Effects of Erythropoietin on Muscle O_2 Transport during Exercise in Patients with Chronic Renal Failure

Ramon M. Marrades,* Josep Roca,* Josep M. Campistol,* Orlando Diaz,* Joan A. Barberà,* Josep V. Torregrosa,* Joan R. Masclans,* Albert Cobos,‡ Robert Rodríguez-Roisin,* and Peter D. Wagner§

**Servei di Pneumologia i Al.lèrgia Respiratòria, Unitat de Transplantament Renal, Hospital Clínic, Departament de Medicina, Universitat de Barcelona; ‡Unit of Biometry, Medical Department, QF Bayer SA, 08036 Barcelona; and §Department of Medicine, Section of Physiology, University of California, San Diego, La Jolla, California 92093-0623*

Abstract

Erythropoietin (rHuEPO) has proven to be effective in the treatment of anemia of chronic renal failure (CRF). Despite improving the quality of life, peak oxygen uptake after rHuEPO therapy is not improved as much as the increase in hemoglobin concentration ([Hb]) would predict. We hypothesized that this discrepancy is due to failure of O_2 transport rates to rise in a manner proportional to [Hb]. To test this, eight patients with CRF undergoing regular hemodialysis were studied pre- and post-rHuEPO ([Hb] = 7.5±1.0 vs. 12.5±1.0 g · dl^{-1}) using a standard incremental cycle exercise protocol. A group of 12 healthy sedentary subjects of similar age and anthropometric characteristics served as controls. Arterial and femoral venous blood gas data were obtained and coupled with simultaneous measurements of femoral venous blood flow ($\dot{Q}$leg) by thermodilution to obtain O_2 delivery and oxygen uptake ($\dot{V}O_2$). Despite a 69% increase in [Hb], peak $\dot{V}O_2$ increased by only 33%. This could be explained largely by reduced peak leg blood flow, limiting the gain in O_2 delivery to 37%. At peak $\dot{V}O_2$, after rHuEPO, O_2 supply limitation of maximal $\dot{V}O_2$ was found to occur, permitting the calculation of a value for muscle O_2 conductance from capillary to mitochondria (DO_2). While DO_2 was slightly improved after rHuEPO, it was only 67% of that of sedentary control subjects. This kept maximal oxygen extraction at only 70%. Two important conclusions can be reached from this study. First, the increase in [Hb] produced by rHuEPO is accompanied by a significant reduction in peak blood flow to exercising muscle, which limits the gain in oxygen transport. Second, even after restoration of [Hb], O_2 conductance from the muscle capillary to the mitochondria remains considerably below normal. (*J. Clin. Invest.* 1996. 97:2092–2100.). Key words: exercise • leg blood flow • muscle O_2 conductance • oxygen delivery • oxygen uptake

Introduction

Anemia is a prominent complication of chronic renal failure (CRF)[1] that requires treatment in a significant number of patients undergoing hemodialysis (1, 2). Since the late 80's, the therapy with recombinant human erythropoietin (rHuEPO) has effectively eliminated the need for red cell transfusions (1–4), thus avoiding commonly associated complications such as infections and iron overload. Patients receiving rHuEPO significantly improve their quality of life, but they exhibit a higher incidence of hypertension seemingly associated with the rHuEPO dosage (5).

Physiological studies on the long-term cardiorespiratory effects of rHuEPO treatment suggest that the increase in hemoglobin concentration results in a suppression of the hyperdynamic cardiac state of these patients together with a modest improvement of the aerobic exercise performance (6–13). However, the effects of increased arterial O_2 content (CaO_2) on muscle O_2 use are not clear in CRF patients, and some of them fail to significantly improve aerobic exercise capacity after rHuEPO, despite near normalization of hemoglobin concentration ([Hb]) (14). This limited exercise response could be explained by one or more of the following factors: (*a*) gains in muscle O_2 delivery, calculated as the product of arterial O_2 content and muscle blood flow, could be less than expected; (*b*) muscle O_2 conductance from the muscle microcirculation to the mitochondria may be abnormally low, reducing the effect of increased O_2 delivery; or (*c*) oxygen uptake ($\dot{V}O_2$) may not be O_2 supply limited, such that enzyme or substrate limitation would play the key role in determining $\dot{V}O_2$max.

The current study was undertaken to assess the role of each of the above-mentioned mechanisms in eight sedentary young previously anemic, erythropoietin-treated patients with chronic renal failure undergoing regular hemodialysis. All performed maximum exercise on a bicycle ergometer at two different inspired O_2 concentrations (F_IO_2), both before and after rHuEPO therapy to assess O_2 supply-dependence of peak $\dot{V}O_2$. Data from 12 matched healthy young sedentary subjects from a previous study (15) following a similar protocol were used as control values. In all subjects, whole-body $\dot{V}O_2$, $\dot{V}O_2$

Address correspondence to Josep Roca, M.D., Servei de Pneumologia, Hospital Clínic, Villarroel 170, Barcelona 08036, Spain. Phone: 343-227-5540; FAX: 343-227-5455; E-mail: roca@medicina.ub.es

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1. *Abbreviations used in this paper:* CaO_2, arterial O_2 content; $C_{fv}O_2$ femoral venous O_2 content; CRF, chronic renal failure; DO_2, muscle O_2 conductance from the capillary to the mitochondria; F_IO_2, inspired O_2 concentration; [Hb], hemoglobin concentration; La_{fv}, lactate levels in femoral venous blood; $\dot{Q}$leg, one-leg femoral venous blood flow; $\dot{Q}O_2$leg, one-leg O_2 delivery; RER, respiratory exchange ratio; rHuEPO, recombinant human erythropoietin; $\dot{V}_E$, minute ventilation; $\dot{V}O_2$, whole-body O_2 uptake; $\dot{V}O_2$leg, one-leg O_2 uptake.

Table I. Anthropometric Data, Lung Function, Adequacy of Dialysis, and Changes in Hemoglobin Concentration

Subject	Age	Height	Weight	FEV_1		FEV_1/FVC	PaO_2	Kt/V	PCR	HD	Dialyzer	[Hb]	[Hb]	Δ[Hb]
	yr	*cm*	*kg*	*liters*	*%pred*	*%*	*mmHg*	*urea*	$g \cdot k^{-1} \cdot d$	$h \cdot wk^{-1}$		*pre-rHuEPO*	*post-rHuEPO*	$g \cdot dl^{-1}$
MNP	20	176	62	4.9	(109)	81	101	1.1	0.9	12	C 1.5	9.2	12.5	3.3
RCS	20	167	58	4.6	(114)	87	109	1.2	1.1	12	C 1.3	7.3	12.5	5.2
VLL	21	166	65	4.6	(114)	87	101	1.1	1.0	12	CA 1.4	7.7	11.0	3.3
JRM	20	172	58	4.3	(99)	93	116	1.0	0.9	10.5	C 1.3	8.5	11.0	2.5
MMB	29	165	80	3.5	(92)	88	113	1.2	1.2	12	CA 1.4	7.5	12.3	4.8
JGG	21	167	83	3.8	(95)	87	87	1.0	1.0	12	C 1.5	7.7	14.2	6.5
FMM	29	172	70	3.6	(85)	81	100	1.3	1.2	12	C 1.7	6.5	13.8	7.3
JMR	29	169	63	2.9	(73)	79	111	1.2	1.1	13.5	C 1.5	5.9	12.7	6.8
Mean	24	169	67	4.0	(98)	85	105	1.1	1.1	12		7.5	12.5	5.0
SD	5	5	10	0.7	15	4	9	0.1	0.1	0.8		1.0	1.0	1.8

Results expressed as mean±SD; FEV_1, forced expiratory volume in the first second in liters and as percentage of predicted value within parentheses (17); FEV_1/FVC, FEV_1 to forced vital capacity ratio; PaO_2, PO_2 in arterial blood; Kt/V(urea), indicates the decrease of BUN during each dialysis treatment, dimensionless; PCR, protein catabolic rate expressed in $g \cdot k^{-1} \cdot d$; HD, duration of dialysis in $h \cdot wk^{-1}$ (three times a wk); Dialyzer, type of membrane of the dialyzer and surface expressed in square meters: C 1.5, Cuprophane 1.5 m^2, CA 1.4, Cellulose Acetate 1.4 m^2; [Hb] pre-rHuEPO and [Hb] post-rHuEPO, hemoglobin concentration in the pre-rHuEPO and post-rHuEPO studies expressed in $g \cdot dl^{-1}$, respectively; Δ[Hb], increase in [Hb] after rHuEPO therapy.

of one leg, and the convective and diffusive components of muscle O_2 transport (16) were assessed.

Methods

Population. Eight sedentary anemic men (age 24±4.5 [mean±SD] yr; height 169±5 cm; and wt 67±10 kg) with chronic renal failure undergoing regular hemodialysis over the preceding 12±18 mo, were enrolled in the study. Anthropometric, lung function data, adequacy of dialysis, and [Hb] before and after rHuEPO therapy using standard dosage (92.5 ± 21 UI $\cdot kg^{-1} \cdot week^{-1}$) are listed in Table I. All were informed of any risks and discomfort associated with the experiment and informed consent was obtained in accordance with the Committee on Investigations Involving Human Subjects at the Hospital Clínic, Universitat de Barcelona. Subject preparation, safety precautions, and technical aspects of the central measurements (arterial and femoral venous blood gases and femoral venous blood flow) have been described in detail elsewhere (15, 18).

Data from twelve healthy sedentary subjects, (11 males, 1 female) (age 22±3.2 y; height 174±8 cm; and wt 71±10 kg; hemoglobin concentration 13.8 $g \cdot dl^{-1}$) selected on the basis of no previous history of regular or even occasional physical exercise above that required for average daily activities, were used as control values. These data have been previously reported (15).

Preliminary measurements. Initially, each subject performed a standard noninvasive incremental cycle exercise test (20-Watt increments every 2 min) until exhaustion. This test, which was carried out breathing room air, served to determine maximal exercise capacity (cycloergometer; E. Jaeger, Würzburg, Germany).

Principal studies. On a single day, each renal failure patient performed two similar exercise tests to exhaustion. The only difference between each test was inspired O_2 concentration (F_IO_2). This series of two tests was carried out twice, once before and once after rHuEPO. In the pre-rHuEPO study, the F_IO_2 values were 0.21 in one test and 1.0 in the other, whereas in the post-rHuEPO study, the corresponding F_IO_2's were 0.13 and 0.21. The order of presentation of the 2 inspirates was randomized but was identical for each patient in the pre- and post-rHuEPO studies. Between the two exercise runs, the subject rested for fully 1 h to ensure an adequate recovery. A target workload for each test was defined as the maximum workload sustained for 2 min in the preliminary noninvasive study. However, an additional 20 W increment was tried to ensure that maximum exercise capacity had in fact been reached in each condition. The same level of daily physical activity (as before rHuEPO) was maintained throughout the period of the study despite the improvement in their quality of life, as assessed using a physical activity questionnaire. Pre- and post-rHuEPO studies in all the patients were done between 18 and 24 h after hemodialysis treatment (Table I). The time elapsed between pre- and post-rHuEPO studies was 7±5 mo.

On-line calculations of whole body $\dot{V}O_2$, CO_2 output ($\dot{V}CO_2$), minute ventilation ($\dot{V}_E$), respiratory exchange ratio (RER), heart rate, and respiratory rate were averaged sequentially over 15-s intervals and displayed on a screen monitor to observe the progress of the tests and confirm a steady state for $\dot{V}O_2$. In each test, in the eight CRF patients in the pre- and post-rHuEPO studies, measurements were made under the following conditions: (*a*) at rest; (*b*) during submaximal workloads (30, 60, and 80% of peak workload); and (*c*) at maximum workload. In the 12 healthy sedentary subjects measurements were done only: (*a*) at rest; (*b*) at 60% of maximum workload; and (*c*) at maximum workload (15). In each instance the following measurements were made: (*a*) PO_2, PCO_2, pH (IL model 1302, pH/Blood gas analyzer and tonometer model 237. Instrumentation Laboratories, Milan, Italy), oxyhemoglobin saturation, [Hb] (IL 482 co-oximeter; Instrumentation Laboratories), and blood lactate concentrations (YSI 23L blood lactate analyzer; Yellow Springs Instruments, Yellow Springs, OH) from simultaneous arterial and femoral venous blood samples; and (*b*) femoral venous blood flow ($\dot{Q}$leg) and arterial pressure. As indicated above, $\dot{V}_E$, F_EO_2, F_ECO_2, and HR were continuously monitored. Technical aspects of these measurements have been previously provided in detail (15).

In the present study, blood O_2 content was calculated as follows: $[(1.39 \cdot [Hb] \cdot \textit{measured oxyhemoglobin saturation}) + (0.003 \cdot PO_2)]$. This was done for arterial (CaO_2) as well as venous ($C_{fv}O_2$) blood. The O_2 delivery to the exercising leg ($\dot{Q}O_2$leg) was calculated as the product of arterial O_2 content and leg blood flow [$\dot{Q}O_2leg = C_aO_2 \cdot \dot{Q}leg$]. Leg O_2 uptake ($\dot{V}O_2$leg) was obtained as the product of Qleg and the arterial − femoral venous difference of O_2 content [$\dot{V}O_2leg = \dot{Q}leg \cdot (C_aO_2 - C_{fv}O_2)$]. Leg O_2 extraction ratio (O_2ER) was calculated as the ratio of the arterial to femoral venous O_2 content difference and the arterial O_2 content [$O_2ER = 100.(C_aO_2 - C_{fv}O_2)/C_aO_2$]. Net lactate output across the leg ($\dot{L}a$) was obtained as the product between $\dot{Q}$leg and the femoral venous to arterial difference in blood lactate concentrations [$\dot{L}a = \dot{Q}leg \cdot (La_{fv} - La_a)$]. In each subject, mea-

Table II. Whole-body Variables during Submaximal and Peak Exercise, $F_IO_2 = 0.21$

		Rest	30% W	60% W	80% W	100% W	*P* pre-post	*P* post-con
Watts	Pre-rHuEPO	0	41±8	83±18	111±23	134±26	NS	0.0001
	Post-rHuEPO	0	40±11	90±19	120±15	145±20		
	Control	0	64±8	115±15	170±21	216±34		
$\dot{V}O_2$	Pre-rHuEPO	0.34±0.06	0.98±0.07	1.26±0.23	1.51±0.23	1.69±0.28	0.001	0.03
liters · min^{-1}	Post-rHuEPO	0.35±0.05	1.02±0.15	1.46±0.35	1.76±0.24	2.18±0.33		
	Control	0.32±0.07	1.05±0.22	1.74±0.22	2.18±0.28	2.66±0.48		
$\dot{V}O_2$	Pre-rHuEPO	4.6±2.0	14.7±1.8	19.1±4.4	22.6±3.4	25.4±4.6	0.003	NS
ml · kg^{-1} · min^{-1}	Post-rHuEPO	5.3±0.8	15.5±1.4	21.9±3.6	26.7±2.1	33.1±4.7		
	Control	4.4±1.4	14.6±2.7	24.3±2.9	30.4±3.9	36.9±5.9		
$\dot{V}CO_2$	Pre-rHuEPO	0.30±0.06	0.89±0.11	1.40±0.22	1.85±0.29	2.21±0.33	0.01	0.03
liters · min^{-1}	Post-rHuEPO	0.31±0.06	0.86±0.12	1.48±0.33	1.96±0.28	2.62±0.33		
	Control	0.29±0.06	0.96±0.28	1.80±0.20	2.52±0.31	3.51±0.63		
RER	Pre-rHuEPO	0.89±0.07	0.91±0.11	1.11±0.06	1.23±0.06	1.32±0.08	NS	NS
	Post-rHuEPO	0.88±0.09	0.84±0.09	1.02±0.11	1.12±0.12	1.21±0.13		
	Control	0.92±0.13	0.91±0.15	1.04±0.07	1.16±0.10	1.30±0.09		
$\dot{V}_E$	Pre-rHuEPO	10±2.4	28±6.7	40±4.3	55±8.2	77±11.7	NS	0.01
liters · min^{-1}	Post-rHuEPO	11±2.1	25±5.7	40±8.9	55±8.7	83±10.7		
	Control	11±2.8	26±10	47±6.6	63±14	115±27.5		
HR	Pre-rHuEPO	88±11	116±16	130±24	146±21	157±16	NS	0.03
min^{-1}	Post-rHuEPO	85±20	109±17	132±23	148±24	162±15		
	Control	99±11	116±17	147±11	164±15	176±12		

Results expressed as mean±SD; 30, 60, 80, and 100% W correspond to measurements carried out at those percentages of peak exercise workload; Pre-rHuEPO, Post-rHuEPO, and Control, results before rHuEPO therapy, Post-rHuEPO therapy, and from the sedentary control subjects respectively; $\dot{V}O_2$, O_2 uptake; $\dot{V}CO_2$, CO_2 production; RER, respiratory exchange ratio; $\dot{V}_E$, minute ventilation (BTPS); HR, heart rate; pre-post, probability of the comparisons between pre- and post-rHuEPO measurements at peak exercise (paired analysis); post-con, probability of the comparisons between post-rHuEPO study and sedentary control subjects at peak exercise (unpaired analysis).

sured O_2 saturation and the corresponding PO_2 from all samples were used to estimate the P_{50} of hemoglobin. Calculations of mean muscle capillary PO_2 ($P_{mc}O_2$) and the corresponding value of muscle O_2 conductance (DO_2) were obtained by numerical integration (16, 19–22); the assumptions involved in this analysis having been previously described in detail (16). It should be noted that DO_2 is a lumped parameter that reflects both diffusional conductance and the effect of any heterogeneity of VO_2 with respect to blood flow. The O_2 conductance from the microcirculation to the muscle cell (DO_2) was calculated at peak exercise from: (*a*) the room air measurement in the pre-rHuEPO study; (*b*) the data of both the hypoxic and room air measurements (F_IO_2 of 0.13 and 0.21) in the post-rHuEPO study; and (*c*) the data of both the hypoxic measurements (F_IO_2 of 0.12 and 0.15) in the control group. Maximum O_2 uptake data breathing room air (control subjects) and 100% O_2 (pre-rHuEPO study) were not used to estimate DO_2 because they did not fulfill the requirements of the analysis, as discussed above and in (15).

Data analysis. Results are expressed as mean±SD. After rHuEPO, changes in $\dot{Q}$leg were compared using an analysis of covariance after demonstrating existence of a linear relationship between $\dot{Q}$leg and whole-body $\dot{V}O_2$ (as work rate was increased) with no variations in the slope from pre- to post-rHuEPO. For the remaining variables in the study, results during submaximal exercise were examined pooling the data obtained at 30, 60, and at 80% of peak work load. Comparisons between pre- and post-rHuEPO studies were done using the Student's paired *t* test, and those between post-rHuEPO and the control group of healthy sedentary subjects were carried out using the Student's unpaired *t* test. Pearson's regression analysis was used to

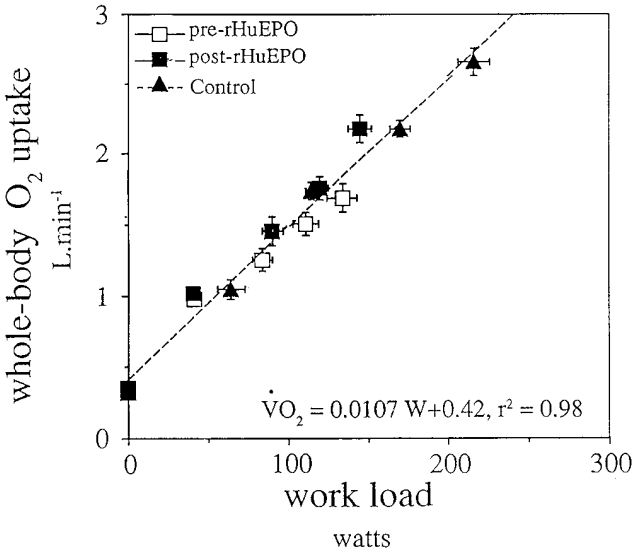

Figure 1. Relationships between whole-body O_2 uptake and external work rate. *pre-rHuEPO*, prior to erythropoietin; *post-rHuEPO*, after erythropoietin therapy; *control*, healthy sedentary subjects. The calculated mechanical efficiency in the overall set of measurements was 26.8%. Results are expressed as mean±SEM.

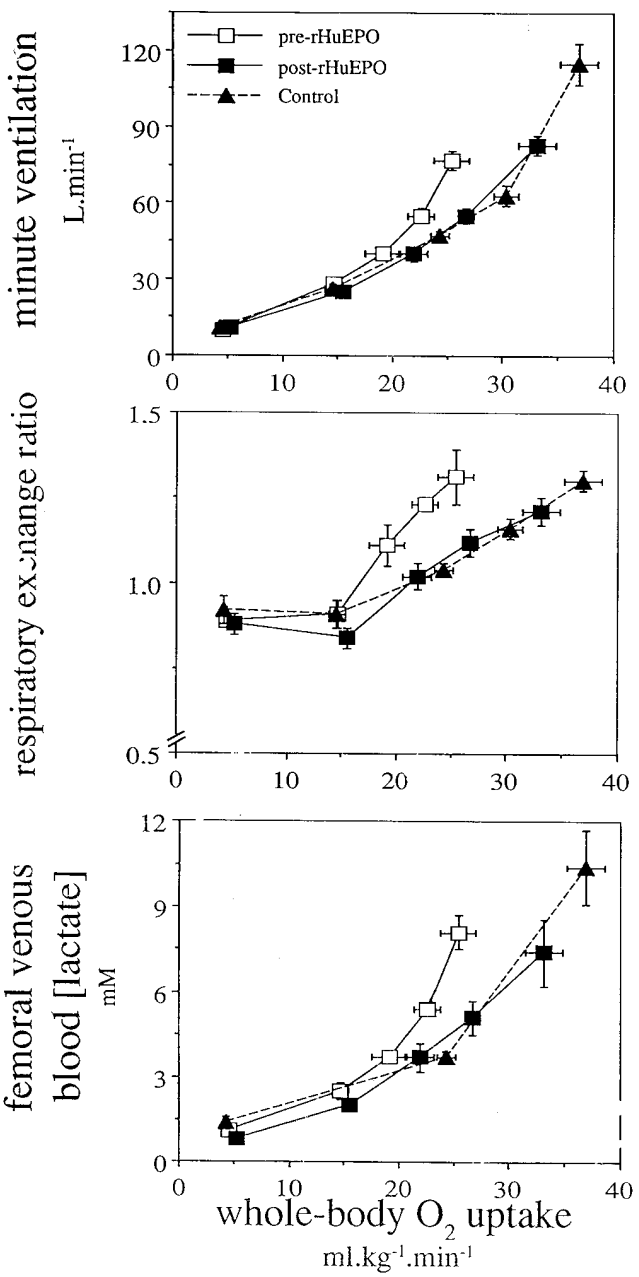

Figure 2. Relationships between whole-body O_2 uptake and, from top to bottom, minute ventilation ($\dot{V}_E$), respiratory exchange ratio, and femoral venous lactate concentration (La_{fv}). At any given $\dot{V}O_2$, $\dot{V}_E$, RER, and La_{fv} decreased after rHuEPO. No differences were found between submaximal exercise data of the post-rHuEPO study and those of the sedentary control group. Results are expressed as mean±SEM.

explore the relationships between variables. Statistical significance was set at $P \leq 0.05$.

Results

After the rHuEPO therapy, [Hb] in the eight renal patients increased by 5.0±1.8 g · dl^{-1}, from 7.5±1.0–12.5±1.0 g · dl^{-1} (Table I). All patients had normal arterial blood gases both at rest and at all exercise workloads, as exemplified by the alveolar-arterial O_2 difference (at peak exercise, 5±8.3 and 3±9.0 mmHg, pre- and post-rHuEPO, respectively). Consequently, at rest, the 69% increase in [Hb] (mean of individual responses) after rHuEPO therapy resulted in a similar (59%) arterial O_2 content change of 6.2±2.4 ml O_2 · 100 ml^{-1}, from 10.8±1.6–17.0±1.4 ml O_2 · 100 ml^{-1}. Similar values were observed during exercise.

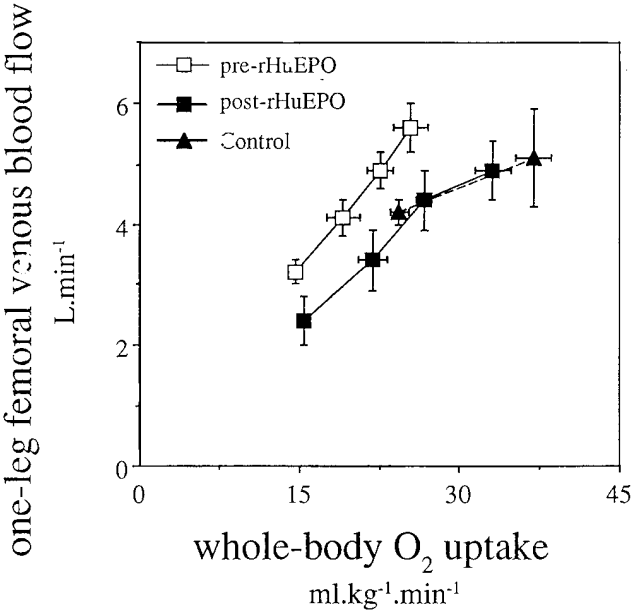

Figure 3. Relationships between femoral venous blood flow and whole-body O_2 uptake. At any given $\dot{V}O_2$, $\dot{Q}$leg significantly decreased after rHuEPO ($P < 0.004$). No differences were detected between the post-rHuEPO and control group. Results are expressed as mean±SEM.

Exercise performance. Exercise data from the patients (pre- and post-rHuEPO) and from the sedentary control subjects are shown in Tables II and III and in Fig. 1–3. Although a plateau in whole-body $\dot{V}O_2$ at peak workload could not be clearly defined in most of the patients, the respiratory exchange ratio (from 1.21 to 1.30) and the elevated blood lactate levels obtained at peak workload suggest that maximal exercise was achieved in both pre- and post-rHuEPO studies. After rHuEPO, peak whole-body $\dot{V}O_2$ increased 30% by 0.49±0.25 liters · min^{-1} but was still lower ($P = 0.03$) than that observed in the control group (33.1±4.7 ml · kg^{-1} · min^{-1}, CRF post-rHuEPO, and 36.9±5.9 ml · kg^{-1} · min^{-1}, normal subjects). As shown in Fig. 1, the patients (pre- and post-rHuEPO) and the control subjects showed a similar linear relationship between whole-body VO_2 and external work.

After rHuEPO therapy (Fig. 2), at any given oxygen uptake, $\dot{V}_E$, RER, and femoral venous blood lactate levels (La_{fv}) were consistently lower. In the post-rHuEPO study, the relationships between these variables and whole-body $\dot{V}O_2$ were similar to those of sedentary control subjects, but maximal values of $\dot{V}O_2$, $\dot{V}_E$, and La_{fv} were less than in control subjects. In contrast, at any given oxygen uptake, no significant changes were observed in the net lactate output across the leg between the patients (both pre- and post-rHuEPO) and the control group.

One-leg blood flow and O_2 delivery. Femoral venous blood flow ($\dot{Q}$leg) was consistently reduced after rHuEPO ($P < 0.004$). On average, $\dot{Q}$leg decreased by 0.70±0.9 liters · min^{-1}

Table III. O_2 Transport Variables during Submaximal and Peak Exercise, $F_IO_2 = 0.21$

		Rest	30% W	60% W	80% W	100% W	*P* pre-post	*P* post-con
PaO_2	Pre-rHuEPO	105±9	109±11	113±11	115±8	118±9	NS	NS
mmHg	Post-rHuEPO	106±9	109±11	110±8	115±10	116±10		
	Control	96±10	—	104±7	—	115±8		
$PaCO_2$	Pre-rHuEPO	36±4	35±4	36±4	34±4	30±5	NS	NS
mmHg	Post-rHuEPO	33±1	37±3	37±3	36±4	32±2		
	Control	35±3	—	38±3	—	31±3		
CaO_2	Pre-rHuEPO	10.8±1.9	10.7±1.4	10.6±1.4	10.8±1.6	11.0±1.7	0.0002	0.01
$ml \cdot 100ml^{-1}$	Post-rHuEPO	17.0±1.3	17.0±1.4	17.0±1.5	17.0±1.5	17.0±1.4		
	Control	18.9±1.4	—	19.2±1.3	—	19.2±1.4		
$PfvO_2$	Pre-rHuEPO	27±6.8	24±3.5	24±3.4	24±2.9	24±2.7	NS	NS
mmHg	Post-rHuEPO	27±5.2	24±4.8	26±5.0	27±5.5	27±5.4		
	Control	25±5.3	—	23±1.7	—	25±4.3		
$PfvCO_2$	Pre-rHuEPO	42±5.5	50±5.7	53±5.1	55±4.6	53±5.4	0.005	NS
mmHg	Post-rHuEPO	42±5.5	57±5.0	62±5.3	62±6.3	63±7.6		
	Control	45±4.1	—	60±5.0	—	65±5.1		
$CfvO_2$	Pre-rHuEPO	4.9±1.6	3.6±0.7	3.6±0.9	3.4±0.7	3.2±5.1	0.01	NS
$ml \cdot 100ml^{-1}$	Post-rHuEPO	8.2±2.5	5.7±1.7	5.7±1.9	5.6±2.1	5.1±1.8		
	Control	8.6±3.3	—	6.0±1.0	—	5.4±1.7		
$\dot{Q}_{leg}$	Pre-rHuEPO	—	3.2±0.5	4.1±0.9	4.9±0.8	5.6±1.1	0.004	NS
$liters \cdot min^{-1}$	Post-rHuEPO	—	2.4±1.1	3.4±1.4	4.4±1.5	4.9±1.5		
	Control	—	—	4.2±0.7	—	5.1±0.8		
$\dot{Q}O_{2leg}$	Pre-rHuEPO	—	0.35±0.07	0.43±0.08	0.52±0.09	0.61±0.12	0.04	0.04
$liters \cdot min^{-1}$	Post-rHuEPO	—	0.40±0.20	0.57±0.08	0.74±0.27	0.83±0.28		
	Control	—	—	0.80±0.15	—	0.98±0.14		
O_2 ER	Pre-rHuEPO	53±18	66±8	66±8	68±7	71±6	NS	NS
$liters \cdot min^{-1}$	Post-rHuEPO	52±13	67±10	67±10	67±11	70±10		
%	Control	55±15	—	69±4	—	72±8		
$\dot{V}O_{2leg}$	Pre-rHuEPO	—	0.23±0.06	0.28±0.07	0.36±0.08	0.44±0.11	0.03	0.04
$liters \cdot min^{-1}$	Post-rHuEPO	—	0.26±0.1	0.37±0.15	0.48±0.15	0.57±0.15		
	Control	—	—	0.56±0.10	—	0.71±0.13		
$\dot{L}a$	Pre-rHuEPO	—	1.7±1.4	1.8±1.8	2.2±1.8	4.4±2.7	NS	NS
$mM \cdot min^{-1}$	Post-rHuEPO	—	1.1±1.6	3.0±3.3	2.3±2.0	3.9±3.1		
	Control	—	—	2.8±1.2	—	4.8±2.9		

Results expressed as mean±SD; 30, 60, 80, and 100% W correspond to measurements carried out at those percentages of peak exercise workload; Pre-rHuEPO, Post-rHuEPO, and Control, results before rHuEPO therapy, post-rHuEPO therapy, and from the sedentary control subjects respectively; PaO_2 and $PaCO_2$, PO_2 and PCO_2 in arterial blood; CaO_2, arterial O_2 content; $PfvO_2$, $PfvCO_2$ and $CfvO_2$, femoral venous PO_2, PCO_2 and O_2 content; $\dot{Q}_{leg}$, femoral venous blood flow; $\dot{Q}O_{2leg}$, one-leg O_2 delivery; O_2 ER, muscle O_2 extraction as a percentage; $\dot{V}O_{2leg}$, one-leg O_2 uptake; $\dot{L}a$, lactate output across the leg; pre-post, probability of the comparisons between pre- and post-rHuEPO measurements at peak exercise (paired analysis); post-con, probability of the comparisons between post-rHuEPO study and sedentary control subjects at peak exercise (unpaired analysis).

(Fig. 3 and Table III), a fall of 13% at peak exercise compared to before rHuEPO. This reduction in $\dot{Q}$leg partially offset the effect of the rHuEPO-induced increase in arterial O_2 content of O_2 delivery ($\dot{Q}O_2$leg). Consequently, the increase in $\dot{Q}O_2$leg after rHuEPO at peak exercise was only 0.22±0.25 $liters \cdot min^{-1}$ (37%).

One-leg O_2 uptake. As shown in Table III, leg $\dot{V}O_2$ significantly increased after rHuEPO at each relative workload ($P = 0.05$). This was because a given relative work load occurred at a higher absolute level post-rHuEPO. At peak exercise, post-rHuEPO $\dot{V}O_2$leg rose by 0.13±0.13 $liters \cdot min^{-1}$ (33%) ($P = 0.03$) but was still 18% lower than that observed in the control group (8.5±1.6 compared to 10±2.7 $ml \cdot kg^{-1} \cdot min^{-1}$, respectively).

Femoral venous PO_2 and O_2 extraction. At peak exercise, no significant changes were observed in femoral venous PO_2 ($P_{fv}O_2$) (24±2.7 mmHg pre-rHuEPO vs. 27±5.4 mmHg post rHuEPO), femoral venous O_2 saturation (28.6±6.8 vs. 27.9±9.0%) or in O_2 extraction ratio (O_2ER) at peak exercise after rHuEPO (from 71±6 to 70±10%, respectively). Post-rHuEPO O_2ER was similar to that observed in the control group (72±8%).

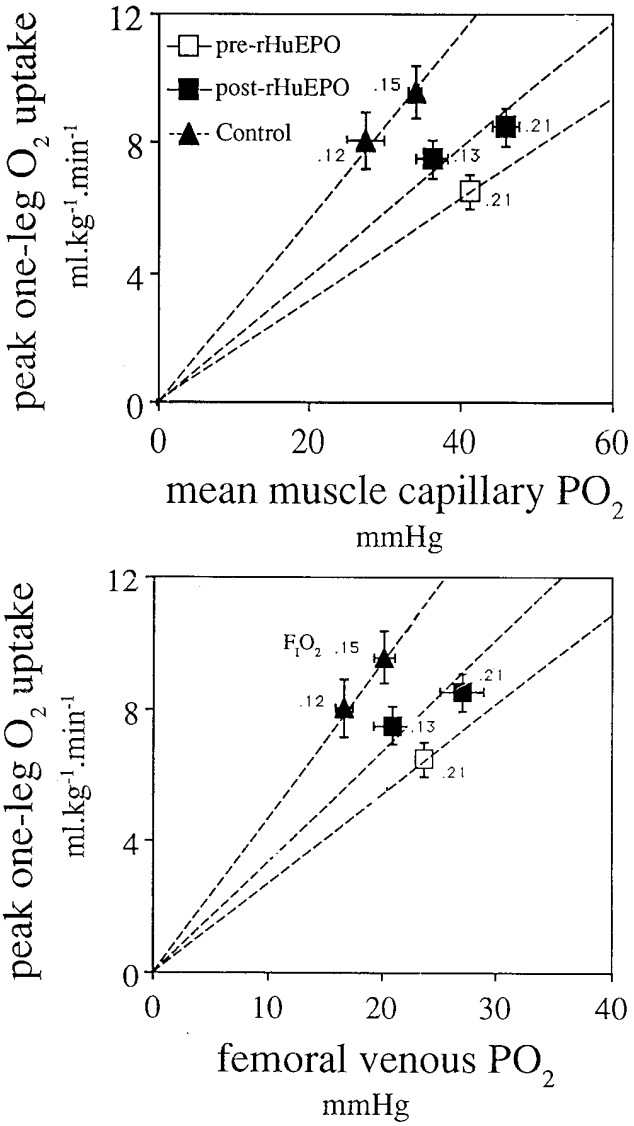

Figure 4. Relationships of one-leg O_2 uptake to mean muscle capillary PO_2 (*top*) and femoral venous PO_2 (*bottom*). The inspired O_2 fraction (F_IO_2) of each measurement is indicated in the figure. Dashed lines indicate muscle O_2 conductance of each study group.

DO_2 at peak exercise. In the post-rHuEPO study, $\dot{V}O_2$leg and mean muscle capillary PO_2 (or femoral venous PO_2) at maximum exercise determined during hypoxia and room air followed a linear and proportional relationship. Hence, O_2 supply dependency of peak $\dot{V}O_2$ constitutes a reasonable assumption, allowing calculation of O_2 conductance, as analyzed in Discussion. Such behavior was also observed in the studies during hypoxia in the control group (15). The slopes of the dashed lines in Fig. 4 reflect this proportionately, and hence indicate the DO_2 of each study group. Based on only the room air data before rHuEPO in which O_2 supply dependency had been assumed, muscle O_2 conductance increased by 31% after erythropoietin, from 10.4±3.3 to 13.0±3.1 ml $O_2 \cdot min^{-1} \cdot mmHg^{-1}$ ($P = 0.02$). However, the post-rHuEPO DO_2 was still 33% lower than that observed in healthy sedentary subjects (19.4±5.4 ml $O_2 \cdot min^{-1} \cdot mmHg^{-1}$) ($P = 0.007$). Most (71%) of the change in muscle O_2 conductance after rHuEPO was explained by the concomitant changes in both hemoglobin concentration and femoral venous blood flow ($\Delta DO_2 = 0.75\ \Delta[Hb] + 1.06\ \Delta\ \dot{Q}leg$, $r^2 = 0.71$).

Discussion

Summary of principal findings. This study shows that after erythropoietin therapy there is a substantial rise (69%) in hemoglobin concentration, and thus in CaO_2 (59%) in patients with chronic renal failure, but at the same time a moderate but consistent fall in femoral venous blood flow (of 13% at peak $\dot{V}O_2$ and even more submaximally) (Fig. 3). The latter is probably due to the reversal of the hyperhemodynamic state provoked by the anemia (23, 24). The fall in $\dot{Q}$leg partially offset the increase in O_2 delivery expected from the rise in arterial O_2 content. Thus, $\dot{Q}O_2$leg rose by only 37%.

While rHuEPO enhanced aerobic capacity (whole-body $\dot{V}O_2$ by 30% and $\dot{V}O_2$leg by 33%, respectively) (Figs. 2 and 3), the relative change was much less than that of arterial O_2 content (59%). This occurred despite increases in the two major components of O_2 transport from the atmosphere to the muscle cell after rHuEPO: oxygen delivery (37%) and muscle O_2 conductance (31%). The relative contributions of these two components of O_2 transport can explain the differences between the gains in O_2 content and $\dot{V}O_2$leg at peak exercise, as discussed below.

Oxygen supply limitation of peak $\dot{V}O_2$ was found to occur after rHuEPO, as indicated in Fig. 4 and discussed below, which allowed an estimate of muscle O_2 conductance. It should be noted that although DO_2 increased 31% with erythropoietin, post-rHuEPO DO_2 was the most severely impaired index of O_2 transport (Fig. 5), being 33% less than that in activity-matched control subjects.

Since 1990, several studies (5–13) have shown a rather

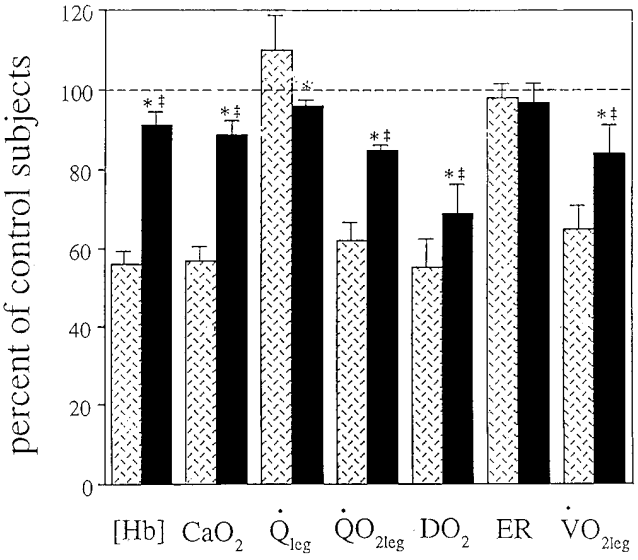

Figure 5. Results of key muscle O_2 transport variables expressed as percentages of the values obtained in the sedentary control subjects (*discontinuous line*). Dashed bars represent the pre-rHuEPO data and black bars correspond to the post-rHuEPO study. Values are mean±SEM. * Indicates statistical significance ($P < 0.05$) between pre- and post-rHuEPO results, and ‡ refers to the comparison between post-rHuEPO and control group($P < 0.05$).

small improvement in $\dot{V}O_2$ peak after rHuEPO therapy despite increases in [Hb] to almost normal levels, as confirmed in the present study. Impairment of O_2 transport to the mitochondria and/or alterations in the regulation of the oxidative phosphorylation in the cell have been invoked as the two potential explanations of the phenomenon, but no measurements have been made to confirm or refute these suggestions. The current study therefore represents the first integrative investigation of the changes with rHuEPO in the factors determining muscle O_2 transport. Recently, Moore et al. (25) reported no differences in cellular oxidative capacity (31-phosphorus nuclear magnetic resonance spectroscopy) among: (*a*) CRF patients under regular hemodialysis; (*b*) patients after renal transplantation; and (*c*) control subjects, indirectly suggesting impairment in O_2 transport in the muscle microcirculation as the primary explanation for the limited increase in $\dot{V}O_2$peak after rHuEPO. Dissociation between systemic hematocrit and microvascular hematocrit (26) can be invoked as an additional factor to explain the differences between gains in CaO_2 and in $\dot{V}O_2$leg at peak exercise after rHuEPO therapy. However, this possibility could not be ruled out since microvascular hematocrit cannot be measured during exercise in intact subjects. Muscle biopsies done in CRF patients (27) indicate that many muscle fibers show abnormally low adjacent capillary supply, which would provide the structural bases for the low muscle O_2 conductance obtained in our study. The results from the current investigation support the contention that abnormal muscle O_2 transport is the key factor limiting increases in $\dot{V}O_2$ in anemic CRF patients after rHuEPO therapy.

Interactions between O_2 delivery and muscle O_2 conductance in the effects of rHuEPO. As proposed by Piiper and Scheid (28), O_2 extraction (under conditions of O_2 supply limitation of $\dot{V}O_2$max) depends on the ratio of muscle O_2 diffusional conductance (DO_2) to perfusional conductance ($\beta \cdot \dot{Q}$leg). The term β corresponds to the slope of the O_2 dissociation curve and is the ratio of the arterial to femoral venous O_2 content difference and the arterial to femoral venous PO_2 difference [$\beta = (C_aO_2 - C_{fv}O_2)/(P_aO_2 - P_{fv}O_2)$]. The most efficient way to improve O_2 transfer to the mitochondria therefore occurs in those circumstances that provide an increase in this ratio. Endurance training for example, increases not only cardiac function, and thus $\dot{Q}$leg, but also DO_2. Because DO_2 increases relatively more than does $\dot{Q}$leg (15), $DO_2/\beta \cdot \dot{Q}$leg increases, raising maximal O_2 extraction. Thus $\dot{V}O_2$max increases with training due to both increased leg blood flow and increased O_2 extraction (15).

In contrast, the patients with chronic renal failure of the present study showed a fall in $\dot{Q}$leg (13%), an increase in DO_2 (31%), and an increase in [Hb] (69%) that raised β by 65%. This led to no significant change in $DO_2/\beta \cdot \dot{Q}$leg ratio (2.22± 0.70 pre-rHuEPO vs. 2.12±0.58 post-rHuEPO) explaining how after rHuEPO, CRF patients do not exhibit any change in muscle O_2 extraction. Had DO_2 not increased by 31%, $DO_2/\beta \cdot \dot{Q}$leg would have fallen to $\sim$ 1.7 and O_2 extraction would have fallen, further reducing the benefits of rHuEPO on $\dot{V}O_2$peak. DO_2 probably increased due to the higher [Hb], consistent with previous observations (29), although the specific mechanism remains obscure.

Analysis of O_2 supply limitation. The use of two levels of F_IO_2 in the pre- and post-rHuEPO studies was adopted because the calculation of DO_2 requires the demonstration of O_2 supply dependence of peak $\dot{V}O_2$ (16, 19, 21), and changing F_IO_2 is the most acceptable way to alter O_2 transport in intact subjects. Oxygen supply limitation would be manifest by a higher $\dot{V}O_2$peak at the higher F_IO_2. Moreover, one would expect the calculated DO_2 at each F_IO_2 to be the same (21). This is equivalent to demonstration of proportionality between $\dot{V}O_2$max and mean muscle capillary PO_2 as F_IO_2 is varied. In the post-rHuEPO studies (F_IO_2 = 0.13 and 0.21) this was indeed observed. However, before rHuEPO, it was not considered ethical to study these patients at any reduced F_IO_2 because of the anemia. Thus, we chose 100% O_2 as the second inspired gas. Because 100% O_2 did not increase $\dot{V}O_2$peak in these patients, we were unable to confirm O_2 supply dependency of $\dot{V}O_2$peak breathing room air before rHuEPO. We have for comparison purposes assumed that $\dot{V}O_2$peak at F_IO_2 = 0.21 is supply limited and computed the associated DO_2 under this condition. To the extent that even at F_IO_2 = 0.21 our patients were not O_2 supply limited at $\dot{V}O_2$peak, this value of DO_2 would be an underestimate (15), such that the relative increase in DO_2 after rHuEPO would be overestimated.

As indicated in Fig. 4, conditions of O_2 supply dependency were satisfied in the post-rHuEPO study and in the hypoxic measurements carried out in the control group. $\dot{V}O_2$peak increased with F_IO_2 in an amount that reflects the corresponding increase in O_2 delivery. However, the CRF patients were not tested in the pre-rHuEPO study since the estimation of muscle O_2 conductance was done only during room air breathing. It should be noted that the arterial O_2 content before rHuEPO at F_IO_2 = 0.21 was similar to that observed post-rHuEPO at an FIO_2 of 0.13 and in the healthy sedentary subjects at F_IO_2 = 0.12. Consequently, it can be reasonably argued that the CRF patients were likely to be O_2 supply dependent breathing room air in the pre-rHuEPO study. However, if anything, pre-rHuEPO O_2 conductance at F_IO_2 = 0.21 would have been underestimated if peak $\dot{V}O_2$ breathing air were not O_2 supply limited (15), so that the calculated 31% change in DO_2 is a maximal value. It should be noted that up to 71% of the variance in DO_2 (calculated assuming O_2 supply dependence) was explained by the concomitant changes in both hemoglobin concentration and femoral venous flow.

Comparisons between the post-rHuEPO study and the control group (Fig. 5). Hemoglobin concentration and $\dot{Q}$leg at peak exercise after rHuEPO were slightly lower than in the healthy sedentary subjects (−9 and −4%, respectively). No such difference was observed in arterial PO_2, and consequently the $\dot{Q}O_2$leg was 15% lower in the patients with CRF. However, muscle O_2 conductance, among all variables determining O_2 transport, was the most severely impaired in the CRF patients. Even after rHuEPO, DO_2 was 33% lower than that of the control group, which was well matched for lack of exercise and for age, height, and weight. The higher O_2 delivery (15%) and the higher $DO_2/\beta \cdot \dot{Q}$leg ratio (8%) observed in the healthy sedentary subjects together explain the higher $\dot{V}O_2$leg at maximum exercise (by 18%) of the control group compared to the post-rHuEPO CRF patients.

The structural basis for the reduced muscle O_2 conductance was not examined in the present study since muscle biopsies were not attempted, but it is likely to reflect a less rich muscle microcirculatory network (27, 30, 31), since a previous study in a dog model without renal disease (32) has highlighted capillary surface area in muscle as the key structural factor that determines O_2 conductance. In contrast, the subsequent diffusion distance to the mitochondria appears to offer little impediment

to O_2 transport, presumably due to diffusion facilitation by myoglobin (33). The structural heterogeneity in the matching of capillaries to muscle fibers observed in CRF patients (27) suggests that functional heterogeneity of $\dot{Q}/\dot{V}O_2$ ratios may constitute an additional factor to explain impairment of muscle O_2 transfer in these patients. Although limitation of aerobic capacity in CRF patients is a complex phenomenon, the present study provides the first direct evidence for impaired O_2 transport out of the muscle microcirculation. This may explain, in part, the subnormal levels of $\dot{V}O_2$peak in chronic renal failure and the poor response to rHuEPO.

Possible exercise training during rHuEPO therapy. In the current study, to preclude training occurring as a result of feeling better after rHuEPO therapy, all CRF patients were repeatedly instructed to maintain the same level of daily physical activity throughout the protocol. However, if any spontaneous training effect had developed as a result of the improvement of both quality of life and exercise tolerance, the end result would have presumably been an increase in $\dot{Q}$leg and muscle O_2 conductance. Thus, this would have decreased the differences between post-rHuEPO measurements and those obtained in the sedentary control subjects. Consequently, the assertion of an abnormally low O_2 conductance even after [Hb] restoration is, if anything, strengthened to the extent that any training took place without our knowledge.

In summary, the hemodynamic response of reduced leg blood flow to the increase in hemoglobin concentration produced by rHuEPO plays a key role in limiting the increase in O_2 uptake at peak exercise. Analysis of the interactions between the convective and diffusive components of muscle O_2 transport shows an increased but still abnormally low muscle O_2 conductance after rHuEPO. However, the even greater net increase in perfusional conductance for O_2 due to the increase in [Hb] contributes to the relatively limited increase in peak $\dot{V}O_2$ because it does not allow O_2 extraction to increase. Muscle O_2 transport conductance in CRF, even after rHuEPO, is $\sim$ 33% lower than that in control subjects (matched for age, size, and activity). This suggests a myopathic alteration, possibly of the microcirculation, due to renal failure that compromises exercise capacity more than anemia and inactivity alone would predict.

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