# Neither Hematocrit Normalization nor Exercise Training Restores Oxygen Consumption to Normal in Hemodialysis Patients

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**Running title:** Mechanisms of impaired exercise capacity in ESRD

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## Detailed Methods

### Study Design

This study was a cross-over design (see Figure 1). Following enrollment and baseline assessment patients were randomized to either Group A or B. Group A participants' hematocrit levels were increased to 42% "UN42", while Group B levels were maintained at 30% "UN30". After approximately 12 weeks the testing battery was repeated before Group A performed ~12 weeks of intradialytic exercise training (36 exercise sessions) while maintaining hematocrit levels at 42% "TR42", and Group B performed 12 weeks of intradialytic exercise training (36 exercise sessions) while maintaining hematocrit levels at 30% "TR30". All subjects then stopped training for 12 week, while Hct decreased to 30% in Group A and increased to 42% in Group B. The testing battery was repeated again after 12 weeks, before both groups completed a further 12 weeks of exercise training.

### Interventions

*Hematocrit management.* Blood samples were collected for hematocrit and hemoglobin concentration on EDTA prior to the second and third dialysis session of each week. During the normalized hematocrit period for Group A and the normalized hematocrit period for Group B, the patient's recombinant ESA dose was increased initially by 150% of the maintenance dose. If the ESA dose was insufficient to raise hematocrit after 2 weeks, the dose was doubled every 2 weeks until the target hematocrit (42±3%) was obtained. The ESA dose was then titrated to maintain the hematocrit within the target range. While hematocrit was maintained at 30% for Group A, a placebo was administered until the patient's hematocrit approached 30±3%, thereafter the original ESA dose

necessary to maintain the desired hematocrit was reintroduced and titrated to maintain the hematocrit in the target range. Hematocrit levels were managed by one of the primary investigators (JT). The investigator did not share this knowledge of the subject's group assignment with any patients, staff or investigator in the study. The staff in the dialysis unit were provided with identically labelled syringes with the patients dosages of ESA adjusted with diluent so the volume was identical, which was administered by a clinical nurse. This investigator (JT) had no involvement with study subject training or exercise testing. While participating in the study, ESA for the patient was provided complimentary by the sponsor. Staff at the dialysis units were unaware of the specific ESA dose (or placebo) delivered to the patients in the study or of the patient's hematocrit.

*Exercise Training.* Supervised exercise training was performed during the second hour of the dialysis session 3 times per week for 12 weeks. Target workloads were based on the initial exercise test and each patient was encouraged to pedal at 75% of their peak power output. Exercise duration and intensity were gradually increased over the 12 weeks, such that by workout 24 participants were cycling for up to 60 min and at the highest tolerable workload that could be sustained. At week 9, high intensity interval training was introduced with every third workout consisting of an interval session. The intervals were either 1 min at 200% of the pre-training peak power output, or 2-3 min intervals at 150-200% of pre-training peak power. Each session was supervised by a trainer and heart rate (HR) (Polar, Finland) was monitored throughout the session as was cadence which was maintained at 50 rpm.

To quantify exercise training the absolute amount of work performed (kilojoules) in each session (kJ/session) was calculated along with training impulse (TRIMPS) of the workout (Trimps/session).[1] The TRIMP method multiplies the duration of a training session by the average heart rate achieved during that session, weighted for exercise intensity as a function of heart rate reserve. Using this method, exercise sessions of longer duration and/or greater intensity such as interval workouts, are assigned relatively higher TRIMP values (higher heart rate, and higher weighting factor), than sessions of shorter duration and/or lower intensity. TRIMP is commonly used technique to assess and monitor exercise training load[2]. The absolute work performed by the Trimps score provided a ratio (kJ/Trimp) to normalize the absolute work to relative effort. The goal for each patient was to complete at least 33 "good" sessions (92% of all sessions) before post-training testing was performed. Post-testing was scheduled at the end of each phase when an individual subject had completed the goal of that phase (see figure 1, either attain the target hematocrit or completion of 33 or more "good" training sessions). The actual time for completion of study phases was 17±10 weeks for Phase 1, 15±2 weeks for Phase 2, 18±10 weeks for Phase 3, and 15±4 weeks for Phase 4. Thus, the patients spent an average of 16 months enrolled in the study.

**Measurements and Outcomes**

**On-Dialysis Day Assessments.**

Approximately 44 hours after the last dialysis session patients attended the dialysis unit, where the arterial side of their vascular access was cannulated and routine blood samples were performed, before being transported to our laboratory. On arrival, body weight was

determined and the patient was instrumented. The patient then rested quietly in the supine position for 60 minutes before resting HR and blood pressure (BP) were measured.

*Blood Volume.* Blood volume was measured using the Evan's Blue dye dilution technique.[3] Values were indexed to body weight and are presented as milliliters per kilogram body mass (ml/kg).

*Exercise testing.* Maximal exercise testing was performed on a cycle ergometer (Monark 818E, Sweden) 44 hours post the last dialysis session. Resistance was increased every 3 minutes until the patient could not maintain 40 rpm. Measures of ventilatory gas exchange were made using the Douglas bag technique. HR (Polar, Finland) and a 3-lead ECG (telemetry unit, Transkinetics) were continuously monitored throughout the test. At the end of each stage BP (STBP-780 Colin Medical Instruments, South Plainfield, NJ), arterial blood gas (ILS-1306 blood gas analyzer and ILS-482 co-oximeter) and cardiac output (Qc; modification of foreign gas rebreathing method[4, 5]) were measured. Patients were then given 2-3 minutes rest and asked to perform a slightly higher workload. This continued until a plateau in $VO_2$ occurred, or the patient could not complete the workload). "Peak" values for oxygen uptake and power were the highest values obtained during the test. Data collected from the exercise test included power output, ventilation, oxygen uptake, carbon dioxide production, Qc, HR, systolic and diastolic BP, arterial oxygen content, arterial pH, and arterial lactate concentration for the assigned "submaximal" and "peak" power outputs. Data calculated from the collected data included respiratory quotient, stroke volume, mean arterial pressure, systemic vascular

resistance (SVR), a-$VO_2$ diff, mixed venous oxygen content and tissue diffusing capacity (peak data only). Briefly, the calculation of tissue diffusing capacity allows the assessment of oxygen transport, $VO_2$ max = diffusing capacity x (mean muscle capillary $PO_2$ – mitochondrial $PO_2$).[6] To calculate tissue diffusing capacity the following variables were imputed, muscle blood flow (assumed to = cardiac output), arterial blood gases ($PO_2$, $PCO_2$, pH, $O_2$ saturation), estimated tissue venous blood gases (based on the Fick principle), hemoglobin and hemoglobin p50 and blood temperature. Muscle capillary PO2 is then calculated using a custom designed computer program[7].

**Off-Dialysis Day Assessments.**

The day following a dialysis session, the patient would report to the laboratory for a series of procedures listed below.

*Quadriceps biopsy.* Muscle biopsies were performed on the right medial vastus lateralis. Prior to incision the biopsy site was anesthetized with 1% Xylocaine. Approximately 50-75 g of muscle was sampled using a 5mm Bergstrom needle under suction. The same general location was biopsied at each time point. The specimen was rolled on blotting paper to remove blood. Any obvious fat was removed, taking care not to distort the muscle architecture. The specimen was then separated into three parts, for light microscopy, electron microscopy and biochemical analyses. The specimen for light microscopy was oriented along the axis of the fibers and then quickly immersed in Freon 22 cooled to liquid nitrogen temperature and then transferred to a -80°C freezer until analysis. The specimen for electron microscopy was immersed and stored in a solution of 10% glutaraldehyde. The specimen for biochemistry was immersed in liquid nitrogen and

transferred to a -80°C freezer until analysis. All specimens from a given subject were analyzed on the same run of a given assay.

*Fiber type and size*: Adjacent 10 μm sections were cut on a Slee cryostat at -20°C, dried overnight, and stained for myosin ATPase at pH of 10.20 and 22°C as previously reported.[8] Fibers were designated Type I or Type II.

*Capillary density*: Adjacent 5μm sections were cut and dried overnight in the same manner as the sections for fiber type. Sections were incubated for one hour with a Ulex lectin, then incubated for another hour in avidin-alkaline phosphatase, then counterstained with Fast Green to provide contrast as previously reported.[9]

*Image analysis*: Percentage of each fiber type and fiber areas was assessed in duplicate from 3-4 full fields at 20X, by two blinded experienced technicians. Capillary density and capillary-to-fiber ratio were read at 40X. Inter and intra observer variability was < 10%. At least 50 fibers of each type were counted and measured utilizing an automated image analysis system. Fiber type is expressed as a percent of total fibers counted. Fiber area is expressed in square microns ($\mu m^2$). Capillary density is expressed as capillaries per square micron ($caps \cdot \mu m^{-2}$) and as a ratio of capillaries to fiber ($caps \cdot fiber^{-1}$).

*Electron microscopy*: After fixing in 2% glutaldehyde, specimens were serially dehydrated in alcohol and contrast enhanced with a silver stain. The specimens were then imbedded in plastic for sectioning. Ten Ångstrom thick sections were prepared and

visualized on an electron microscope at 5,000X. Capillaries that had been cut at 90° to the plane of the section were photographed with the interstitial space and some surrounding muscle. The photographs were then scanned into the image analysis system described above. Two blinded experienced technicians, measured capillary endothelium and capillary basement membrane thickness in six radial directions, taking care to avoid areas including endothelial nuclear material. Measurements were averaged and expressed in nanometers. Seven subjects (5 male/ 2 female) with normal renal function and similar age to our patients had biopsies analyzed in the same manner for comparison.

*Enzyme activities*: All specimens from a given subject were analyzed at the same time. Five to 10mg pieces of frozen muscle were pulverized in tissue grinders mixed with homogenizing buffers to the desired dilutions. Activities for citrate synthase, acyl-coA Dehydrogenase and phosphofructokinase were measured with a flurometer on muscle homogenates at pH 7.0 and room temperature. Five reactions for each biopsy, for each enzyme were performed. Reaction slopes were averaged. Values are expressed as micromoles of NADH per minute per gram dry weight of tissue ($\mu m \cdot min^{-1} \cdot drygm^{-1}$).

*Tissue buffering capacity*: Tissue buffering capacity was measured on muscle homogenates by the method described by Mizuno et al.[10] Five mg pieces of frozen muscle were dehydrated by vacuum at liquid nitrogen temperatures. Then the dry specimens were pulverized in tissue grinders and diluted in a Na solution. The pH was measured on a Accumet 915 pH meter (Fisher Scientific) equipped with a microelectrode and then adjusted to 7.00 by addition of 0.01N sodium hydroxide solution. The pH of the

solution was then carefully titrated to pH 6.00 with 0.01N hydrochloric acid. The amount of HCl acid required to lower the pH of the specimen from 7.00 to 6.00 is defined as the tissue buffering capacity. Values are expressed as micromoles of $H^+$ per pH unit per gram dry weight of tissue ($\mu mH^+ \cdot pHunit^{-1} \cdot drygm^{-1}$).

*Muscle myoglobin concentration*: Myoglobin concentrations were measured on muscle homogenates by RIA using a standardized kit designed to measure plasma levels of myoglobin. Cross reactivity/sensitivity values are expressed as nanograms of myoglobin per gram wet weight of tissue ($ng \cdot wetgm^{-1}$).

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