Exercise-induced lactate increase in relation to muscle substrates in patients with chronic obstructive pulmonary disease

Exercise-Induced Lactate Increase in Relation to Muscle Substrates in Patients with Chronic Obstructive Pulmonary Disease

MARIËLLE P. K. J. ENGELEN, ANNEMIE M. W. J. SCHOLS, JOAN D. DOES, HARRY R. GOSKER, NICOLAAS E. P. DEUTZ, and EMIEL F. M. WOUTERS

Departments of Pulmonology and Surgery, University Hospital Maastricht, Maastricht; and Asthmacentre Hornerheide, Horn, The Netherlands

Early lactic acidosis has been suggested as negatively influencing the exercise capacity of patients with chronic obstructive pulmonary disease (COPD). We conducted a study to investigate whether the early lactate (La) response to exercise in COPD is related to alterations in exercise-related substrate levels in resting muscle, associated with physical inactivity. Twenty-seven COPD patients and 22 controls (physically inactive [PI] subjects, n = 15; and physically active [PA] subjects, n = 7) performed an incremental cycle test. Venous blood was sampled for La analyses, and the oxygen uptake at which the La level began to rise (La threshold) was calculated. Vastus lateralis biopsy specimens were obtained at rest. In the PA group, muscle glutamate (GLU) and glycogen were higher, but muscle La, pyruvate, and glucose were not different than in the PI group. Moreover, the La threshold was higher in the PA group. The COPD group had lower values for La threshold and muscle GLU, and higher values for muscle La and pyruvate levels than did the PI group. Stratification of patients into those with and without macroscopic emphysema (EMPH+, EMPH−, respectively), with comparable physical activity levels on the basis of previous observations, revealed lower values for La threshold and GLU in EMPH+ patients. Diffusing capacity for carbon monoxide ($D_{L_{CO}}$) and arterial oxygen tension (Pa_{O_2}) in the four study groups were positively related to GLU and La threshold. Moreover, La threshold was positively related to GLU. This study illustrates that the early lactic acidosis during exercise in patients with COPD is associated with the physical inactivity-related reduction in these patient's muscle GLU. However, factors other than physical inactivity, such as Pa_{O_2} or $D_{L_{CO}}$, play a role in the different La responses during exercise in subjects with different subtypes of COPD.

It is generally known that a substantial portion of patients with chronic obstructive pulmonary disease (COPD) develop lactic acidosis early in exercise and at very low work rates (1, 2). Lactic acidosis is unfavorable, since it puts additional stress on these patients' limited ventilatory system during exercise.

Recently, evidence has become available that a reduced oxidative capacity of skeletal muscle correlates with the accelerated lactate (La) response to exercise in COPD (3), as indicated by the inverse relationship between the steepness of the increase in La concentration and the activity of muscle oxidative enzymes. The significant correlation found between the decreased percentage of type 1 muscle fibers and diffusing capacity for carbon monoxide ($D_{L_{CO}}$) (4) suggests that emphysema patients in particular are prone to a reduced muscle oxidative capacity and thus to an increased La response to exercise.

(*Received in original form on October 15, 1999 and in revised form on June 5, 2000*)

Supported by a research grant from the University Hospital Maastricht and the Netherlands Asthma foundation.

Correspondence and requests for reprints should be addressed to Mariëlle P. K. J. Engelen, Department of Pulmonology, University Hospital Maastricht, P.O. Box 5800, 6202 AZ Maastricht, The Netherlands. E-mail: M.Engelen@Pul.Unimaas.NL

Am J Respir Crit Care Med Vol 162. pp 1697–1704, 2000
Internet address: www.atsjournals.org

Until now, no information has been available about the extent to which intrinsic changes in exercise-related substrate metabolism in COPD patients (i.e., previously reported increased levels of muscle La and reduced levels of glycogen [5, 6]) are responsible for these patients' accelerated La response to exercise. Recently, we observed severely decreased glutamate (GLU) levels in two different muscle groups of COPD patients (7, 8). GLU is high in the free amino acid pool of human skeletal muscle, and takes part in numerous important metabolic processes at rest and during exercise. Studies have shown that in healthy human muscle, the GLU pool functions to generate tricarboxylic acid (TCA) intermediates during the first minutes of exercise (9, 10), which is achieved via the alanine aminotransferase reaction (pyruvate + GLU → alanine + α-ketoglutarate) at the cost of GLU. Moreover, this reaction can shunt the pyruvate accumulated during exercise toward alanine instead of La, suggesting a possible role of the intracellular GLU level in the La response to exercise.

Physical inactivity is known to play a role in the early lactic acidosis in COPD (1), but the relative contributions of physical inactivity and disease-related muscle myopathy are still not clear. Recently, we studied the physical activity level of 20 COPD patients, recruited from a pulmonary rehabilitation center, who were able to participate in and finish the center's intensive physical training program. The average physical activity score of the COPD group was lower than that of 10 healthy sedentary controls ($p < 0.05$), but no difference was found between COPD patients with and without macroscopic emphysema (EMPH− and EMPH+, respectively) (Voorrips score: controls = 19 ± 6; EMPH+ patients = 11 ± 5; EMPH− patients = 7 ± 1). This indicates that patients with these COPD subtypes are comparable in their level of physical inactivity, and we therefore used this patient population to study the relative contribution of disease-related myopathy to possible differences in the La response to exercise. Additionally, in order to obtain greater insight into the effect of physical inactivity on muscle substrate metabolism, we used two control groups with a difference in their physical training status.

The first purpose of our study was to investigate whether the early La response to exercise in patients with COPD was associated with a reduced intramuscular GLU level and with intrinsic alterations in other exercise-related substrate levels (i.e., La, glucose, glycogen). The second purpose was to examine possible differences in the exercise-induced La increase in patients with the EMPH+ and EMPH− subtypes of COPD. The third purpose of our study was to investigate differences in intrinsic exercise-related muscle substrate levels in a sedentary and a physically active control group.

METHODS

Study Population

A group of 27 patients with moderate to severe airflow obstruction (21 males and six females; FEV_1 = 45 ± 14% [mean ± SE] predicted)

and 22 healthy, age-matched volunteers (17 males and five females) was studied. The control group was stratified by level of physical activity in daily life into a physically inactive (PI) group (n = 15) and a physically active (PA) group (n = 7), using the modified Baecke questionnaire (11). Controls were defined as PA when they had a total Baecke index > 8.5. All patients had COPD according to American Thoracic Society guidelines (12), and had chronic airflow limitation, defined as a measured FEV_1 < 70% of reference FEV_1. Furthermore, the patients had irreversible obstructive airway disease (< 10% improvement of predicted baseline FEV_1 after inhalation of β_2-agonist), and were in clinically stable condition and without respiratory tract infection or exacerbation of their disease for at least 4 wk before the study. Exclusion criteria were malignancy; cardiac failure; distal arteriopathy; recent surgery; severe endocrine, hepatic, or renal disorder; and use of anticoagulant medication. Written informed consent was obtained from all subjects, and the study was approved by the medical ethics committee of the University Hospital Maastricht.

Exercise Capacity

Patients and healthy volunteers underwent a symptom-limited incremental exercise test on an electronically braked cycle ergometer (Cornival 400; Lode, Groningen, The Netherlands). After a period of rest and 2 min of unloaded pedaling, a progressively increasing work-rate (WR) test was started in order to determine each subject's peak WR (WR_{peak}), and maximal oxygen uptake ($V_{O_{2peak}}$). The work rate increase was set at 10 Ws/min for each patient and 15 to 30 Ws/min for the healthy volunteers, depending upon their training status. A pedaling frequency of 60 to 70 rpm was selected by the subjects and held constant throughout the test. Breath-by-breath gas exchange was measured throughout the test with a ventilated hood system (Oxycon-β; Jaeger, Bunnik, The Netherlands). An infrared electrode (Fasttrac; Sensormedics, Anaheim, CA) was placed on a finger to measure Sa_{O_2}. During rest, at every minute during exercise, and at 3 min into the recovery period, blood samples were drawn from an antecubital vein, put into heparinized syringes, and subsequently centrifuged to obtain plasma for determination of La content, using an enzymatic method (13) in an automated system (Cobas Mira; Roche, Bazel, Switzerland). La threshold was determined according to the method of Beaver and colleagues (14). Plots of log La concentration versus log V_{O_2} were constructed, and two straight-line segments were fitted to each plot. The point of intersection of these two line segments was defined as the La threshold.

Collection and Analysis of Peripheral Skeletal Muscle Biopsy and Arterial Blood Samples

In the early morning, a peripheral skeletal muscle biopsy specimen was collected from all subjects in the postabsorptive state and at rest.

The muscle biopsy specimen, from the lateral part of the quadriceps femoris, was obtained under local anesthesia, using the needle biopsy technique (15). The muscle tissue was immediately frozen in liquid nitrogen and stored at −80° C until analysis. After the addition of glass beads (1 mm), the muscle tissue was homogenized with a Minibeater (Biospec Products, Bartlesville, OK). Muscle tissue was deproteinized with sulfosalicylic acid for determination of the citric acid cycle-related amino acids GLU and alanine (ALA), and with trichloric acid for determination of the glycolytic substrates La, pyruvate, and glucose. One part of muscle was freeze-dried in order to determine glycogen. This muscle specimen was kept at 100° C for 3 h after addition of 1.0 ml of 1 M HCl to hydrolyze glycogen, which was subsequently neutralized with Tris (0.12 M)–KOH (2.1 M) saturated with KCl. The glucose residues were measured fluorometrically as de-

TABLE 1

GENERAL CHARACTRISTICS AND PULMONARY FUNCTION OF PATIENTS WITH SUBTYPES OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE AND PHYSICALLY INACTIVE AND ACTIVE HEALTHY VOLUNTEERS

	EMPH+	EMPH−	Physically Inactive Healthy Volunteers	Physically Active Healthy Volunteers
General characteristics				
Sex, M/F	10/2	11/4	10/5	7/0
Age, yr	64 ± 9	64 ± 10	67 ± 3	63 ± 2
Height, cm	170 ± 10	170 ± 8	167 ± 8	172 ± 9
Weight, kg	66.2 ± 11.4	76.5 ± 10.1*	73.3 ± 11.2	74.3 ± 12.1
pH	7.41 ± 0.02	7.41 ± 0.02	7.41 ± 0.01	7.41 ± 0.02
Pa_{O_2}, mm Hg	70.8 ± 6.8	78.5 ± 9.0*	81.3 ± 9.5†	97.5 ± 10.0‡‖††
Pa_{CO_2}, mm Hg	42.4 ± 6.6	40.9 ± 3.4	42.0 ± 3.0	40.3 ± 2.2
HCO_3, mM	27.0 ± 3.0	25.6 ± 2.6	26.0 ± 1.2	25.3 ± 1.4
BE, mM	2.1 ± 2.1	1.2 ± 2.3	1.3 ± 0.8	0.9 ± 1.3
Sa_{O_2}, %	94.0 ± 1.6	95.2 ± 1.5*	95.6 ± 1.2*	97.3 ± 1.0‡‖**
Pulmonary function				
FEV_1, % pred	34 ± 8	54 ± 12‡	111 ± 21‡¶	110 ± 8‡¶
FVC, % pred	89 ± 23	94 ± 12	118 ± 18†¶	117 ± 6†¶
$D_{L_{CO}}$, % pred	43 ± 8	79 ± 15‡	107 ± 11‡¶	135 ± 17‡¶††
K_{CO}, % pred	48 ± 9	87 ± 21‡	107 ± 18‡§	128 ± 19‡¶**
TLC, % pred	125 ± 13	111 ± 11†	114 ± 11*	113 ± 4
RV, % pred	144 ± 31	186 ± 43*	113 ± 18‡‖	107 ± 13‡‖
ITGV, % pred	170 ± 26	126 ± 18‡	104 ± 19‡‖	104 ± 11‡§
R_{aw}, % pred	249 ± 99	222 ± 111	110 ± 49‡‖	65 ± 13‡¶‡‡

Definition of abbreviations: BE = base equivalents; $D_{L_{CO}}$ = diffusing capacity of carbon monoxide; EMPH+ = patients with emphysema; EMPH− = patients without emphysema; F = female; ITGV = intrathoracic gas volume; K_{CO} = diffusing capacity for carbon monoxide corrected for alveolar volume; M = male; Pa_{CO_2} = arterial carbon dioxide tension; Pa_{O_2} = arterial oxygen tension; Raw = airway resistance; Sa_{O_2} = arterial oxygen saturation.

Values are mean ± SD.

* $p < 0.05$ versus EMPH+.

† $p < 0.01$ versus EMPH+.

‡ $p < 0.001$ versus EMPH+.

§ $p < 0.05$ versus EMPH−.

‖ $p < 0.01$ versus EMPH−.

¶ $p < 0.001$ versus EMPH−.

** $p < 0.05$ versus physically inactive healthy volunteers.

†† $p < 0.01$ versus physically inactive healthy volunteers.

‡‡ $p < 0.001$ versus physically inactive healthy volunteers.

scribed elsewhere (16). The values obtained were corrected for the amount of free glucose already present at the time of tissue sampling.

Arterial blood was obtained by puncture of the radial artery while the subject breathed room air. One sample was used for determination of blood gases (ABL 330; Radiometer, Copenhagen, Denmark). A second sample was put in a heparinized syringe, immediately put on ice, and subsequently centrifuged at 4° C for 10 min to obtain plasma. The plasma was deproteinized with sulfosalicylic acid for determination of GLU and ALA, and with trichloric acid for determination of glucose, La, and pyruvate. The samples were frozen in liquid nitrogen and stored at −80° C until analyses.

The glycolytic substrates and the TCA-related amino acids in muscle tissue and arterial plasma were analyzed with a fully automated high-performance liquid chromatograph (HPLC) (17), and muscle glycogen was analyzed by enzymatic techniques (18).

Assessment of Macroscopic Emphysema

In all patients, evaluation of the presence of parenchymal destruction, the hallmark of emphysema (19), was done with high-resolution computed tomography (HRCT), using a commercial scanner (Somatom Plus; Siemens, Erlangen, Germany) at 137 kVp, 220 mA, a collimation of 1.0 mm, and a 1.0-s scanning time. Five HRCT scans were obtained of each subject in the supine position during breathholding at end-expiration: these consisted of two scans of the upper and two scans of the lower lung zones at 3 and 6 cm above and below the carina, respectively, and one scan at the carina. Images were made at a level of −800 HU and a window width of 1,600 HU, which is appropriate for lung detail. The severity and extent of emphysema in each scan were scored visually on a four-point scale by two independent observers according to the direct observational method developed by Sakai and colleagues (20). For each of the 10 lung sections, the score for severity was multiplied by the score for extent of emphysema, and the resultant scores were subsequently summed to give the total HRCT score. Visual HRCT scores ranged from 0 (no macroscopic emphysema) to 120 (severe macroscopic emphysema). Patients were stratified by HRCT score into two groups, as follows: HRCT score < 30: no or trivial macroscopic emphysema (EMPH-); HRCT score ≥ 30: macroscopic emphysema (EMPH+).

Pulmonary Function Tests

All patients and healthy volunteers underwent spirometry with determination of FEV_1 and FVC, with the highest value from at least three technically acceptable maneuvers being used. Static and dynamic lung volumes (TLC, intrathoracic gas volume [ITGV], and airway resistance [R_{aw}]) were assessed through whole-body plethysmography (Masterlab; Jaeger, Wurzburg, Germany). Diffusing capacity for carbon monoxide (D_{LCO}) was measured according to the single-breath method (Masterlab). Subtracting estimated deadspace from inspiratory volume gives an estimate of alveolar volume (V_A), and the D_{LCO} was then corrected for V_A (transfer factor: K_{CO}). All values obtained were related to a reference value and expressed as percentages of this predicted value (21).

Statistical Analysis

Results for muscle and arterial plasma determinations are expressed as mean ± SE, and those for other characteristics as mean ± SD. Analysis of covariance (ANCOVA) followed by Tukey's pairwise multiple comparison procedure was used to determine differences between the EMPH+, EMPH−, PI, and PA in pulmonary function, exercise capacity, and muscle and arterial plasma variables, with sex as the covariate. In addition, to adjust for the differences between the EMPH+ and EMPH− groups in FEV_1, ANCOVA was used with FEV_1 as the covariate. A two-tailed value of $p < 0.05$ was considered statistically significant.

RESULTS

Physically Inactive versus Active Healthy Volunteers

Twenty-two healthy age-matched controls (15 PI and seven PA) participated in the study (Table 1). All of these PA controls were men. Age, height, and weight of the PI and PA groups were not significantly different. The pulmonary function of both groups was within the normal range. The PA group had higher values for D_{LCO} ($p < 0.01$) and lower values for R_{aw} ($p < 0.001$) than did the PI group. Moreover, the PA group had higher values for arterial oxygen tension (Pa_{O_2}) ($p < 0.01$) and Sa_{O_2} ($p < 0.05$).

Exercise capacity (Figure 1), reflected by $\dot{V}O_{2peak}$ and work rate (WR_{peak}), was higher in the PA than in the PI group ($p < 0.01$). No significant difference between the two groups was found in venous La concentration at rest, peak exercise, or recovery (Table 2). La threshold (Table 2, Figure 2) was higher in the PA than in the PI group ($p < 0.05$).

In the PA group, higher values were found for muscle GLU ($p < 0.05$) and glycogen ($p < 0.01$) than in the PI group (Table 3), but no significant differences were found in muscle glucose, pyruvate, La, or the ratio of La to pyruvate. The observed differences between the PI and PA groups in lung function, exercise capacity, and muscle substrates remained after adjustment for the variation in sex.

Total COPD Group Stratified into EMPH+ and EMPH− Subgroups, versus Physically Inactive Healthy Volunteers

Twenty-seven COPD patients (12 EMPH+ and 15 EMPH−) participated in the study (Table 1). Age, height, and sex in the

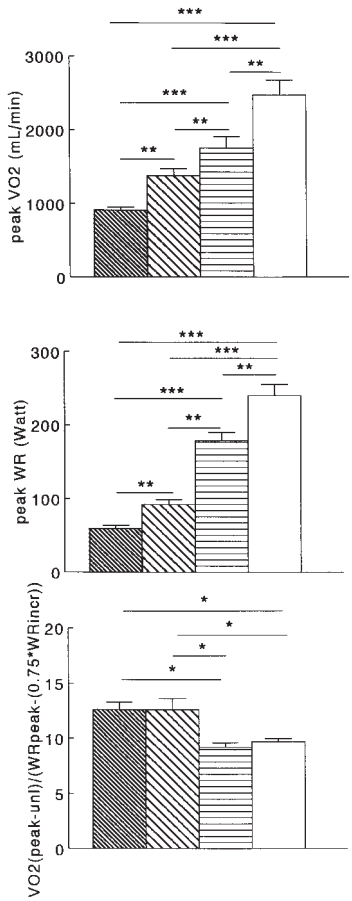

Figure 1. Bar diagram of the mean ± SE values for peak $\dot{V}O_2$ (*top*), WR_{peak} (*middle*), and ratio of $\dot{V}O_{2(peak\ exercise-unloaded)}$ to WR_{peak} − (0.75 * WR increment), where 0.75 is the (assumed) $\dot{V}O_2$ time constant (*bottom*) of the EMPH+ group (*small crosshatched bar*), the EMPH− group (*large crosshatched bar*), the healthy PI volunteers (*horizontally striped bar*), and the healthy PA volunteers (*open bar*). A portion of the difference between COPD patients and healthy controls in WR_{peak} is due to the different increases in WR. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ for differences between the groups.

TABLE 2

VENOUS LACTATE RESPONSE TO EXERCISE IN PATIENTS WITH SUBTYPES OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE AND PHYSICALLY INACTIVE AND ACTIVE HEALTHY VOLUNTEERS

	EMPH+	EMPH−	Physically Inactive Healthy Volunteers	Physically Active Healthy Volunteers
Rest, mM lactate	1.6 ± 0.7	1.8 ± 0.7	1.2 ± 0.2*§	1.4 ± 0.4§
Peak exercise, mM lactate	4.0 ± 1.3	5.4 ± 2.1	5.2 ± 1.4	6.4 ± 3.0
Recovery, mM lactate	4.6 ± 1.2	6.4 ± 2.3*	8.1 ± 2.3‡	8.9 ± 2.5‡§
$\Delta La/W_{peak}$, mM/W	0.03 ± 0.02	0.04 ± 0.02	0.02 ± 0.01*§	0.02 ± 0.01*§
La threshold, L/min	0.56 ± 0.04	0.76 ± 0.04†	0.94 ± 0.07‡‖	1.49 ± 0.16‡‖¶

Definition of abbreviations: EMPH+ = patients with emphysema; EMPH− = patients without emphysema; La = lactate; La threshold = $\dot{V}O_2$ (oxygen uptake) at which blood lactate begins to increase.

* $p < 0.05$ versus EMPH+, corrected for sex of patients.

† $p < 0.01$ versus EMPH+, corrected for sex of patients.

‡ $p < 0.001$ versus EMPH+, corrected for sex of patients.

§ $p < 0.05$ versus EMPH−, corrected for sex of patients.

‖ $p < 0.001$ versus EMPH−, corrected for sex of patients.

¶ $p < 0.05$ versus physically inactive healthy volunteers, corrected for sex of patients.

COPD and PI groups did not differ significantly. Body weight of the total COPD group and the PI controls was not significantly different, but was lower in the EMPH+ group than in the EMPH− group ($p < 0.05$). On average, the total COPD group was characterized by severe airflow obstruction (FEV_1 = 45 ± 14% predicted), reduced D_{LCO} (63 ± 22% predicted), moderate hyperinflation (TLC = 117 ± 14% predicted; RV = 163 ± 42% predicted), and increased R_{aw} (235 ± 104% predicted). The EMPH+ group (mean HRCT score = 81.8 ± 23.8) had more severe airflow obstruction and hyperinflation ($p < 0.01$) and lower values for Pa_{O_2} and Sa_{O_2} ($p < 0.05$) than did the EMPH− group (mean HRCT score = 3.3 ± 6.6). No significant differences between the EMPH+ and EMPH− groups were found in arterial pH, arterial carbon dioxide tension (Pa_{CO_2}), HCO_3, or base equivalents (BE).

$\dot{V}O_{2peak}$ and WR_{peak} ($p < 0.01$) (Figure 1) were lower in all COPD patients than in the controls, the lowest values being found in the EMPH+ group. The lower values for $\dot{V}O_{2peak}$ and WR_{peak} in the EMPH+ than in the EMPH− group ($p < 0.01$) remained after adjustment for the variation in FEV_1. Moreover, the ratio of $\Delta\dot{V}O_2$ to WR_{peak}, corrected for differences in WR increase ($\dot{V}O_{2(peak-unloaded)}/WR_{peak} - [0.75 \cdot WR_{increment}]$), was higher in both COPD subtype groups than in the PI group ($p < 0.05$), but not significantly different between the EMPH+ and the EMPH− groups. Transcutaneous Sa_{O_2} at peak exercise was lower in the EMPH+ group than in the EMPH− group (89.4 ± 2.5% versus 93.8 ± 2.1%, $p < 0.001$).

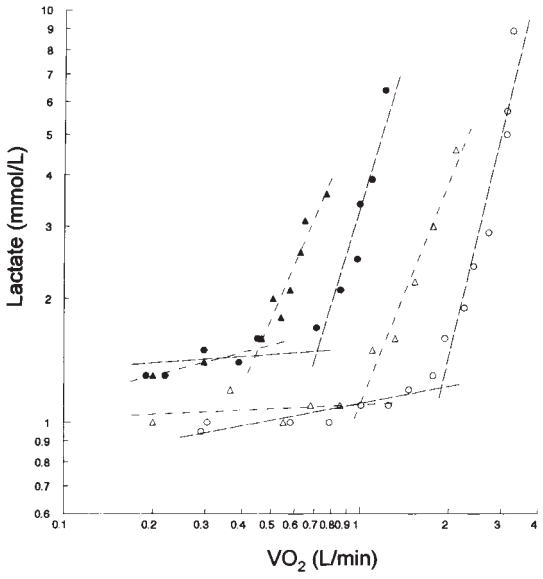

Figure 2. Effect of incremental exercise on venous La levels in one of the EMPH+ (*closed triangles*) and EMPH− (*closed circles*) patients, and in one of the PI (*open triangles*) and PA (*open circles*) volunteers. Venous La is plotted versus oxygen uptake on a log-log scale. The La threshold is the point of intersection of the two line segments. Use of antecubital venous blood will yield a significant delay in La increase as compared with arterial (or femoral venous) La values.

At rest, venous La (Table 2) was higher in both COPD subtype groups than in the PI group ($p < 0.05$), but no significant difference was found between the EMPH+ and EMPH− group. At peak exercise, no significant difference between the study groups was present in venous La. During recovery, venous La was lower in the total COPD group than in the PI controls ($p < 0.01$), being more reduced in the EMPH+ than in the EMPH− subgroup ($p < 0.05$). The La increase per maximal achieved workload ($\Delta lactate/WR_{peak}$) was higher in both COPD subtype groups than in the PI group ($p < 0.05$), but not different in the EMPH+ and EMPH− groups. La threshold (Table 2, Figure 2) was lower in the EMPH+ subgroup than in the EMPH− subgroup ($p < 0.01$) or PI group ($p < 0.001$). Moreover, La threshold was lower in the EMPH− subgroup than in the PI group ($p < 0.01$).

Resting muscle GLU (Table 3) was significantly lower in the total COPD group than in the PI group ($p < 0.01$), whereas muscle pyruvate and La and the muscle-to-plasma gradient for pyruvate and La were higher ($p < 0.05$). Muscle GLU was significantly lower in the EMPH+ than in the EMPH− subgroup ($p < 0.05$), whereas glycolysis-related substrates in both peripheral skeletal muscle and arterial plasma (data not shown) were not significantly different in the two COPD subgroups. Muscle glycogen and glucose, and the La-to-pyruvate gradient, were not significantly different among the total COPD group, EMPH+ and EMPH− subgroups, and PI group.

The significance of the differences between the EMPH+ and EMPH− subgroups in body weight, exercise capacity, La threshold, venous La concentration at recovery from exercise, and muscle GLU remained after adjustment for the two subgroups' difference in FEV_1.

In Figure 3, mean D_{LCO}, muscle GLU, and La threshold during incremental exercise are plotted against each other for the four study groups (EMPH+, EMPH−, PI, and PA). A positive relationship was visible between D_{LCO} and muscle GLU (Figure 3, *top panel*), and between D_{LCO} and La thresh-

TABLE 3

RESTING GLUTAMATE AND GLYCOLYSIS-RELATED SUBSTRATE LEVELS OF PATIENTS WITH SUBTYPES OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE AND PHYSICALLY INACTIVE AND ACTIVE HEALTHY VOLUNTEERS

	EMPH+	EMPH−	Physically Inactive Healthy Volunteers	Physically Active Healthy Volunteers
Glutamate, $\mu mol/kg_{ww}$	1,262 ± 64	1,682 ± 122*	1,908 ± 84‡	2,150 ± 126‡¶
Glycogen, $\mu mol/kg_{ww}$	325 ± 39	269 ± 15	334 ± 27	504 ± 43†‖**
Glucose				
Muscle, $mmol/kg_{ww}$	1.3 ± 0.1	1.5 ± 0.3	1.3 ± 0.2	1.2 ± 0.2
Muscle/plasma, $mmol/kg_{ww}$	0.3 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.3 ± 0.1
Pyruvate				
Muscle, $mmol/kg_{ww}$	0.12 ± 0.02	0.14 ± 0.01	0.05 ± 0.01§	0.05 ± 0.01§
Muscle/plasma, $mmol/kg_{ww}$	4.9 ± 1.3	4.8 ± 2.1	1.6 ± 0.4§	2.2 ± 0.5
Lactate				
Muscle, $mmol/kg_{ww}$	3.8 ± 0.5	3.8 ± 1.0	1.7 ± 0.1†§	1.9 ± 0.2*§
Muscle/plasma, $mmol/kg_{ww}$/mM	6.9 ± 1.1	5.8 ± 1.5	2.9 ± 0.4†§	3.1 ± 0.5*
Lactate/pyruvate				
Muscle	43.7 ± 6.5	38.8 ± 6.8	37.2 ± 3.3	34.3 ± 4.1
Plasma	22.1 ± 1.3	22.7 ± 2.9	18.1 ± 1.7	23.9 ± 2.9

Definition of abbreviations: EMPH+ = patients with emphysema; EMPH− = patients without emphysema; La = lactate; La threshold = $\dot{V}O_2$ (oxygen uptake) at which blood lactate begins to increase.

* $p < 0.05$ versus EMPH+, corrected for sex of patients.

† $p < 0.01$ versus EMPH+, corrected for sex of patients.

‡ $p < 0.001$ versus EMPH+, corrected for sex of patients.

§ $p < 0.05$ versus EMPH−, corrected for sex of patients.

‖ $p < 0.001$ versus EMPH−, corrected for sex of patients.

¶ $p < 0.05$ versus physically inactive healthy volunteers, corrected for sex of patients.

** $p < 0.01$ versus physically inactive healthy volunteers, corrected for sex of patients.

Values are mean ± SE.

old (Figure 3, *middle panel*). Moreover, a positive relationship was visible between muscle GLU and La threshold during exercise (Figure 3, *bottom panel*). The relationships with muscle GLU and La threshold were also seen when using Pa_{O_2} instead of $D_{L_{CO}}$ (Figure 4). No relationship was found between La threshold during incremental exercise and resting muscle glycogen, glucose, pyruvate, or La levels.

DISCUSSION

This study shows an early La response to incremental exercise in EMPH− patients, but to a greater extent in EMPH+ patients, as compared with control subjects. The La threshold during exercise was related to resting muscle GLU status, the latter being most severely depleted in EMPH+ patients. No association was found between La threshold and alterations in resting muscle glycogen, pyruvate, or La level.

Physical inactivity is often seen as the main cause or one of the main causes of the early lactic acidosis during exercise in COPD. In the present study, we compared two control groups with different physical training status in order to gain greater insight into the effect of physical activity level on exercise related muscle substrate levels. The PA controls, characterized by higher values for WR_{peak} and La threshold during exercise than were the PI subjects, had higher resting values for muscle GLU and glycogen, but showed no differences from the PI subjects in glucose, La or pyruvate. This indicates that the role of physical inactivity has to be taken into account when studying muscle glycogen and GLU metabolism in patients with COPD.

Since premature lactic acidosis represents a misbalance between La production and the body's ability to clear La, it is of importance to discern the factors that define both the rate of La production and La clearance in COPD. In this way, greater insight can be obtained about the potential causes of the different La responses to exercise in COPD patients and controls, and between patients with the subtypes of COPD.

Under normal conditions and in the presence of adequate O_2 delivery, muscle La production may increase when the rate of pyruvate production by glycolysis exceeds the rate of pyruvate oxidation by the TCA cycle. Since it is unlikely that respiratory muscles contribute appreciably to the early increase in La in COPD patients (22), an impaired oxidative muscle metabolism of the exercising peripheral skeletal muscles is likely to be an important source of this increase in La. Indeed, in a recent study by Maltais and coworkers (3), the enhanced increase in La in COPD patients was associated with decreased activities for the oxidative enzymes citrate synthase and 3 hydroxyacyl CoA. In accord with this is the observed increased proportion of type 2b/x fibers in quadriceps femoris of COPD patients (4, 23). Moreover, Satta and associates showed that increased levels of type 2b/x fibers were particularly present in COPD patients with a reduced $D_{L_{CO}}$ (4), suggesting that patients with emphysema are particularly prone to a decreased muscle oxidative capacity.

The fiber type distribution in peripheral skeletal muscle is important, since the ratio of oxidative to glycolytic fibers may control the range over which lactic acidosis can shift during exercise. The metabolic profile of type 2b/x fibers, with a high concentration of glycolytic enzymes and La dehydrogenase-M isozyme and a low mitochondrial content, favors glycolytic energy production. These fibers produce La when stimulated. In contrast, the high mitochondrial density and enzyme activity of type 1 fibers favor oxidative energy production. Furthermore, type 1 fibers have a greater La and pyruvate-oxidative capacity than do type 2b/x fibers during contraction, owing to a high La dehydrogenase-H isozyme concentration. Therefore, it is likely that the oxidative type 1 fibers play a role in the clearance of La produced by the glycolytic type 2b/x fibers during exercise. Since COPD patients, and probably EMPH+ patients in particular, have a reduced percentage of type 1 fibers, the capacity of these patients to reduce the enhanced La levels produced by type 2b/x fibers during low-intensity exercise may be diminished.

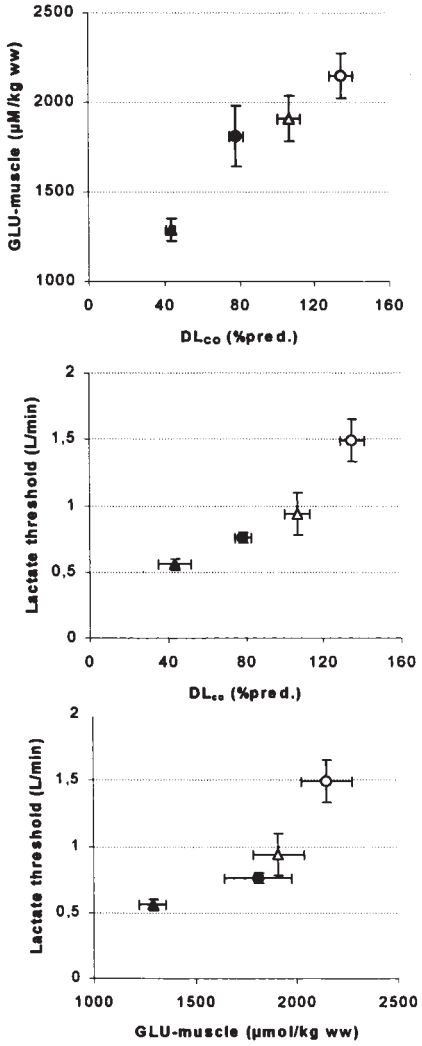

Figure 3. Scatter plot of the relationships between muscle glutamate versus D_{LCO} (*top*), lactate threshold versus D_{LCO} (*middle*), and lactate threshold versus muscle GLU at rest (*bottom*) for the EMPH+ group (*closed triangles*), the EMPH− group (*closed circles*), the PI volunteers (*open triangles*), and the PA volunteers (*open circles*). Values are mean ± SE.

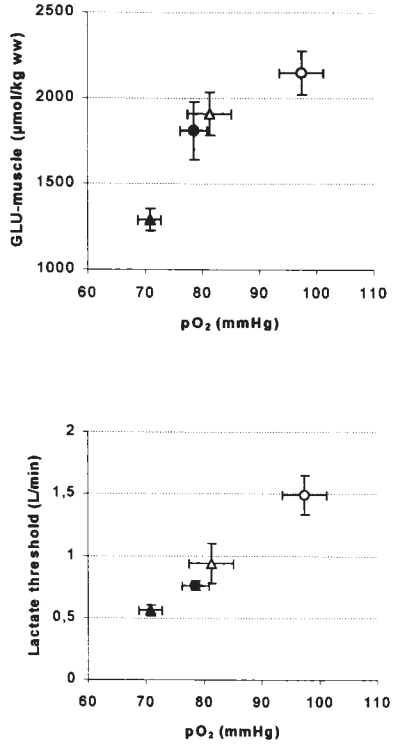

Figure 4. Scatter plot of the relationships between muscle GLU and Pa_{O_2} at rest (*top*), and lactate threshold versus Pa_{O_2} at rest (*bottom*) for the EMPH+ subgroup (*closed triangles*), the EMPH− subgroup (*closed circles*), the PI volunteers (*open triangles*), and the PA volunteers (*open circles*) (*bottom*). Values are shown as mean ± SE.

In the present study, significantly lower values for transcutaneous Sa_{O_2} were found at peak exercise in the EMPH+ group than in the EMPH− group (89% versus 94%, respectively). In agreement with this is the finding that desaturation during exercise, a potential contributor to hypoxemia, occured in 68% of the emphysema patients (D_{LCO} < 55% predicted). When D_{LCO} fell below 55% predicted, a further increase took place in both the percentage of COPD patients with desaturation during exercise and in the magnitude of desaturation. It is therefore very possible that a reduced O_2 supply to the peripheries is present during exercise in EMPH+ patients, contributing to these patients' early La response to exercise. However, despite normal resting Pa_{O_2} values and the absence of oxygen desaturation during exercise in the EMPH− patients, it is also possible that O_2 availability to the skeletal muscles is compromised during exercise in these patients as compared with controls. This may be due to more severe redistribution of blood flow toward the respiratory apparatus. Evidence for this is provided by a recent study which found that breathing of helium significantly increased values of $V_{O_{2max}}$ in COPD patients, possibly because of a reduced work of breathing and increased skeletal muscle blood flow (24).

Hypoxic episodes are also suggested to have a detrimental effect on La clearance in the livers of patients with COPD. Recently, acute exposure to hypoxia in rats was found to inhibit liver gluconeogenesis through an abolishing effect on phosphoenolpyruvate carboxykinase activity and transcription, which was independent of food intake (25). Moreover, hypoxic episodes influence catecholamine levels in blood. Epinephrine is known to increase blood La by activating phosphorylase. During exercise, the concentration of epinephrine in the blood rises as $\dot{V}_{O_2}$ exceeds 50% of $V_{O_{2max}}$. This rise causes an increase in La output of the working muscles, and it may stop the uptake of lactate (26). Furthermore, the rising concentration of epinephrine may contribute to the rising La concentrations in other tissues by reducing the effective distribution volume of La from La-producing tissues (i.e., working muscles). Earlier studies showed that patients with advanced emphysema (27) and patients with chronic respiratory failure (28) had higher levels of plasma norepinephrine than did control subjects in the resting state. Although no difference was found in epinephrine levels at rest, it is still unknown whether exercise induces an alteration in the epinephrine response of these patients as compared with controls, thereby contributing to the difference in La response.

In the present study, the relationship between resting muscle GLU status and La threshold during exercise suggests that depleted GLU levels in resting peripheral skeletal muscle of COPD patients play a role in these patients' early La response to exercise. We found no association between La threshold during exercise and other intrinsic changes in muscle substrates (i.e., La, pyruvate, glucose, or glycogen). Earlier re-

duced intracellular GLU levels are shown to negatively influence the alanine aminotransferase (AAT) reaction, since the muscle in which this occurs is less able to shunt a proportion of pyruvate for conversion into alanine than to lactate. In this way the capacity of the muscle to resist lactate production is diminished. Moreover, an adequate level of muscle GLU in peripheral skeletal muscle is necessary, since the AAT reaction occurs at the cost of GLU. The potential causes of the decreased intracellular GLU levels in COPD patients may be related to a decreased capacity for membrane transport of GLU into the muscle, and/or to increased intramuscular GLU degradation. The positive relationship between Pa_{O_2} (and/or $D_{L_{CO}}$) and muscle GLU in the present study indicates that a decrease in oxygen availability negatively influences muscle GLU metabolism, leading to a reduction in muscle GLU status. This is in accord with several studies done *in vivo* and *in vitro* that have shown an accelerated GLU breakdown in muscle (mainly heart muscle) and mitochondria in situations in which oxygen deprivation is present (30–32). The myocardial tissue content of GLU was depleted during hypoxia and by underperfusion of isolated heart preparations, and tended to decrease during cardioplegic arrest in humans. Together, these results indicate that ischemia as well as hypoxia causes an increased degradation and/or utilization of intracellular GLU. In patients with chronic heart failure, an augmented cardiac consumption of GLU was found even in the resting asymptomatic state, suggesting a specific metabolic adaptation induced by repetitive or chronic ischemia. Lower resting Pa_{O_2} values and chronic intermittent hypoxia are often also present during daily living in COPD patients. However, it is not clear to what extent the lower resting Pa_{O_2} levels and/or the presence of chronic intermittent hypoxia (possibly related to the reduced $D_{L_{CO}}$) in the COPD group in our study contributed to their decreased muscle GLU status. The PA group in our study had significantly higher values of $D_{L_{CO}}$, Pa_{O_2}, and Sa_{O_2} at rest than did the PI group. We are unaware of any studies indicating that physical training has a positive effect on Pa_{O_2}. The exact reason for the higher Pa_{O_2} in the PA group than in our PI group is not yet clear.

The increased levels of GLU in the PA as compared with the PI subjects in the present study are in accord with the increased GLU levels found in muscle of trained as compared with untrained men (33). It has also been shown that trained muscle can increase its capacity for resisting La production by shunting a greater proportion of pyruvate to alanine than to La (29). Although we did not have physical activity scores for the COPD group in our study, the 50% higher WR_{peak} in the PI group than in the COPD group may suggest that we were not completely successful in recruiting controls with a comparably low physical activity level to that of our COPD patients. This indicates that physical inactivity may have been at least partly responsible for the decreased resting muscle GLU levels in our COPD group. However, the EMPH− and EMPH+ patients in the present study were able to participate in and complete the intensive training part of the pulmonary rehabilitation program, and had pulmonary function characteristics comparable to those of the group of COPD patients described at the beginning of this article, in whom we observed decreased GLU levels, indicating comparable physical activity levels in both subtypes of COPD patients. More evidence for the expectation of comparable physical activity levels in patients with the subtypes of COPD comes from a large data base obtained from our pulmonary rehabilitation center ($n = 61$), in which we did not find a significant correlation between $D_{L_{CO}}$ (the indirect measure of emphysema) and physical activity as scored by Voorrips and coworkers (34) or the Physical Activity Scale for the Elderly (35) questionnaire in COPD patients ($r = 0.04$ and $r = 0.13$, respectively). This indicates that other factors, rather than physical inactivity, contributed to the lower resting GLU level found in the EMPH+ group as compared with the EMPH− group in our study.

In conclusion, the present study indicates that the early La response in patients with COPD is associated with the reduction in muscle GLU related to physical inactivity in COPD. However, other factors, such as a reduced Pa_{O_2} or $D_{L_{CO}}$ play a role in the early La response during exercise in EMPH+ as opposed to EMPH− patients.

References

1. Casaburi R, Patessio A, Ioli F, Zanaboni S, Donner CF, Wasserman K. Reductions in exercise lactic acidosis and ventilation as a result of exercise training in patients with obstructive lung disease. *Am Rev Respir Dis* 1991;143:9–18.
2. Sue DY, Wasserman K, Moricca RB, Casaburi R. Metabolic acidosis during exercise in patients with chronic obstructive pulmonary disease. *Chest* 1988;94:931–938.
3. Maltais F, Simard AA, Simard C, Jobin J, Desgagnes P, LeBlanc P. Oxidative capacity of the skeletal muscle and lactic acid kinetics during exercise in normal subjects and in patients with COPD. *Am J Respir Crit Care Med* 1996;153:288–293.
4. Satta A, Migliori GB, Spanevello A, Neri M, Bottinelli R, Canepari M, Pellegrino MA, Reggiani C. Fibre types in skeletal muscles of chronic obstructive pulmonary disease patients related to respiratory function and exercise tolerance. *Eur Respir J* 1997;10:2853–2860.
5. Gertz I, Hedenstierna G, Hellers G, Wahren J. Muscle metabolism in patients with chronic lung disease and acute respiratory failure. *Clin Sci Mol Med* 1977;52:395–403.
6. Jakobsson P, Jorfeldt L, Brundin A. Skeletal muscle metabolites and fibre types in patients with advanced chronic obstructive pulmonary disease (COPD), with and without chronic respiratory failure. *Eur Respir J* 1990;3:192–196.
7. Pouw EM, Schols AMWJ, Deutz NEP, Wouters EFM. Plasma and muscle amino-acid levels in relation to resting energy expenditure and inflammation in stable COPD. *Am J Respir Crit Care Med* 1998;158: 797–801.
8. Engelen MPKJ, Schols AMWJ, Does JD, Deutz NEP, Wouters EFM. Altered glutamate metabolism is associated with reduced muscle glutathione levels in patients with emphysema. *Am J Respir Crit Care Med* 2000;161:98–103.
9. Van Hall G, Saltin B, Van der Vusse GJ, Soderlund K, Wagenmakers AJM. Deamination of amino acids as a source for ammonia production during prolonged exercise in man. *J Physiol* 1995;1995:251–261.
10. Van Hall G, MacLean DA, Saltin B, Wagenmakers AJM. Mechanisms of activation of muscle branched-chain alpha-keto acid dehydrogenase during exercise in man. *J Physiol* 1996;494:899–905.
11. Pols MA, Peeters PHM, Bueno-de-Mesquita HB, Ocke MC, Wentink CA, Kemper HCG, Collette HJA. Validity and repeatability of a modified Baecke questionnaire on physical activity. *Int J Epidemiol* 1995;24:381–388.
12. American Thoracic Society. Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease (COPD) and asthma. *Am Rev Respir Dis* 1987;137:225–228.
13. Noll F. L-(+)-lactate, determination with LDH, GTP and NAD. Bergmeyer, editor. Methods of enzymatic analysis. New York: 1974: p. 1475.
14. Beaver WL, Wasserman K, Whipp BJ. Improved detection of lactate threshold during exercise using a log-log transformation. *J Appl Physiol* 1985;59:1936–1940.
15. Bergstrom L. Muscle electrolytes in man. Determination by neutron activation analysis on needle biopsy specimens. A study on normal subjects, kidney patients, and patients with chronic diarrhea. *Scand J Clin Lab Invest* 1962;68:7–110.
16. Van der Vusse GJ, Coumans WA, Van der Veen FH, Drake A, Flameng W, Suy R. ATP, creatine phosphate and glycogen content in human myocardial biopsies: markers for the efficacy of cardioprotection during aortocoronary bypass surgery. *Vasc Surg* 1984;18:127–134.
17. Van Eijk HMH, Rooyakkers DR, Deutz NEP. Rapid routine determination of amino acids in plasma by high-performance liquid chromatography with a 2–3 μm Sperisorb ODS 2 column. *J Chromatogr* 1993; 620:143–148.
18. Van der Vusse GJ, Janssen GME, Coumans WA, Kuipers H, Does RJMM,

ten Hoor F. Effect of training and 15-, 25-, and 42-km contests on the skeletal muscle content of adenine and guanine nucleotides, creatine phosphate, and glycogen. *Int J Sports Med* 1989;10:S146–S152.
19. American Thoracic Society. Chronic bronchitis, asthma, and pulmonary emphysema by the committee on diagnostic standards for nontuberculosis respiratory disease. *Am Rev Respir Dis* 1962;85:762–812.
20. Sakai F, Gamu G, Im JG, and Ray CS. Pulmonary function abnormalities in patients with CT-determined emphysema. *J Comput Assist Tomogr* 1987;11:963–968.
21. Quanjer P, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernault JC. Standardized lung function testing. *Eur Respir J* 1993;6:5–40.
22. Engelen MPKJ, Casaburi R, Rucker R, Carithers E. Contribution of the respiratory muscles to the lactic acidosis of heavy exercise in COPD. *Chest* 1995;108:1246–1251.
23. Whittom F, Jobin J, Simard PM, Leblanc P, Simard C, Bernard S, Belleau R, Maltais F. Histochemical and morphological characteristics of the vastus lateralis muscle in patients with chronic obstructive pulmonary disease. *Med Sci Sports Exerc* 1998;30:1467–1474.
24. Richardson RS, Sheldon J, Poole DC, Hopkins SR, Ries AL, Wagner PD. Evidence of skeletal muscle metabolic reserve during whole body exercise in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1999;159:881–885.
25. Pison CM, Chauvin C, Perrault H, Schwebel C, Lafond JL, Boujet C, Leverve XM. *In vivo* hypoxic exposure impairs metabolic adaptations to a 48 hour fast in rats. *Eur Respir J* 1998;12:658–665.
26. Stainsby WN, Sumners C, Eitzman PD. Effects of catecholamines on lactic acid output during progressive working contractions. *J Appl Physiol* 1985;59:1809–1814.
27. Hofford JM, Milakofsky L, Vogel WH, Sacher RS, Savage GJ, and Pell S. The nutritional status in advanced emphysema associated with chronic bronchitis: a study of amino acid and catecholamine levels. *Am Rev Respir Dis* 1990;141:902–908.
28. Keller R, Lohmann FW, Schuren KP. Catecholamines in chronic respiratory insufficiency. *Respiration* 1971;28:273–279.
29. Mole PA, Baldwin KM, Terjung RL, Holloszy JO. Enzymatic pathways of pyruvate metabolism in skeletal muscle: adaptations to exercise. *Am J Physiol* 1973;224:50–54.
30. Pisarenko OI, Solomatina ES, Studneva IM, Ivanov VE, Kapelko VI, Smirnov VN. Effect of glutamic acid and aspartic acids on adenine nucleotides, nitrogenous compounds and contractile function during underperfusion of isolated rat heart. *J Mol Cell Cardiol* 1983;15:53–60.
31. Pisarenko OI, Studneva IM, Solomatina ES, Kapelko VI. Adenine nucleotides, glutamate and respiratory function of heart mitochondria during acute hypoxia. *Biochem J* 1986;13:51–58.
32. Wiesner RJ, Deussen A, Borst M, Schrader J, Grieshaber MK. Glutamate degradation in the ischemic dog heart: contribution to anaerobic energy production. *J Mol Cell Cardiol* 1989;21:49–59.
33. Henriksson J. Effect of exercise on amino acid concentrations in skeletal muscle and plasma. *J Exp Biol* 1991;160:149–165.
34. Voorrips LE, Ravelli AC, Dongelmans PC, Deurenberg P, Van Staveren WA. A physical activity questionnaire for the elderly. *Med Sci Sports Exerc* 1991;23:974–979.
35. Washburn RA, McAuley E, Katula J, Mihalko SL, Boileau RA. The physical activity scale for the elderly (PASE): evidence for validity. *J Clin Epidemiol* 1999;52:643–651.