Blood flow in the microcirculation is regulated according to local metabolic demands of the tissue; however, the mechanism for this regulation is not entirely known. The purpose of this investigation is to analyze the effects of metabolic flow regulation by signals derived from red blood cells (RBCs), from the vessel wall, and from surrounding tissue, in response to changes in oxygen (O2) demand and delivery. A theoretical model is used to simulate blood flow, O2 transport, and flow regulation in microvascular networks with realistic heterogeneous structures. If O2 demand is increased or O2 delivery is decreased, the initial effects are increased regions of poor tissue oxygenation. If the metabolic signal is assumed to originate solely from a RBC-dependent mechanism, the model predicts that flow regulation will cause a reduction in blood flows and further worsening of tissue oxygenation. If the metabolic signal is assumed to originate instead from a wall- or tissue-dependent mechanism, flow regulation causes an increase in flow, resulting in improved tissue oxygenation. These findings suggest that a RBC-independent mechanism of metabolic blood flow regulation is required for an appropriate physiological response to changing O2 demand and delivery. NIH grant HL070657. (Received September 23, 2012)