Ischemia is defined as inadequate blood supply to a part of the body, usually caused by partial or total blockage of an artery. Reperfusion injury occurs when tissue perfusion and oxygenation are restored to an area that has been affected by an ischemic event.

Ischemia/reperfusion (I/R) injury is a complex cascade of events resulting in devastating effects on the body, sometimes including death. Despite more than 70 years of research, I/R injury is not fully understood. Events such as gastric dilatation–volvulus (GDV), mesenteric torsion, or strangulation of a limb can lead to I/R injury. It is important for all veterinary personnel to understand I/R injury so that treatment and prevention can begin as early as possible.

The Ischemic Cascade

The chain of events involved in I/R injury can be broken down into the ischemic cascade (BOX 1) and reperfusion injury (BOX 2). An ischemic episode involves a series of events called the ischemic cascade. Within 5 minutes of the development of ischemia, the electrolyte balance within cells becomes disturbed. The ischemic cascade usually continues for 2 or 3 hours but can last for days, even after perfusion is restored to the affected area. Although the term cascade suggests that events always follow a sequential pattern, in the ischemic cascade, events can occur linearly or simultaneously.

To fully understand the ischemic cascade, it is important to understand the role of adenosine triphosphate (ATP) in the body. ATP is a multifunctional nucleotide (a structural component of DNA and RNA) that is considered to be the most important nucleotide responsible for transporting energy for metabolism within cells. One of the fastest ways that ATP is produced is by oxidative phosphorylation, which, as the name implies, requires oxygen. Despite the importance of ATP, cells do not stockpile ATP. They only make what they need for a particular time. When ischemia occurs, oxygenation of cells ceases, resulting in anaerobic ATP production, which is less efficient.

When oxygen becomes unavailable, cells begin anaerobic glycolysis. This process can be a lifesaving way for cells to obtain energy; however, it is extremely wasteful. During anaerobic glycolysis, pyruvic acid and hydrogen atoms combine with nicotinamide adenine dinucleotide (NAD) to form NADH and H+. If the buildup of NADH and H+ within cells becomes too great, the anaerobic process stops, terminating energy production. However, NADH and H+ combine to form lactic acid, which diffuses from cells rapidly so that the process can continue. Although this is not ideal, the body can safely continue anaerobic glycolysis for several minutes. If the process continues for too long, as in ischemia, lactic acid can build up (lactic acidosis), indicating worsening illness. As a consequence of lactic acidosis, pH decreases, injuring and inactivating mitochondria. These mitochondria then break down, releasing toxins that cause apoptosis. Some researchers think that lactic acid may also interfere with the recovery of aerobic ATP production after ischemia. A lactate level should be obtained for all ischemic patients. Values <2 mmol/L are normal. In patients with GDV, a level >6 mmol/L is associated with increased gastric necrosis. In 1999, a study of 102 dogs with GDV found that 58% of dogs with a blood lactate level >6 mmol/L survived, whereas 99% of dogs with a level <6
Death is an ischemic event because it causes oxygen deprivation at the tissue level. Lactic acid buildup is the major cause of rigor mortis.\textsuperscript{3} When ATP fails to form, cells become depolarized, allowing calcium and sodium (normal extracellular electrolytes) to enter cells.\textsuperscript{5} Excessive intracellular calcium overexcites cells, creating free radicals and many enzymes, such as xanthine dehydrogenase (XDH) and xanthine oxidase (XO). The extent of ischemic damage is related to the amount of calcium that enters cells and the duration for which the intracellular calcium level remains elevated.\textsuperscript{6} The longer calcium stays in cells, the more harmful compounds it can create.

One of the most important events involving calcium is the conversion of XDH to XO.\textsuperscript{4} XO requires oxygen for activation. During ischemia, oxygen is not present, so XO accumulates without getting used. Later, during reperfusion, XO can damage cells.

Another important event during ischemia is the activation of nuclear factor–κB (NFκB), leading to the production of inflammatory mediators.\textsuperscript{1} NFκB becomes activated during stress.\textsuperscript{3} NFκB activates inflammatory cytokines and their receptors as well as platelet-activating factor.\textsuperscript{1} This allows neutrophils to pass through the vascular endothelium. Activated neutrophils are generally rigid because of hypoxia and acidosis, which accompany ischemia. Because of the alteration of the cell membrane and the high number of neutrophils, capillaries may become plugged or clogged by neutrophils.\textsuperscript{7} Even after reperfusion, the redistribution of blood to affected areas may not produce enough force to clear clogs.\textsuperscript{7} The full pathway of NFκB is still not understood.\textsuperscript{1}

### Reperfusion Injury

It would seem that simply reintroducing oxygen into an ischemic area would be beneficial. In patients with GDV, oxygen is restored when the stomach is decompressed or untwisted, allowing oxygen and blood to flow back into the stomach wall. However, the reintroduction of oxygen into affected areas initiates a complex chain of events. The harsh effects of ischemia alone do not cause nearly as much damage as reperfusion does.\textsuperscript{1} The longer the duration of the ischemic event, the greater the insult from reperfusion injury.\textsuperscript{1} An ischemic event may not be long enough to produce a reperfusion injury.

One of the first events in reperfusion is that oxygen binds with XO that has built up during ischemia. The combination of XO, oxygen, and hypoxanthine forms superoxide (O$_2^–$), a radical.\textsuperscript{5} Superoxide is not that damaging but can inactivate iron–sulfur–containing enzymes, liberating free iron and generating highly reactive hydroxyl (OH) radicals. Hydroxyl is considered to be a reactive oxygen species (ROS).

An ROS is an oxygen-containing molecule that is very chemically reactive. ROS molecules react quickly with other molecules. If present in high levels, they can damage cellular macromolecules such as DNA and RNA or cause endothelial injury, microvascular dysfunction, and apoptosis.\textsuperscript{8} ROS molecules can form within 10 to 30 seconds after the onset of reperfusion.\textsuperscript{5}

During ischemia, neutrophils leak into the endothelium because of the activation of NFκB and XO. The inflammatory response to reperfusion accelerates the influx of neutrophils to the affected area.\textsuperscript{9} Neutrophil activation alone can lead to even more ROS formation.\textsuperscript{5} The inflammatory cascade accelerates during reperfusion. In short, neutrophils and macrophages attack reperfused tissues. Inflammatory cytokines are released as neutrophils are activated.\textsuperscript{1} When the body becomes overwhelmed with inflammatory cells, cytokines can be overproduced, resulting in massive cytokine influx (hypercytokinemia) into the affected tissue.\textsuperscript{1} The exact mechanism behind this phenomenon is not fully understood.\textsuperscript{1}

### Complications

The ischemic insult sets up the body for a damaging chain of events that is initiated by reperfusion. Although some researchers debate the exact relationship between I/R injury...
and these events, the end result can be the death of the patient.

Disseminated Intravascular Coagulation
In patients with I/R injury, damaged endothelial cells release substances that activate the clotting cascade. Eventually, the balance between clotting and bleeding becomes disturbed, resulting in disseminated intravascular coagulation (DIC). DIC is a pathologic process in which blood coagulates throughout the body. The result is depletion of platelets and coagulation factors, creating a risk for increased bleeding.1 Petechiae, ecchymoses, and excessive bleeding are often noted in patients with DIC. DIC is generally triggered when there is a major disruption in the intravascular system.4 Anything that causes ischemia creates a major change in the intravascular system. DIC can also occur because of a low pH, which can result when lactate accumulates.

Systemic Inflammatory Response Syndrome
Cytokines produced during reperfusion injury act as mediators of systemic inflammatory response syndrome (SIRS), which is an inflammatory response by the entire body that can result in death. SIRS can be diagnosed if the patient has two or more of the following clinical criteria10:

- Heart rate: >160 bpm in dogs; <140 or >250 bpm in cats
- Respiratory rate: >20 breaths/min in dogs; >40 breaths/min in cats
- Body temperature: <100°F (37.8°C) or >103.5°F (39.7°C) in dogs and cats
- White blood cell count: >12,000 or <4000 cells/µL or >10% bands in dogs and cats

Multiple-Organ Dysfunction Syndrome
As the name implies, multiple-organ dysfunction syndrome (MODS) is altered function of two or more organ systems. MODS usually results from endothelial cell damage caused by overwhelming numbers of cytokines. MODS is usually a complication of sepsis or SIRS.10 If MODS occurs in conjunction with SIRS, the prognosis is very poor.10 As the number of affected organs increases, the patient’s chance of survival decreases.10

Rhabdomyolysis
Rhabdomyolysis is the rapid breakdown of muscle fibers resulting from traumatic injury to skeletal muscles. The principal result is the release of muscle fiber contents, such as myoglobin, into the bloodstream. Myoglobin then circulates through the bloodstream and eventually through the kidneys, where it blocks the structures of the kidneys, causing acute tubular necrosis or kidney failure. Ischemia of the muscles can predispose the body to rhabdomyolysis.1 In children and adolescents, hypoxia associated with propofol administration has been shown to cause rhabdomyolysis.11 Treatment must be aggressive to help the kidneys eliminate myoglobin.

Treatment
There is no known definitive cure for I/R injury. Many doctors and scientists agree that stopping the key components of the ischemic cascade would likely stop the effects of I/R injury. Most also agree that stopping the cascade at the earliest possible point would produce the best results.

It is difficult to test whether an ischemic episode has occurred.3 One of the greatest hindrances to testing is that ischemia occurs when there is a lack of oxygen, and most test samples are exposed to air at some point. This makes obtaining an accurate sample very difficult.

Scientists have tested thousands of treatment methods, drugs, and other interventions to help prevent I/R injury in humans.3 Very few studies have been performed in animals. Treatment has focused on blocking ROS formation, blocking calcium production, and stopping neutrophil activation.3 Much of the research into limiting the activation of neutrophils has had poor results.3 The use of multimodal treatments directed at limiting damage during reperfusion appears to be most effective.12 Specifically, the main focus is on how to either stop ROS production or scavenge the ROS that are formed.3

Focusing on Reactive Oxygen Species
Dimethyl sulfoxide (DMSO) is an effective scavenger of the hydroxyl radical.5,12,13 DMSO can penetrate membranes,
such as mitochondrial membranes, to act at intracellular sites of free radical production. Animal studies have had mixed conclusions regarding the efficacy of DMSO. In cat and rat models, pretreatment with DMSO resulted in a decrease in neutrophil infiltration during reperfusion. Conversely, in a study in 2008, the use of intravenous DMSO at the start of reperfusion injury of equine jejunal mucosa caused by arteriovenous or venous obstruction did not significantly change the severity of the injury after 1 hour of reperfusion. This is because DMSO can create methyl radicals and methyl peroxy radicals when it scavenges hydroxyl radicals. While these other radicals are not as potent as hydroxyl radicals, they can still injure cell membranes. Conclusions about the efficacy of DMSO for treating I/R injury will remain elusive until more is known about these other radicals.

In the past several years, human and veterinary researchers have conducted several promising studies using N-acetylcysteine, which is a powerful scavenger of the hydroxyl radical. While most studies involving rats, rabbits, and mice have produced promising results with this drug, the most recent (2008) veterinary-related study showed that the use of N-acetylcysteine failed to decrease I/R injury of the liver in a canine model. It is clear that more research is needed.

Deferoxamine has been studied for more than 20 years in humans and is now being studied in animals. The research in humans and animals has been promising; in a study in 2009, deferoxamine significantly protected rats with ischemic stroke. This is because deferoxamine is an iron chelator and, therefore, inhibits hydroxyl radical formation.

Allopurinol can inhibit XO formation and neutrophil infiltration during reperfusion. The best results have been obtained when allopurinol has been used as a pretreatment against I/R injury. Because predicting I/R injury is almost impossible, it would be difficult to obtain optimal results on the use of allopurinol in a clinical setting.

Other Options

Lidocaine may prove to be a cost-effective treatment option for I/R injury in animals. Lidocaine may act as a sodium and calcium channel blocker, ROS scavenger, and inflammatory mediator. Because lidocaine also has beneficial analgesic properties, some literature supports administering lidocaine to all at-risk postoperative veterinary patients until they no longer have signs of pain, shock, or ileus (as in GDV).

Ketamine may also prove to be a cost-effective treatment for I/R injury. Ketamine inhibits N-methyl-D-aspartate receptors, reduces neutrophil adhesion, and decreases cytokine production. A study in 2009 concluded that the use of ketamine as an anesthetic reduced intestinal I/R injury in rats. As with lidocaine, ketamine has analgesic properties and is frequently used in veterinary medicine in postsurgical constant-rate infusion analgesic combinations such as morphine–lidocaine–ketamine. Perhaps ketamine could be used similarly to lidocaine in postsurgical patients at risk for I/R injury.

The benefit of steroids in treating I/R injury has long been debated. A study in 2008 suggested that prednisolone may suppress hepatic I/R injury. Steroid therapy has proven to help decrease liver injury by increasing tissue blood flow and suppressing production of oxygen free radicals and cytokines. This study showed that prednisolone can help to prevent I/R injury in rat livers.

The use of colloids may have some benefit in reducing the effects of I/R injury. This is likely because high-molecular-weight colloids can help to decrease microvascular permeability.

Therapeutic hypothermia has been shown to help minimize harmful effects of the inflammatory cascade and

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**Glossary**

- **Aerobic**—pertaining to a process that requires oxygen
- **Anaerobic**—pertaining to a process that does not require oxygen
- **Apoptosis**—the body’s way of safely disposing of dead cell parts by autolysis (self-destruction) of cells
- **Coagulation**—a process in which blood becomes sticky, forming clots
- **Cytokine**—small, secreted proteins that help to mediate an inflammatory response; they are released by activated monocytes, macrophages, and neutrophils
- **Ecchymosis**—a bruise-like spot, larger than a petechia, caused by bleeding from broken blood vessels under the skin into surrounding tissue
- **Free radicals**—radicals that move from where they were created; they are highly reactive and are usually involved in chemical reactions
- **Mitochondrion**—a small body in the cytoplasm of most cells that is responsible for metabolic conversion of energy
- **Petechia**—a tiny red or purple spot on the skin caused by broken blood vessels
decrease ROS production during reperfusion.\textsuperscript{23,24} While therapeutic hypothermia has not yet been used in veterinary medicine, it is a promising possibility. One of the most common I/R injuries in people follows cardiac arrest, which cuts off oxygen to the heart. In 2007, Dr. Lance Becker at the University of Pennsylvania showed that cooling the body after cardiac arrest increases the chance of survival by 16%.\textsuperscript{23} This prompted the American Heart Association to recommend cooling of every cardiac arrest patient.\textsuperscript{23} Since 2007, an injectable ice–salt mixture has allowed emergency personnel to quickly cool people to help slow or even prevent I/R injury.

Conclusion
The pathology of I/R injury is extensive and not fully understood, even in people. More research is needed to help develop tests and treatments for I/R injury. To improve medical and veterinary knowledge, it is important to identify I/R injury and record treatments used.

References
Article #1 FREE CE Test

The article you have read qualifies for 1.0 credit hour. To receive credit from Alfred State College, choose the best answer to each of the following questions. Take the test online at Vetlearn.com.

1. ATP is produced by
   a. an anaerobic process.
   b. phosphorylation.
   c. an aerobic process.
   d. all of the above

2. An increase in lactic acid
   a. decreases pH.
   b. activates mitochondria.
   c. increases pH.
   d. causes neutrophil infiltration.

3. When ATP fails to form and cells become depolarized, which two electrolytes enter cells?
   a. sodium and potassium
   b. calcium and potassium
   c. calcium and magnesium
   d. sodium and calcium

4. Calcium plays a role in the conversion of
   a. XDH to XO.
   b. XDH to XA.
   c. XO to XDH.
   d. XO to XA.

5. Inflammatory mediators are produced because of
   a. ATP production.
   b. XO production.
   c. sodium.
   d. NFkB activation.

6. ________ is a highly reactive radical that causes damage during I/R injury.
   a. Hypoxanthine
   b. XO
   c. Hydroxyl
   d. Superoxide

7. ROS can
   a. directly cause DIC and SIRS.
   b. damage DNA and RNA.
   c. increase lactic acid.
   d. decrease ATP formation.

8. The rapid breakdown of muscle fibers resulting from traumatic injury to the muscles is known as
   a. MODS.
   b. SIRS.
   c. DIC.
   d. rhabdomyolysis.

9. Most therapies for I/R injury have focused on
   a. decreasing neutrophil infiltration.
   b. increasing ATP production.
   c. scavenging or blocking the formation of ROS.
   d. increasing hydroxyl radical production.

10. ________ is an effective scavenger of the hydroxyl radical.
    a. DMSO
    b. Allopurinol
    c. Ketamine
    d. Hydroxyethyl starch