Ischemia/Reperfusion Injury in Skeletal Muscle

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Ischemia/reperfusion injury to skeletal muscle may be explained by a cascade of cellular and systemic events initiated by an ischemic period followed by reperfusion. During the period of ischemia there is a gradual reduction of intracellular energy stores. Adenosine triphosphate is gradually depleted despite the buffering effect of creatine phosphate which is present in large stores in muscles. As well, glycogen stores are depleted with resultant production of small amounts of energy and large accumulations of lactate. Upon reperfusion there is a reactive hyperemia, resulting in an overall increase in muscle blood flow, despite the fact that areas may continue to be underperfused. Results of this blood flow are mixed with the beneficial effects of removing metabolic by-products and supplying exogenous substrates and oxygen. However, this blood flow also causes harmful effects by washing out necessary precursors for adenine nucleotide resynthesis. Production of oxygen free radicals occurs with resultant membrane lipid peroxidation, and calcium influx occurs upon reoxygenation with resultant disruption of oxidative rephosphorylation in the mitochondria. The sequestration of white blood cells in the muscle due to up regulation of both neutrophil receptors and endothelial leukocyte adhesion molecules results in a prolongation of the reperfusion injury. This subsequently results in damage to remote organs, including lung, heart, and kidneys. The future for therapeutic interventions aimed at reducing this injury lie mostly in the ability to modulate the reperfusion effect. (Ann Vasc Surg 1991;5:399-402).

KEY WORDS: ischemia/reperfusion injury; skeletal muscle; intracellular energy; reperfusion.

Skeletal muscle cell damage follows successful revascularization of a lower extremity that has suffered a prolonged period of absent or reduced circulation. The clinically evident sequelae of local swelling, muscle dysfunction, or frank necrosis and remote complications of renal, pulmonary and cardiac injury can be explained by a cascade of cellular and systemic events initiated by a period of ischemia followed by reperfusion.

Skeletal muscle represents 95% of the metabolic activity of the lower extremity, and its response to a period of ischemia most determines the final outcome of the lower extremity [1]. Cellular events during the ischemic phase represent an attempt at preservation of energy stores necessary for metabolic activity in the absence of oxygen. Intracellular energy stores are present in the form of adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), and creatine phosphate (CP). Energy demands for ATP at rest are relatively low in muscle, and under normal circumstances are met by the oxidation of free fatty acids [2], resulting in the aerobic conversion of ADP to ATP. During ischemia, energy stores are
used predominantly to maintain membrane potential and ion compartmentalization. Restricted circulation results in the absence of exogenous substrates, particularly oxygen and free fatty acids, which in turn induces an increased flux along anaerobic pathways for energy production that, in skeletal muscle, are well developed [3].

Continued anaerobic production of ATP can occur along two different pathways in skeletal muscle, through creatine phosphate depletion and by glycolgen metabolism. Large stores of creatine phosphate exist in skeletal muscle, and these can donate a high energy phosphate to an ADP molecule converting it to ATP, a reaction catalyzed by the enzyme creatine kinase. In skeletal muscle large stores of glycogen are present and cytoplasmic enzymes capable of producing ATP by glycolysis exist. Glycogen is broken down through multiple steps, leading to the production of pyruvate. In order to maintain this pathway, pyruvate is converted to lactate with the liberation of a hydrogen ion. As this inefficient production of ATP continues, intracellular pH falls, an event which eventually serves to limit the process of glycolysis by negative feedback upon the rate limiting enzyme phosphofructokinase. We have documented continued lactate production in skeletal muscle for up to six hours during normothermic ischemia [4].

The rate of fall of ATP stores during normothermic ischemia is related to the rate of consumption of ATP and the rate of production through these two pathways. Adenosine triphosphate falls at a very slow rate during the first three hours as it is buffered by the depletion of creatine phosphate stores. At the end of three hours, creatine phosphate stores are completely exhausted, and there is an accelerated rate of decline in ATP stores since glycolysis alone is unable to maintain an adequate rate of ATP production [5]. After six or seven hours there is extensive ATP depletion which correlates with almost complete death of skeletal muscle. There is a good correlation between the fall in energy stores during the period of ischemia as determined by changes in the energy charge potential (the ratio of phosphorylated adenine nucleotides) and the extent of ultimate muscle necrosis [6].

In addition to the dephosphorylation of the adenine nucleotides, ischemia-induced breakdown continues. Adenosine monophosphate is broken down into precursors such as inosine monophosphate, adenosine, hypoxanthine, and xanthine. The significance of this continued adenine nucleotide breakdown during the ischemic phase is related to the fact that these breakdown products are lipid soluble, and when the reperfusion occurs these necessary precursors are washed out of the muscle cells and are unavailable for adenine nucleotide restoration [7]. It is noteworthy that the metabolic pathways for adenine nucleotide synthesis through either salvage pathways or de novo resynthesis are slow and energy dependent themselves [8]. Hence, the ability to replenish energy stores to preischemic levels is severely limited, and the ultimate survival of the muscle depends upon the degree of energy depletion during the phase of ischemia.

In summary, during a period of normothermic ischemia there is gradual depletion of intracellular stores of high energy phosphate bonds and glycogen stores. There is a gradual buildup of products of glycolysis, particularly lactic acid with accompanying hydrogen ion accumulation as well as an increase in intracellular reducing agents. These alterations in normal metabolic function set the stage for events that will occur with the sudden reintroduction of circulation to the lower extremity.

Reperfusion, by providing necessary substrates and the washing out of metabolic by-products, may lead to the restoration of normal muscle metabolic activity and a return to viability in muscle cells. Some cells, which have suffered overwhelming ischemic insults have no chance for restoration yet others, experiencing intermediate degrees of ischemia, may survive, depending upon events that occur during reperfusion. It is well accepted that, with the restoration of blood flow, there are new processes initiated which can lead to the extension of cellular damage, with the potential for worsening injury in otherwise salvageable muscle cells [9]. Understanding these adverse effects of reperfusion may allow selective therapeutic interventions which would lessen the damaging aspects of flow restoration.

Upon revascularization, blood flow is returned to the ischemic extremity, but many of the usual controls are not functioning. A local vasodilation is present, resulting in an overall reactive hyperemia to the extremity. The peak and duration of the increased rate of blood flow are usually related to the time of ischemia in an inverse manner [10], with longer periods of ischemia resulting in reduced reactive hyperemia. The magnitude of the increase in blood flow is not related to the necessity of delivery but rather is an example of ischemia-induced ablation of normal autoregulation [11]. In addition to this overall increase in muscle blood flow, regions of decreased flow may be present due to endothelial swelling and plugging of capillaries by white cells [12].

The effects of this sudden and overwhelming increase in blood flow are multiple. The delivery of substrates is increased dramatically, and, in addition, the larger than normal blood flow accelerates the rate of washout of lipid soluble intracellular metabolites. These include such small molecules as lactate as well as the precursors of adenine nucleotide metabolism, clearly a detrimental effect. This increased blood flow may precipitate an increase in