Erythropoietin Treatment Improves Peak VO$_2$ and Oxygen Uptake Efficiency Slope without Changing VE vs. VCO$_2$ Slope in Anemic Patients

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Abstract

**Background:** While the improvement of anemia with erythropoietin (EPO) treatment increases peak VO$_2$ in anemic patients, the effects of EPO on minute ventilation (VE), VO$_2$- and VCO$_2$- kinetics are not well described.

**Objectives:** The aim of this study was to evaluate the improvement in hemodynamic, metabolic, and ventilatory response during exercise along with the improvement of anemia with EPO treatment in anemic hemodialysis patients.

**Methods:** Thirty-seven hemodialysis patients with anemia (48.8 ± 13.6 years) received EPO (1500 or 3000 unit, 3 times a week). Parameters measured prospectively before and after EPO treatment included hemoglobin, cardiac output by dye dilution method, echocardiography, and cardiopulmonary exercise parameters.

**Results:** With EPO treatment, hemoglobin increased from 6.4 ± 0.9 to 10.3 ± 0.9g/dl (p<0.001), with concomitant improvements of O$_2$ delivery at rest (from 663.8 ± 161.1 to 793.4 ± 188.5ml/min, p=0.004) and high output state (from 8.0 ± 1.6 to 5.6 ± 1.2l/min, p<0.001). Peak heart rate, peak systolic blood pressure, and peak VE did not change. Peak VO$_2$ increased from 15.7 ± 5.3 to 18.8 ± 5.3ml/min/kg (p=0.017), and oxygen uptake efficiency slope (OUES) improved from 1,255 ± 375 to 1,517 ± 357 (p<0.001). However, VE vs. VCO$_2$ slope (from 34.3 ± 7.3 to 31.8 ± 7.2, p=0.86) and VE-VCO$_2$ curve did not change.

**Conclusion:** The treatment of anemia improved peak VO$_2$ and OUES without affecting VE vs. VCO$_2$ slope significantly. In evaluating the efficacy of treatment for patients with renal failure and anemia, the VE vs. VO$_2$ relation rather than the VE vs. VCO$_2$ should be used.

**Keywords**

Anemia, Exercise tolerance, Ventilation, Chronic renal failure

Introduction

Exercise tolerance in anemic patients is markedly reduced, and shortness of breath is a common symptom in these patients. The mechanism of shortness of breath is thought that reduction of hemoglobin concentration may result in exercise intolerance by decreasing oxygen (O$_2$) delivery. It has been reported that erythropoietin (EPO) treatment for anemia improves exercise tolerance (i.e. peak oxygen uptake (peak VO$_2$)) [1-6]. In chronic renal failure (CRF) patients with anemia and reduced cardiac function, treatment of the anemia with EPO has improved many of abnormalities seen in chronic heart failure (CHF), reducing left ventricular hypertrophy, preventing left ventricular dilatation, and increasing the left ventricular ejection fraction (LVEF) [7-10].

Minute ventilation (VE) vs. CO$_2$ output (VCO$_2$) slope, which reflects a ventilatory efficiency during exercise, has been emphasized an importance in patients with CHF [11,12]. Recently, the oxygen uptake efficiency slope (OUES), a new expression of the relationship between VE and VO$_2$ during exercise has been described [13,14]. Peak VO$_2$ [15], OUES and VE vs. VCO$_2$ slope are not only indexes of exercise tolerance but also powerful predictors of mortality. Although EPO treatment improves peak VO$_2$, the effects of EPO on VE, VO$_2$, and VCO$_2$ kinetics, OUES and VE vs. VCO$_2$ slope are not well recognized in anemic patients. The aim of this study was to evaluate the improvement in hemodynamic, metabolic, and ventilatory response during exercise along with the improvement of anemia with EPO treatment in anemic hemodialysis patients.

Methods

**Study patients**

Thirty-seven (14 males, 23 females; 48.8 ± 13.6 years) ambulatory anemic patients with CRF on regular hemodialysis (3 times a week) without apparent heart disease were eligible for study. Patients were excluded if they had exercise limitation by peripheral vascular or lung disease, moderate to severe valvular heart disease, left ventricular
dysfunction, ischemic heart disease who stopped exercise testing due to chest pain or significant ST depression in exercise electrocardiogram (ECG), or a history of EPO treatment within the past 6 months. The underlying renal diseases included chronic glomerulonephritis in 26 patients, diabetic nephropathy in 6 patients, and other chronic renal disease in 5 patients. All the patients were on the 4 hours, 3 times a week regular hemodialysis. The averaged hemodialysis period was 6.6 ± 5.2 years.

This study was approved by the committee on clinical study and ethics. The purposes and risks of the study were explained to the patients, and informed consent was obtained from each patient.

Study protocol

EPO (1500 or 3000 unit) was administrated intravenously 3 times per week, after each dialysis session. For the patients whose serum ferritin was less than 1000ng/ml, oral or intravenous iron was supplied. Hemoglobin and hematocrit were measured before dialysis once a week. The patients were evaluated measurement before treatment and after the hematocrit reached the target range of 30% to 35%. Serum EPO was measured before and after treatment.

Echocardiography

M-mode and 2-dimensional echocardiographic studies were performed before and after EPO treatment in all patients. We used an ultrasound system with a 2-dimensional mechanical sector scanner (SSD-2000; Aloka; Tokyo, Japan). Left ventricular end-diastolic dimension (LVDd) and end-systolic dimension (LVDs) were obtained from the apical 4- and 2-chamber views from which LVEF was automatically calculated by a modified Simpson’s method [16].

Blood gas analysis and hemodynamic measurements

Arterial blood gases were measured before and after EPO treatment in all patients at rest before hemodialysis. The partial pressure of arterial O₂ (PaO₂), arterial CO₂ (PaCO₂), and the pH were measured with a blood gas analyzer (ABL 300, Radiometer, Copenhagen, Denmark). Cardiac output was measured by the dye dilution method using an earpiece with a dye densitometer (MCL-4200, Nihon Coden, Tokyo, Japan) [17]. A 19G cannula was placed in the antecubital vein, and 5 mg of indocyanine green dissolved in 5 ml distilled water was injected through this cannula at rest. O₂ delivery was calculated using the formula as follows; hemoglobin X 1.36 X O₂ saturation in arterial blood X cardiac output.

Cardiopulmonary exercise test

Cardiopulmonary exercise test was performed before and after EPO treatment on the next day to the first hemodialysis of the week. An incremental symptom-limited exercise test using to ramp protocol was performed with a treadmill. After 4 min standing on the treadmill, exercise began with a 4-min warm-up at the speed of 1.0km/h, followed by an increase in grade and speed according to treadmill-ramp protocol. Treadmill-ramp protocol was developed in Japan which was designed to increase VO₂ linearly at the rate of 3/ min/kg based on the formula as follows; VO₂=0.15S² + 0.14 SG + 0.45 S + 0.4 G + 0.423, where S is speed (km/hr) and G is grade (%) [18]. Heart rate and ECG were monitored continuously during the test with a Stress System ML-5000 (Fukuda Denshii Co. Ltd.; Tokyo, Japan). Cuff blood pressure was measured at rest, and every minute during the test.

Respiratory gas analysis

VO₂, VCO₂, VE, respiratory rate (R-R), and tidal-volume (TV) were measured throughout the test using an Aeromonitor AE-280S (Minato Medical Science; Osaka, Japan). Prior to calculating the parameters from respiratory gas analysis, an eight-point moving average of the breath-by-breath data was obtained. Peak VO₂ was defined as the average value obtained during the last 30 seconds. The anaerobic threshold (AT) was determined by the V-slope method [19]. VE vs. VCO₂ slope was calculated from the start of incremental exercise to the respiratory compensation point by least squares linear regression, as previously described [20]. The oxygen uptake efficiency slope (OUES) describes as the gradient of the linear relationship between VO₂ and VE during incremental exercise via a single-segment logarithmic expression of VE and is defined as the regression slope “a” in VO₂ = a log10 VE + b [13].

Statistics

Clinical and exercise variables for before and after treatment were compared through the use of and paired t tests. Statistical comparisons were considered significant for probability value <0.05. Results

Laboratory data

All thirty-seven patients responded to the treatment with EPO without apparent side effect. Duration of therapy averaged 160 ± 49 days (between 86 and 275 days). Mean values of hemoglobin and hematocrit increased significantly during the study period from 6.4 ± 0.9 to 10.8 ± 1.0g/dl (p<0.001) and from 19.0 ± 2.5 to 32.9 ± 3.1 % (p<0.001), respectively (Table 1). Serum EPO did not change significantly by during the treatment (from 17.3 ± 4.5 to 19.3 ± 5.7mIU/ml, p=0.128). Among 37 patients, 31 patients prescribed elemental oral or intravenous iron.

Echocardiography

By the treatment with EPO, LVDd significantly decreased from 53.0 ± 8.4 to 53.1 ± 8.6 (p=0.976). Peak VO₂ was obtained from the apical 4- and 2-chamber views from which LVEF was automatically calculated by a modified Simpson’s method [16].

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Echocardiography

By the treatment with EPO, LVDd significantly decreased from 53.0 ± 8.4 to 53.1 ± 8.6 (p=0.976), while LVDs unchanged from 30.9 ± 4.6 to 29.5 ± 4.5mm (p=0.219), and LVEF unchanged from 77.6 ± 6.7 to 75.8 ± 7.4 % (p=0.299).
Therefore, VE/VO2 curve shifted downward after the treatment. VE decreased at given exercise time after the treatment (Figure 1). There was weak correlations between change in Hb and change in OUES (r=0.395, p=0.017). In contrast, VE/VCO2 curve and VE vs. VCO2 slope (from 34.3 ± 7.3 to 31.8 ± 7.2, p=0.86) did not change (Figure 3).

### Discussion

In the present study, treatment for anemia resulted in 1) increasing O2 delivery at rest, Peak VO2, and OUES, 2) decreasing cardiac output at rest, VE, and VE vs. VO2 relationship, and an 3) altering VE vs. VCO2 relationship. The result of the present study has revealed that anemia affected VE vs. VO2 but not VE vs. VCO2 relationship in CRF patients.

### Anemia and exercise tolerance

The treatment of anemia with EPO improves exercise tolerance, which has previously been described in many studies [1-6]. In our present study, peak VO2 represented a mean increase of 20% over the baseline measurements in agreement with the other reports. The mechanism by which EPO improves exercise tolerance has been presumed to be increased hemoglobin concentration leading to increased VO2 delivery. In CRF patients, EPO has been shown to improve skeletal muscle function and O2 use, as well as endothelial function [21]. In our present study, instead of skeletal muscle function and O2 use, cardiac output was actually measured by the dye dilution method, and O2 delivery was calculated. We demonstrated that O2 delivery increased along with the improvement of anemia.

### OUES

A non-linear measure of the ventilatory response to exercise (i.e. OUES) has been described, initially in young patients with cardiac disease [13]. The OUES is derived from the single-segment logarithmic relation between VO2 and VE during incremental exercise. OUES incorporates in a single index not only cardiovascular and peripheral factors that determine VO2 but also pulmonary factors that influence the ventilatory response to exercise. It has been shown that OUES is significantly correlated to peak VO2 in patients with CHF [22] and is not influenced by the duration of the exercise test, or by achieved exercise intensity. However, the effect of anemia on OUES hasn’t been well known. In our present study, OUES improved along with improvement of anemia. Its mechanism might be similar in peak VO2, due to increasing O2 delivery and VO2 and decreasing VE.

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**Table 2: Cardiopulmonary exercise testing parameters at rest and exercise between Pre and Post EPO treatment**

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>Post</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>At Rest</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest HR (bpm)</td>
<td>88.8 ± 18.2</td>
<td>84.8 ± 13.1</td>
<td>0.362</td>
</tr>
<tr>
<td>Rest BP (mmHg)</td>
<td>148.5 ± 24.7</td>
<td>141.0 ± 18.9</td>
<td>0.147</td>
</tr>
<tr>
<td>Rest VE, (l/min)</td>
<td>9.4 ± 2.2</td>
<td>8.7 ± 1.7</td>
<td>0.110</td>
</tr>
<tr>
<td>Rest R-R, (n/min)</td>
<td>16.3 ± 4.0</td>
<td>16.9 ± 4.2</td>
<td>0.510</td>
</tr>
<tr>
<td>Rest TV, (ml)</td>
<td>617.1 ± 228.4</td>
<td>566.8 ± 187.6</td>
<td>0.304</td>
</tr>
<tr>
<td>Rest VO2, (ml/min)</td>
<td>202.9 ± 42.1</td>
<td>207.7 ± 36.2</td>
<td>0.604</td>
</tr>
<tr>
<td>Rest VCO2, (ml/min)</td>
<td>172.2 ± 41.5</td>
<td>165.5 ± 31.4</td>
<td>0.436</td>
</tr>
<tr>
<td>Rest VE/VCO2, (ml/ml)</td>
<td>46.9 ± 6.4</td>
<td>43.8 ± 5.5</td>
<td>0.028</td>
</tr>
<tr>
<td>Rest RCO2/VCO2, (ml/ml)</td>
<td>55.6 ± 8.1</td>
<td>55.2 ± 8.1</td>
<td>0.824</td>
</tr>
</tbody>
</table>

Data presented are the mean value ± SD. HR: Heart Rate, BP: Systolic Blood Pressure, VE: Ventilation, R-R: Respiratory Rate, TV: Tidal Volume, VCO2: CO2 output, AT: Anaerobic Threshold, AT time: Time at AT after starting ramp exercise, OUES: Oxygen Uptake Efficiency Slope.

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TV and R-R both at rest and peak exercise did not change (Table 2). VE decreased at given exercise time after the treatment (Figure 1). Therefore, VE/VO2 curve shifted downward after the treatment and OUES improved significantly from 1,255 ± 375 to 1,517 ± 357 (p<0.001) (Figure 2). There was weak correlations between change in Hb and change in OUES (r=0.395, p=0.017). In contrast, VE/VCO2 curve and VE vs. VCO2 slope (from 34.3 ± 7.3 to 31.8 ± 7.2, p=0.86) did not change (Figure 3).

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**Figure 1:** Changes in Oxygen Uptake and Minute Ventilation during Exercise
Anemia and VE vs. VCO₂ relationship

VE vs. VCO₂ slope, which reflects a ventilatory efficiency during exercise, has been emphasized as a powerful predictor of prognosis and severity of CHF [11,12]. Ventilatory efficiency depends on pulmonary hemodynamics and related parameters such as ventilation-perfusion mismatch, skeletal muscle ergoreceptor and peripheral chemoreceptor sensitivity, and heightened sympathetic activity. Until now, the effects of EPO on VE, VO₂, and VCO₂ kinetics and VE vs. VCO₂ slope are not well recognized in anemic patients.

On the patients of iron deficiency anemia, Davies et al. [23] revealed that improvement of anemia decreased VE at the same exercise stage. Robertson et al. [1] reported that correction of anemia significantly by decreased VE in CRF patients. But they did not mention about the physiologic mechanism of this changing. On the patients of HbSC sickle cell patients, Oyono-Enguelle et al. [24] reported VE in anemic patients (HbSC patients) drifted upward than that in normal patients (HbAA patients). They expected that caused by changes of the body temperature, epinephrine and norepinephrine, acidosis, and lactate metabolism.

Similarly, in our present study, VE decreased by improving anemia. Treatment for anemia might improve tissue O₂ supply and muscle function in terms of lactate production and/or utilization, then improvement of acidosis in the tissue during exercise lead the decreasing the VE in the given exercise intensity. That would be
explained by the delay of AT appearance after treatment and decrease of heart rate at AT. VE is mainly determined by the rate of CO₂ production, the physiologic and anatomic dead space, and the level at which PaCO₂ during exercise. VE vs. VCO₂ slope was steeper in CHF patients than normal subjects, because it is considered to be derived from the ventilation-perfusion mismatch and the increased ratio of physiologic dead space to TV, which was due mainly to the inappropriate increase in cardiac output during exercise [25]. However, in case of anemia without CHF, VE vs. VCO₂ relationship are parallel, because dead space ventilation doesn’t increase.

**Ventilatory efficacy for VO₂ and VCO₂**

Improvement of anemia decreases VE at the same exercise stage, which results in improvement in OUES. In contrast, the kinetics of VE and VCO₂ are parallel, which results in unchanging VE vs. VCO₂ slope. In the point of O₂ transport capacity during exercise, anemic state is similar with the state of decreased pulmonary blood flow with normal hemoglobin concentration. In contrast, in the point of CO₂ kinetics, differ from O₂ kinetics, CO₂ is transported across alveolar membrane by diffusion mechanism, which means CO₂ excretion is not disturbed when pulmonary blood flow is maintained even in anemic state. In summary, hemoglobin affects O₂ but not CO₂ transport. The improvement of CHF results in the improvement of both VE vs. VCO₂ slope and OUES, however, the improvement of anemia results in unchanging of the VE vs. VCO₂ slope and changing of the OUES. That is the difference between ventilatory efficacy for VO₂ and VCO₂ in the treatment of anemia.

**Limitation**

In the interpretation of our study results, some limitations should be considered. The major limitations of this study were the small number of patients and the lack of a control group. In case of CRF patients, improvement of anemia induces improvement of hyperdynamic state. However, the physiological mechanism at the pulmonary alveolar membrane level is similar with CHF patients, so it was worth in considering about VE, VO₂ and VCO₂ kinetics in anemia in this model.

**Conclusions**

The treatment of anemia improved peak VO₂ and OUES without affecting VE vs. VCO₂ slope significantly. In evaluating the efficacy of treatment for CRF patients with anemia the VE vs. VO₂ relation rather than the VE vs. VCO₂ should be used.

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**References**