late sodium current inhibitor ranolazine. Heart 2006 Jul;92(Suppl 4):iv6–iv14.

Bell RM, Mocanu MM, Yellon DM. Retrograde heart perfusion: The Langendorff technique of isolated heart perfusion. J Mol Cell 2011. 50: 940-950.

Blanchard EM, Solaro RJ. Inhibition of the activation and troponin calcium binding of dog cardiac myofibrils by acidic pH. Circ Res 1984;55:382-391.

Boengler, K., Konietzka, I., Buechert, A., Heinen, Y., Garcia-Dorado, D., Heusch, G., et al. Loss of IP's cardioprotection in aged mouse hearts is associated with reduced gap junctional and mitochondrial levels of connexin 43. Am. J. Physiol. Heart Circ. Physiol. 2007:292;H1764–H1769

Boland R, Martonosi A, Tillack TW. Developmental changes in the composition and function of sarcoplasmic reticulum. J Biol Chem 1974 Jan 25;249(2):612-23.

Bolling K, Kronon M, Allen BS, Ramon S, Wang T, Hartz RS, et al. Myocardial protection in normal and hypoxically stressed neonatal hearts: the superiority of hypocalcemic versus normocalcemic blood cardioplegia. J Thorac Cardiovasc Surg 1996 Nov;112(5):1193-200.

Boucher F, Tanguy S, Besse S, Tresallet N, Favier A, de LJ. Age-dependent changes in myocardial susceptibility to zero flow ischemia and reperfusion in isolated perfused rat hearts: relation to antioxidant status. Mech Ageing Dev 1998 Jul 15;103(3):301-16.

Bove EL, Stammers AH. Recovery of left ventricular function after hypothermic ischemia: Age-related differences in the isolated working rabbit heart. J Thorac Cardiovasc Surg. 1986;91:115–22.

Braunwald E, Kloner RA. Myocardial reperfusion: A double-edged sword? J Clin Invest. 1985;76:1713–9.

Buckberg GD, Brazier JR, Nelson RL, Goldstein SM, McConnell DH, Cooper N. Studies of the effects of hypothermia on regional myocardial blood flow and metabolism during cardiopulmonary bypass. I. The adequately perfused beating, fibrillating, and arrested heart. J Thorac Cardiovasc Surg 1977 Jan;73(1):87-94.

Buckberg GD. Update on current techniques of myocardial protection. Ann Thorac Surg 1995;60(3):805-14.

Caldarone CA, Krukenkamp IB, Burns PG, Gaudette GR, Schulman J, Levitsky S. Blood cardioplegia in the senescent heart. J Thorac Cardiovasc Surg 1995 Feb;109(2):269-74.

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. Ann Thorac Surg 2002; 74:530–535.

Catinella FP, Cunningham JN Jr, Spencer FC. Myocardial protection during prolonged aortic cross-clamping. Comparison of blood and crystalloid cardioplegia. J Thorac Cardiovasc Surg 1984; 88:411–423.

Chambers DJ, Hearse DJ. Developments in cardioprotection: "polarized" arrest as an alternative to "depolarized" arrest. Ann Thorac Surg 1999 Nov;68(5):1960-6.

Charette K, Gerrah R, Quaegebeur J, Chen J, Riley D, Mongero L, et al. Single dose myocardial protection technique utilizing del Nido cardioplegia solution during congenital heart surgery procedures. Perfusion 2012 Mar; 27(2):98-103.

Chen Jc, Warshaw Jb, Sanadi Dr. Regulation Of Mitochondrial Respiration In Senescence. J Cell Physiol 1972; 80: 141-148.

Chen S, Li G, Long L. Clinical research of ischemic preconditioning on lung protection. Hunan Yi Ke Da Xue Xue Bao 1999; 24: 357–9.

Chiu Yj, Richardson A. Effect Of Age On The Function Of Mitochondria Isolated From Brain And Heart Tissue. Exp Gerontol 1980; 15: 511-517.

Chocron S, Kaili D, Yan Y, Toubin G, Latini L, Clement F, et al. Intermediate lukewarm (20 degrees c) antegrade intermittent blood cardioplegia compared with cold and warm blood cardioplegia. J Thorac Cardiovasc Surg 2000 Mar;119(3):610-6.

Chouker A, Schachtner T, Schauer R, et al. Effects of Pringle manoeuvre and ischaemic preconditioning on haemodynamic stability in patients undergoing elective hepatectomy: a randomized trial. Br J Anaesth 2004; 93: 204–11.

Clavien PA, Selzner M, Rudiger HA, et al. A prospective randomized study in 100 consecutive patients undergoing major liver resection with versus without ischemic preconditioning. Ann Surg 2003; 238: 843–50.

Cremer J, Steinhoff G, Karck M, Ahnsell T, Brandt M, Teebken OE, Hollander D, Haverich A. Ischemic preconditioning prior to myocardial protection with cold blood cardioplegia in coronary surgery. Eur J Cardiothorac Surg 1997;12:753—8.

Crompton M. Mitochondrial intermembrane junctional complexes and their role in cell death. J Physiol 2000;529:11–21.

Dobson GP, Jones MW. Adenosine and lidocaine: A new concept in nondepolarizing surgical myocardial arrest, protection, and preservation. J Thorac Cardiovasc Surg. 2004;127:794–805.

Dohi Y, Kojima M, Luscher TF. Age-related changes in vascular smooth muscle and endothelium. Drugs Aging 1995;7:278-291.

Driamov SV, Bellahcene M, Butz S, Buser PT, Zaugg CE. Bradykinin is a mediator, but unlikely a trigger, of antiarrhythmic effects of ischemic preconditioning. J Cardiovasc Electrophysiol 2007 Jan;18(1):93–9.

Fallouh HB, Kentish JC, Chambers DJ. Targeting for cardioplegia: arresting agents and their safety. Current Opinion in Pharmacology 2009; 9:220-226.

Fannin Sw, Lesnefsky Ej, Slabe Tj, Hassam Mo, Hoppel Cl. Aging Selectively Decreases Oxidative Capacity In Rat Heart Interfibrillar Mitochondria. Arch Biochem Biophys 1999; 372: 399-407.

Faulk EA, McCully JD, Hadlow NC, Tsukube T, Krukenkamp IB, Federman M, et al. Magnesium cardioplegia enhances mRNA levels and the maximal velocity of cytochrome oxidase I in the senescent myocardium during global ischemia. Circulation 1995 Nov 1;92(9 Suppl):II405-II412.

Feng J, Rosenkranz ER. Bradykinin pretreatment improves ischemia tolerance of the rabbit heart by tyrosine kinase mediated pathways. Ann Thorac Surg 1999 Nov;68(5):1567–72.

Feng J, Sellke ME, Ramlawi B, Boodhwani M, Clements R, Li J, et al. Bradykinin induces microvascular preconditioning through the opening of calcium-activated potassium channels. Surgery 2006 Aug;140(2):192–7.

Folkow B, Svanborg A. Physiology of cardiovascular aging. Physiol Rev 1993;73:725-764.

Fremes SE, Christakis GT, Weisel RD, Mickle DA, Madonik MM, Ivanov J, et al. A clinical trial of blood and crystalloid cardioplegia. J Thorac Cardiovasc Surg 1984 Nov;88(5 Pt 1):726-41.

Fremes SE, Weisel RD, Mickle DA, Ivanov J, Madonik MM, Seawright SJ, et al. Myocardial metabolism and ventricular function following cold potassium cardioplegia. J Thorac Cardiovasc Surg 1985 Apr;89(4):531-46.

Frolkis Vv, Frolkis Ra, Mkhitarian Ls, Fraifield Ve. Age-Dependent Effects Of Ischemia And Reperfusion On Cardiac Function And Ca^{2+} Transport In Myocardium. Gerontology 1991; 37:233-239.

Garcia-Dorado D, Gonzalez MA, Barrabes JA, et al. Prevention of ischemic rigor contracture during coronary occlusion by inhibition of Na+-H+ exchange. *Cardiovasc Res*. 1997;35:80-9.

Gaudel Y, Duvelleroy MA. Role of oxygen radicals in cardiac injury due to reoxygenation. J Mol Cell Cardiol 1984;16:459-470.

Gay WA, Jr. Potassium-induced cardioplegia. Ann Thorac Surg 1975 Jul;20(1):95-100.

Gerdin E, Tyden O, Eriksson U. The development of antioxidant enzymatic defense in the perinatal rat lung. Activities of superoxide dismutase, glutathione peroxidase and catalase. Pediatr Res 1985;19:687-691.

Gombosova I, Boknik P, Kirchhefer U, Knapp J, Luss H, Muller FU, et al. Postnatal changes in contractile time parameters, calcium regulatory proteins, and phosphatases. Am J Physiol 1998 Jun;274(6 Pt 2):H2123-H2132.

Gray CC, Amrani M, Smolenski RT, Nakamura K, Yacoub MH. Cold cardioplegic arrest enhances heat shock protein 70 in the heat-shocked rat heart. J Thorac Cardiovasc Surg 2001; 121(6):1130-1136.

Guru V, Omura J, Alghamdi AA, et al. Is blood superior to crystalloid cardioplegia? A meta-analysis of randomized clinical trials. Circulation 2006; 114(1 Suppl):1331–1338.

Haigney MC, Lakatta EG, Stern MD, Silverman HS (1994). Sodium channel blockade reduces hypoxic sodium loading and sodiumdependent calcium loading. Circulation 90: 391–399.

Halestrap A. Mitochondria and reperfusion injury of the heart – a holey death but not beyond salvation. J Bioenerg Biomembr 2009; 41:113-121.

Hattori Y, Yang Z, Sugimura S, Iriyama T, Watanabe K, Negi K, et al. Terminal warm blood cardioplegia improves the recovery of myocardial electrical activity. A retrospective and comparative study. Jpn J Thorac Cardiovasc Surg 2000 Jan;48:1-8.

Hausenloy, D.J., Yellon, D.M. Survival kinases in IP and postconditioning. Cardiovasc. Res. 2006:70; 240–253.

Hearse DJ, Garlick PB, Humphrey SM. Ischemic contracture of the myocardium: mechanisms and prevention. Am J Cardiol 1977;39:986-993.

Hess ML, Manson NH. Molecular oxygen: friend and foe. J Mol Cell Cardiol 1984;16:969-985.

Hiramatsu T, Zund G, Schermerhorn ML, Shinoka T, Minura T, Mayer JE Jr. Age differences in effects of hypothermic ischemia on endothelial and ventricular function. Ann Thorac Surg. 1995;60:S501– 4.

Hirose H, Amano A, Yoshida S, Takahashi A, Nagano N, Kohmoto T. Coronary artery bypass grafting in the elderly. Chest 2000 May;117(5):1262-70.

Hoerter J, Mazet F, Vassart G. Perinatal growth of the rabbit cardiac cell: possible implications for the mechanism of relaxation. J Mol Cell Cardiol 1981;13:725-740.

Holman WL, Vicente WVA, Spruell RD, Digerness SB, Pacifico AO. Effect of postcardioplegia reperfusion rhythm on myocardial blood flow. Ann Thorac Surg 1994;58:351– 8.

Homeister JW, Hoff PT, Fletcher DD, Luchester BR. Combined adenosine and lidocaine administration limits myocardial reperfusion injury. Circulation 1990;82:592-608.

Ibrahim MF, Venn GE, Young CP, Chambers DJ. A clinical comparative study between crystalloid and blood based St Thomas' hospital cardioplegic solution. Eur J Cardiothorac Surg 1999 Jan;15(1):75-83.

Illes RW, Swoyer KD. Prospective, randomized clinical study of ischemic preconditioning as an adjunct to intermittent cold blood cardioplegia. Ann Thorac Surg 1998;65:748—52.

Imahashi K, Kusuoka H, Hashimoto K, Yoshioka J, Yamaguchi H, Nishimura T. Intracellular sodium accumulation during ischemia as the substrate for reperfusion injury. Circ Res 1999;84:1401–6.

Iseri LT, French JH. Magnesium: Nature's physiologic calcium beneficial in hypothermic crystalloid cardioplegia. Am Heart J. 1984;108:188–93.

Jenkins DP, Pugsley WB, Alkhulaifi AM, Kemp M, Hooper J, Yellon DM. Ischaemic preconditioning reduces troponin T release in patients undergoing coronary artery bypass surgery. Heart 1997; 77: 314–8.

Jennings RB, Reimer KA. Lethal myocardial ischemic injury. Am J Pathol. 1981;102:241–55.

Kane JJ, Murphy ML, Bisset JK, DeSoyza N, Doherty JE, Staub KD. Mitochondrial function, oxygen extraction, epicardial ST segment changes and tritiated digoxin distribution after reperfusion of ischemic myocardium. Am J Cardiol 1975;36:218-224.

Kaneko S, Okumura K, Numaguchi Y et al. Melatonin scavenges hydroxyl radical and protects isolated rat hearts from ischemic reperfusion injury. Life Sciences 2000; 67:101-112.

Kaukoranta PK, Lepojarvi MV, Kiviluoma KT, Ylitalo KV, Peuhkurinen KJ. Myocardial protection during antegrade versus retrograde cardioplegia. Ann Thorac Surg 1998;66:755—61.

Kawasuji M, Tomita S, Yasuda T, Sakakibara N, Takemura H, Watanabe Y. Myocardial oxygenation during terminal warm blood cardioplegia. Ann Thorac Surg 1998;65:1260-1264.

Kempsford RD, Hearse DJ. Protection of the immature myocardium during global ischemia. A comparison of four clinical cardioplegic solutions in the rabbit heart. J Thorac Cardiovasc Surg. 1989;97:856 – 63.

Kinoshita T, Asai T. Preservation of myocardium during coronary artery bypass surgery. Curr Cardiol Rep 2012 Aug;14(4):418-23.

Kiyosue T, Arita M. Late sodium current and its contribution to action potential configuration in guinea pig ventricular myocytes. Circ Res 1989;64:389-397.

Klein HH, Pich S, Bohle RM, Lindert-Heimberg S, Nebendahl K. Na+/H+ exchange inhibitor cariporide attenuates cell injury predominantly during ischemia and not at onset of reperfusion in porcine hearts with low residual blood flow. *Circulation*. 2000;102:1977-82.

Klug G, Mayr A, Mair J, Schocke M, Nocker M, Trieb T et al., Role of biomarkers in assessment of early infarct size after successful p-PCI for STEMI. Clin. Res. Cardiol. 2011 Jun;100:501-510.

Kohman LJ, Veit LJ. Single-dose versus multidose cardioplegia in neonatal hearts. J Thorac Cardiovasc Surg. 1994;107:1512–8.

Kwong Lk, Sohal Rs. Substrate And Site Specificity Of Hydrogen Peroxide Generation In Mouse Mitochondria Arch Biochem Biophys 1998: 350; 118-126.

Ladilov Y, Efe O, Schafer C, Rother B, Kasseckert S, Abdallah Y, et al. Reoxygenation-induced rigor-type contracture. J Mol Cell Cardiol 2003 Dec;35(12):1481-90.

Larach DR, Solina AR. Cardiovascular drugs. In: Hensley FA, Martin DE, eds. A Practical Approach to Cardiac Anesthesia. 2nd ed. Boston: Little, Brown and Company; 1995:32–95.

Leesar MA, Stoddard MF, Manchikalapudi S, Bolli R. Bradykinin-induced preconditioning in patients undergoing coronary angioplasty. J Am Coll Cardiol 1999 Sep;34(3):639–50.

Lesnefsky Ej, Gudz Ti, Migita Ct, Ikeda-Saito M, Hassan Mo, Turkaly Pj, Hoppel Cl. Ischemic Injury To Mitochondrial Electron Transport In The Aging Heart: Damage To The Iron-Sulfur Protein Subunit Of Electron Transport Complex Iii. Arch Biochem Biophys 2001; 385: 117-128.

Lesnefsky EJ, Hoppel CL. Ischemia-reperfusion injury in the aged heart: role of mitochondria. Archives of Biochemistry and Biophysics 2003; 420(2):287-297.
Li G, Chen S, Lu E, Li Y. Ischemic preconditioning improves preservation with cold blood cardioplegia in valve replacement patients. Eur J Cardiothorac Surg 1999;15:653—7.

Liu L, Azhar G, Gao W, Zhang X, Wei Jy. Bcl-2 And Bax Expression In Adult Rat Hearts After Coronary Occlusion: Age-Associated Differences. Am J Physiol 1998; 275: R315-R322.

Loiselle DS. The effect of temperature on the basal metabolism of cardiac muscle. European Journal of Physiology 1985;405:163-169.

Lu EX, Chen SX, Yuan MD, Hu TH, Zhou HC, Luo WJ, Li GH, Xu LM. Preconditioning improves myocardial preservation in patients undergoing open heart operations. Ann Thorac Surg 1997;64:1320—4.

Lucas Dt, Szweda Li. Cardiac Reperfusion Injury: Aging, Lipid Peroxidation, And Mitochondrial Dysfunction. Proc Natl Acad Sci Usa 1998; 95:510-514.

Magovern JA, Pae WE, Waldhausen JA. Protection of the immature myocardium. An experimental evaluation of topical cooling, singe-dose, and multiple-dose administration of St Thomas' Hospital cardioplegic solution. J Thorac Cardiovasc Surg 1988 Sep;96(3):408-13.

Martin M, Macias M, Leon J et al. Melatonin increases the activity of the oxidative phosphorylation enzymes and the production of ATP in rat brain and liver mitochondria. Int J Biochem Cell Biol 2002;4:348–357.

Matte GS, del Nido PJ. History and use of delNido cardioplegia solution at Boston Children's Hospital. J Extra Corpor Technol 2012 Sep;44(3):98-103.

McCully JD, Toyoda Y, Wakiyama H, Rousou AJ, Parker RA, Levitsky S. Age- and gender related differences in ischemia/reperfusion injury and cardioprotection: effects of diazoxide. Ann Thorac Surg 2006 Jul;82(1):117-23.

Murry CE, Jennings RB, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. Circulation 1986;74:1124–36.

Neely JR, Liebermeister H, Battersby EJ, Morgan HE. Effect of pressure development on oxygen consumption by isolated rat heart. Am J Physiol 1967. Apr;212(4):804-14.

Neely JR, Rovetto MJ, Whitmer JT, Morgan HE. Effects of ischemia on function and metabolism of the isolated working rat heart. Am J Physiol 1973. Sep;225(3):651-8. neonatal hearts. J Thorac Cardiovasc Surg. 1994;107:1512–8.

Nishioka K, Jarmakani JM. Effect of ischemia on mechanical function and high-energy phosphates in rabbit myocardium. Am J Physiol 1982;242:H1077-H1083.

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.

O'Brien SM, Shahian DM, Filardo G, Ferraris VA, Haan CK, Rich JB, et al. The Society of Thoracic Surgeons 2008 cardiac surgery risk models: part 2--isolated valve surgery. Ann Thorac Surg 2009 Jul;88(1 Suppl):S23-S42.

O'Blenes SB, Friesen CH, Ali A, Howlett S. Protecting the aged heart during cardiac surgery: the potential benefits of del Nido cardioplegia. J Thorac Cardiovasc Surg. 2011 Mar;141(3):762-70.

Ohkado A, Cao-Danh H, Sommers KE, del Nido PJ. Evaluation of highly buffered low-calcium solution for long-term preservation of the heart: Comparison with the University of Wisconsin solution. J Thorac Cardiovasc Surg. 1994;108:762 –71.

O'Neill MJ, Francalancia N, Wolf PD, Parr GV, Waldhausen JA. Resistance differences between blood and crystalloid cardioplegic solutions with myocardial cooling. J Surg Res 1981 Apr;30(4):354-60.

Oldenburg O, Qin Q, Krieg T, Yang XM, Philipp S, Critz SD, et al. Bradykinin induces mitochondrial ROS generation via NO, cGMP, PKG, and mitoKATP channel opening and leads to cardioprotection. Am J Physiol Heart Circ Physiol 2004 Jan;286(1):H468–76.

Oldenburg O, Qin Q, Sharma AR, Cohen MV, Downey JM, Benoit JN. Acetylcholine leads to free radical production dependent on K(ATP) channels, G(i) proteins, phosphatidylinositol 3-kinase and tyrosine kinase. Cardiovasc Res 2002 Aug 15;55(3):544–52.

Otani H, Engelman RM, Rousau JA, Breyer RH, Lemesaw S et al. Alteration of antioxidant and lipogenic enzyme activities during ischemia and reperfusion in neonatal pig heart. Circulation 1985;72:33-36.

Paradies G, Petrosillo G, Pistolese M, et al. Lipid peroxidation and alterations to oxidative metabolism in mitochondria isolated from rat heart subjected to ischemia and reperfusion. Free Radical Biology and Medicine 199;27: 42-50.

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. Circ Res 1987 Nov;61(5):609-15.

Patent 5,407,793. 1995. U.S. Patent and Trademark Office. Alexandria, VA.

Perlmutter NS, Wilson RA, Edgar SW, Sanders W, Greenberg BH, Tanz R. Vasodilatory effects of lidocaine on epicardial porcine coronary arteries. Pharmacology 1990;41(5):280-5.

Perrault LP,MenascheP, Bel A,deChaumarayT, Peynet J,Mondry A,Olivero P, Emanoil-Ravier R, Moalic JM. Ischemic preconditioning in cardiac surgery: a word of caution. J Thorac Cardiovasc Surg 1996;112:1378—86.

Petrosillo G, Colantuomo C, Moro N, Ruggiero FM et al. Melatonin protects against heart ischemia-reperfusion injury by inhibiting the mitochondrial permeability transition pore opening. Am J Physiol Heart Circ Physiol 2009; 297:H1487-H1493.

Piper HM, Abdallah Y, Schafer C. The first minutes of reperfusion: a window of opportunity for cardioprotection. Cardiovascular Research 2004;61:365-371.

Piper HM, Garcia-Dorado D, Ovize M. A fresh look at reperfusion injury. Cardiovascular Research 1998;38:291-300.
Piper HM, Garcia-Dorado D. Prime causes of rapid cardiomyocyte death during reperfusion. Ann Thorac Surg 1999 Nov;68(5):1913-9.

Piper HM, Meuter K, Schafer C. Cellular mechanisms of ischemia-reperfusion injury. Ann Thorac Surg 2003 Feb;75(2):S644-S648.

Powell WJ, DiBona DR, Flores J. The protective effect of hyperosmotic mannitol in myocardial ischemia and necrosis. Circulation. 1976;54:603 –15.

Qin Q, Yang XM, Cui L, Critz SD, Cohen MV, Browner NC, et al. Exogenous NO triggers preconditioning via a cGMP- and mitoKATP-dependent mechanism. Am J Physiol Heart Circ Physiol 2004 Aug;287(2):H712–8.

Radnoti Working Heart Rat System 120101BEZ Instruction Manual. www.radnoti.com

Randomised trial of normothermic versus hypothermic coronary bypass surgery. The Warm Heart Investigators. Lancet 1994 Mar 5;343(8897):559-63.

Rebeyka IM, Hanan SA, Borges MR, Lee KF, Yeh T, Jr., Tuchy GE, et al. Rapid cooling contracture of the myocardium. The adverse effect of prearrest cardiac hypothermia. J Thorac Cardiovasc Surg 1990 Aug;100(2):240-9.

Rudd DM, Dobson GP. Toward a new cold and warm nondepolarizing, normokalemic arrest paradigm for orthotopic heart transplantation. J Thorac Cardiovasc Surg 2009 Jan;137(1):198-207.

Rupprecht HJ, vom DJ, Terres W et al. Cardioprotective effects of the Na+/H+ exchange inhibitor cariporide in patients with acute anterior myocardial infarction undergoing direct PTCA. Circulation 2000;101:2902-2908.

Saint DA (2006). The role of the persistent Na current during cardiac ischemia and hypoxia. J Cardiovasc Electrophysiol 17 (Suppl 1): S96–S103.

Sakai A, Miya J, Sohara Y, Maeta H, Ohshima N, Hori M. Role of red blood cells in the coronary microcirculation during cold blood cardioplegia. Cardiovasc Res 1988 Jan;22(1),62-66.

Sawa Y, Matsuda H, Shimazaki Y, et al. Comparison of single dose versus multiple dose crystalloid cardioplegia in neonate. J Thorac Cardiovasc Surg. 1989;97:229–34.

Schafer C, Ladilov YV, Inserte J, Schafer M, Haffner S, Garcia-Dorado D. Role of the reverse mode of the Na+/Ca2+ exchanger in reoxygenation-induced cardiomyocyte injury. Cardiovasc Res 2001;51:241-250.

Shahian DM, O'Brien SM, Filardo G, Ferraris VA, Haan CK, Rich JB, et al. The Society of Thoracic Surgeons 2008 cardiac surgery risk models: part 1--coronary artery bypass grafting surgery. Ann Thorac Surg 2009 Jul;88(1 Suppl):S2-22

Shahian DM, O'Brien SM, Filardo G, Ferraris VA, Haan CK, Rich JB, et al. The Society of Thoracic Surgeons 2008 cardiac surgery risk models: part 1—coronary artery bypass grafting surgery. Ann Thorac Surg 2009 Jul;88(1 Suppl):S2-22.

Shahian DM, O'Brien SM, Filardo G, Ferraris VA, Haan CK, Rich JB, et al. The Society of Thoracic Surgeons 2008 cardiac surgery risk models: part 3--valve plus coronary artery bypass grafting surgery. Ann Thorac Surg 2009 Jul;88(1 Suppl):S43-S62.

Siegmund B, Ladilov YV, Piper HM. Importance of Na+ for the recovery of Ca2+ control in reoxygenated cardiomyocytes. Am J Physiol 1994;267:H506-H513.

Siegmund B, Schlack W, Ladilov YV, Balser C, Piper HM. Halothane protects cardiomyocytes against reoxygenation-induced hypercontracture. Circulation 1997;96:4372-4379.

Siegmund B, Zude R, Piper HM. Recovery of anoxic-reoxygenated cardiomyocytes from severe Ca2+ overload. Am J Physiol 1992;263:H1262-H1269.

Sink JD, Currie WD, Pellom GL, Hill RC, Chitwood R et al. Correlation of mitochondrial function and ischemic contracture. J Thorac Cardiovasc Surg 1980;79:570-578.

Skulachev Vp. The Programmed Death Phenomena, Aging, And The Samurai Law Of Biology. Exp Gerontol 2001; 36: 995-1024.

Statistics Canada. The Canadian Population in 2011: Age and Sex. Catalogue #: 98-311-X2011001. 2012.

Steenbergen C, Murphy E, Watts JA, London RE. Correlation between cytosolic free calcium, contracture, ATP, and irreversible ischemic injury in perfused rat heart. Circ Res 1990 Jan;66(1):135-46.

Suaudeau J, Shaffer B, Dagget WM, Austen WG, Erdman AJ. Role of procaine and washed red cells in the isolated dog heart perfused at 5 degrees C. J Thorac Cardiovasc Surg. 1982;84:886–96.

Sun J, Picht E, Ginsburg KS, Bers DM, Steenbergen C, Murphy E. Hypercontractile female hearts exhibit increased S-nitrosylation of the L-type Ca2? channel alpha1 subunit and reduced ischemia/reperfusion injury. Circ Res 2006 Feb 17;98(3):403–11.

Sun J. Protein S-nitrosylation: a role of nitric oxide signaling in cardiac ischemic preconditioning. Sheng Li Xue Bao 2007 Oct 25;59(5):544–52.

Sutherland FJ, Hearse DJ. The isolated blood and perfusion fluid perfused heart. Pharmacol Res Jun. 2000;41(6):613–27.

Tani M, Suganuma Y, Hasegawa H, Shinmura K, Ebihara Y, Hayashi Y, Guo X, Takayama M. Decrease In Ischemic Tolerance With Aging In Isolated Perfused Fischer 344 Rat Hearts: Relation To Increases In Intracellular Na^+ After Ischemia. J Mol Cell Cardiol 1997; 29:3081-3089.

Tani, M., Honma, Y., Hasegawa, H., Tamaki, K. Direct activation of mitochondrial K [ATP] channels mimics preconditioning but protein kinase C activation is less effective in middle-aged rat hearts. Cardiovasc. Res. 2001:49; 56–68.

Tanswell AK, Freeman BA. Pulmonary antioxidant enzyme maturation in the fetal aand neonatal rat. Pediatr Res 1984;18:584-587.

Tapuria N, Kumar Y, Habib MM, Amara MA, Seifalian AM, Davidson BR. Remote Ischemic Preconditioning: A Novel protective method from ischemia reperfusion injury – A review. Journal of Surgical Research 2008;150:304-330.

Teoh KH, Christakis GT, Weisel RD, Fremes SE, Mickle DAG, Romashvin AD, et al. Accelerated myocardial metabolic recovery with terminal warm blood cardioplegia. J Thorac Cardiovasc Surg 1986;91:888-95.

Teoh LK, Grant R, Hulf JA, Pugsley WB, Yellon DM. A comparison between ischemic preconditioning, intermittent cross-clamp fibrillation and cold crystalloid cardioplegia for myocardial protection during coronary artery bypass graft surgery. Cardiovasc Surg 2002;10:251—5.

Theroux P, Chaitman BR, Danchin N et al. Inhibition of the sodium-hydrogen exchanger with cariporide to prevent myocardial infarction in high-risk ischemic situations. Main results of the GUARDIAN trial. Guard during ischemia against necrosis (GUARDIAN) Investigators. Circulation 2000;102:3032-3038.

Transonic Surgical Protocol #75. Transonic Systems Inc. Coronary Blood Flow: Isolated Perfused Heart Preparation: Langendorff & Working Heart Models; Surgical Methods Protocol RL-75-sp. 2011.

Tsien RW. Possible effects of hydrogen ions in ischemic myocardium. Circulation 1976:55;382-391.

Tsukube T, McCully JD, Federman M, Krukenkamp IB, Levitsky S. Developmental differences in cytosolic calcium accumulation associated with surgically induced global ischemia: optimization of cardioplegic protection and mechanism of action. J Thorac Cardiovasc Surg 1996 Jul;112(1):175-84.

Tsukube T, McCully JD, Metz KR, Cook CU, Levitsky S. Amelioration of ischemic calcium overload correlates with high-energy phosphates in senescent myocardium. Am J Physiol 1997 Jul;273(1 Pt 2):H418-H425.

Van-Remmen H, Richardson A. Oxidative Damage To Mitochondria And Aging. Exp Gerontol 2001; 36:957-968.

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.

Wallace Dc. A Mitochondrial Paradigm For Degenerative Diseases And Ageing. Novartis Found Symp 2001; 35: 247-263.

Walsh SR, Tang T, Sadat U, Dutka DP, Gaunt ME.Cardioprotection by remote ischemic preconditioning. British Journal of Anaesthesia 2007. 99(5): 611-616.

Walters AM, Porter GA, Brookes P. Mitochondria as a drug target in ischemic heart disease and cardiomyopathy, 2012. Circ Res; 111:1222-1236.

Wei Jy. Age And The Cardiovascular System. N Engl J Med 1992; 327: 1735-1739.

Weisel RD, Mickle DA, Finkle CD, Tumiati LC, Madonik MM, Ivanov J. Delayed myocardial metabolic recovery after blood cardioplegia. Ann Thorac Surg 1989 Oct;48(4):503-7.

Werns SW, Shea MJ, Lucchesi BR. Free radicals and myocardial injury: Pharmacologic implications. Circulation. 1986;74:1–5.

Willems L, Zatta A, Holmgren K, Ashton KJ, Headrick JP. Age-related changes in ischemic tolerance in male and female mouse hearts. J Mol Cell Cardiol 2005 Feb;38(2):245-56.

Wittnich C, Peniston C, Ianuzzo D, Abel JG, Salerno TA. Relative vulnerability of neonatal and adult hearts to ischemic injury. Circulation. 1987;76:V156– 60.

Wu ZK, Tarkka MR, Pehkonen E, Kaukinen L, Honkonen EL, Kaukinen S. Ischaemic preconditioning has a beneficial effect on left ventricular haemodynamic function after a coronary artery bypass grafting operation. Scand Cardiovasc J 2000;34:247—53.

Xiong J, Wang Q, Xue FS, Yuan YJ, Li S, Liu JH Liao X et al. Comparison of cardioprotective and anti-inflammatory effects of ischemia pre and postconditioning in rats with myocardial ischemia-reperfusion injury. Inflamm Res 2011 Jun;60(6):547-554.

Yamaguchi S, Watanabe G, Tomita S, Tabata S. Lidocaine-magnesium blood cardioplegia was equivalent to potassium blood cardioplegia in left ventricular function of canine heart. Interact Cardiovasc Thorac Surg 2007 Apr;6(2)172-6.

Yarbrough WM, Mukherjee R, Escobar GP, Minoiga JT, Sample JA, Hendrick JM, Dowdy KB, McLean JE, Stroud RE, Spinale FG. Direct inhibition of the sodium/hydrogen exchanger after prolonged regional ischemia improves contractility on reperfusion independent of myocardial viability. *Cardiopulmonary Support and Physiology*. 2003;126:1486-1497.

Yellon DM, Alkhulaifi AM, Pugsley WB. Preconditioning the human myocardium. Lancet 1993; 342: 276–7.

Yellon, D.M., Downey, J.M. Preconditioning the myocardium: from cellular physiology to clinical cardiology. Physiol. Rev. 2003:83; 1113–1151.

Zeymer U, Suryapranata H, Monassier JP et al. The Na+/H+ exchange inhibitor eniporide as an adjunct to early reperfusion therapy for acute myocardial infarction. Results of the evaluation of the safety and cardioprotective effects of eniporide in acute myocardial infarction (ESCAMI) trial. J Am Coll Cardiol 2001;38:1644-1650.

Zimmer HG. The isolated perfused heart and its pioneers. News Physiol Sci Aug. 1998;13:203–10.

Zipes DP, Jalife J. Cardiac Electrophysiology, From Cell to Bedside. Elsevier Inc.; Philadelphia, 2009.

Zygmunt AC, Eddlestone GT, Thomas GP, Nesterenko VV, Antzelevitch C. Larger late sodium conductance in M cells contributes to electrical heterogeneity in canine ventricle. Am J Physiol Heart Circ Physiol 2001;281:H689–97.