
Ischemia–Reperfusion Injury

Cell injury mechanisms and clinicopathologic consequences

Restored flow: cellular rescue and paradoxical damage

Dr Abu Suraih Sakhri

MBBS, MD (Pathology)

www.abusuraihsakhri.com

Learning objectives

By the end of the lecture, distinguish:

- Ischemic injury from injury generated after restoration of flow.
- The four proposed mechanisms of reperfusion-associated damage.
- How oxidants, calcium, inflammation and complement converge on cell death.
- Why restoring perfusion remains essential despite its potential for added injury.

Reperfusion can rescue—and injure—the same tissue

Potential benefit

- Reoxygenation supports metabolism in viable cells.
- Blood flow brings needed substrates and removes accumulated metabolites.
- Reversibly injured cells may recover.

Paradoxical consequence

- New damaging processes begin with reperfusion.
- Previously salvageable cells may be lost.
- Net tissue damage includes ischemic and reperfusion components.

Reperfusion injury means additional damage attributable to the restoration phase.

Perfusion maintains more than oxygen delivery

Oxygen

Supports mitochondrial oxidative phosphorylation.

Metabolic substrates

Maintains cellular energy generation.

Metabolite clearance

Limits accumulation of products that inhibit glycolysis.

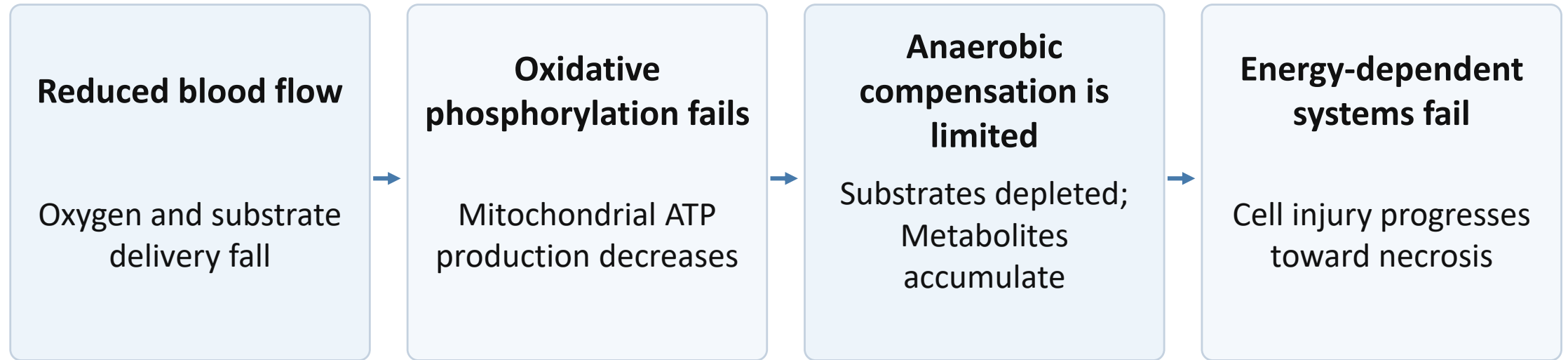
Circulating components

Can also deliver leukocytes and complement during reperfusion.

Ischemia and hypoxia are not synonymous

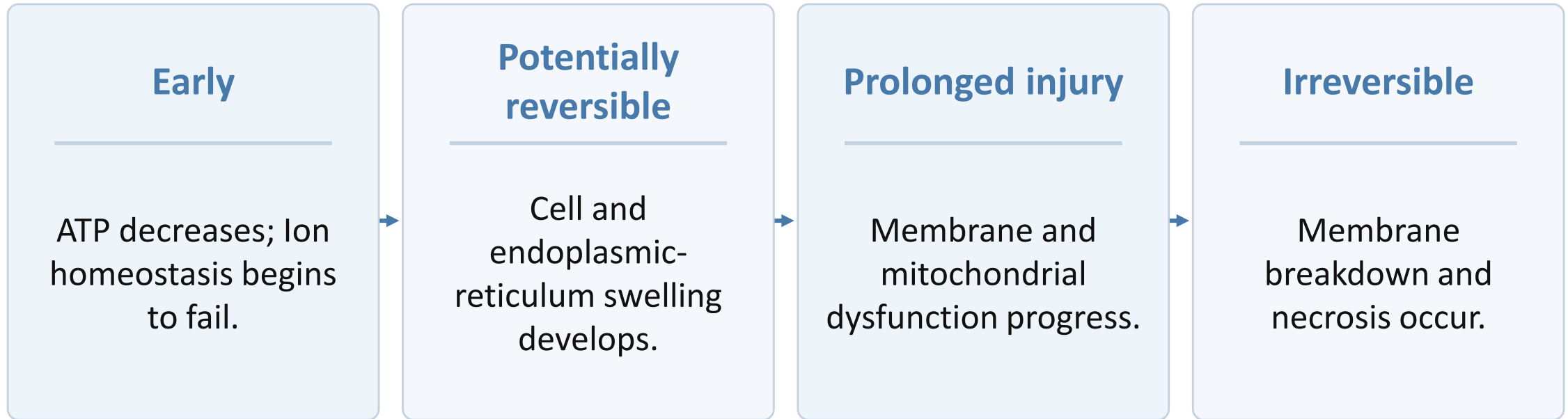
Feature	Hypoxia with maintained flow	Ischemia
Blood flow	Maintained	Reduced or obstructed
Oxygen availability	Reduced	Reduced due to impaired flow
Glycolytic substrate delivery	May continue	Compromised
Removal of metabolites	Continues	Impaired
Typical injury	May be less rapid	Often more rapid and severe

Why ischemia produces faster injury



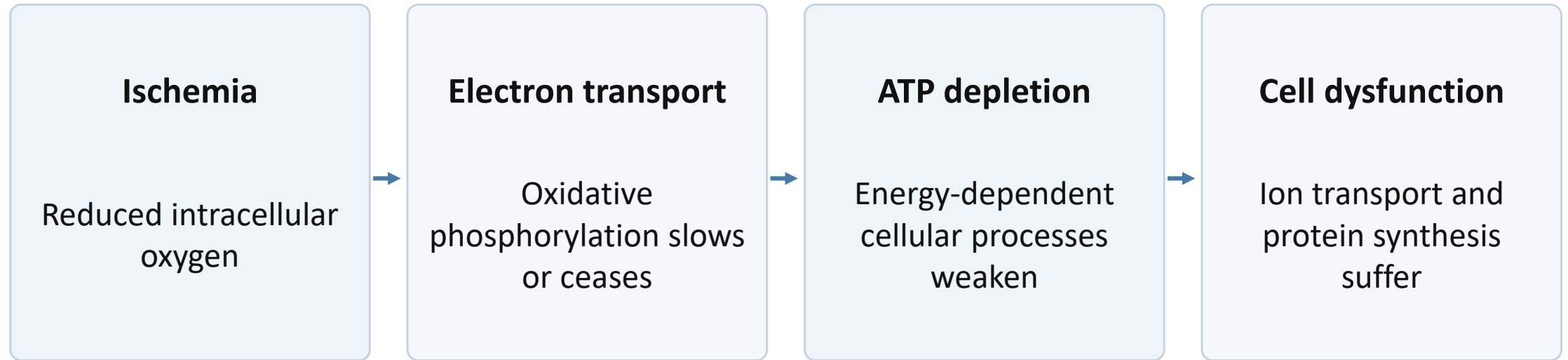
The shortage is both oxygen and usable metabolic support.

The chronology of ischemic cell injury



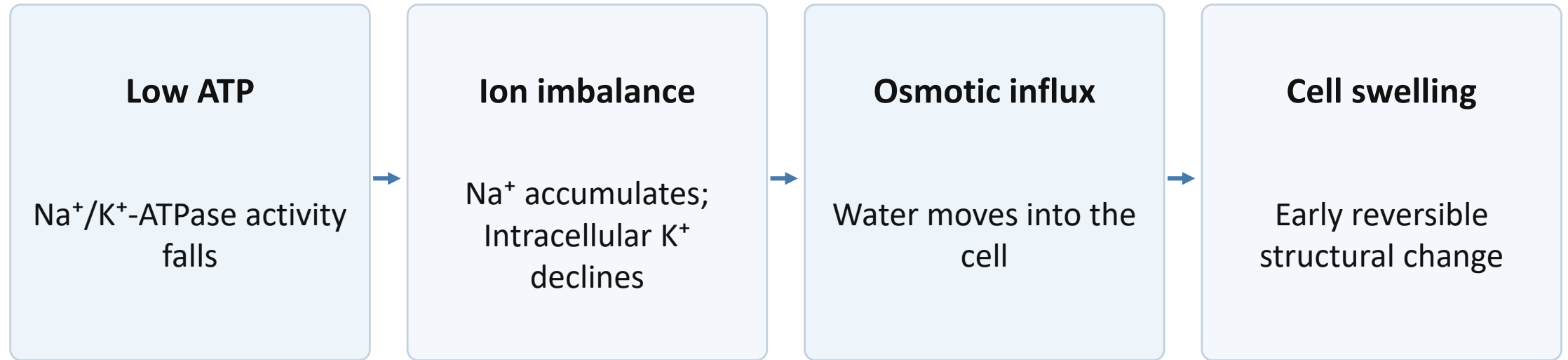
Sequence shown conceptually; Cellular outcomes depend on the severity and duration of ischemia.

Mitochondrial respiration is an early casualty



This pathway explains injury even before reperfusion occurs.

ATP depletion first disrupts ion pumping



Cell swelling is a consequence of energy failure, not proof of irreversibility.

Cell swelling signals an initially reversible phase

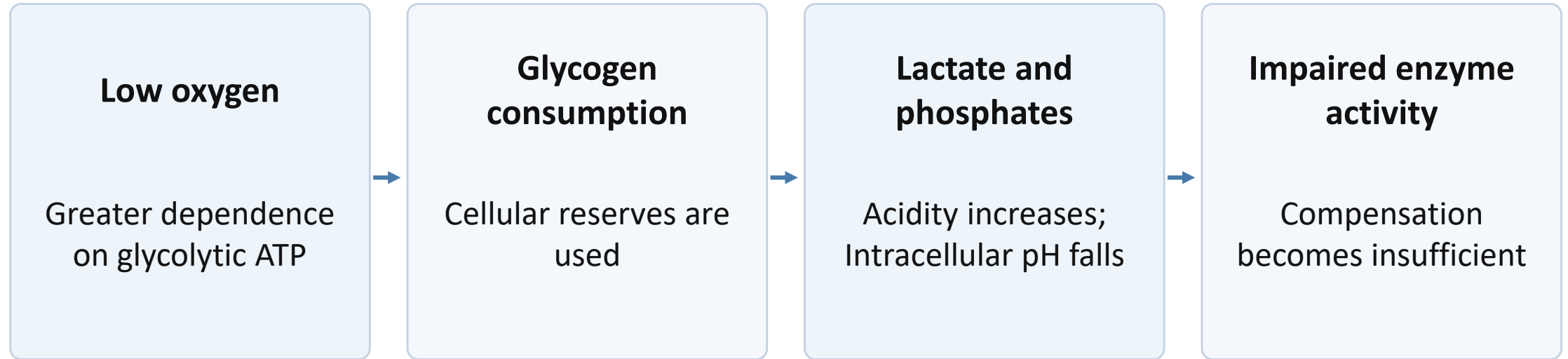
What develops

- Intracellular sodium increases.
- Osmotically driven water accumulation expands cells.
- Endoplasmic reticulum may dilate.

Why it matters

- The plasma membrane can remain functionally recoverable.
- Prompt restoration of adequate perfusion may permit recovery.
- Further mitochondrial and membrane damage threatens viability.

Anaerobic glycolysis offers limited rescue



Reduced flow prevents adequate substrate replenishment and waste removal.

Energy failure eventually becomes irreversible

Progression during ischemia

- ATP depletion persists or worsens.
- Mitochondrial and lysosomal membranes are injured.
- Cellular integrity cannot be maintained.

Pathologic endpoint

- Loss of membrane integrity culminates in necrosis.
- Restoring flow cannot revive cells already irreversibly injured.
- Other reversibly injured cells may still be at risk during reperfusion.

Reversibility determines the possible benefit

Ischemic tissue at the moment flow returns

Reversibly injured cells

Potential recovery if adequate perfusion is restored

Irreversibly injured cells

Necrosis is not reversed by blood flow

Vulnerable salvageable cells

May undergo additional damage during reperfusion

Two processes coexist after restoration of flow

Restoration of homeostasis

- Oxygen and substrates become available.
- Recoverable cells may restore ATP generation.
- Metabolic waste can be cleared.

New injury pathways

- Oxidants are generated during reoxygenation.
- Calcium dysregulation is aggravated.
- Inflammatory and complement pathways can intensify damage.

Four proposed reperfusion-injury mechanisms

Oxidative stress

Reactive oxygen and nitrogen species rise.

Calcium overload

Cytosolic and mitochondrial Ca^{2+} accumulation worsens.

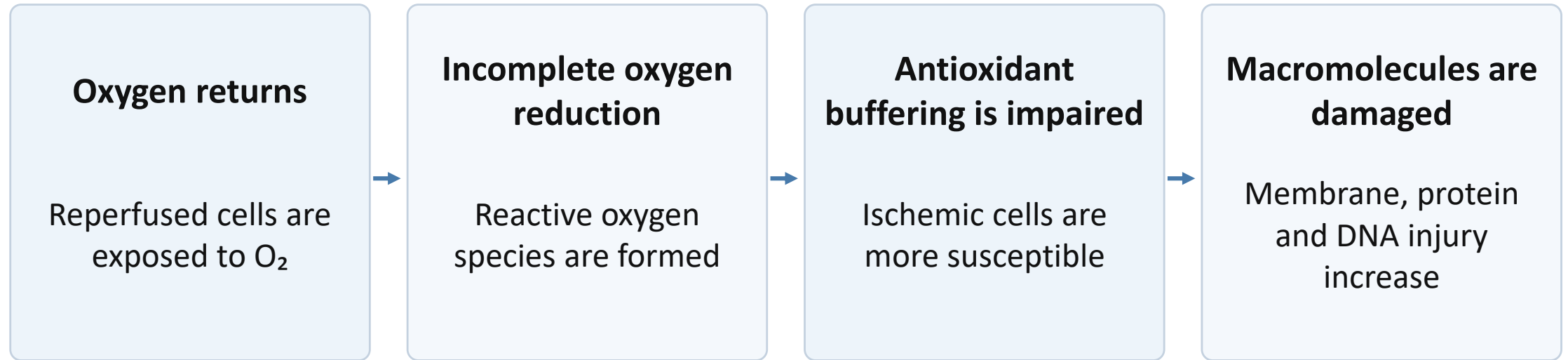
Inflammation

Danger signals, cytokines and neutrophils amplify injury.

Complement activation

Antibody-associated complement activation adds damage.

Reoxygenation can create a burst of oxidants



The oxygen required for recovery can also participate in oxidative injury.

Oxidants arise in multiple reperfused cell types

Leukocytes

Activated inflammatory cells generate oxidants.

Endothelial cells

Previously damaged cells can produce reactive species.

Parenchymal cells

Injured resident cells participate in oxidant generation.

Antioxidant defenses

Reduced capacity during ischemia raises vulnerability.

Oxidative stress is a balance, not a single molecule

Amount of reactive species in injured tissue

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graph TD; A[Amount of reactive species in injured tissue] --> B[Increased generation]; A --> C[Decreased removal]; A --> D[Net accumulation]; B --> E[Reoxygenation, leukocytes and damaged cells]; C --> F[Compromised scavenging and antioxidant defenses]; D --> G[Oxidative stress and cellular macromolecule injury];
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Increased generation

Reoxygenation, leukocytes and
damaged cells

Decreased removal

Compromised scavenging and
antioxidant defenses

Net accumulation

Oxidative stress and cellular
macromolecule injury

ROS and reactive nitrogen species converge on injury

Reactive oxygen species

- Oxygen-derived oxidants include superoxide and hydroxyl radical.
- Hydrogen peroxide is a reactive oxygen species but not a free radical.
- Incomplete oxygen reduction increases oxidant burden.

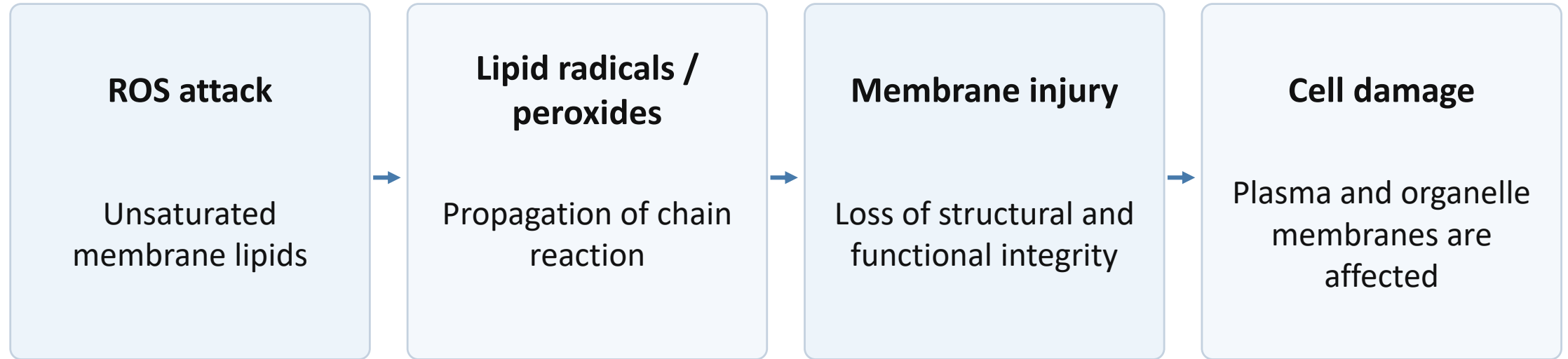
Reactive nitrogen species

- Nitric oxide can participate in radical reactions.
- Superoxide can react with nitric oxide to form peroxynitrite.
- These species can damage membrane lipids, proteins and DNA.

Principal reactive species: Essential distinctions

Species	Biochemical distinction	Injury relevance
Superoxide ($\text{O}_2\bullet^-$)	Oxygen-derived radical	Precursor of additional oxidants
Hydrogen peroxide (H_2O_2)	ROS; not itself a radical	Can generate more reactive products
Hydroxyl radical ($\bullet\text{OH}$)	Very reactive oxygen radical	Damages lipids, proteins and DNA
Peroxynitrite (ONOO^-)	Reactive nitrogen-derived oxidant	Can oxidatively damage macromolecules

Lipid peroxidation undermines membranes



Lipid damage can propagate beyond the initial oxidative reaction.

Oxidative protein changes impair cell function

Enzyme modification

Oxidation can disturb catalytic sites.

Protein conformation

Oxidation alters folding and stability.

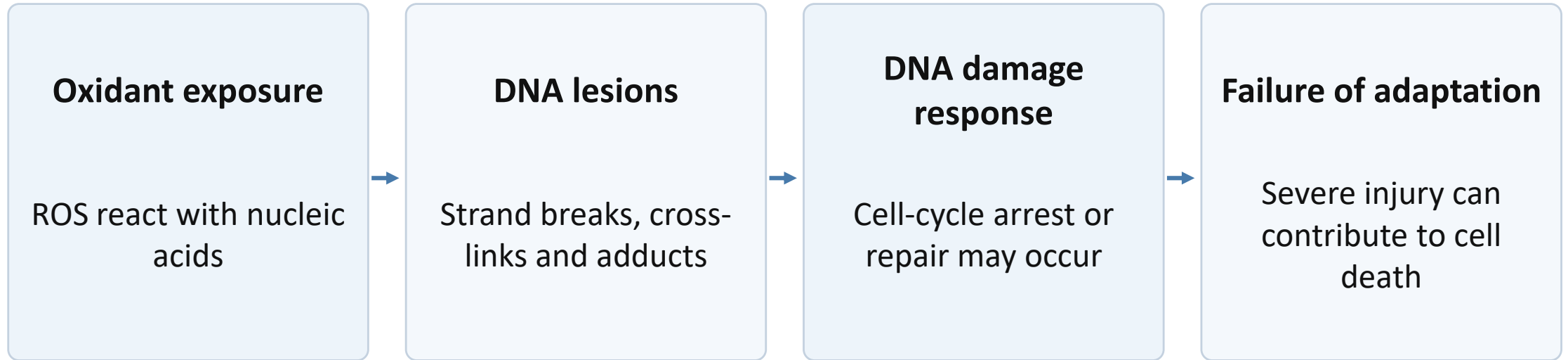
Cross-linking

Protein–protein bonds may be modified.

Proteostasis burden

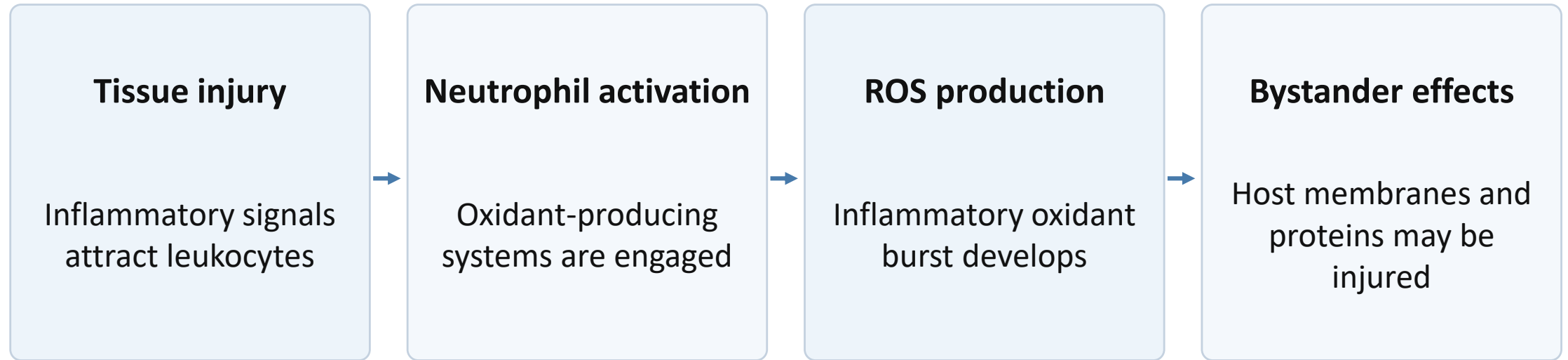
Abnormal proteins may undergo increased degradation.

Reactive species can also damage nuclear DNA



DNA injury is one of several macromolecular effects; It is not a specific test for reperfusion injury.

Activated leukocytes are an oxidant source



A response meant to clear damaged tissue can aggravate local injury.

Depleted antioxidant defenses increase susceptibility

Normal protection

- ROS are produced in normal cellular metabolism.
- Enzymatic and nonenzymatic systems neutralize oxidants.
- Protection depends on scavenging near oxidant sources.

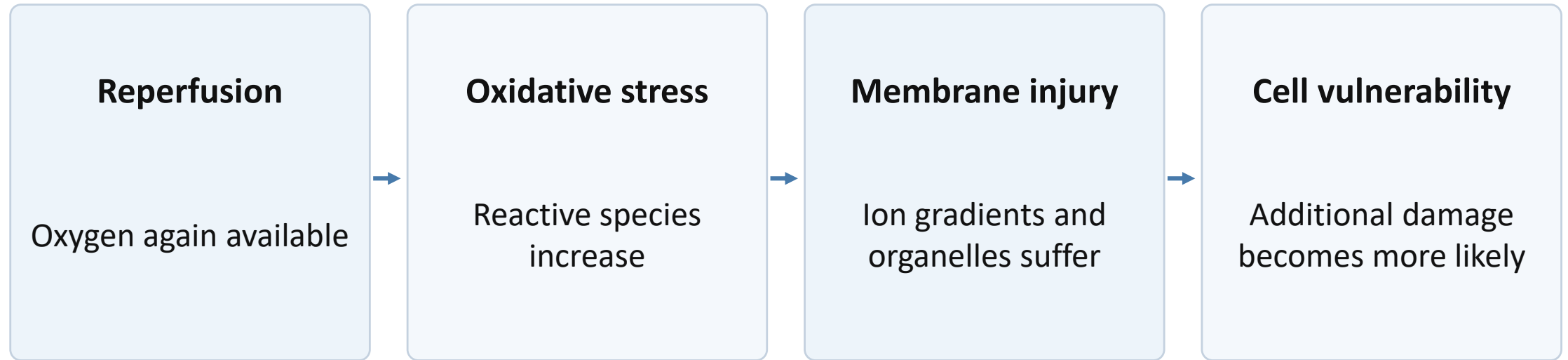
After ischemia

- Protective buffering may be compromised.
- New ROS production can exceed available removal.
- Oxidative damage therefore rises during reoxygenation.

Enzymatic antioxidant defenses act at different steps

Defense	Principal role	Teaching implication
Superoxide dismutases	Convert superoxide into H_2O_2 and O_2	Affects the superoxide burden
Catalase	Converts H_2O_2 into water and oxygen	Detoxifies hydrogen peroxide
Glutathione peroxidase	Reduces H_2O_2 using glutathione	Links ROS removal to redox reserve
Antioxidants / metal binding	Scavenge radicals or restrict metal catalysis	Lower generation and propagation of oxidative damage

Oxidants increase susceptibility to further injury



Membrane injury can heighten susceptibility to further oxidant-associated damage.

Cytosolic calcium is normally tightly regulated

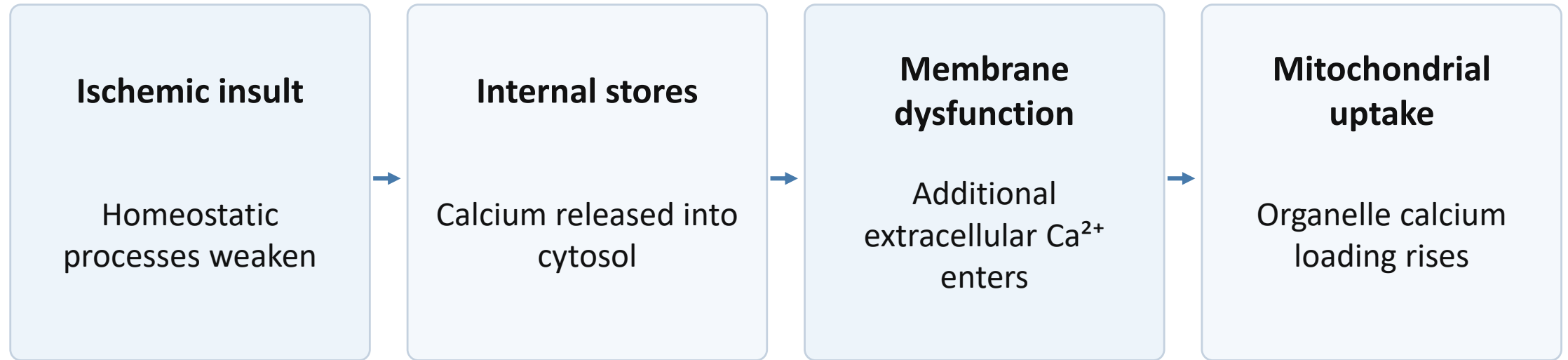
Normal organization

- Free cytosolic Ca^{2+} is kept much lower than extracellular Ca^{2+} .
- Endoplasmic reticulum and mitochondria store much intracellular calcium.
- Controlled Ca^{2+} signals regulate cell function.

Injury state

- Excess cytosolic calcium is toxic.
- Ischemia releases calcium from internal stores.
- Later membrane injury permits additional calcium influx.

Calcium loading begins before reperfusion



Calcium overload is initiated by ischemia and is exacerbated by reperfusion.

Reperfusion magnifies pre-existing Ca^{2+} overload

Injured cell exposed to restored perfusion

**Damaged plasma
membrane**

Allows additional Ca^{2+} influx

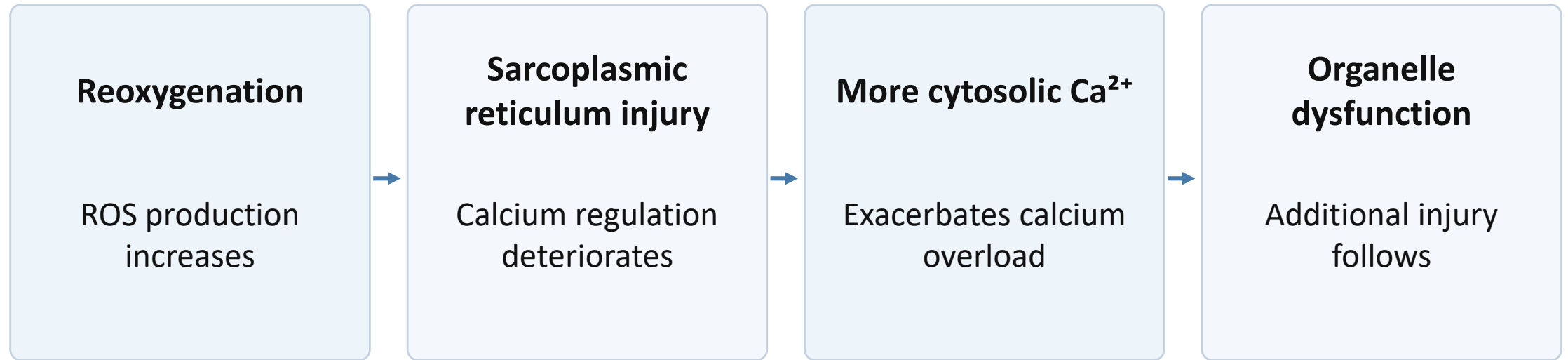
**ROS-injured sarcoplasmic
reticulum**

Aggravates intracellular Ca^{2+}
dysregulation

Ca^{2+} -loaded mitochondria

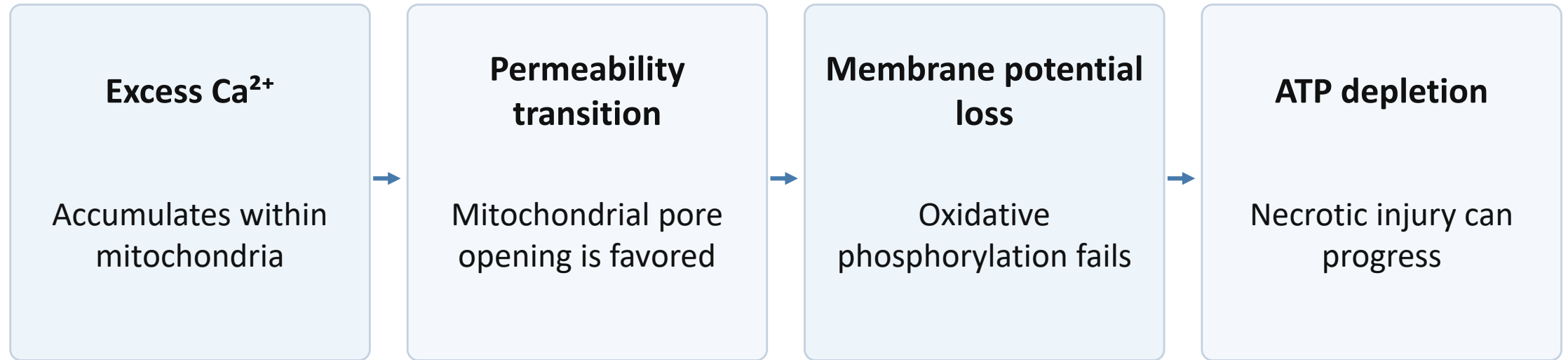
Become vulnerable to
permeability transition

The sarcoplasmic reticulum links ROS to Ca^{2+}



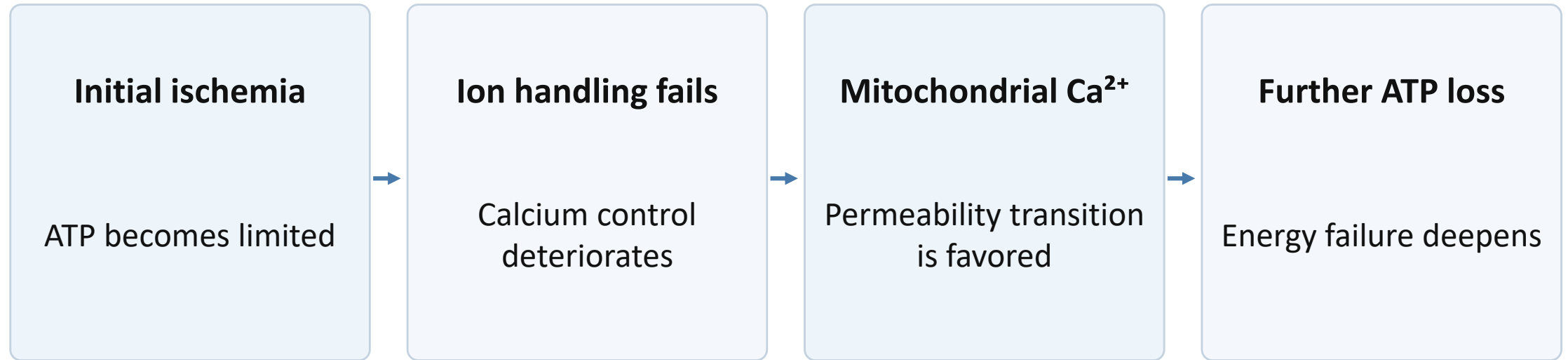
This coupling is especially relevant to understanding the reperfusion process in muscle cells.

Mitochondrial calcium overload promotes pore opening



Mitochondrial permeability transition is a mechanistic link between calcium and energy failure.

Calcium overload perpetuates ATP depletion



Additional ATP loss further impairs ion handling and calcium regulation.

Excess Ca^{2+} activates destructive enzymes

Enzyme class	Main target	Damage caused
Phospholipases	Membrane phospholipids	Membrane disruption
Proteases	Membrane / cytoskeletal proteins	Structural degradation
Endonucleases	DNA and chromatin	Nuclear fragmentation
ATPases	Cellular ATP stores	Accelerated energy depletion

ROS and calcium pathways are mechanistically coupled

ROS → membrane

Oxidative damage facilitates disordered Ca^{2+} influx.

ROS → intracellular stores

Sarcoplasmic-reticulum injury increases calcium dysregulation.

Ca^{2+} → mitochondria

Permeability transition weakens ATP synthesis.

Common endpoint

Energy failure and membrane injury reinforce cell death.

Reperfusion injury can be sterile inflammation

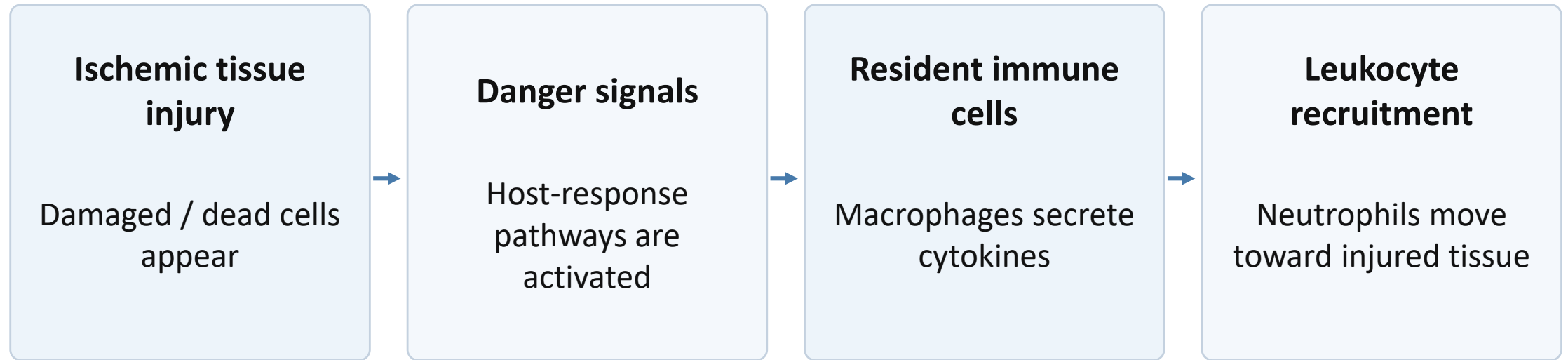
Trigger without an infection

- Dead and damaged cells release danger signals.
- Resident macrophages can secrete cytokines.
- Hypoxic tissue cells display adhesion-related signals.

Tissue consequence

- Circulating leukocytes are recruited to injured tissue.
- Recruited neutrophils add to local damage.
- The inflammatory response extends injury beyond the initial insult.

Danger signals connect cell death to inflammation



Inflammation follows tissue damage and can increase it.

Resident macrophages contribute to recruitment

Local sentinel cells

Resident macrophages detect injury-associated signals.

Cytokine output

Inflammatory mediators are released.

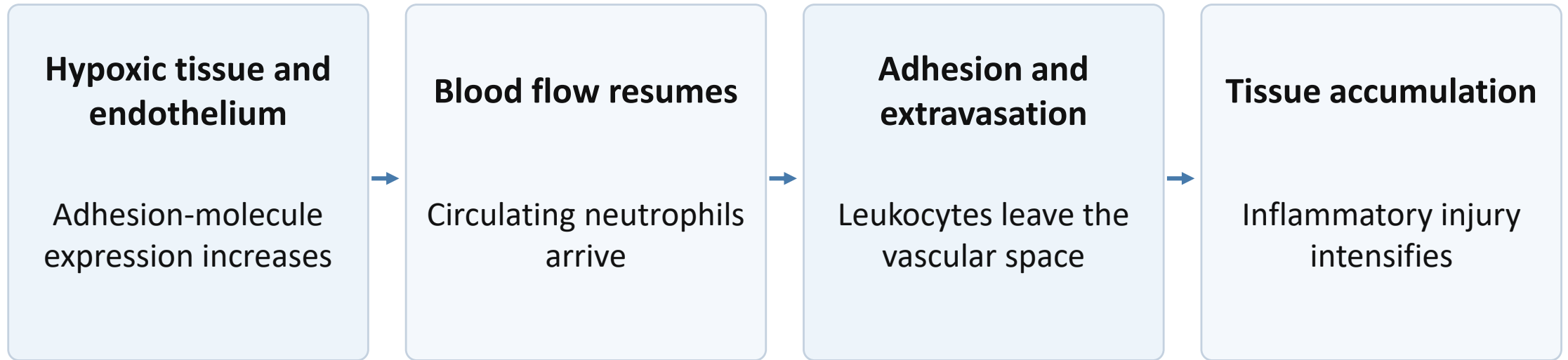
Vascular communication

Cytokine activity supports leukocyte recruitment.

Amplification

Infiltrating leukocytes subsequently increase injury.

Endothelial adhesion facilitates leukocyte influx



Vascular reopening supplies the cellular participants of inflammation.

Recruited neutrophils cause secondary injury

Why neutrophils are recruited

- Cytokines and adhesion molecules signal tissue injury.
- Neutrophils enter the reperfused tissue.
- Their normal role includes response to damaged tissue.

Why their arrival can be harmful

- Activated leukocytes release reactive products.
- Inflammatory activity damages viable neighboring cells.
- Net injury includes both primary ischemia and secondary inflammation.

Reperfusion supplies oxygen and inflammatory cells

Restoration of blood flow

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graph TD; A[Restoration of blood flow] --> B[Oxygen and metabolic substrates]; A --> C[Leukocytes and plasma proteins]; A --> D[Damaged microenvironment]; B --> E[Recovery of salvageable cells]; C --> F[New cellular and humoral injury pathways]; D --> G[Inflammatory signals favor localized amplification];
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Oxygen and metabolic substrates

Recovery of salvageable cells

Leukocytes and plasma proteins

New cellular and humoral injury pathways

Damaged microenvironment

Inflammatory signals favor localized amplification

Neutrophil recruitment has experimental support

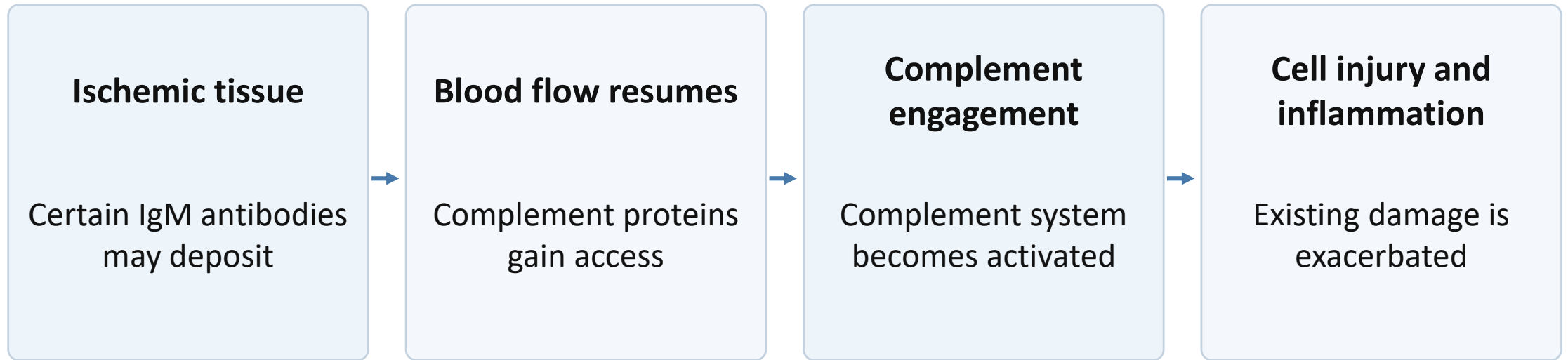
What Robbins reports

- Experimental antibodies blocking cytokines or adhesion molecules reduce neutrophil extravasation.
- These interventions had beneficial effects in reperfusion-injury models.

Proper interpretation

- This supports a causal role for recruited neutrophils.
- It does not establish an approved clinical antibody regimen.
- Experimental protection must not be equated with proven patient benefit.

Complement can be activated by ischemic-tissue IgM



The reason for preferential IgM deposition is unknown.

Complement amplifies more than one injury process

Antibody deposition

Some IgM preferentially localizes to ischemic tissue.

Complement proteins

Restored circulation supplies the humoral components.

Inflammatory amplification

Activated complement augments local inflammation.

Additional injury

Tissue damage can increase after blood flow returns.

Complement: Distinguish claim from uncertainty

Supported in Robbins	Not established by this passage
IgM may deposit in ischemic tissues.	Why the IgM preferentially deposits.
Complement can bind after reperfusion.	A universal mechanism in every patient or organ.
Activation may worsen injury and inflammation.	A clinically proven complement-blocking therapy.

The four mechanisms act as an interconnected network

Oxidants

Injure membranes and calcium-regulating compartments.

Ca²⁺ overload

Drives permeability transition and ATP loss.

Neutrophils

Generate additional oxidants and inflammatory damage.

Complement

Contributes to tissue injury and inflammatory amplification.

Multiple pathways converge on a few lethal endpoints

Oxidative stress + Ca²⁺ overload + inflammation + complement

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graph TD; A["Oxidative stress + Ca²⁺ overload + inflammation + complement"] --> B["Mitochondrial dysfunction"]; A --> C["Membrane injury"]; A --> D["Cell death"];
```

Mitochondrial dysfunction

Failure of oxidative phosphorylation; ATP depletion

Membrane injury

Disturbed compartment barriers and enzyme leakage

Cell death

Additional necrosis in already vulnerable tissue

Necrosis reflects failure of cellular integrity

Interpretation of tissue damage:

- Irreversible ischemic damage already commits some cells to necrosis.
- Reperfusion injury can cause additional cells to lose viability.
- Mitochondrial ATP failure and membrane damage are convergent mechanisms.
- Histologic changes reflect cell death, but routine morphology alone does not apportion damage between the two phases.

Ischemia versus reperfusion: Causal attribution

Question	Ischemic phase	Reperfusion phase
Primary trigger	Reduced perfusion	Restoration of perfusion
Principal energy effect	Less oxidative phosphorylation	Recovery or continued mitochondrial failure
Distinct contributors	Substrate depletion, metabolite buildup	Reoxygenation ROS, Ca ²⁺ aggravation
Inflammatory contribution	Danger signals begin	Neutrophil and complement pathways intensify
Cell viability	Depends on insult severity/duration	Recovery and new injury can coexist

Myocardial infarction provides a clinical setting

Before flow is restored

- Coronary ischemia jeopardizes oxygen-dependent ATP generation.
- Viable cells may be reversibly injured.
- Other cells may already be irreversibly damaged.

After restoration of flow

- Salvageable myocardium has an opportunity to recover.
- Oxidative, calcium and inflammatory mechanisms may injure additional cells.
- Reperfusion injury does not negate the need to restore perfusion.

Cerebral infarction provides a second setting

Ischemic cerebral tissue

- Reduced blood flow compromises oxygen and metabolic substrate delivery.
- Mitochondrial dysfunction and ATP depletion threaten cell survival.
- The extent of irreversible injury varies with the ischemic insult.

After restored cerebral perfusion

- Recovery is possible in still-viable tissue.
- Oxidative stress and inflammatory recruitment may worsen injury.
- Cerebral infarction as clinically relevant to reperfusion injury.

Restoring blood flow remains the therapeutic priority

Goal of restoring perfusion

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graph TD; A[Goal of restoring perfusion] --> B[Save salvageable tissue]; A --> C[Recognize a trade-off]; A --> D[Avoid false inference]; B --> B1[Support surviving cells before irreversible injury occurs]; C --> C1[Reperfusion may contribute additional cellular injury]; D --> D1[A secondary hazard does not make ongoing ischemia desirable];
```

Save salvageable tissue

Support surviving cells before irreversible injury occurs

Recognize a trade-off

Reperfusion may contribute additional cellular injury

Avoid false inference

A secondary hazard does not make ongoing ischemia desirable

Experimental mechanisms are not clinical directives

Observation from the chapter	Legitimate inference	What cannot be concluded
Antibody blockade limits neutrophil influx in experiments	Neutrophils contribute mechanistically	Routine antibody treatment is effective in patients
Calcium overload favors mitochondrial pore opening	Mitochondria are an injury hub	A specific calcium-targeted therapy is established
Oxidants cause macromolecular damage	Oxidative stress is an injury pathway	Any antioxidant necessarily improves clinical outcome

Hypoxic adaptation is distinct from reperfusion injury

HIF-1 during hypoxic stress

- A response to reduced oxygen availability.
- Supports glycolysis, survival pathways and new vessel formation.
- Described in the adjacent ischemic-cell-injury section.

Reperfusion injury

- Arises with restoration of blood flow.
- Includes ROS, Ca²⁺, inflammation and complement activation.
- Cannot be reduced to a failure of one hypoxic adaptive pathway.

Case: The reperfused myocardium

Clinical reasoning scenario

A patient with coronary arterial obstruction receives treatment that restores myocardial blood flow. Despite successful restoration of perfusion, injury may extend beyond cells already lost during ischemia.

Identify two mechanisms that could increase cell loss after reperfusion.

Case analysis: Separate injury phases

Damage already present

- Ischemia reduced oxidative phosphorylation.
- ATP depletion and ion-pump failure injured cells.
- Prolonged damage may have produced irreversible necrosis.

Additional reperfusion injury

- ROS can oxidize membranes and proteins.
- Calcium overload favors mitochondrial permeability transition.
- Inflammatory cells and complement can amplify damage.

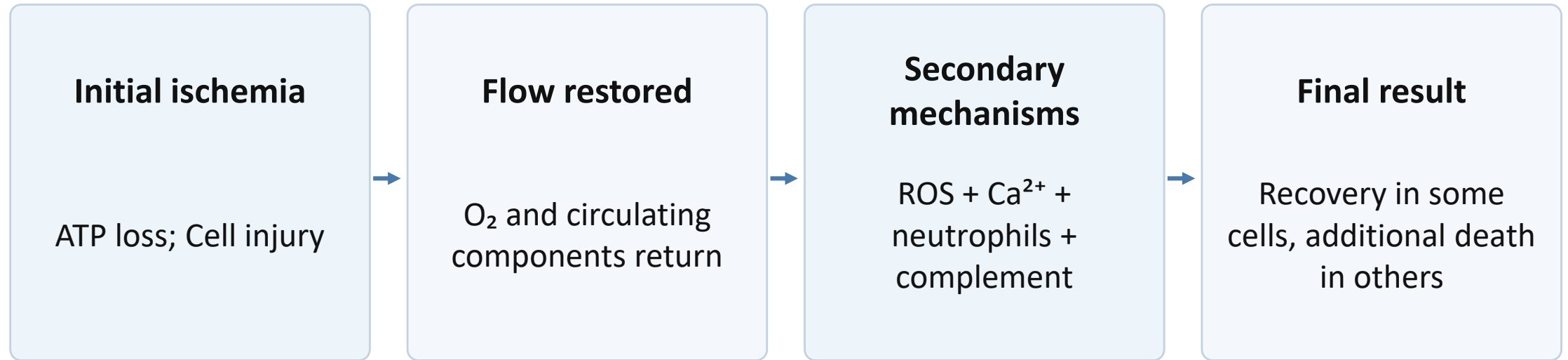
Case: After cerebral blood flow returns

Clinical reasoning scenario

Cerebral perfusion is re-established after a period of ischemia. Some cells recover, while inflammatory cells appear in the injured region and oxidative stress increases.

Why is the presence of recovered perfusion not proof that all remaining injury was caused by the original ischemic period?

Case analysis: A causal explanation



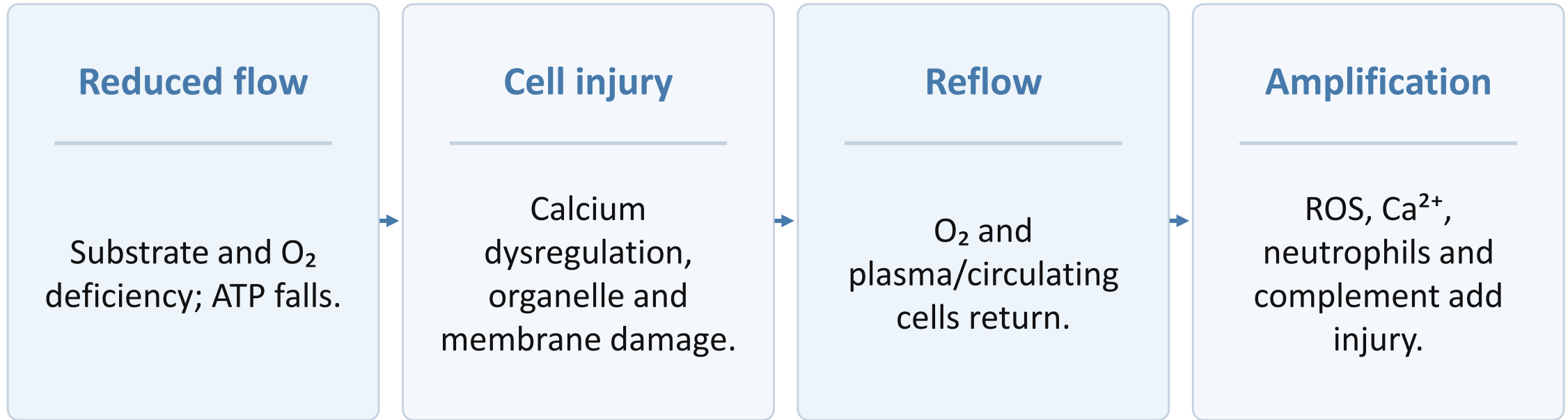
The contribution of each mechanism need not be identical in every tissue.

What cannot be diagnosed from this excerpt alone

Keep the evidentiary boundary clear:

- No tissue-specific histologic criterion uniquely identifies reperfusion injury.
- No diagnostic biomarker cutoff or validated test is specified here.
- No proportion of final infarct size attributable to reperfusion is supplied.
- Mechanistic plausibility does not establish the efficacy of a particular treatment.

Integrating the chronology and mechanistic pathways



Sequence shown conceptually; Cellular outcomes depend on the severity and duration of ischemia.

Mechanistic knowledge check

1. Why does ischemia usually injure faster than isolated hypoxia?

It compromises substrate delivery and metabolite clearance as well as oxygen.

2. What does mitochondrial Ca^{2+} overload favor?

Opening of the permeability transition pore with ATP failure.

3. What finding supports a role for neutrophils?

Experimental benefit from limiting cytokine / adhesion-molecule-mediated extravasation.

Five durable points for clinical pathology

Synthesis of the mechanism:

- Reperfusion is necessary to salvage reversibly injured ischemic tissue.
- A subset of reperfused cells can incur additional injury rather than recover.
- Oxidants, intracellular Ca^{2+} , inflammation and complement are principal proposed mechanisms.
- Mitochondrial failure and membrane damage are important convergent endpoints.
- Mechanistic evidence must be separated from established clinical interventions.

Reference

Robbins, Cotran & Kumar: Pathologic Basis of Disease. 11th edition. 2026.

Chapter 2: Cell Injury, Cell Death, and Adaptations.

Sections: Mechanisms of Cell Injury; Oxidative Stress; Disturbance in Calcium Homeostasis; Hypoxia and Ischemia; Ischemia-Reperfusion Injury.