Although immediate transplantation provides the best prognosis for solid organ survival and function, limb storage and preservation is usually required prior to surgical intervention. Hypothermic ex-situ perfusion (HESP) systems and Static Cold Storage (SCS) methods are both clinically employed to prolong allograft survival.

In this study, we investigated the long-term effects of HESP and SCS preservation on skeletal muscle metabolism, structure, and force generation and compared it to immediate transplantation in a rat model.

**Experimental Methods**

Forty male Lewis rats were divided into 5 study groups as follows: naïve control, sciatic nerve transection/repair, immediate transplantation, SCS, and HESP. For the last three study groups, donor limbs were amputated at the mid-femoral level and fixed to the recipient femur using an 18-gauge needle as an intramedullary rod. In the SCS group, donor hind limbs were preserved at 4°C for 6 hours. In the HESP group, limbs were continuously perfused with oxygenated Histidine-Tryptophan-Ketoglutarate (HTK) solution at 10-15°C for 6 hours, with continuous monitoring of hemodynamic and biochemical parameters.

At 12 weeks post-surgery, all limbs underwent (A), electromyography, and (B), force measurements, followed by muscle sample harvests for histology and metabolomics analysis.

**Results**

Histology demonstrated 49% myocyte injury in the immediate transplantation group, compared to 48% injury in the HESP, and 74% in the SCS groups (p<0.05). Figure 1 and 2. Latency, a measure of demyelination, was preserved better in the immediate transplantation group and the static cold storage groups, than those in the hypothermic ex-situ perfusion group (p<0.05). Amplitude, a measure of depolarizing muscle fibers, demonstrated reduced number of axons on both static cold storage and the hypothermic ex-situ perfusion groups compared to immediate transplantation group (p<0.05). Figure 3.

The maximum twitch and tetanic force measurements were significantly higher in the immediate transplantation group, and 40% to 50% lower in the SCS and HESP groups, respectively, suggesting that both limb preservation methods experience similar amounts of axonal loss and demyelination. Figure 4.

Muscle metabolomic profiling of energy markers was similar between the HESP and SCS groups, with the exception of increased phosphocreatine levels, as well as significantly increased amino acid levels in HESP preserved transplants, indicating better energy storage, and lower reperfusion injury.

**Conclusion**

While both SCS and HESP limb storage methods exhibit similar physiological preservation of peripheral nerves, HESP improves skeletal muscle viability in transplanted limbs.