MYOCARDIAL ISCHAEMIC
PRE-CONDITIONING in Open Heart surgery.

An Essay
Submitted for fulfillment of master degree in anesthesiology and Intensive care.

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<tr>
<td>AIF</td>
<td>Apoptosis-inducing factor</td>
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<td>ANT</td>
<td>Adenine nucleotide translocator</td>
<td></td>
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<tr>
<td>AP-1</td>
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<tr>
<td>AST</td>
<td>Aspartate aminotransferase</td>
<td></td>
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<tr>
<td>ATP</td>
<td>Adenosine-triphosphate</td>
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<tr>
<td>Bad</td>
<td>Proapoptotic protein</td>
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<tr>
<td>Bax</td>
<td>Regulator of apoptosis</td>
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<td>CAD</td>
<td>Coronary artery disease</td>
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<tr>
<td>CK</td>
<td>Creatine kinase</td>
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<tr>
<td>CPK-MB</td>
<td>Creatine phosphokinase-MB</td>
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<tr>
<td>CPB</td>
<td>Cardiopulmonary bypass.</td>
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<tr>
<td>CRP</td>
<td>C-reactive protein.</td>
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<td>DADLE</td>
<td>D-Ala2-D-Leu5-enkephalin</td>
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<td>DPDPE</td>
<td>D-Pen2-D-Pen5-enkephalin</td>
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<tr>
<td>EGFR</td>
<td>Epidermal growth factor receptor</td>
<td></td>
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<tr>
<td>eNOS</td>
<td>Endothelial nitric oxide synthetase</td>
<td></td>
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<tr>
<td>ERK</td>
<td>Extracellular signal-regulated protein kinase</td>
<td></td>
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<tr>
<td>FADH</td>
<td>Flavin adenine dinucleotide</td>
<td></td>
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<tr>
<td>GC</td>
<td>Guanylyl cyclase</td>
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<tr>
<td>GSK-3β</td>
<td>Glycogen synthase kinase-3β</td>
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<td>I/R</td>
<td>Ischemia/reperfusion</td>
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<td>IFN</td>
<td>Interferons</td>
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<td>Interleukin-1b</td>
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<td>IMM</td>
<td>Inner mitochondrial membrane</td>
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<td>iNOS</td>
<td>Inducible nitric oxide synthase</td>
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<td>IPC</td>
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<td>IVRT</td>
<td>Isovolumic relaxation time</td>
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<td>LDH</td>
<td>Lactate dehydrogenase</td>
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<tr>
<td>LDL</td>
<td>Low-density lipoprotein</td>
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<tr>
<td>LTB4</td>
<td>Leukotriene</td>
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<td>LVEDP</td>
<td>Left ventricular end-diastolic pressure</td>
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<td>MAPK</td>
<td>Mitogen-activated protein kinase</td>
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<td>mitoK&lt;sub&gt;ATP&lt;/sub&gt;</td>
<td>Mitochondrial ATP sensitive potassium channels</td>
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<td>MPT</td>
<td>Mitochondrial permeability transition</td>
<td></td>
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<tr>
<td>MPTP</td>
<td>Mitochondrial permeability transition pore</td>
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<tr>
<td>NADH</td>
<td>Nicotinamide adenine dinucleotide levels</td>
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<tr>
<td>NADH</td>
<td>Nictotinamide adenine dinucleotide</td>
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<tr>
<td>NF&lt;sub&gt;K&lt;/sub&gt;B</td>
<td>Nuclear factor kappa B</td>
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</tr>
<tr>
<td>NO</td>
<td>Nitric oxide</td>
<td></td>
</tr>
<tr>
<td>NOS</td>
<td>Nitric oxide synthase</td>
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<tr>
<td>O&lt;sub&gt;2&lt;/sub&gt;−</td>
<td>Superoxide anion</td>
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<tr>
<td>OH</td>
<td>Hydroxide radical</td>
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<tr>
<td>OMM</td>
<td>Outer mitochondrial membrane</td>
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<tr>
<td>ONOO&lt;sup&gt;-&lt;/sup&gt;</td>
<td>Peroxynitrite</td>
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<tr>
<td>OPCAB</td>
<td>Off-pump CABG</td>
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<td>PARP</td>
<td>Poly (ADP-ribose) polymerase.</td>
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<td>PI3-K</td>
<td>Phosphatidylinositol3-kinase</td>
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<td>PKC</td>
<td>Protein kinase C</td>
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<td>PKG</td>
<td>Protein kinase G</td>
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<tr>
<td>PTP</td>
<td>Permeability Transition Pore</td>
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<td>PVD</td>
<td>Peripheral vascular disease</td>
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<td>ROS</td>
<td>Reactive oxygen species</td>
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<td>sICAM-1</td>
<td>The soluble form of ICAM-1</td>
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<td>SIRS</td>
<td>Systemic inflammatory response syndrome</td>
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<td>Sarcoplasmic reticulum</td>
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<td>SWMA</td>
<td>Segmental wall motion abnormalities</td>
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<td>Tropomyosin</td>
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<td>Troponin</td>
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<td>Tumour necrosis factor-α</td>
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<td>TXA2</td>
<td>Thromboxan A2</td>
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<td>VCAM-1</td>
<td>Vascular cell adhesion molecule-1</td>
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<td>VDAC</td>
<td>Voltage-dependent anion channel</td>
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<td>VEGF</td>
<td>Vascular endothelial growth factor</td>
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<tr>
<td>VM</td>
<td>Membrane potential</td>
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INTRODUCTION
INTRODUCTION

Optimal results in cardiac surgery not only require a rapid and perfect surgical repair, but also prevention of myocardial damage and maintenance of normal cellular integrity during cardiopulmonary bypass (CPB). Myocardial protection during cardiac surgery aims to preserve myocardial function while providing a bloodless and motionless operating field to make surgery easier (Mickleborough et al., 2001).

The term "myocardial protection" refers to strategies and methodologies used either to attenuate or to prevent post-ischemic myocardial dysfunction that occurs during and after heart surgery. Post-ischemic myocardial dysfunction is attributable, in part, to a phenomenon known as ischemia/reperfusion-induced injury (Das et al., 1993).

In 1986 Murry and co-workers introduced the term ‘Ischemic Preconditioning’ (Murry et al., 1986). In this classical paper the authors referred to ischemic preconditioning as an adaptation to ischemic stress induced by repetitive short periods of ischemia and reperfusion. The protective effect comprised a reduction of energy consumption and a delay of the onset of lethal cell injury during ischemia resulting in a limitation of infarct size (Lukas, 1998).

A potential and very desirable spin-off of research on ischaemic preconditioning may be the development of pharmacological interventions that could mimic or induce myocardial endogenous states and could be applied at the time of reperfusion. This development was not included as a potential clinical implication in the original report by Murry et al. When cell death secondary to ischaemia/reperfusion is seen as a consequence of active signalling processes that can be interfered with (David et al., 2011).

The mechanically perfect heart cannot undergo early or late survival if operative damage from protection is severe. An example is the development of “stone” heart after 30min of normothermic aortic clamping for aortic stenosis, or late dilatation from
evolving scar from intraoperative ischemic damage. Conversely, the normal myocardium on bypass, with preserved structural and biochemical integrity, cannot maintain cardiac output if there is a technical operative error, such as a closed coronary anastomosis or iatrogenic valvular insufficiency (Ahd-Elfattah et al., 1993).

In addition, our patient’s vulnerability to injury has increased, so we need to improve our methods of protection as well as learn new operative techniques. The early and late success of a cardiac surgical procedure is related to how well the operation corrected, the mechanical problem and how carefully myocardial protection avoided the secondary dysfunctional effects of aortic clamping for technical repair, and there is no separation between these two central events (Mickleborough et al., 2001).

This study aims to review the mechanism and different techniques of ischemic preconditioning during cardiac surgery for better clinical outcome particularly in high risk patients.
( 1 )

Physiologic Anatomy Of Cardiac Muscle
Physiologic Anatomy Of Cardiac Muscle

The heart is composed of three major types of cardiac muscle: atrial muscle, ventricular muscle, and specialized excitatory and conductive muscle fibers. The atrial and ventricular types of muscle contract in much the same way as skeletal muscle except that the duration of contraction is much longer. On the other hand, the specialized excitatory and conductive fibers contract only feebly because they contain few contractile fibrils; instead, they exhibit rhythmically and varying rates of conduction, providing an excitatory system for the heart (Arthur C. Guyton et al., 1996).

Cardiac muscle is striated in the same manner as typical skeletal muscle. Furthermore, cardiac muscle has typical myofibrils that contain actin and myosin filaments almost identical to those found in skeletal muscle, and these filaments interdigitate and slide along one another during contraction in the same manner as occurs in skeletal muscle (Arthur C. Guyton et al., 1996).

The dark areas crossing the cardiac muscle fibers are called intercalated discs; they are actually cell membranes that separate individual cardiac muscle cells from one another. That is, cardiac muscle fibers are made up of many individual cells connected in series with one another. Yet electrical resistance through the intercalated disc is only 1/400 the resistance through the outside membrane of the cardiac muscle fibers because the cell membranes fuse with one another in such a way that they form permeable “communicating” junctions (gap junctions) that allow relatively free diffusion of ions. Therefore, from a functional point of view, ions move with ease along the longitudinal axes of the cardiac muscle fibers, so that action potentials travel from one cardiac muscle cell to another, past the intercalated discs, with one cardiac muscle cell to another, past
the intercalated discs, with only slight hindrance. Thus, cardiac muscle is a syncytium of many heart muscle cells, in which the cardiac cells are so interconnected that when one of these cells becomes excited, the potentials spreads to all of them, spreading from cell to cell as well as throughout the latticework interconnections (*Wit Al et al, 1997*).

The heart is actually composed of two syncytiums: the atrial syncytium that constitutes the walls of the two atria and the ventricular syncytium that constitutes the walls of the two ventricles. The atria are separated from the ventricles by fibrous tissue that surrounds the valvular openings between the atria and ventricles. Normally, action potentials can be conducted from the atrial syncytium into the ventricular syncytium only by way of specialized conductive system, the atrioventricular bundle, a bundle of conductive fibers several millimeters in diameter. This division of the muscle mass of the heart into two functional syncytia allows the atria to contract a short time ahead of ventricular contraction, which is important for the effectiveness of heart pumping (*Arthur C. Guyton et al, 1996*).

**Physiologic consideration:**

**Electrophysiology:**

In the normal heart the action potential originates at the SA node. Contraction of the heart is initiated by the action potential, of which two types present within the heart: fast-response action potentials, which occur in most myocardial tissue, including the atria, ventricels, and purkinje cells of the conduction system; and slow-response action potentials, which are found in the specialized cells responsible for the internal automaticity or pacemaker activity of the heart, namely the SA and AV nodes. The difference between the two types of action potential lies in the resting membrane potential (Vm) present in the various cells and the rate of rise of upstroke of the action
potential, which subsequently determines the propagation velocity of the action potential through the heart (Wit Al et al, 1997).

The action potential can be divided into four phases (Figure.1), in the fast response action potential, the resting (Vm) in a cardiac muscle cell is –80 to –90 mV, and phase 0 represents initial depolarization of the cell through the voltage-dependent fast sodium (Na) channels. These sarcolemmal Na channels operate in a double-gated fashion (Figure.2) in which initially the outer, or m-gate (activation gate) is closed until a threshold Vm of –60 to –70 mV is achieved. The gate then opens, and the sodium is allowed to enter the cell along its concentration and electrostatic gradient, contributing to the brisk upstroke seen in the Vm. As the Vm reaches +30 mV, the inner or h-gate (inactivation gate) closes, preventing further influx of Na⁺ (inhibition of Na⁺ channels) and effectively ending phase 0. At a Vm of 0, there are no further electrostatic forces pulling Na⁺ into the cell, but Na⁺ nonetheless continues to enter the cell because of the persistent concentration gradient, which accounts for the overshoot seen in the Vm. These fast Na⁺ channels are inhibited by tetrodotoxin (Cohen CJ et al, 1989).

Partial repolarization (phase 1) and the plateau (phase 2) at a Vm of around 0 are dominated by the influx of Ca²⁺ through slow L-type voltage-dependent Ca²⁺ and to a lesser extent, of Na⁺ along its slow channel. These channels open initially at a Vm of –30 mV during the rapid depolarization upstroke of phase 0 and allow Ca²⁺ and to a lesser extent Na⁺, to enter the cell along its concentration gradient (Jeel A. Kaplan, 1999).
Figure 1. The four phases of action potential and the various ion movements (Jeel A. Kaplan, 1999).

Figure 2. Sodium channel dynamics that occur during a cardiac cycle (Jeel A. Kaplan, 1999).
The entry of Ca\(^{2+}\) via these channels triggers further release of Ca\(^{2+}\) from the SR. Free intracellular Ca\(^{+}\) is then able to bind contractile proteins and to initiate contractile force. Catecholamines, such as epinephrine and norepinephrine, increase slow inward Ca\(^{2+}\) currents, and this represents one of the mechanisms by which Catecholamines increase contractile force. These slow Ca\(^{2+}\) channels are inhibited by manganese and by dihydropyridine and phenylalkylamine-type Ca\(^{2+}\) channel antagonists (Zobrist RH et al., 1998).

Repolarization, or phase 3, occurs as the ionized potassium (K\(^{+}\)) permeability increases, leading to an efflux of K\(^{+}\) along its concentration gradient out of the cell. This has the effect of lowering Vm to its resting potential and causing the closure and inactivation of the slow Ca\(^{2+}\) and Na\(^{+}\) channels. During this period, which is known as the effective or absolute refractory period, no further depolarization of the cell can take place. Restoration of Na\(^{+}\) and K\(^{+}\) to their preexcitation concentration gradients occurs via an active transport Na\(^{+}\)/K\(^{+}\) membrane adenosine triphosphatase (ATPase) pump at ratio of 6 Na\(^{+}\) ions out for every 3 K\(^{+}\) ions in. Ca\(^{2+}\) homeostasis is achieved by the SR, Ca\(^{2+}\), Mg\(^{2+}\) ATPase and the sacrolemmal Ca\(^{2+}\) ATPase and Na\(^{+}\), Ca\(^{2+}\) exchange mechanism (Zobrist RH et al., 1998).

Finally phase 4 represents the period between completion of repolarization and initiation of the next action potential. During this period, K\(^{+}\) continues to leak slow from the cell along its concentration gradient. In contrast the slow-response action potential, cell-resting V\(_m\) are approximately \(-60\) mV (Sapsford RN et al. 1997).

Fast Na\(^{+}\) channel activation is virtually absent, and depolarization occurs in a manner similar to that of phase 2 of the fast-response action potential, with the predominance of slow inward Ca\(^{2+}\) and Na\(^{+}\) currents. Phase 3 repolarization and phase 4 are virtually identical between the two types of action potentials, although the absolute
refractory period in cells exhibiting slow-type action potentials is much longer (Ruiz-Meana M et al.,1995).

At this point, we must ask the question: Why is the action potential of cardiac muscle so long and why does it have a plateau, whereas that of the skeletal muscle does not? At least two major differences between the membrane properties of cardiac and skeletal muscle account for the prolonged action potential and the plateau in cardiac muscle (Arthur C et al.,1996).

First the action potential of skeletal muscle is almost entirely by sudden opening of large numbers of so-called fast sodium channels that allow tremendous numbers of sodium ions to enter the skeletal muscle fiber. These channels are called “fast” channels because they remain open for only a few 10,000ths of a second and then abruptly close. At the end of this closure repolarization occurs and the action potential is over within another 10,000th of a second or so, in cardiac muscle, on the other hand, the action potential is caused by the opening of two types of channels (1) the same fast sodium channels as those in skeletal muscle and (2) another entire population of so-called slow calcium channels, also called calcium-sodium channels. This second population of channels differs from the fast sodium channels in being slower to open; but more important, they remain open for several 10ths of a second. During this time, a large quantity of both calcium and sodium ions flows through these channels to the interior of the cardiac muscle fiber, and this maintains a prolonged period of depolarization, causing the plateau in the action potential (Arthur C et al.,1996).
The second major functional difference between cardiac muscle and skeletal muscle that helps account for the prolonged action potential and its plateau is this: Immediately after the onset of the action potential, the permeability of the cardiac muscle membrane for potassium decreases about fivefold, an effect that does not occur in skeletal muscle. This decreased potassium permeability may be caused by the excess calcium influx through the calcium channels just noted. Regardless of the cause, the decreased potassium permeability greatly decreases the outflux of potassium ions during the action potential plateau to its resting level. When the slow calcium-sodium channels do close at the end of 0.2 to 0.3 second and the influx of calcium and sodium ions ceases, the membrane permeability for potassium increases rapidly; this rapid loss of potassium from the fiber returns the membrane potential to its resting level, thus ending the action potential (Arthur C et al., 1996).
Mechanisms Of Myocardial Contraction

Biochemical Components:

The heartbeat is initiated at the SA node, which is a strip of fine muscle fibers located posteriorly near the junction of the superior vena cava with the RA the nodal cells undergo spontaneous depolarization the impulse passes out from the SA node to the atria and down the conduction system through the AV node into the ventricular cells (Ronald D. Miller, 2000).

Sarcoplasmic Reticulum:

The SR is the intracellular storage site of Ca\(^{2+}\) used for contraction. The Ca\(^{2+}\) entering through the sarcolemma is scant and acts solely as a trigger to initiate Ca\(^{2+}\) release from the SR. The rise of Ca\(^{2+}\) as measured by luminescent or fluorescent intracellular Ca\(^{2+}\) dyes indicates that little, if any, measurable lag exists between the initiation of the action potential and the increase in intracellular Ca\(^{2+}\) (Mcperson PS et al., 1993).

The other equally important function of the SR is the reaccumulation of Ca\(^{2+}\) this is accomplished by a 105,000 molecular weight Ca/Mg-ATP ase pump, which is imbedded in the membrane of the longitudinal SR. Because removal of free intracellular Ca\(^{2+}\) from the myoplasm to generate a Ca\(^{2+}\) gradient is an active, energy-consuming process, hydrolysis of ATP is required (Mcperson PS et al., 1993).

Contractile Elements:

The purpose of the elaborate system of Ca\(^{2+}\) delivery and removal is to control the availability of Ca\(^{2+}\) for the contractile process for cardiac muscle to contract actin and
myosin must interact. These two contractile proteins make up. Respectively, the thin and thick filaments seen in electron micrographs of cardiac and skeletal muscle action is a small protein with a molecular weight of 43,000 individual actin molecules combine to form long polymer chains in a double- helical structure that make up the thin filament. Interposed at a regular spacing along the thin filament is a complex of tropomyosin ( Tm ) and troponin (Tn). Tm is a linear protein of approximately 70,000 molecular weight that lies within the sulcus of the thin filament. The thick filament observed in electron micrographs of cardiac muscle is made up of myosin, which is a large, asymmetric molecule consisting of two heavy chains, and four light chains, the functions of the light chains are still uncertain but may include regulation of cross-bridge formation (Eisenerge E et al.,1995).

A complex cycle of actin-myosin interactions, shortening, and tension development occurs on binding of Ca\(^{2+}\) to Tn. This active process requires ATP and utilizes approximately 70 percent of ATP available in the myocyte. The hydrolysis of ATP to adenosine diphosphate (ADP) and the formation of a high-energy phosphate intermediate of actinmyosin ATP ase drives the three parts of the contractile cycle:

- detachment of the myosin head from actin,
- reattachment of myosin to actin with a different conformation and higher free energy at the beginning of the work stroke and
- development of force and the performance of mechanical work( Eisenerge E et al.,1995).

**Metabolic considerations:**

The metabolic function of the cardiac myocyte serves a 2-fold purpose: to generate energy to maintain cell integrity by maintaining ionic gradients across membranes and to generate energy to maintain a physiologic pump that must work nonstop for, on average, 73 years (Ronald D.Miller,2000).
Substrates and Energy Sources

The heart is able to metabolize glucose, carbohydrates, lactate, and fatty acids to form its metabolic energy source, ATP. During fasting, free fatty acids (FFA) are elevated in the serum, and the substrates utilized by the heart in fasting patients are mainly FFA and glucose. In fact, FFA in these circumstances inhibits the utilization of glucose. These substrates are used to form ATP and creatine phosphate, molecules with high energy, which are then used by the heart to perform mechanical and chemical work, such as contraction and ion transport (Ronald D. Miller, 2000).

Mitochondria

The mitochondrion contains the enzymatic constituents to synthesize the ATP used by the cell for all its energy requirements. Approximately 23 percent of the volume of the myocardial cell is occupied by mitochondria, a finding that reflects the importance of a continuous supply of ATP for continuous cardiac function. The inner membrane system consists of a multiplex of folds, which contain the enzymes for aerobic metabolism and the cytochromes involved in electron transport. Modified FFA and the intermediary products of glucose metabolism are further metabolized by the mitochondria, yielding reduced nicotinamide adenine dinucleotide (NADH) and reduced flavin adenine dinucleotide (FADH), which are fed into the electron transport chain (Ronald D. Miller, 2000).
Coronary blood supply

Myocardial cells receive an abundant blood supply, contain numerous mitochondria in which energy is produced, and have an increased myoglobin content which act as oxygen store. The great majority of the energy used by cardiac cells is provided by aerobic metabolism in the form of ATP. At rest, less than 1% of energy is produced anaerobically although this proportion can increase to 10% under hypoxic conditions. The main metabolic substrates are free fatty acids, glucose and ketones (Przyklenk K, 1997).

Anatomy of coronary circulation:

The coronary circulation consists of:

- Arterial blood supply: The two coronary arteries arise from the coronary ostia just above the respective cusps of the aortic valve.

  - Left coronary artery, which gives off the following branches:
    - Left circumflex artery supplies posterior free wall of left ventricle
    - Left anterior descending artery supplies the anterior free wall of the left Ventricle; a septal branch supplies the upper inter-ventricular septum

  - Right coronary artery supplies the free wall of the right ventricle and right atrium and the posterior free wall of the left ventricle.

- Venous drainage: epicardial veins; coronary sinus; Thebesian veins (Jeel A. Kaplan, 1999).
Figure 3. (A) Anatomy of the left coronary artery

(B) Anatomy of the right coronary artery.

LAD. left anterior descending, SA. sinoatrial., RCA. right coronary artery, AV. atrioventricular, LV. left ventricle (Jeel A. Kaplan, 1999).
Regulation of coronary blood flow:

At rest, approximately 250 ml min⁻¹ of blood perfuse the coronary arteries, 5% of cardiac output. Because of the limited capacity of the heart to provide energy anaerobically, any increased myocardial oxygen demand must be met by improving the oxygen supply by altering coronary blood flow (Downey et al., 1992).

At rest, the coronary vasculature is relatively constricted. Increased metabolic demands are therefore met by appropriate coronary vasodilatation, which increases coronary flow up to fivefold. There are two mechanisms involved in the regulation of coronary blood flow, localized metabolic control and neurohumoral control (Downey et al., 1992).

1) Metabolic control of coronary blood flow:

Inadequate blood supply to areas of the heart results in hypoxia and accumulation of myocardial metabolites (such as carbon dioxide, phosphate, adenosine, prostaglandins, hydrogen ions and potassium ions). Some of these metabolites have a major effect on the coronary vasculature, dilating small arterioles and precapillary sphincters to increase local coronary blood flow. Local metabolism thus has a major role in regulating coronary blood flow (Downey et al., 1992).

2) Neurohumoral control of coronary blood flow:

The coronary vessels are innervated by both sympathetic and parasympathetic fibres, but the role of the autonomic nervous system in controlling coronary blood flow is probably minor compared with local effects (Downey et al., 1992).
Distribution of coronary blood flow:

During left ventricular systole, myocardial contraction results in the production of a large intraventricular pressure. This pressure is transmitted across the ventricular wall, progressively increasing from the epicardium to the endocardium to the subendocardium. The pressure in the subendocardial layer of the myocardial muscles exceeds systolic arterial blood pressure. Consequently, there is no subendocardial blood flow during systole, and flow occurs in the arteries supplying the subendocardium of the left ventricle only during diastole. In the right ventricle, the lower intraventricular pressures result in flow to all areas of the heart throughout the cardiac cycle (Downey et al, 1992).

Determinants of Coronary Perfusion:

Coronary perfusion is unique in that it is intermittent rather than continuous, as it is in other organs. During contraction, intramyocardial pressures in the left ventricle approach systemic arterial pressure. The force of left ventricular contraction almost completely occludes the intramyocardial part of the coronary arteries; in fact, blood flow may transiently reverse in epicardial vessels. Even during the later part of diastole, left ventricular pressure eventually exceeds venous (right atrial) pressure (Figure 4). Thus, coronary perfusion pressure is usually determined by the difference between aortic pressure and ventricular pressure. Moreover, as a determinant of myocardial blood flow, arterial diastolic pressure is more important than mean arterial pressure (Piper HM et al, 1996).
Decreases in aortic pressure or increases in ventricular end-diastolic pressure can reduce coronary perfusion pressure. Increases in heart rate also decrease coronary perfusion because of the disproportionately greater reduction in diastolic time as heart rate increases (Figure 5). Because it is subjected to the greatest intramural pressures during systole, the endocardium tends to be most vulnerable to ischemia during decreases in coronary perfusion pressure (Piper HM et al., 1996).
It is to conclude that, a better understanding of the physiology of myocardial cell function helps the correction of cellular defects. The ultimate success of new treatment modalities is, however, still constrained by an inadequate understanding of the underlying pathophysiological events. These recent developments point to a need for the re-examination of the concept and application of metabolic treatment for the failing myocardium in defined clinical settings, such as reperfusion after an acute ischemic event, controlled hypothermic ischemic arrest, or acute myocardial infarction (Mickleborough et al., 2001).
(2)

Myocardial Ischemia, Injury and Infarction
Myocardial Ischemia, Injury and Infarction

Ischemia, injury and infarction: these three terms are often referred to as “the 3I’s” of coronary artery events (*Libby, 1995*).

**Ischemia:**

Ischemia occurs with a mismatch between the amount of blood flowing to a section of the heart and the amount of oxygen needed by that section of the heart. Regional myocardial ischemia secondary to coronary occlusion is frequently a heterogenous event in the portion of the heart fed by the involved artery. Global myocardial ischemia induced by aortic cross clamping also causes heterogenous injury, in that the subendocardium is more vulnerable to irreversible injury than the epicardial layer. The consequences of myocardial ischemia depend on its severity, duration and conditions of reperfusion (*Libby, 1995*).

Myocardial oxygen demand is normally the most important determinant of myocardial blood flow. Relative contributions to oxygen requirements include basal requirements (20%), electrical activity (1%), volume work (15%), and pressure work (64%). The myocardium normally extracts (65%) of the oxygen in arterial blood. Compared with (25%) in most other tissues. Coronary sinus oxygen saturation is normally (30%). Therefore, the myocardium (unlike other tissues) cannot compensate for reductions in blood flow by extracting more oxygen from hemoglobin. Any increases in myocardial metabolic demand must be met by an increase in coronary blood flow. Table (1) lists the most important factors in myocardial oxygen demand and supply (*Morgan et al., 2002*).

<table>
<thead>
<tr>
<th>O2 Supply</th>
<th>O2 Demand</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate</td>
<td>Basal requirements</td>
</tr>
<tr>
<td>Diastolic time</td>
<td>Heart rate</td>
</tr>
<tr>
<td>Coronary perfusion pressure</td>
<td>Wall tension</td>
</tr>
<tr>
<td>Aortic diastolic blood pressure</td>
<td>Preload (ventricular radius)</td>
</tr>
<tr>
<td>Ventricular end-diastolic pressure</td>
<td>Afterload</td>
</tr>
<tr>
<td>Arterial oxygen content</td>
<td>Contractility</td>
</tr>
<tr>
<td>Arterial oxygen tension</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin concentration</td>
<td></td>
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<tr>
<td>Coronary vessel diameter</td>
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</tr>
</tbody>
</table>

**Ischemic Cascade:**

The ischemic cascade is a chronologic series of events occurring after an ischemic insult (Figure 6). When a coronary artery is occluded, the first changes are biochemical. Oxygen deficit results in an intracellular acidosis and increased intracellular calcium. The cells become anaerobic and lactate production increases. The next changes are mechanical. In severe ischemia, all these events may occur. If ischemia is mild, only a few signs in the cascade may occur (*Comunale et al.*, 1998).

Most of the signs resolve in reverse order. After treatment, ST segment changes usually resolve within 5 minutes, systolic function within 25 minutes, and diastolic function within 1 hour (*Barnes et al.*, 2000).
Electrocardiographic (ECG) Changes of Ischemia:

The ECG hallmarks of Ischemia are ST-segment depression and changes in T waves. Significant ST-segment depression is present with horizontal or downsloping ST-segment depression of 0.1mV and with slowly upsloping depression of 0.2 mV (all measured from 60 to 80 ms after the J point). The T-wave changes of ischemia are less
specific and can be either T-wave inversion (relative to the axis of QRS complex) or T waves that are tall, peaked and symmetrical. Giant, hyperacute T-wave changes are sometimes the only changes seen in early acute myocardial ischemia (Okin et al., 2001).

Non-ischemic causes of ST-segment depression and T wave changes:

Some non-ischemic conditions that can cause ST-segment depression and T-wave changes are:

- **Digoxin:**
The administration of digoxin causes T wave inversion or flattening, characteristically with coved depression of ST segment. It is helpful to record an ECG before giving digitalis, to save later confusion about the significance of T wave changes (Braunwald et al., 1999).

- **Ventricular hypertrophy:**
Left ventricular hypertrophy causes inverted T waves in the leads looking at the left ventricle (V₅, V₆, II and aVL). Right ventricular hypertrophy causes T wave inversion in the leads looking at the right ventricle (T wave inversion is normal in V₁, but in white adults is abnormal in V₂ or V₃) (Braunwald et al., 1999).

- **Bundle branch block (BBB):**
The abnormal path of depolarization in bundle branch block is usually associated with an abnormal path of repolarization. Therefore, inverted T waves associated with QRS complexes which have a duration of 0.16 seconds or more have no significance in themselves (Braunwald et al., 1999).

- **Electrolyte abnormalities:**
Abnormalities in the plasma levels of potassium, calcium and magnesium affect the ECG. A low potassium level causes T wave flattening or inversion and the appearance of a
hump on the end of the T wave called a “U” wave. A high potassium level causes tall, wide T waves with disappearance of ST segment. High plasma calcium level causes shortening of QT interval, the proximal limb of the T wave abruptly slopes to its peak and the ST segment may disappear. A low plasma calcium level causes prolongation of the QT interval (Braunwald et al., 1999).

- **Intracranial hemorrhage:**
  Hemorrhage into either the intracerebral or subarachnoid spaces can produce T wave widening and usually inversion, prolonged QT interval and bradyarrhythmias (Braunwald et al., 1999).

**Injury:**

Injury occurs when the period of ischemia is prolonged more than just a few minutes. Usually this occurs within 40 minutes. Injured myocardium does not function normally but serum markers are not yet released from the injured cells. If reperfusion is initiated rapidly and effectively, the resulting myocardial injury is reversible and is characterized, functionally by depressed myocardial contractility, which eventually recovers completely. Myocardial tissue necrosis is not detectable in the previously ischemic region although functional impairment of contractility may persist for a variable period, a phenomenon known as myocardial stunning (Schaper, 1997).

Chronic incomplete reduction in blood flow to the myocardium may also lead to prolonged ventricular dysfunction “hibernating myocardium” which resolves when adequate flow is reestablished (Guest and Mazer, 1996).
ECG changes of injury:

The ECG hallmark of injury is ST-segment elevation, which is considered to be significant when the ST-segment measures 1mm or more (0.1mV=1mm) above the PT-baseline at a point 1mm past the J-point.

Other causes of ST-segment elevation:

- **Pericarditis:**
ST-segment elevation is present in all leads.

- **Left bundle branch block (LBBB):**
LBBB pattern is best seen in V₁ (RSR`) with wide QRS complex (> 120 milliseconds). Left ventricular activation delay hides ST-segment elevation. If known to be new, it is treated as ischemia (*Okin et al., 2001*).

- **Pacemaker with paced beats originating from the right ventricle:**
LBBB pattern is seen in V₁, pacemaker spike is seen.

- **Early repolarization:**
ST elevation is seen in the lateral leads (V₅, V₆, I, aVL). Elevation is coved (*Okin et al., 2001*).

Infarction:

Infarction refers to the actual death of injured myocardial cells. The extent of tissue necrosis that develops during reperfusion is related directly to the duration of the ischemic event. Myocardial injury evolves into infarction within one to two hours. In most total occlusions, infarction is 90% complete within six hours. Tissue necrosis
originates in the subendocardial regions of the ischemic myocardium and extends to the subepicardial regions of the area at risk, often referred to as the wave-front phenomenon (Ganz, 1997).

Intracellular components such as creatine phosphokinase, troponins and myoglobin begin to leak out into the bloodstream. They can then be measured as serum markers of infarction (Libby, 1995).

**ECG Changes of Infarction:**

The ECG hallmark of infarction is the presence of abnormal Q waves. Q waves are considered abnormal if they are >1mm (0.04 second) wide and the height is greater than 25% of the height of the R wave in that lead. They can appear within hours of onset of symptoms. The presence of Q waves, in the absence of clinical signs and symptoms, indicates only dead myocardium. Q waves do not reveal when the infarction of myocardium occurred. The time of infarction may have been days, weeks and even years in the past. When combined with changes in T waves or ST segments, however, the Q waves indicate a recent (acute) myocardial infarction. If the infarction is not full-thickness and so does not cause an electrical window, there will be T wave inversion but no Q waves. This is called a “non-Q wave infarction” pattern. The term “subendocardial infarction” pattern is sometimes used (Berger et al., 2002).
**Myocardial Stunning and Hibernation:**

In patients with CAD, but without chronic kidney disease, transient myocardial ischemia may lead to left ventricular (LV) dysfunction that can persist after the return of normal perfusion. This prolonged dysfunction is known as myocardial stunning. Repetitive episodes of ischemia can be cumulative and have been shown to lead to prolonged LV dysfunction. Myocardial stunning has been well described, in the non-dialysis patient population, as a causative mechanism for heart failure (Barnes et al., 2002).

Repeated episodes of myocardial ischemia lead to a spectrum of disease encompassing myocardial stunning through to myocardial hibernation and ending in myocardial remodeling and scarring, with irreversible loss of contractile function. Repetitive myocardial stunning might lead to such a process, resulting in chronic LV dysfunction. Myocardial hibernation may represent a functional adaptation to chronic hypoperfusion that can be reversed with restoration of regional blood flow (the 'smart heart' hypothesis) (Braunwald et al., 1986).

There is evidence to suggest that hibernating myocardium is still highly vulnerable to increases in demand or reductions in oxygen supply. Therefore, ongoing recurrent episodes of ischemia precipitated by coronary artery occlusion may have negative consequences on this adaptive balance leading to further myocardial injury and eventual non-viable myocardium with irreversible reduction in LV function (Barnes et al., 2002).
(3)

Ischemia reperfusion injury
Ischemia reperfusion injury

Introduction:

Heart diseases are nowadays an important health problem in our society, where such diseases constitute a leading cause of death. A shortage of oxygen and nutrients supply to cardiac cells due to a coronary perfusion insufficiency (ischemia) followed by its reposition (reperfusion) causes serious cardiac tissue damage and can compromise survival (Hearse D J, 1990).

Myocardial ischemia can occur under several forms and persist for a few seconds or minutes (angioplasty or angina), for hours (cardiac surgery or transplantation) or for years (chronic ischemia). Independently of the way it is manifested, myocardial ischemia is characterized by a marked imbalance between the provision of oxygen and nutrients to myocardial cells and their needs (Hearse D J, 1990).

The imbalance may arise for any of the three following reasons:

- an increase in the myocardial demand for oxygen and nutrients (demand ischemia).
- A decrease in the supply of oxygen and nutrients (supply ischemia).
- A mixed situation in which the above two factors are simultaneously present (cardiac surgery or transplantation) (Holleyman et al, 2001).

Whatever is the underlying mechanism, when such an imbalance of supply and demand occurs, biochemical and physiological repercussions can even result in cell death (Holleyman et al, 2001).
Ischemia/Reperfusion-Induced Cardiac Damage:

The series of biochemical phenomena by which myocardial ischemia can lead to functional depression of myocardial contractility is not yet fully understood. One possibility is that I/R (ischemia/reperfusion) is associated with increased oxidative stress in the myocardium, especially occurring during the reperfusion phase, when the affected tissue is suddenly confronted with a burst of oxygen (Ferrari et al., 1989).

The role of mitochondria in I/R damage, including the role on the modulation of cardiomyocyte life and death has been widely explored. In a metabolically hyperactive tissue such as the heart, mitochondria play a very important role in the energy supply for the myocyte. Cardiac cells have two distinct mitochondrial populations, one beneath the plasma membrane and the other between the myofibrils, which present differences regarding calcium accumulation and respiratory activity (Palmer et al., 1985).

It has been reported that both cardiac mitochondrial subpopulations differ in their resistance to I/R. For example, it has been described that sub-sarcolemmal mitochondria are more susceptible to ischemia-induced loss of cardiolipin, which explains the reported inhibition of mitochondrial complex IV and the mitochondrial adenine nucleotide translocator activity in that mitochondrial subpopulation during I/R (Duan et al., 1989).

There is evidence that multiple pathways of cell death participate simultaneously in promoting mitochondria-mediated cardiac damage (Fig. 7). Apoptosis and autophagy are considered forms of programmed cell death that involve the activation of regulated pathways leading to cell death while necrosis is considered an irreversible and non-regulated process of cell killing (Loos et al., 2009).
Cardiac apoptosis is a cellular death program extremely regulated and with great efficiency, involving an interaction of innumerable factors. Loss of cardiomyocytes via apoptosis is believed to contribute to the continuous decline of ventricular function during I/R. Cardiac myocytes are terminally differentiated and are not replaced after loss. With fewer myocytes, the ability of the myocardium to sustain contractile function may be compromised (Mani K., 2008).

Autophagy plays a critical and seemingly double role in cardiomyocytes, being implicated as a mechanism for both cellular survival during I/R injury and cell death when repaired of the injured myocardium is impossible. Although several doubts remain, the upregulation of autophagy in response to I/R stress may serve as a protective response by removing damaged mitochondria, thus preventing activation of apoptosis (Dhesi et al., 2009).
Fig. 7 Apoptotic signaling pathways involved in I/R damage. Intrinsic, extrinsic and ER stress-induced pathways are shown. Caspase-12 activity induced by endoplasmic reticulum (ER) stress has not been identified in humans; therefore, its existence is controversial. Recently, Caspase-4 was considered as a gene homologous to Caspase-12 and seems to be localized predominantly to the ER and to mitochondria → Stimulatory/Activating effect; → Translocation events; → Inhibitory effect (Loos et al., 2009).
**Apoptosis and Ischemia/Reperfusion induced cardiac damage:**

As described earlier, apoptosis is a highly regulated, energy-requiring process that follows well-defined time-dependent signaling pathways, resulting in cell shrinkage, changes in plasma membrane, proteolysis of intracellular proteins, loss of mitochondrial function and DNA fragmentation (Fig. 8). The immediate objective of apoptotic signaling pathway is the activation of pro-caspases and the “safe” dismantling of intracellular components (*Gupta S., 2003*).

**Apoptosis is mediated by two central pathways:**

1) *Extrinsic pathway:*

It occurs in response to activated death receptors present in the cell surface. This pathway is triggered by the linkage of specific ligands to one group of membrane receptors that belong to the tumor necrosis factor receptor family (αTNF) or Fas receptor (*Gupta S., 2003*).

2) *Intrinsic pathway:*

It occurs in response to signals originated inside the cell and which involve mitochondria as either an initiator or a magnifier of apoptotic signals. Mitochondria are deeply involved in the intrinsic apoptotic signaling. Protein release from mitochondria, including cytochrome *c*, may be associated with the opening of the mitochondrial permeability transition pore (MPTP) located at contact sites between the inner and outer membranes (*Baines C.P., 2009*).
Fig. 8 Mitochondrial mechanisms of cell dysfunction and key events in cardiac I/R injury. The fate of mitochondria determines the fate of the cell. Ischemia leads to ATP dissipation, with consequent rises in cell Ca\(^{2+}\) and Pi. On reperfusion, these three factors, together with oxidative stress, trigger MPTP opening. If widespread, MPTP opening results into ATP depletion and cell necrosis. If MPTP opening is more limited enabling the cell to maintain sufficient ATP, then outer membrane rupture leads to apoptosis. The degree of MPTP opening may determine the balance between necrotic and apoptotic cell death and whether it might leads to contractile dysfunction at reperfusion (Gupta S., 2003).
The key step in the intrinsic pathway is a disturbance in mitochondrial membrane permeability (Fig. 9) that is regulated by a variety of pro-apoptotic and anti-apoptotic Bcl-2 proteins such as Bax, Bad and Bid, or Bcl-2, respectively. The mitochondrial release of cytochrome $c$ leads to the apoptosome formation, a macromolecular complex that includes cytochrome $c$ itself, Apaf-1, dATP and pro-caspase-9 (Riedl et al., 2007).

It has been described that myocardial apoptosis is dominant in the pathogenesis of cardiac I/R and in the maintenance of persistent myocardial dysfunction after reperfusion. Prolonged periods of myocardial ischemia are related with an increase in necrosis rate, whereas reperfusion leads to enhancement of apoptosis. During reperfusion, the energy required for sequential apoptosis steps is supplied because oxygen and glucose supply are restored (Buja L. M., 2005).

As metabolic processes are restored, ATP becomes available and the apoptotic cascade may proceed. Genetic approaches and pharmacological studies suggest that cardiomyocyte apoptosis plays a crucial role in the pathogenesis of many cardiac syndromes and pathologies. For instance, the inhibition of cardiac myocyte apoptosis reduces infarct size up to 50–70% and decreases cardiac dysfunction after I/R (Chen et al., 2001).

Increased generation of free radicals without concomitant increase in antioxidant protection has been shown to induce apoptosis during I/R. The resulting oxidative stress leads to peroxidation of the phospholipid cardiolipin in the inner mitochondrial membrane, which contributes to induce cytochrome $c$ release from the inner mitochondrial membrane (IMM) to the extramitochondrial environment and trigger apoptosis (Kagan et al., 2006).
Mitochondrial apoptotic signaling during cardiac I/R injury. ETC.—Electron transport chain. → Stimulatory/Activating effect; ← Translocation events and ↓ Inhibitory effect (Riedl et al., 2007).
Mitochondrial dysfunction during I/R:

During ischemia, an overall deterioration of cardiac cell function occurs, which is assessed by the loss of intracellular components, including proteins such as troponins and lactate dehydrogenase. Intracellular levels of ATP are also reduced, while ADP, AMP and phosphate levels increase as mitochondria hydrolyzes ATP during ischemia in a useless attempt to preserve the mitochondrial membrane potential (St-Pierre et al., 2000).

Ischemia has already been shown to damage several mitochondrial components, including proteins involved in oxidative phosphorylation and membrane lipids, which may predispose mitochondria for increased free radical generation during reperfusion. Although mitochondria generate reactive oxygen species (ROS) during normal physiological conditions, it is accepted that during cardiac reperfusion, the production of ROS is greatly increased as a sudden supply of oxygen becomes available to the reduced components of the respiratory chain (Ferrari et al., 1992).

Also, a decrease in internal antioxidant defences including superoxidase dismutase, glutathione peroxidase and reduced glutathione occurs during reperfusion, which further exacerbates oxidative stress. Nevertheless, it is considered that a small amount of oxygen free radical production may also occur during ischemia, probably due to the existence of “oxygen pockets” in the cardiac tissue (Ferrari et al., 2004).

Several sources of free radicals during I/R include the mitochondrial respiratory chain, the oxidation of catecholamines, neutrophils or the enzyme xanthine oxidase. From increased ROS production and decreased efficacy of antioxidant defences, oxidative alterations to biomolecules occur. One consequence is protein oxidation, particularly in the respiratory chain, leading to structural alterations and consequent inhibition of mitochondrial respiration with consequent decreased ATP synthesis (Powers et al., 2007).
Besides oxidative damage per se, reperfusion has also a negative impact on intracellular pH and Ca\(^{2+}\) homeostasis. Excessive influx of Ca\(^{2+}\) into the mitochondrion during reperfusion can also lead to inhibition of oxidative phosphorylation and increased permeability of the IMM by means of increased mitochondrial permeability transition (Baines C.P.,2009).

**Calcium, the Permeability Transition Pore (PTP) and Cell Death:**

Various studies have shown that apoptosis is preceded by alterations in the mitochondrial membrane that result in the loss of the normal electrochemical gradient and lead to the phenomenon known as the mitochondrial permeability transition (MPT), production of ROS and release of apoptotic factors, such as cytochrome c and the apoptosis-inducing factor (AIF), into the cytosol (Skulachev V. P.,2000).

Two mechanisms have been proposed to explain the release of cytochrome c from the mitochondrial intermembrane space:

1) **The first mechanism:**

   It implies the MPT, with consequent rupture of the outer mitochondrial membrane (OMM) after mitochondrial swelling, together with loss of transmembrane potential and loss of several molecules (Halestrap A. P.,2009).

2) **The second mechanism:**

   It is the passage of cytochrome c and other pro-apoptotic proteins through specific pores formed from complexes between pro-apoptotic proteins, such as Bax, and the voltage-dependent anion channel (VDAC), at the OMM (outer mitochondrial
membrane). It is thought that some pro-apoptotic signals may induce the translocation of Bax to mitochondria and/or the activation of Bax by another pro-apoptotic protein, Bid (Fig. 7) (Halestrap A. P., 2009).

In either case, this mechanism does not require any change in the properties of the IMM (Inner mitochondrial membrane) (Borutaite et al, 2003).

One important aspect of the MPT is that it may play a central role in cell death by either necrosis or apoptosis. It has been demonstrated that the MPT can be induced by conditions that may occur during I/R, such as the accumulation of inorganic phosphate, oxidation of pyridine nucleotides, oxidative stress, decrease/oxidation of GSH (Reduced glutathione) and lower matrix pH (Baines C. P., 2009).

Although MPTP (Mitochondrial permeability transition pore) opening is strongly inhibited by acidosis during ischemia, it is favoured by ATP depletion, oxidative stress and high intramitochondrial Ca\(^{2+}\) concentrations, conditions all concurrent during myocardial reperfusion. Another critical agent in the induction of apoptosis is Ca\(^{2+}\). In the heart tissue, physiological Ca\(^{2+}\) spikes increase intramitochondrial Ca\(^{2+}\) concentration that acts as a signal for increased ATP production because of several inter-connected mechanisms (Dedkova et al., 2008).

However, a concerted action of the Na\(^+\)/H\(^+\) (due to increase in acidosis during the ischemic period) and Ca\(^{2+}\)/Na\(^+\) antiporters will cause cytosolic calcium overload during the reperfusion phase as acidic intracellular pH recovers toward normal values, causing a defective contractile function and mitochondrial dysfunction, though several pathways, including activation of the MPTP and calpain stimulation (Chen et al., 2002).
Cellular and molecular basis of ischemia-reperfusion injury:

These are summarized in the table below:

*Table 2. Effects of ischemia-reperfusion injury (Buja, L. M., 2005).*

<table>
<thead>
<tr>
<th>Molecular effects</th>
<th>Cellular effects</th>
</tr>
</thead>
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<tr>
<td>ATP=Adenosine-triphosphate depletion.</td>
<td>Endothelial cell dysfunction/swelling.</td>
</tr>
<tr>
<td>Defective ATP-resynthesis.</td>
<td>Leukocyte (PMN) recruitment.</td>
</tr>
<tr>
<td>Increase in hypoxanthine.</td>
<td>Oxidative burst (PMN)= Neutrophil granulocytes.</td>
</tr>
<tr>
<td>Activation of xanthine oxidase.</td>
<td>Impaired vasodilatation (NO-mediated).</td>
</tr>
<tr>
<td>Generation of ROS (Reactive oxygen species)</td>
<td>Enhanced vasoconstriction (endothelin-mediated).</td>
</tr>
<tr>
<td>(O2−=superoxide anion, H2O2=hydrogen peroxide, OH=hydroxide radical, ONOO− =peroxynitrite).</td>
<td>Endothelial barrier disruption.</td>
</tr>
<tr>
<td>Antioxidant (glutathione) depletion.</td>
<td>Expression of adhesion molecules (P/L-selectin, ICAM-1/2).</td>
</tr>
<tr>
<td>Intracellular Na+ and Ca++-overload.</td>
<td>Liberation of matrix-degrading proteases (Elastase, MMP-9).</td>
</tr>
<tr>
<td>Activation of NHE=sodium (Na+)-hydrogen (H+)-exchanger.</td>
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<tr>
<td>Activation of PLA2.</td>
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<tr>
<td>Activation of PARP=Poly (ADP-ribose) polymerase.</td>
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<tr>
<td>Activation of NFκB.</td>
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<td>Activation of Toll-like receptor-signaling.</td>
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<td>Subcellular effects</td>
<td>Mediators</td>
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<td>----------------------------------------------------------</td>
<td>--------------------------------------------------------------------------------</td>
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<tr>
<td>Mitochondrial dysfunction/swelling.</td>
<td>Arachidonic acid metabolites</td>
</tr>
<tr>
<td>Translocation of bax=Regulator of apoptosis.</td>
<td>(LTB4=leukotriene, TXA2=thromboxan).</td>
</tr>
<tr>
<td>Efflux of cytochrome c.</td>
<td>Cytokines (IL-1b=Interleukin-1b, IL-6, TNF-a=Tumor necrosis factor).</td>
</tr>
<tr>
<td>Lipid peroxidation.</td>
<td>Chemokines (IL-8, MCP-1).</td>
</tr>
<tr>
<td>DNA strand breaks.</td>
<td>Activated complement (C3a, C5a, C5b-9).</td>
</tr>
<tr>
<td>Cell membrane damage.</td>
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</tr>
<tr>
<td>Increased cell membrane permeability.</td>
<td></td>
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<tr>
<td>Cytoskeletal derangements.</td>
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</tbody>
</table>

**Endothelial cell-dysfunction and leukocyte adhesion:**

Endothelial cell dysfunction occurs as a consequence of cell injury during I/R and is likely caused by the concert of oxidative damage to membranes, dysregulation of ion homeostasis and osmotic stress. In addition to endothelial cell swelling, IRI (ischemia reperfusion injury) is known to cause many additional changes in endothelial cells including increased membrane permeability, cytoskeletal derangements and recruitment of inflammatory cells *(Riedl et al., 2007).*

One of the most sensitive indicators of EC (endothelial cell) dysfunction is impaired endothelium-dependent vasodilatation that is mediated by NO(Nitric oxide) and during IRI synthesis of NO by eNOS (endothelial nitric oxide synthase) or iNOS (Inducible nitric oxide synthase) may be decreased by reduced availability of the
precursor L-arginine or by depletion of the cofactor tetrahydrobiopterin (BH4) (Riedl et al., 2007).

The release of endothelin-1, the most powerful vasoconstrictor, is dramatically increased following reperfusion and further capacity to cause vasospasm is conferred by leukotriene B4, activated complement components and thromboxan A2 that are liberated during the inflammatory reaction induced by reperfusion. In addition, the acute endothelial dysfunction may result in endothelial swelling a result of influx of water and sodium during ischemia and enhanced vasoconstriction, all of which can work in concert narrowing the capillary lumen and elevating hydraulic resistance to impair perfusion at the microvascular level despite adequate restoration of blood flow (Menger et al., 1997).

Additionally, disruption of the endothelial barrier due to disorganization of junctional adhesion proteins, increased fluid filtration at the capillary level and macromolecular leakage results in reduced capillary perfusion. The pathological sequence of endothelial integrity disruption and fluid loss with the consequence of intravascular hemoconcentration seems to be the most likely cause for the capillary “no-reflow” phenomenon, whereas blood coagulation, platelet aggregation and leukocyte plugging appear not to be a significant mechanism, although early reports have attributed the so called “no-reflow” phenomenon to blockage of capillaries by neutrophils preventing reperfusion and resulting in extensive capillary damage and myocardial cell swelling (Kloner RA et al., 1974).

The process of leukocyte adhesion is initiated by P-selectin that is expressed on endothelial cells and is primarily responsible for the initial tethering of granulocytes (PMN) in the microvessels (Springer TA., 1994).
Release of Inflammatory Mediators (Cytokines, Chemokines, Activated Complement):

Conclusive evidence demonstrates the involvement of proinflammatory cytokines such as Tumor necrosis factor (TNF-a), Interleukin-1b (IL-1b) and Interleukin-6 (IL-6) in the postischemic response. This is corroborated by the finding, that both defective IL-1b signaling as well as TNF-a signaling resulted in decreased chemokine upregulation and attenuated neutrophil infiltration and that in a selection of patients with episodes of ischemia/reperfusion (major blunt trauma, ruptured aortic aneurysm) increased levels of TNF-a, IL-1b and IL-6 are associated with increased mortality and increased risk for ARDS and MOF (Roumen RMH et al., 1993).

Clinical Manifestations of Reperfusion Injury:

The clinical manifestations of I/R are diverse and may include:

- Myocardial hibernation/stunning.
- Reperfusion arrhythmias.
- Impaired cerebral function.
- Breakdown of the gastrointestinal barrier.
- Systemic inflammatory response syndrome (SIRS).
- Multiorgan dysfunction syndrome (MODS) (Collard CD et al., 2001).
(4)
SURGICAL TECHNIQUES IN CARDIAC SURGERY
SURGICAL TECHNIQUES IN CARDIAC SURGERY

Surgeons returned from the second world-war after exposure to military surgery, and had developed an interest in the treatment of traumatic chest wounds. This renewed interest in cardiac surgery led to great expansion of the specialty in 1950. Cardiac surgery developed later than other surgical specialties, largely due to the technical difficulties of operating on the heart. The surgeon could not support the circulation while working on the heart and this limited the kind of surgery that could be done upon the heart (Wang et al., 1999).

Myocardial revascularization:

It was only when coronary arteriography was accidentally discovered at the cleveland clinic by Sones in 1958 that a rational basis for surgical myocardial revascularization with a coronary artery bypass grafting (CABG) procedure was established by Effler and Favaloro (Diegler et al., 2000).

Off pump beating heart surgery:

By using innovative techniques to manipulate and stabilize the heart and achieve myocardial protection, it is possible to achieve total revascularization for a patient with multivessel coronary artery disease. This technique, also popularly known as OPCAB (off-pump coronary artery bypass), has the most versatility for surgeons wanting to avoid extracorporeal circulation for coronary bypass operations (Diegler et al., 2000).

The feasibility of performing total myocardial revascularization on the beating heart is largely dependent on the ability to expose all coronary targets and to minimize myocardial ischemia and hemodynamic instability during the operation (Imasaka et al., 2000).
Manipulation of the heart is necessary during beating heart coronary artery bypass grafting, and this makes the myocardium more susceptible to ischemia. After completion of the coronary grafting, reperfusion injury in the revascularized myocardium needs to be avoided (Imasaka et al., 2000).

Intraoperative ischemia in the unprotected myocardium can lead to perioperative myocardial infarction with all its attendant complications, intraoperative arrhythmias and hemodynamic alterations, all of which may lead to inability to complete revascularization and thus an incomplete operation. It may also lead to intraoperative hemodynamic collapse necessitating the use of extracorporeal circulation for assistance (Wang et al., 1999).

The role of the anesthesiologist during this operation cannot be overemphasized. The anesthesiologist is a critical member of the operative team and must be closely involved throughout the procedure. Routine monitoring of the patient’s hemodynamic status, the ECG, and the intraoperative transesophageal echocardiogram (TEE) are just some of the important roles of the anesthesiologist. He/she must be aware of every step of the operation to be able to cooperate with the surgeon during the procedure (Bull et al., 2001).

Maintenance of stable hemodynamic indices using mechanical or pharmacological means of support at appropriate time during the operation is critical to the success of the procedure. Mechanical support may mean as little as positioning of the operative table to allow easier manipulation of the heart and improved exposure of the target vessel (Sabik et al., 2002).

Pharmacological support during OPCAB may take many different forms. Preoperative administration of beta-blockers has been shown to conclusively reduce the incidence of perioperative myocardial infarction in patient with coronary artery disease
undergoing any kind of cardiac or non-cardiac surgery. Most patients undergoing CABG would be already on beta-blocker therapy, but if they are not, it should be started (Eagle et al., 2002).

Fig. (9): Attachment of an apical suction device to the epicardial surface to facilitate exposure of the posterior circulation without compression or distortion of the right ventricle (Eagle et al., 2002).
Beta-blocker drugs are thought to have a cardioprotective effect, thus reducing the overall incidence of perioperative myocardial infarction. Ultra short acting beta-blockers were used commonly to induce bradycardia during the early stages of development of stabilizing devices to help achieve a less mobile target during performance of distal anastomosis. Recognition of improved outcomes in selected patients with the elimination of cardiopulmonary bypass serves as the impetus to develop off-pump coronary artery bypass (OPCAB) as a treatment option for multivessel coronary artery disease (*Trehan et al.*, 2001).

**On pump beating heart surgery:**

OPCAB can be performed for the large majority of patients with coronary artery disease to achieve complete revascularization using the basic principles of exposure and myocardial protection. However, persistent hemodynamic instability may occasionally not allow progression of the procedure in an off-pump fashion (*Guyton et al.*, 2000).

This may be due to several factors. Global ventricular dysfunction may occur from sequential periods of regional ischemia or the heart may not do well even with minimum manipulation, necessitating abandonment of the off-pump procedure. If the patient arrives to the operative room in a relatively unstable condition, further manipulation of a sick, ischemic heart may injure the myocardium and set the stage for the development of malignant ventricular arrhythmias, cardiogenic shock, or intraoperative cardiac arrest. A vicious circle is set up in this circumstance due to the decrease in cardiac output, leading to further deterioration of coronary perfusion superimposed on already ischemic myocardium (*Puskas et al.*, 2001).

In these situations, the risk of global myocardial ischemia overshadows the use of extracorporeal circulation. One approach is to initiate cardiopulmonary bypass with standard aortic and venous cannulas, and perform the grafts with the heart beating but
supported, the operation can still be carried out on the beating heart to achieve complete revascularization of all target intended coronary arteries. The heart can be emptied, the myocardium can be rested, and systemic perfusion is maintained, no cross clamping of the aorta is necessary and it is not necessary to give cardioplegia (Perrault et al., 1997).

Since the heart is kept beating and the perfusion is maintained by extracorporeal circulation, the demands of the myocardium will be met till such time that adequate revascularization can be completed (Perrault et al., 1997).

Another way to perform OPCAB with some adjunctive help is using perfusion assisted direct coronary artery bypass (PADCAB), with this technique distal anastomoses are constructed first, as is done in OPCAB, after which the grafts are proximally connected to the outflow of a small pump circuit, where the inflow of the circuit is provided by a small cannula placed in the ascending aorta or the femoral artery, this circuit comprises a pump system called the Quest Medical MPS (Quest Medical, Allen, TX) which allows accurate control of coronary artery perfusion pressure as well as allowing the addition of various chemical additives in exact concentration at specified temperature. This enables maintenance of the coronary circulation despite changes in systemic pressure, but only after the construction of the distal anastomosis (Steele et al., 2000).

Once the proximal and distal anastomoses have been completed, it is extremely important to know that these anastomoses are patent and allow the grafts to function as they are supported to. This can be done by measuring the flows in all grafts prior to closure of the chest to document patency of the anastomoses (D’Ancona et al., 2001).
On pump coronary artery bypass grafting:

Conventional CABG with CPB is still the most commonly performed cardiac surgical procedure. This method results in high quality anastomoses and excellent clinical results (de Jaegere et al., 2002).

Nevertheless, complications may be associated with the systemic inflammatory response following CABG. The inflammatory response has been related to anesthesia, the surgical trauma per se, cardioplegia, ischemia-reperfusion and use of the heart–lung machine. Thus, the increasing number of CABG without CPB (off-pump operations, OPCAB), has renewed the scientific interest in the systemic inflammatory response following heart surgery (Shennib, 2001) (Czerny et al., 2000).

Recent publications on randomized studies comparing on- and off-pump procedures, showed comparable cardiac outcome, and significantly lowered in-hospital morbidity without compromising outcome in OPCAB patients (Angelini et al., 2002) (Nathoe et al., 2003).

In addition, reduced cytokine responses and less myocardial injury are observed in patients undergoing OPCAB compared to CABG with CPB (Wan et al., 1999).
(5)

Myocardial Protection
Myocardial Protection

Myocardial protection is a multifactorial problem that extends well beyond the scope of cardioplegic delivery techniques alone. Factors preceding the operation, including the patient’s age, disease and degree of illness, determine the environment within which myocardial ischemia and reperfusion occur. Events in the operating room, including anesthetic induction in preparation for cardiopulmonary bypass, the process of separation from bypass and stabilization thereafter, also have a direct impact on the patient’s tolerance of the perioperative period of myocardial ischemia (Murashoita et al., 1990).

Myocardial protection Techniques:

The anesthesiologist and cardiac surgeon must be familiar with alternative methods of myocardial protection to allow flexibility when standard techniques are either ineffective or not applicable to a specific clinical circumstance (Lu et al., 1997).

**Noncardioplegic Techniques**

1. Systemic Hypothermia with Ventricular Fibrillation:

   Systemic hypothermia with induced fibrillation of the heart was introduced as a method of myocardial preservation in the earliest years of cardiac surgery. Although this method has largely been replaced by chemical cardioplegic arrest, it continues to be used by some with satisfactory results. This technique is an alternative to cardioplegic arrest when aortic clamping is not possible or is dangerous (i.e., with a heavily plaqued or calcified aorta). The technique can be also used when patent internal mammary grafts cannot be temporarily occluded and therefore prevent adequate regional administration of
cardioplegia by antegrade or retrograde techniques. It is commonly employed for procedures requiring only a short period of aortic occlusion (i.e. atrial septal defect closure, single coronary bypass). It has been also employed for multiple coronary artery bypass grafting where only the distal anastomosis is done with complete aortic cross clamping and ventricular fibrillation and the proximal anastomosis done with beating heart (Akins, 1987).

2. 

Ischemic Arrest with Hypothermia:

This technique finds its origin in the earliest days of cardiac surgery. It was the method of choice for many years before the popularization of chemical cardioplegic arrest. In contrast to fibrillatory arrest, ischemic arrest requires aortic clamping (Buckberg, 1991).

3. Continuous Coronary Perfusion:

This method of myocardial protection provides continuous blood perfusion of the beating, non-working heart. Perfusion is carried out either by aortic root infusion or by direct coronary ostial intubation and perfusion with blood from the pump oxygenator. This technique is considered unsafe for open heart repairs because of the risk of air embolism. It also represents a significant technical disadvantage for coronary bypass surgery because of the continuous flow of blood in the coronary system and because of the beating, nonarrested heart. It may, however, have specific utility in arrhythmia surgery in which normal atrioventricular conduction is desirable to detect possible injury to the conduction system or His bundle and to map the location of ectopic arrhythmogenic foci. Prolonged periods of normothermic perfusion are to be avoided, however, owing to evidence that this enhances myocardial edema formation. Direct coronary ostial perfusion presents the added risk of ischemic pressure injury of the ostial endothelium, with resultant coronary ostial stenosis (Buckberg, 1986).
4.Ischemic Preconditioning:

Single or repeated brief periods of myocardial ischemia after reperfusion increase myocardial tolerance to subsequent long-term ischemic insult. It has been discovered that the heart has its own systems of protection against ischemia-reperfusion injury. Studies of preconditioning revealed that two 3-minute ischemic periods each followed by 2 minutes of reperfusion produced a higher adenosine triphosphate content in the myocardium than no exposure to preceding brief ischemic periods after 10 minutes of aortic cross-clamping in a coronary artery bypass program. The precise mechanism of ischemic preconditioning remains unknown (Zhong-kai et al., 2000).

A variety of agents have been proposed to mediate the protection of preconditioning; however, two signaling pathways have emerged in animal models: adenosine activation and alpha₁-adrenergic activation. Adenosine is released by ischemic myocytes and has protective effects when infused before ischemia (Cleveland et al., 1996).

Pharmacologic preconditioning might offer an alternative method to protect the myocardium with adenosine or an α₁-adrenergic agonist (phenylephrine) before ischemia-reperfusion injury (Lu et al., 1997).

Preconditioning can be used for protection during prolonged hypothermic storage of a donor heart, a measure that may increase the availability of donor organs and make transplantation of the heart even more safe (Engelman et al., 1995).
**Chemical Cardioplegic Techniques**

The main reason for using cardioplegia is to maintain the heart in diastolic arrest while the aorta is clamped and there is no coronary blood flow. By chemically inducing diastolic arrest and immediately terminating electromechanical work, heart’s energy requirements are lowered, and waste of myocardial adenosine triphosphate and substrates needed to sustain basal cell metabolism and integrity during the period of ischemia is avoided. In addition, the cardioplegic solution can be used to assist in the uniform cooling or warming of the arrested heart, to maintain appropriate pH, provide substrates, avoid myocardial edema and to avoid cellular reperfusion damage (Booker, 1998).

A growing number of cardioplegic solutions with various components and methods of administration have been promoted. Despite variation in formulation, two broad types of cardioplegic solutions can be defined; crystalloid cardioplegia and blood cardioplegia (Morgan and Mikhail, 1996).

A. Crystalloid Cardioplegia:

Crystalloid cardioplegic solutions can principally be divided into the intracellular and extracellular types. This division is based on the relative content of sodium and potassium in the solution, and therefore the means of inducing myocardial arrest (Langer, 1993).

Intracellular Solutions:

Intracellular solutions, initially designed for long-term organ preservation, have a low sodium content and a moderate potassium content, much like one would find in the cell cytoplasm. Diastolic arrest is induced with these solutions by rapid extracellular sodium depletion, causing a loss in the transmembrane sodium gradient. Intracellular
solutions are rarely used, mainly because of the concern of secondary calcium influx resulting in poorer mitochondrial preservation (Langer, 1993).

**Extracellular Solutions:**

Extracellular solutions are composed of sodium and chloride concentrations that are similar to those of plasma, with exogenous potassium added to induce and maintain diastolic arrest of the heart. The resulting increase in extracellular potassium concentration reduces the transmembrane potential (less negative inside). The latter progressively interferes with the normal sodium current during depolarization, decreasing the rate of rise, amplitude and conduction velocity of subsequent action potentials. Eventually, the sodium channels are completely inactivated, action potentials are abolished and the heart is arrested in diastole (Rosenkranz, 1994).

**Table (3):** Typical components of cardioplegia solutions (Arnold, 1991).

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
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<tbody>
<tr>
<td>Potassium</td>
<td>15-40mEq/L</td>
</tr>
<tr>
<td>Sodium</td>
<td>100-120meq/L</td>
</tr>
<tr>
<td>Chloride</td>
<td>110-120mEq/L</td>
</tr>
<tr>
<td>Calcium</td>
<td>0.7 mEq/L</td>
</tr>
<tr>
<td>Magnesium</td>
<td>15 mEq/L</td>
</tr>
<tr>
<td>Glucose</td>
<td>28 mmol/L</td>
</tr>
<tr>
<td>Bicarbonate</td>
<td>27mmol/L</td>
</tr>
</tbody>
</table>

A large variety of solutions have been described. By far, the most widely used solutions have the following components:
**Potassium:**

Most clinically used solutions employ potassium concentrations of 20-40meq/L. Excessively high potassium concentrations are potentially injurious to the myocardium. Myocardial contracture or “stone heart” could be induced in hearts exposed to solution containing more than 100meq/L of potassium (Rich and Brady, 1994).

**Sodium:**

Intracellular sodium and calcium concentrations are interrelated. These two ions can be exchanged via a common channel termed the sodium-calcium exchanger. Perfusion of isolated hearts with low sodium perfusate causes calcium-dependent increase in diastolic pressure, energy consumption and dissociation of oxygen consumption and work. The ideal sodium concentration for standard cardioplegia is probably between 100-120meq/L and chloride concentration is 110-120meq/L (Morgan and Mikhail, 1996).

**Calcium:**

Loss of cell membrane control over calcium influx during postischemic reperfusion leads to a rise in intracellular calcium, increasing energy utilization and adenosine triphosphate consumption, producing myocardial contracture. Excessive calcium deposition can be avoided by reducing calcium content in the cardioplegic solution. On the other hand, very low calcium content (less than 0.05mmol/L) must be avoided to prevent destabilization of cell membrane (Rosenkranz, 1994).

Hypothermia (18°C) prevents the membrane damage seen with calcium depletion. Tolerance to calcium depletion diminishes with increasing temperature (Langer, 1993).

**Magnesium:**

Magnesium is lost from the myocardium during ischemic arrest. This loss may result in impairment of myocardial recovery. It is hypothesized that the inclusion of
magnesium in cardioplegia improves myocardial function because of its major role as a cofactor in several myocardial enzyme systems and in controlling excessive influx of calcium intracellularly. A magnesium concentration of 15meq/L was found to be optimum for myocardial recovery (*Morgan and Mikhail, 1996*).

**Buffers:**

Aerobic metabolism during aortic occlusion induces tissue acidosis, which can be adequately buffered by adjusting the pH of the cardioplegic solution. The bicarbonate ion concentration commonly used in cardioplegic solution is 20-30mmol/L. However, the bicarbonate is considered ineffective buffer of intracellular pH change. Phosphate is more effective in buffering intracellular pH changes. Tromethamine (THAM) and histidine are also effective in increasing intracellular pH. However, THAM is toxic to certain tissues. Histidine buffering maintains adenosine triphosphate levels during ischemia and prevents glycogenolysis by promoting utilization of glucose (*Del Nido, 1995*).

**Substrates:**

Aerobic glycolysis of glucose from limited myocardial glycogen stores is the main source of myocardial adenosine triphosphate generation during ischemia. Therefore, glycolysis could be maintained and energy stores preserved by inclusion of glucose in the cardioplegic solution. However, profound hyperglycemia may increase patient susceptibility to neurologic injury mediating a cautious use of glucose (*Ning et al., 1996*).

Laboratory and clinical evidence clearly documents a marked increase in aerobic amino acid metabolism and adenosine triphosphate generation during ischemia and the period following reperfusion. Hearts arrested using glutamate-aspartate cardioplegia achieved earlier metabolic recovery (*Cohen et al., 1999*).

Adenine nucleotides that result from adenosine triphosphate utilization are freely diffusible from the myocardial cell and are lost in the coronary venous blood. Supplying
the heart with adenine nucleotide precursors (e.g. adenosine, ribose) was associated with enhanced postischemic function (Rosenkranz, 1994).

**Local anesthetics:**

Local anesthetics have been added to cardioplegia for membrane stabilization. Adding lidocaine (0.1mg/ml) to cardioplegia was associated with spontaneous return of sinus rhythm in 74% of patients versus 22% of controls. Left ventricular function was minimally depressed 30 minutes after reperfusion, but this effect was transient and of no clinical importance (Fiore et al., 1990).

**B. Blood Cardioplegia:**

It is usual to use a 4:1 mix of blood to crystalloid cardioplegia. The crystalloid solution is prepared beforehand to achieve the desired final compositions. Blood for blood cardioplegia is obtained from the arterial line of the CPB. Blood for blood cardioplegia is obtained from the cardioplegia cannula inserted in the aortic root and from the arterial line of CPB (Barner, 1991).

**Temperature of cardioplegic solution :**

There are 3 levels of temperature commonly used in cardioplegia: cold (8°C), tepid (29°C) and warm (37°C). Intermediate lukewarm (20°C) cardioplegia is recently being evaluated for the quality of myocardial protection as a temperature halfway between the cold and tepid levels (Chocron et al., 2000).

The lowest oxygen demands occur when the heart is arrested and decompressed, since electromechanical work is stopped completely. Normothermic arrest (i.e. at 37°C) reduces oxygen demands by approximately 90% to only 1ml/100gm/min. Hypothermia
reduces oxygen demands further, so that myocardial oxygen requirements in the arrested heart at 22°C is only 0.3ml/100gm/min or 97% below the requirements of the beating working heart (and 60% less than fibrillating or beating heart at 22°C). Reducing myocardial temperature to 10°C lowers oxygen requirements to approximately 0.14ml/100gm/min, which is only slightly less than the 97% reduction achieved at 22°C. Since reduction of myocardial temperature below 15°C has an almost immeasurable effect on myocardial oxygen requirements, concentrated efforts at achieving deeper levels of cardiac cooling make little physiologic sense (Chocron et al., 2000).

**Cardioplegic Delivery:**

Cardioplegic solution in adequate amounts must be distributed to all segments and layers of the myocardium to provide adequate myocardial protection. Several factors are important in determining regional cardioplegic delivery. The most obvious problem is coronary artery obstruction that results in nonhomogeneous delivery of antegrade cardioplegia to the myocardium. Although substantial collateral vessels may exist in patients with coronary artery disease, these vessels may not adequately supply cardioplegic solution to protect the myocardial tissue distal to significant coronary obstruction (Quintilio et al., 1995).

Similarly, aortic valve insufficiency may prevent adequate filling of the aortic root and therefore may compromise adequate antegrade cardioplegic administration. If the aortic root is open, as during aortic valve replacement, then direct cannulation of the coronary ostia is required for antegrade cardioplegic delivery. During mitral valve surgery, retraction of the atrium often distorts the aortic root, resulting in aortic valve incompetence, which prevents adequate antegrade cardioplegic delivery without intermittent removal of the retraction apparatus (Quintilio et al., 1995).
The hypertrophied heart presents additional problems with cardioplegic delivery to the subendocardium, despite unobstructed coronary arteries. This is so because of increased coronary vascular resistance, especially when the ventricle is empty and its volume is decreased, as in the beating empty or arrested states *(Rosenkranz, 1994).*
Ischemic preconditioning
Ischemic preconditioning

Introduction:

'Hearts live by being wounded'

Oscar Wilde ("A Woman Of No Importance", Act 3)

Whereas it was initially believed that repeated episodes of angina may result in a worsened cardiac insult, there is evidence that brief episodes of transient ischemia may actually have a protective effect. Lange et al. first demonstrated that the rate of ATP depletion is less upon repeated ischemic episodes compared to those associated with a single ischemic episode (Lange et al., 1984).

Patients with preinfarction angina were less likely to experience severe congestive heart failure or shock (1%) versus patients without preinfarction angina. There is also another study which further confirmed that preinfarction angina is protective in the setting of acute Q wave or ST segment elevation myocardial infarction, resulting in decreased infarct size, and better clinical outcome (Rezkalla et al., 2004).

Since irreversible damage begins to occur within approximately 20 min of occlusion and is complete within 6 h, reperfusion should be accomplished as soon as possible. It should be noted that there is concern that reperfusion itself may cause tissue injury. Thus, while reperfusion is necessary to salvage the myocardium, it may also kill some of the tissue in the process (Rezkalla et al., 2004).

Finding a way to render the heart resistant to ischemia and/or reduce reperfusion injury would undoubtedly improve the outcome following acute myocardial infarction.
(MI). While much of current treatment is devoted to treating arrhythmias and remodelling associated with infarction, surprisingly little is being done to promote myocardial salvage during the acute phase in these patients other than simply reducing the delay before reperfusion (Zhang et al., 2012).

In 1986, Murry et al. published a remarkable study showing that it was indeed possible to render the myocardium less susceptible to ischemia-induced infarction. They protected hearts against infarction by subjecting them to several brief periods of ischemia prior to the prolonged lethal ischemic insult. They reported that this “ischemic preconditioning” (IPC) resulted in decrease of infarct sizes that were approximately 25% of those observed in untreated hearts (Murry et al., 1986).

This not only demonstrated that protection of the myocardium from ischemia/reperfusion is possible, but it also provided a model with which to study ways to protect the ischemic heart. All that remained was to determine IPC’s mechanism (Rezkalla et al., 2004).

**Adenosine, bradykinin and opioids trigger IPC:**

In 1991, Liu et al. discovered that stimulation of the $G_i$-coupled adenosine $A_1$ receptor was necessary to trigger IPC’s protection. They showed that IPC’s protection could be blocked by an adenosine receptor antagonist (Liu GS et al., 1991).

Two other endogenously released trigger substances, bradykinin and opioids, were subsequently found to be involved in the protective effect of IPC and appeared to work in parallel. Inhibition of any one of these three receptors blocked IPC’s protection from a single preconditioning cycle. However, it was found that protection could again be realized if the number of preconditioning cycles was increased (Schultz et al., 1995).
This led Goto et al. to suggest that the three receptors had an additive effect necessary to reach a hypothetical protective threshold. It was theorized that the additional brief ischemia/reperfusion cycles produced more of the trigger substances so that the two receptors that were not inhibited could eventually reach the protective threshold without the participation of the third (Rezkalla et al., 2004).

All of these three trigger substances work through G\textsubscript{i}-coupled receptors. The multiple trigger theory requires that all triggers converge on a common target. It was found that protection afforded by all of the trigger substances could be blocked by protein kinase C (PKC) inhibitors and PKC is thought to be this common target. Ligands to several other G\textsubscript{i}-coupled receptors in the heart were also found to have the ability to mimic preconditioning through PKC activation including catecholamines, angiotensin II, and endothelin. This indicates that virtually all G\textsubscript{i} protein-coupled receptors (G\textsubscript{i};PCR) in the heart can trigger the IPC phenotype (Wang et al., 1996).

**IPC’s trigger pathway:**

Pain et al. proposed an overview of IPC’s trigger mechanism. They hypothesized that receptor stimulation led to the opening of mitochondrial ATP sensitive potassium channels (mitoK\textsubscript{ATP}), causing oxygen-based free radicals (reactive oxygen species or ROS) to be made by the mitochondria which then in turn activated PKC. This pathway is called the “trigger phase” (Fig. 11) because after a brief exposure to the ligand the heart remains protected for about an hour even after the trigger substance has been washed out (Pain et al., 2000).
The mechanism of this memory is still unknown but is thought to be related to PKC. In preconditioning the receptors are occupied during the brief periods of ischemia and ROS are thought to be made during the reperfusion periods following reintroduction of oxygen. The requirement for ROS production explains why the trigger ligands do not protect the heart with a simple coronary occlusion: oxygen is not available for ROS to be produced (*Pain et al.*, 2000).

The discovery of ROS involvement in IPC provided us with an easily measured parameter for a cell-based model with which to study IPC’s trigger pathway. When isolated adult cardiomyocytes are exposed to trigger ligands their ROS production can be observed with a ROS-sensitive fluorochrome (*Oldenburg et al.*, 2003).
Fig. 11 Flow chart of trigger (left) and mediator (right) phases of preconditioning. The former is operative prior to the index ischemia, while the latter occurs during the ischemia and following reperfusion. Abbreviations: $A_1R$, $A_2bR$, $A_3R$ = adenosine $A_1$, $A_2b$, $A_3$ receptors EGFR = epidermal growth factor receptor ERK = extracellular signal-regulated protein kinase GC = guanylyl cyclase GSK-3β = glycogen synthase kinase-3β mPTP = mitochondrial permeability transition pore NOS = nitric oxide synthase PI3-K = phosphatidylinositol 3-kinase PKC, PKG = protein kinase C, G (Van et al., 1991).
1) **PI3-kinase:**

Tong et al. showed that PI3-kinase (phosphatidylinositol 3-kinase) activation is involved in triggering preconditioning and that it occurs upstream of PKC activation, and Mocanu et al. saw attenuation of IPC’s cardioprotective effect on infarction following PI3-kinase inhibition with either wortmannin or LY 294002 (*Mocanu et al., 2002*) (*Tong et al., 2000*).

2) **Nitric oxide synthetase and PKG:**

Induction of nitric oxide synthase (NOS) was shown to be important in second window preconditioning. Lochner et al. had evidence that NOS was also involved in acute IPC although its location in the signaling pathway was unknown (*Lochner et al., 2000*).

NO is a known activator of guanylyl cyclase (GC) which stimulates the production of cGMP and activates PKG (protein kinase G). Oldenburg et al. found that protection from bradykinin could be aborted by administering the soluble GC inhibitor ODQ (*Oldenburg et al., 2004*).

In 2005, Costa et al. showed that addition of exogenous PKG and cGMP to isolated mitochondria resulted in the opening of mitoK\textsubscript{ATP}. PKG-dependent channel opening could be blocked by mitoK\textsubscript{ATP} inhibitors 5-hydroxydecanoate, glibenclamide, and tetraphenylphosphonium. The mitoK\textsubscript{ATP} are located on the inner mitochondrial membrane, and cytosolic PKG would not be able to penetrate the outer membrane (*Costa et al., 2005*).
3) $K_{\text{ATP}}$ and ROS:

The opening of mito$K_{\text{ATP}}$ channels results in an influx of potassium that causes swelling of the mitochondria and this is thought to somehow lead to production of ROS. Baines et al. and Tritto et al. simultaneously showed that ROS are involved in IPC’s trigger pathway (Baines et al, 1997) (Tritto et al, 1997).

IPC exerts its protection at reperfusion:

Jennings’s group originally proposed that IPC exerted its protection by preserving ATP during the ischemic period, and that reperfusion simply allowed the cells that survived to recover from the damage caused by ischemia (Murry et al., 1991).

The mitochondrial permeability transition pore (MPTP):

Hausenloy et al. recently proposed that the final step of the signaling pathway is inhibition of the MPTP. The pore is thought to be formed by alignment of the adenine nucleotide translocator (ANT) on the inner membrane and the voltage-dependent anion channel (VDAC) on the outer membrane. The pore connects the matrix directly to the cytosol and is formed during the first few minutes of reperfusion as a result of calcium overload, oxidative stress, and ischemic injury singly or in combination (Hausenloy et al., 2004).

After ischemia/reperfusion, it is proposed that there are three different populations of cells in the heart: those that are dead from ischemic injury, those that have suffered a sub-lethal injury and will recover, and those that are alive but will be killed at reperfusion after MPTP formation. Preconditioning protects by promoting survival of the third
population of cells following suppression of MPTP formation. MPTP formation results in a collapse of the mitochondrial membrane potential which blocks the cells’ ability to produce ATP at the critical time when the cells need ATP the most. Loss of energy for the sodium/potassium ATPase pumps leads to intracellular sodium accumulation along with water. This loss of volume regulation results in cellular swelling and rapid cell lysis (necrosis) (Ramnik et al., 2010).

MPTP also causes swelling of the mitochondria with many of them rupturing their outer membranes causing release of cytochrome C into the cytosol. If only a small percentage of a cell’s mitochondria experience MPTP formation, the cell may well survive reperfusion, but may later experience apoptosis triggered by cytochrome C. It has been noted that IPC does indeed reduce the incidence of apoptosis in the heart (Zhao et al., 2002).

Inactivation of the signaling kinase GSK-3β has been shown to strongly inhibit MPTP in cardiomyocytes. Phosphorylation of GSK-3β (glycogen synthase kinase-3β) can be accomplished in several ways. PKC can either directly phosphorylate it, or can act on it indirectly through ERK and PI3-kinase which in turn can phosphorylate sites on GSK-3β (Juhaszova et al., 2004).
Fig. 12 Signaling pathways involved in the pathogenesis of ischemia-reperfusion induced myocardial injury. PARP - poly (ADP Ribose) polymerase, MAPK - Mitogen-activated protein kinase, TNF-α - Tumour necrosis factor-α, JAK/STAT - janus kinase/signal transducer and activator of transcription, IL-6 - interleukin-6, PARG - poly (ADP Ribose) glycohydrolase, MEKI/2 - Mitogen-activated protein kinase kinase, JNK1/2-cjun-N-terminal kinase (Ramnik et al., 2010).
**Possible Clinical Indications of Preconditioning in Cardiac Surgery:**

Indeed, experimentally, the only situations in which preconditioning has been shown to confer additional protection to that of hypothermia and cardioplegia are long ischemic time, and inhomogenous delivery of cardioplegia due to proximal coronary artery blockade (*Galinanes et al, 1995*).

It is likely that in these two settings, the beneficial effects of preconditioning are due to a reduction in the amount of necrosis resulting from suboptimal cardioplegic protection (and, in fact, in the studies documenting an added benefit of preconditioning, the early postischemic leakage of creatine kinase was consistently greater in control than in preconditioned hearts) (*Cave et al, 1992*).

Thus, in clinical practice, high risk situations that could benefit from preconditioning may include:

- extensive coronary artery disease with poor collaterals that increases the risk of cardioplegia maldistribution even with the use of combined ante grade / retrograde perfusion;
- severe left ventricular hypertrophy (where subendocardial perfusion is problematic);
- anticipated long ischemic times including those incurred by cardiac allografts during cold storage;
- the senescent myocardium, more prone to develop tissue-damaging calcium overload, although the ability of the aged heart to respond to preconditioning remains controversial; and
- beating heart minimally invasive coronary’ artery’ bypass operations where local occlusion of the target vessel results in an unprotected distal ischemia that closely
mimics the experimental models of regional ischemia where the infarct limiting effect of preconditioning has been the most clearly demonstrated (David G.D. et al., 2011) (PG et al.,1996).

**Clinical implications of ischemic preconditioning:**

Two situations appear to lack the normal tissue adaptive process to ischemia, namely older age and diabetes. Patients who were older than 65 years of age did not show any clinical benefit from preinfarction angina. Only patients younger than 65 years with preinfarction angina had a lower incidence of cardiogenic shock, congestive heart failure, and in-hospital death. Preconditioning in the elderly was only preserved in patients who maintained a high level of exercise (Mocanu et al.,2002).

The lack of ischemic preconditioning effect does not seem to be limited to non-insulin diabetes mellitus but extends to all patients with hyperglycemia. Notably, use of sulfonylurea hypoglycemic agents may interfere with ischemic preconditioning, and their use is associated with incidence of increased cardiovascular events (Meier et al.,2004).

**Preconditioning and left ventricular function:**

Since preconditioning results in smaller infarction size, it preserves left ventricular function with less incidence of congestive heart failure. It was found that, preinfarction angina patients had smaller infarction size and the combined incidence of significant congestive heart failure or shock were 1% versus 7% in the control group (Zhang et al.,2012).
Ischemic preconditioning and arrhythmias:

Since, ischemic preconditioning results in smaller infarction size and less compromise to cardiac function. **Would the incidence of cardiac arrhythmias decrease concomitantly?**

Two controlled studies were conducted to test the hypothesis that ischemic preconditioning may decrease the incidence of ventricular arrhythmias:

1) Wu et al. 2002 (*Lochner et al., 2000*).

2) Gheeraert et al. 2001 (*Gheeraert et al., 2001*).

Both studies concluded that; ischemic preconditioning resulted in a lower incidence of cardiac arrhythmias in various clinical settings.

Preconditioning during percutaneous coronary intervention:

In the late 1970s, Andreas Gruntzig introduced percutaneous coronary angioplasty. A major limitation to sustained balloon inflation, in order to optimize balloon outcome, was the development of significant regional ischemia and consequent severe chest pain, hypotension, and cardiac arrhythmias. To avoid these complications, an alternative protocol was instituted consisting of multiple balloon inflations with each inflation lasting for only 60–90 s. Notably, ischemic changes observed during the first inflation were usually attenuated with subsequent inflations and initially this was attributable to collateral recruitment that occurs with the first inflation (*Costa et al., 2005*).

**Could preconditioning mimetic drugs be beneficial in the clinical setting for use during coronary intervention?** We believe it is unlikely since the majority of procedures are done with one brief inflation during stent development. A notable exception may be during acute coronary syndromes where the drug nicorandil may have
some potential as a preconditioning mimetic drug, as well as a known treatment for no-reflow phenomenon (Costa et al., 2005).

**Preconditioning and coronary artery bypass surgery:**

*(On-pump bypass surgery)*

Preconditioning mimetics were investigated during on-pump bypass surgery. However, the data did not always show a consistent benefit. Mentzer et al. showed that patients who received adenosine required less frequent use of postoperative dopamine or nitroglycerin. However, Belhomme et al. failed to show a benefit. Nicorandil also did not consistently prove to be useful when used during coronary bypass surgery (Blanc et al., 2001) (Matthias et al., 2012).

None of these conditioning mimetic drugs are now being utilized routinely in conjunction with on-pump coronary bypass surgery. A plethora of studies reported on the benefit of some volatile anesthetics. However, the availability of such drugs currently in various hospitals depends on their safety profile and cost. The only reliable technique to achieve preconditioning is to perform an aortic cross clamp, followed by 1 or 2 min of reperfusion prior to the start of surgery (Kloner & Rezkalla, 2006).

While in clinical studies that technique was of benefit in achieving a higher cardiac index, its current use is not widespread. Cross clamping always carries a risk of causing embolic stroke during the surgery, and is unlikely to be popular, particularly in elderly patients with significant aortic atherosclerosis. Thus, currently there is no clinically useful preconditioning tool that is routinely utilized during on-pump bypass surgery (Wu et al., 2000).
Remote ischemic preconditioning by using anesthetic agents for myocardial protection
Remote ischemic preconditioning by using anesthetic agents for myocardial protection

Introduction:

Preconditioning is a biological process that can be observed in multiple organs and it can be induced by brief ischemic episodes or by drugs such as volatile anesthetics. This protection has two phases: an early phase, immediately operating after the application of the preconditioning stimulus and lasting for 2–3 h, and a late phase, evident after 12–24 h but lasting for up to 3 days (Kristin et al., 2012).

Although early preconditioning is predominantly based on multiple, fast-acting intracellular phosphorylation signaling steps, the second window is a result of transient altered gene activity and depends on novel protein expression. Volatile anesthetic-induced preconditioning was reported to be effective in various cell types, including cardiac myocytes, and endothelial and smooth muscle cells. In addition, several experimental studies provide evidence of a "second window of protection" elicited by volatile anesthetics in mouse, rat, and rabbit hearts (Chiari et al., 2004).

The role of the following anesthetic in preconditioning will be discussed:

1) Volatile anesthetics.
2) I.V. anesthetics.
3) Opioids.
1) Volatile anesthetics:

The administration of some anesthetics produces a preconditioning-like effect, protecting the myocardium from the effects of myocardial infarction and myocardial dysfunction. The potential cardiac protective effects of volatile anesthetics were already recognized before the introduction of the concept of anesthetic preconditioning. Warltier et al. described a better recovery of myocardial function after a 15-min coronary artery occlusion when a volatile anesthetic was administered before the occlusion (Warltier et al., 1988).

In dogs anesthetized with isoflurane or halothane, myocardial function returned to baseline levels within 5 h after the start of reperfusion, whereas awake dogs that received the same treatment without anesthesia still had a 50% decrease in myocardial function at the same time point (Warltier et al., 1988).

A little attention has been directed toward exploration of the potential beneficial effects of volatile anesthetics when administered immediately before or during reperfusion (termed “anesthetic postconditioning”). Nevertheless, some experimental evidence indicated that volatile anesthetics are capable of exerting cardioprotective effects under these conditions. For example, halothane prevented reoxygenation induced hypercontracture of cardiac myocytes in vitro, a potential cause of myocyte necrosis during early reperfusion (Suraphong et al., 2008).

a) Advantages:

I) Volatile anesthetics are commonly used in general anesthesia to induce and maintain hypnosis, analgesia, amnesia, and muscle relaxation, improve postischemic recovery at the cellular level, in isolated hearts, and in animals, mainly through pharmacologic preconditioning. Few studies have been performed on human patients undergoing CABG surgery with
cardiopulmonary bypass (CPB), and only 2 small single-center randomized studies have evaluated the effects of volatile anesthetics in off-pump CABG (OPCAB), with conflicting results as far as cardiac biomarker release is concerned (Bein et al., 2005).

II] It was showed that patients receiving volatile anesthetics for OPCAB had less myocardial damage than patients receiving standard total intravenous anesthetics. This could lead to a reduced use of postoperative inotropes and reduced postoperative hospitalization (Bein et al., 2005).

III] Volatile anesthetics can also trigger an acute cardioprotective memory effect that lasts beyond their elimination, called “anesthetic or pharmacologic preconditioning.” Volatile anesthetics have been used with success for decades and may play a useful role when patients with coronary artery disease need to undergo surgery (Riess et al., 2004).

IV] Volatile anesthetics also have postconditioning effects that may contribute to protection when administered after the onset of ischemia, such as mitigation of Ca2+ overload, free-radical production, and neutrophil adhesion.

V] Volatile anesthetics also, decreased postoperative release of brain natriuretic peptide and long-term incidence of cardiac events during the first post-CABG year (Garcia et al., 2005).

VI] Volatile anesthetics have low costs and very few risks. This may support their routine use in patients undergoing OPCAB to reduce perioperative myocardial injury (Riess et al., 2004).

b) **Mechanisms of action:**

I] Volatile **anesthetics** mediate their effects by either priming or indirectly opening the mitochondrial K_{ATP} channels. However, the actual intracellular
effects of mitochondrial $K_{\text{ATP}}$ channel opening are not well understood. One response may be slight depolarization of the mitochondrial membrane potential; another response may be swelling of the mitochondrial matrix (Zaugg et al., 2003).

**II** Either of these responses can result in altered mitochondrial bioenergetics (respiration state). Consequences of mitochondrial $K_{\text{ATP}}$ channel opening are reduced cytosolic and mitochondrial calcium loading and improved myocardial oxygen efficiency during ischemia and reperfusion. Other observed effects include decreased mitochondrial respiration (increased NADH = nicotinamide adenine dinucleotide levels), modulation of mitochondrial energetic and calcium homeostatic capacity, ATP sparing, and decreased mitochondrial energy consumption during ischemia (Tanaka et al., 2002).

**III** Recently, Kevin et al, reported a direct increase in ROS (reactive oxygen species) in response to sevoflurane administration that did not appear to be dependent on mitochondrial $K_{\text{ATP}}$ channels. This observation suggested that the initial increase in ROS observed with volatile anesthetics may in fact precede the mitochondrial $K_{\text{ATP}}$ channel opening. However, as both $K_{\text{ATP}}$ channel blockade and ROS scavengers also prevent anesthetic preconditioning, this effect seems mediated by an intimate feedback interaction between ROS formation and $K_{\text{ATP}}$ channel opening; i.e., both are necessary components of the mechanism (Novalija et al., 2003).

**IV** Ischemic and anesthetic preconditioning effects have also been described in the vasculature, where they protect coronary endothelial cells against ischemia and reperfusion. This phenomenon seems also mediated at least partially by adenosine receptors and $K_{\text{ATP}}$ channels. This protective effect
against ischemia-reperfusion-induced coronary constriction was reported to be greater than its protective effect on contractility (Kevin et al., 2003).

c) Examples:

1) Sevoflurane:

Recruitment of inflammatory cells to sites of ischemic injury contributes significantly to organ dysfunction. Focal accumulation of leukocytes is mediated by the interaction of selectins with their endothelial counterligands, while firm attachment of leukocytes and transmigration requires activation of \( \beta_2 \)-integrin (CD11b). It was recently shown that sevoflurane inhalation at subanesthetic concentrations decreases activation of \( \beta_2 \)-integrin (CD11b) on granulocytes and monocytes after ischemia-reperfusion of the forearm in healthy volunteers, consistent with an early preconditioning of the endothelium (Lucchinetti et al., 2007).

It was also discovered that sevoflurane inhalation would modulate blood transcripts potentially involved in late protection and reduce the expression of L-selectin (CD62L) and \( \beta_2 \)-integrin (CD11b) 24–48 h later in humans. It was shown that sevoflurane inhalation, even at low subanesthetic concentrations, rapidly altered the blood transcriptome on a genome-wide scale in healthy subjects, a prerequisite for late preconditioning, which depends on de novo protein synthesis. The observed transcriptional changes specifically involved genes with known biological significance in the context of late preconditioning or organ protection (Lucchinetti et al., 2007).

On exposure to sevoflurane, genes involved in fatty acid oxidation, regulated by the PCG1\( \alpha \)-pathway (peroxisome proliferator activated receptor \( \gamma \)coactivator-1\( \alpha \)) , were similarly down-regulated in the blood. Second, 24 to 48 h after sevoflurane exposure, i.e., consistent with the occurrence of a late or second window of preconditioning, the expression of L-selectin (CD62L), a key inflammatory adhesion molecule responsible for
the tethering of leukocytes to the endothelium, was reduced by approximately 25% on granulocytes, which further exhibited an increased resistance to inflammatory stimulation (Barlic et al., 2006).

The transcriptional changes in fatty acid oxidation after subanesthetic sevoflurane inhalation may be due to down-regulation of the PGC-1\(\alpha\) pathway. This nuclear receptor coactivator critically controls cellular energy metabolism, and thus determines oxygen consumption. Moreover, inhibition of the PGC-1\(\alpha\) pathway was recently shown to reduce uptake of oxidized low-density lipoprotein into macrophages and to prevent their retention in atherosclerotic vessel walls, thereby stabilizing vulnerable plaques (Barlic et al., 2006).

Hence, modulation of the PGC-1\(\alpha\) pathway, as observed after sevoflurane administration, may be a novel antiischemic and plaque-stabilizing strategy in perioperative medicine. Also, when sevoflurane at 2.5 MAC (minimum alveolar concentration) given during the first 10 minutes of CPB in 20 patients, PKC (protein kinase-c) and p38 MAPK (mitogen activated protein kinase) were increased with either sevoflurane or CPB alone, suggesting anesthetic preconditioning with sevoflurane may overlap with the preconditioning effects of CPB. However, in the sevoflurane group, tyrosine kinase was also increased, suggesting a greater preconditioning (Barlic et al., 2006).

The addition of sevoflurane to an IV anesthesia regimen for cardiac surgery consistently decreased troponin T levels, with less need for inotropic support for weaning from CPB and a reduced incidence of low cardiac output (Van et al., 2003).
The administration of sevoflurane after ischemia also improved contractile and metabolic function concomitant with reduced myoplasmic Ca$_2^+$ loading in isolated guinea pig hearts. These data suggested that volatile anesthetics may reduce myocardial necrosis and enhance function when administered exclusively during reperfusion (Varadarajan et al., 2002).

Fig. 13 proposed mechanisms of remote ischemic preconditioning (Kristin et al., 2012).
II) Desflurane:

Like all other volatile anesthetics, desflurane induces preconditioning in various experimental models, including rabbits, rats, and isolated human atria. The clinical application of anesthetic-induced preconditioning is a possibly powerful means to reduce sequelae of perioperative ischemic events in patients at cardiac risk. However, with the exception of cardiac and vascular surgery, perioperative ischemic events usually cannot be anticipated. Thus, it is important to investigate if cardioprotection can be achieved by the administration of volatile anesthetics during ischemia or reperfusion, time periods that might be suitable for therapeutic application of volatile anesthetics after the onset of ischemia (Van et al., 2003).

Desflurane-induced postconditioning is equally effective as desflurane-induced preconditioning. NOS (nitric oxide synthase) -derived NO is crucial for desflurane-induced preconditioning in the rabbit model. Desflurane-induced postconditioning is mediated by NO (Van et al., 2003).

Mean arterial blood pressure was reduced during desflurane administration and coronary artery occlusion. Therefore, a reduction in myocardial oxygen consumption during the administration of desflurane might have conferred cardioprotection (Van et al., 2003).

III) Isoflurane:

In contrast to the straight forward data obtained in the experimental studies, results from clinical studies using preconditioning protocols show highly variable results. The first, limited, study on this issue was published by Belhomme et al. in 1999 (Belhomme et al., 1999).
Where Isoflurane preconditioning resulted in an increase in cytosolic activity of 5’ nucleotidase, a surrogate marker for activation of PKC (protein kinase c). However, postoperative release of creatine kinase MB and troponin I were not different from the control group. Similar results were found in a subsequent study that investigated the effect of CPB alone and sevoflurane at 2.5 MAC (minimum alveolar concentration) given during the first 10 minutes of CPB in 20 patients (Pouzet et al., 2002).

In a study of 40 patients, Tomai et al. administered isoflurane, 1.5%, for 15 min, followed by a washout period of 10 min before the start of CPB. No differences were observed between the treatment group and the control group in postoperative cardiac function and peak troponin I values. However, in the subgroup of patients with a left ventricular ejection fraction <50%, troponin I levels 24 h postoperatively were slightly lower in the isoflurane treatment group than in the control group (Tomai et al., 1999).

In another study of 49 patients, Haroun-Bizri et al. administered isoflurane, 0.5%–2%, until the start of CPB and observed a higher postoperative cardiac index in the isoflurane group than in the control group (Haroun-Bizri et al., 2001).

Isoflurane pretreatment before 30 min of anoxia resulted in a greater recovery of force after restoration of oxygen administration compared with untreated controls. Conversely, halothane pretreatment was not associated with a cardioprotective effect; indeed it even seemed to inhibit the cardioprotection provided by hypoxic preconditioning. In this in vitro study, the preconditioning effect of the volatile anesthetics was not related to their cardiac and systemic vascular actions; therefore, they appeared to be exerting additional actions that cause this phenomenon. Isoflurane enhanced the functional recovery of isolated rat hearts when administered solely during reperfusion (Schlack et al., 1998).
Chiari et al showed that using 1.0 minimum alveolar concentration (MAC) isoflurane administered during the final 3 minutes of coronary artery occlusion and the first 2 minutes of reperfusion reduced myocardial infarct size in rabbits, by establishing a plasma concentration of isoflurane and thereby producing a pharmacologic effect immediately at the onset of reperfusion (Chiari et al., 2005) (Tanaka et al., 2003).

Interestingly, postconditioning with 0.5 MAC isoflurane (an anesthetic concentration that did not decrease myocardial necrosis alone) also reduced the time threshold required for ischemic postconditioning (reduction in each brief ischemia and reperfusion episode from 20 to 10 seconds). The beneficial actions of isoflurane before and during early reperfusion were abolished by pretreatment with the selective PI3K (phosphotidylinositol-3-kinase) antagonist wortmannin (Wymann et al., 1996).

These data showed for the first time that activation of the PI3K-signaling pathway directly mediates the protective effects of isoflurane postconditioning. PI3K is responsible for the phosphorylation of many subcellular targets implicated in protein synthesis, metabolism, and cell survival (Hawkins et al., 2007).

Phosphorylation of Bad (proapoptotic protein) by PI3K results in its sequestration from mitochondria and inhibits apoptosis. These data provided the first direct evidence indicating that isoflurane postconditioning preserves myocardial integrity in part by attenuating apoptosis (Zha et al., 1996).

IV) Enflurane:

In a study of 22 patients, the effects of enflurane, 1.3% (range, 0.5%–2%), administered using a vaporizer connected to the mechanical ventilation fresh gas flow for 5 min immediately before CPB were investigated. In that study group, enflurane enhanced
postoperative left ventricular function, but postoperative creatine kinase-MB and troponin I release were not different from the control group (*Penta et al., 1999*).

**V) Halothane:**

Halothane also reduced reperfusion injury after regional myocardial ischemia in rabbit hearts (*Schlack et al., 1997*).

Halothane abolished reoxygenation-induced attenuation of sarcoplasmic reticulum-dependent oscillations of intramyoplasmic Ca$_{2+}$ concentration in isolated cardiac myocytes. These and other findings indicated that volatile anesthetics may prevent intracellular Ca$_{2+}$ overload during early reperfusion, presumably by virtue of their actions as voltage-dependent Ca$_{2+}$ channel antagonists (*Varadarajan et al., 2002*).

d) **Future directions:**

Several questions still remain unanswered about the role of ischemic and anesthetic preconditioning. The first question relates to any possible differences among the available volatile anesthetics in eliciting cardioprotective effects. An investigation of the contractile function of human atrial trabeculae dissected from atrial appendages acquired from patients undergoing coronary surgery demonstrated different preconditioning effects of halothane and isoflurane (*Roscoe et al., 2000*).

Although the use of a volatile anesthetic regimen appears related to a better and earlier recovery of myocardial function, its implications for outcome remain to be established. The authors of a very recent study observed that the length of stay in the intensive care unit seemed to be related to the choice of anesthetic regimen. The use of a volatile anesthetic regimen during coronary surgery was associated with a decreased
incidence of prolonged stay (>48 h) in the intensive care unit compared with use of a total IV anesthetic regimen (De Hert et al., 2004).

The individual variables responsible for a prolonged length of stay were occurrence of atrial fibrillation, increase in postoperative troponin I levels >4 ng/mL, and the need for prolonged inotropic support (>12 h). Although the incidence of atrial fibrillation was similar with all anesthetics studied, the number of patients with an increased troponin I level >4 ng/mL and those receiving prolonged inotropic support were significantly less with the volatile anesthetic regimens compared with the total IV anesthetic regimens (Matthias et al., 2012).

Isoflurane and sevoflurane also reduced postischemic adhesion of neutrophils, an important source of oxygen derived free radicals during reperfusion that are known to be critical mediators of reperfusion injury. However, the precise mechanisms by which volatile anesthetics act to reduce intracellular Ca$_{2+}$ overload or attenuate the adverse consequences of large quantities of reactive oxygen intermediates during early reperfusion were not specifically elucidated by these previous studies nor were endogenous signal transduction pathways previously identified in anesthetic preconditioning initially implicated in postconditioning by volatile agents (Tanaka et al., 2004).

e) **Comparison between different inhalational anesthetics:**

Currently available data show that desflurane and sevoflurane do not cause clinically significant coronary steal. Halothane and enflurane should be avoided in patients with impaired myocardial function, whereas sevoflurane has been reported to depress cardiac function less than either halothane or isoflurane. Many studies have indicated that desflurane and sevoflurane allow better control of hemodynamic and
sympathetic response to different stimuli, when compared with halothane, enflurane, or isoflurane (Bell et al., 2000).

Searle et al reported a lower incidence of postoperative MI in patients anesthetized with sevoflurane for CABG surgery when compared with patients anesthetized with isoflurane (2.2% in the sevoflurane group vs. 4.5% in the isoflurane group), although this difference was not statistically significant. Halogenated anesthetics may also have different potencies as far as pre- and postconditioning are involved; the reason for that is unknown (Chiari et al., 2005).

In rabbits undergoing a 30-minute coronary artery occlusion, desflurane was the most powerful preconditioning agent; desflurane and sevoflurane, when administered during early reperfusion, markedly reduced infarct size, whereas enflurane had only a marginal effect and isoflurane no effect at all (Piriou et al., 2002).

However, Boldt et al performed a cost analysis of anesthetic techniques, concluding that the use of inhaled anesthetics was associated with lower costs compared with TIVA using propofol (Boldt et al., 1998).

2) I.V. anesthetics:

a) Propofol:

Propofol (2, 6-diisopropylphenol) has a chemical structure similar to that of phenol-based free radical scavengers such as vitamin E. It is being used increasingly for cardiac anesthesia and has been shown to decrease postischemic myocardial dysfunction, infarct size, and histologic degeneration (Ko SH et al., 1997).

A study in isolated rat hearts showed that propofol infusion during the reperfusion period attenuates myocardial dysfunction and apoptosis after 4 hours of global cold (4°C) ischemia. Propofol is known to be a free radical scavenger. It also inhibits calcium influx across plasma membranes and reduces the opening of mitochondrial permeability.
transition pores. These features suggest that propofol may directly intervene at the critical phase of reperfusion injury by reducing free radicals and calcium influx (Ko SH et al., 1997).

But propofol induced preconditioning is a dose-dependent protection of cardiac function during ischemia and early reperfusion in the rat. Studies have validated the need for reducing propofol concentrations during the late phase of reperfusion when antioxidant therapy is the major consideration. Infact, the continuous use of high-dose propofol beyond the early phase of reperfusion compromised or prevented myocardial function recovery (Ko SH et al., 1997).

b) **Barbiturates:**

I) **Mechanisms of myocardial protection:**

i. Interference in neutrophil function: supported by published studies.

ii. Blockage of Ca\(^{2+}\) overload in the cardiac myocytes: published studies are conflicting.

iii. Antioxidant like effect: supported by published studies (Ko SH et al., 1997).

II) **Timing of administration:**

i. Pre-ischemia: published studies are conflicting.

ii. During or post-ischemia: supported by published studies (Ko SH et al., 1997).

c) **Ketamine:**

**Mechanisms of myocardial protection:**

I) Interference in neutrophil function: supported by published studies.

II) Blockage of Ca\(^{2+}\) overload in the cardiac myocytes: supported by published studies (Ko SH et al., 1997).
d) **Benzodiazepines:**

**Mechanisms of myocardial protection:**

1) Interference in neutrophil function: published studies are conflicting.

2) Blockage of Ca\(^{2+}\) overload in the cardiac myocytes: supported by published studies (*Ko SH et al.*, 1997).

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3) **Opioids:**

a) **Evidence of protection by opioids:**

Protection by opioid receptor agonists against ischemia-reperfusion injury has been demonstrated during the last several years. Contribution of endogenous opioid peptides to adaptation to hypoxia had been suggested when Mayfield and D’Alecy, in 1994, examined whether exogenous opioid receptor agonists can increase tolerance to hypoxia. They found that D-Pen2-D-Pen5-enkephalin (DPDPE), a \(\delta\)-opioid agonist, can extend survival time of mice under severe hypoxia (*Mayfield et al.*, 1994).

Meanwhile the \(\delta\)-agonist D-Ala2-D-Leu5-enkephalin (DADLE), which had been identified as a hibernation-inducing trigger in nature, showed protective effects in multi-organ preparations, including the heart, preserved for transplant (*Chien et al.*, 1994).

In 1996, Schultz and colleagues were the first to demonstrate that an opioid could attenuate ischemia-reperfusion damage in the heart. Morphine at the dose of 300 \(\mu g\cdot kg^{-1}\) was given before LAD occlusion for 30 min in rats in vivo. Infarct area/area at risk was diminished from 54 to 12% by this treatment. The infarct-reducing effect of morphine has
been shown in hearts *in situ*, isolated hearts and cardiomyocytes *(Takasaki et al,1999)* *(Sawada et al,2003)*.

Consequently, it is now well accepted that morphine provides **protection** against ischemia-reperfusion injury. Schwartz et al. demonstrated that fentanyl enhances postischemic mechanical function in isolated rat hearts *(Schwartz et al.,1999)*.

Pentazocine and buprenorphine improved postischemic contractility in rabbits in *vitro* *(Springer TA,1994)*.

The concomitant use of opioids, cardioplegia and hypothermia has been investigated. Morphine, pentazocine and buprenorphine improved mechanical function after global total ischemia for two hours at 34°C *(Schwartz et al.,1999)*.

**b) Mechanisms behind the protection by opioids:**

1) **Preconditioning:**

The involvement of opioid receptors in ischemic preconditioning has been demonstrated in various animal species and humans. Among opioid receptor subtypes, there is evidence that δ-opioid receptors are responsible for ischemic preconditioning in rats and humans. Opioid receptor subtype distribution in the heart appears to differ between species. δ- and κ-, but not μ-opioid receptors are expressed in the rat heart *(Schultz et al.,2001)*.

In human atrium, δ- and μ- have been shown to be dominant compared to κ- receptors. Quaternary naloxone, which does not cross the blood-brain barrier, eliminated the protection by ischemic preconditioning in *in vivo* models. Therefore it is suggested that it is in the heart itself that opioid receptors play a role in protection by ischemic preconditioning *(Chien et al.,1999)*.
In the study by Bell et al. cardiac myocytes were treated with morphine followed by incubation in drug-free media, before ischemia was induced. The survival rate of morphine-treated cells was higher. This treatment diminished infarct size. A similar effect was observed in human trabeculae (Bell et al., 2000).

It appears that the δ-opioid receptor subtype is responsible for opioid-induced protection. Selective δ- and δ₁-agonists have shown protection. The role of κ-receptors remains controversial. Whether μ-receptors contribute to cardioprotection in humans is not known. The beneficial effects were eliminated by a Gᵢ protein inhibitor, a PKC inhibitor, and a selective mitochondrial Kᵦᵦ channel blocker (Wang et al., 2001).

The effect of "classic" or "early" ischemic preconditioning is transient and lost within 0.5–2 hr after brief ischemic episodes, but the protection exhibits a biphasic time course and returns after 24 hr. TAN-67, a selective δ₁-agonist, also provoked "late" preconditioning. The opioid showed protection one hour after its iv administration, but not after 12 hr. A κ-agonist also showed late preconditioning-like effect (Wang et al., 2001).

1) Neutrophil adhesion and migration:

The neutrophil hypothesis is much less convincing than the preconditioning hypothesis due to the lack of supporting evidence. Hofbauer et al. reported that remifentanil decreased neutrophil adhesion and transmigration, and ICAM-1 expression in a dose-dependent manner. Fentanyl also reduced migration of neutrophils (Hofbauer et al., 2000).
**Comparison between anesthetics:**

Several halogenated anesthetics, administered at the same minimum alveolar concentration (MAC) and in the same preparation, have been assessed for their protective effect in a number of investigations. Overall, there is little difference in the *extent* of protection among halogenated anesthetics. The results are not consistent with respect to which anesthetic agents are more protective than others. In addition, an explanation for the discrepancies between anesthetics is not readily available (*Conradie et al., 1999*).

Benedict et al. examined the effect of several opioids of equal potency on postischemic myocardial contractility. Morphine, buprenorphine and pentazocine improved postischemic function to a similar degree, while fentanyl did not (*Benedict et al., 1999*).

Cope et al. reported that infarct size was smaller when rabbits were anesthetized with halothane, enflurane or isoflurane compared to pentobarbital, ketamine/xylazine or propofol. Because the volatile anesthetics caused blood pressure to be lower than the *iv* anesthetics, the question was raised whether the difference in blood pressure was a determinant of infarct size, but there was no correlation between the two parameters within the halothane-anesthetized group (*Cope et al., 1997*).

However, in this study, the extent of ventricular wall dyskinesia seemed worse (although not statistically different) in the propofol-anesthetized group during the ischemic period; it is possible that the intensity of ischemic insult differed between the anesthetic groups (*Cope et al., 1997*).
CONCLUSION
CONCLUSION

Numerous studies demonstrated the cardioprotective potential of ischemic preconditioning as evidenced by the implication of cardioprotective pathways including cell surface receptors, protein kinases and Mito KATP channels.

In addition, few pharmacological agents have been shown to mimic the cardioprotective effects of ischemic preconditioning and thus infusion of these agents in patients with ischemic heart disease undergoing surgery could improve the outcome of myocardial function by reducing I/R-induced myocardial injury.

However, the cardioprotective potential of preconditioning has been suppressed in animals suffered from various cardiovascular disorders like hyperglycemia, hypertrophy, hypercholesterolemia, hypertension, obesity and ageing. This may reduce the clinical application of preconditioning since most of the patients undergoing cardiac surgery are associated with various metabolic disorders such as hyperglycemia, obesity and hypercholesterolemia.

Further studies are warranted to elucidate the potential culprits involved in metabolic disorders associated with attenuation of cardioprotective potential of preconditioning. Thus, there is clearly a need to improve cardioprotection, and a better understanding of the mechanisms involved in ischemia and especially reperfusion injury might help to define new therapeutic strategies.
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