INTRODUCTION: Despite our identification and incorporation into the clinical operating room of a number of techniques to interrupt the chain of events leading to ischemia/reperfusion (I/R) injury, the rate of failure of replanted and transferred tissue due to inadequate reperfusion is still unacceptable high. During recent years, it has been recognized that nitric oxide (NO) may play a predominant role in I/R injury, both in microcirculation and function. Because skeletal muscle is sensitive to ischemia, resulting in disturbance of microcirculation and loss of contractile function of the muscle, this study was designed to observe the effects of a selective inducible NO synthase (iNOS) inhibitor on microcirculation of denervated I/R skeletal muscle that is more relevant to the clinical practice.

METHODS (approved by IACUC): Under anesthesia, the left cremaster muscles of 24 male rats weighing 90-110 g were isolated and opened on the ventral side via an incision of the scrotum. The testis was separated from the muscle and then severed and discharged. The pudic-epigastric artery and vein and the genito-femoral nerve were isolated along the inguinal canal. All of the vessel branches except the external spermatic artery and vein were divided. The artery, vein and nerve were then separated from each other at the proximal end, and the neck of the cremaster sac cut circumferentially, thereby totally isolating the muscle except for its main neurovascular pedicle. The isolated muscle was then spread onto the surface of a transparent acrylic microscope stage fixed by peripheral sutures, kept moist using Ringer’s solution, and covered with an O2 impermeable polymer. Three hours of ischemia was achieved by clamping the main vascular pedicle of the isolated muscle and denervation by resecting a 3 mm segment of the genitofemoral nerve.

Animals were randomly divided into two groups of 12 rats each. Rats in the experimental group subcutaneously received selective iNOS inhibitor dihydrochloride (1400W, 3mg/kg) at 10 min prior to reperfusion. Rats in the control group received the same volume of phosphate buffering saline. For each group, vessel diameter changes in a selected arterial tree containing vessel sizes of 10-70 µm were measured in 4 rats by using an intravital microscope that connected to a recording and measuring system. Overall blood flow of the muscle was measured in the remaining 8 rats by using a laser Doppler flowmetry. Measurements were recorded at 10 min intervals over a 90 min reperfusion period. The data acquired from each muscle at each time point were compared to the baseline value of each muscle and were expressed as a percentage of baseline. One-way analysis of variance (ANOVA) and then repeated measures analysis of variance were performed. Bonferroni correction was applied where appropriate. A p<0.05 was taken as significant.

RESULTS: At 10 min of reperfusion, the mean blood flow was 30.2±16% of baseline in the control group and 76.3±16% in the 1400W-treated group, with a significant (p<0.01) 2.5-fold difference. The blood flow gradually increased to 67.4±32% in the control group at 60 min and remained at this level throughout the remainder of the 90 min reperfusion period. In the 1400W-treated group, the mean blood flow reached its maximum of 106.3±18% at 50 min of reperfusion. When compared to the controls, the 1400W-treated cremaster muscles had a significantly (p<0.01) greater flow at all time points, except at 60 and 90 min (Fig. 1).

The average diameters of three vessel diameter categories (10-20, 21-40, and 41-70 µm) in the controls were between 54% and 62% of baseline at 10 min of reperfusion and gradually increased to the maximum level of 78.8±11% in 10-20 µm, 73.2±14% in 21-40 µm, 85.3±3% in 41-70 µm vessels at 90 min of reperfusion. In contrast, the diameter in the 1400W-treated group sharply increased to over baseline level in all three vessel categories at 10 min of reperfusion and remained at this level throughout 90 min of observation. There was a significant (p<0.001) difference in mean vessel diameter between the two groups throughout the experiment (Fig. 2).

DISCUSSION: The results showed that treatment with dihydrochloride (1400W) remarkably magnified blood flow and increased vessel diameter in the denervated cremaster muscle during early reperfusion following 3 hrs of warm ischemia, thereby suggesting improved microcirculation in the reperfused muscle. These events occurred as early as at the first 10 minutes of reperfusion and lasted at least 90 minutes. Dihydrochloride is a highly selective inhibitor of iNOS. It is known that tissue damage, including ischemia and reperfusion injury, induces iNOS activity to produce a large amount of NO, which further exerts toxic effects on the tissue. The data from this study suggest that 1) Ischemia/reperfusion results in an increase of iNOS activity and/or production in the reperfused tissues. 2) Selective inhibition of iNOS improves microcirculation in the reperfused tissue, thereby reducing reperfusion injury and improving the “no-reflow” phenomenon. 3) Inhibition of iNOS by dihydrochloride is independent of invervation. Our data suggest a NO mechanism in reperfusion injury and offer a potential pathway of inhibiting iNOS for future clinical use.