Physiological effects of intravenous fructose 1.6-diphosphate on diaphragmatic function in malnourished patients with COPD

S. Nava[1], L.M. Fuccella[2], B.Viglianti[2]

ABSTRACT: *Physiological effects of intravenous fructose 1.6-diphosphate on diaphragmatic function in malnourished patients with COPD. S. Nava, L.M. Fuccella, B.Viglianti.*

Background. A low body mass index is one of the strongest predictors of mortality in Chronic Obstructive Pulmonary Disease (COPD) patients. Under-nutrition is often associated with skeletal muscle wasting and hypophosphatemia.

Aim and Methods. In a pilot, randomised, double-blind placebo-controlled study, we assessed the physiological effects of phosphorous *administration* in 17 stable undernourished COPD patients, on diaphragmatic function, breathing pattern, neuromuscular drive (P0.1) and dyspnea score. Fructose 1.6-diphosphate (FDP) or placebo was administered i.v. for 7 consecutive days.

Results. FDP administration was associated with a marked increase in inspiratory time (Ti) that induced a significant rise ($p<0.05$) in the Pressure Time Product of the diaphragm per breath (PTPdi/b). However, since breathing frequency also decreased, the Pressure Time Product per minute of the diaphragm (PTPdi/min), index of diaphragmatic energy expenditure was markedly reduced. The efficiency of the respiratory pump in clearing CO_2 was also improved, although not significantly, in the FDP group ($p=0.09$) as well as the maximal transdiaphragmatic pressure during the sniff manoeuvre (Pdi,sniff).

Conclusions. This pilot physiological study showed that phosphorus replacement in undernourished, stable COPD patients, may be associated with a complex modification in respiratory pattern and diaphragmatic functions, leading to a marked although not significant reduction in PTPdi/min.

Monaldi Arch Chest Dis 2004; 61: 4, 203-208.

[1] *Respiratory Unit Fondazione S. Maugeri, I.R.C.C.S. Istituto Scientifico di Pavia.*
[2] *Medical Department, Biomedica Foscama, Ferentino, Italy.*

Correspondence: Stefano Nava, Respiratory Unit, Fondazione S. Maugeri, Istituto Scientifico di Pavia; Via Ferrata 8; 27100 Pavia, Italy; e-mail: snava@fsm.it

Introduction

Chronic Obstructive Pulmonary Disease (COPD) is a complex pathology whose prognosis is determined not only by the degree of airway obstruction, but also by residual exercise capacity and nutritional status [1].

Undernourished patients are often affected by hypophoshatemia and phosphorus (P) depletion that may affect the performance of the skeletal muscles [2]. Fiaccadori et al [3] have demonstrated low levels of P in peripheral skeletal muscles of COPD patients. Later they [4] also showed that P depletion, which can be present also in respiratory muscles, could depend on renal P wasting and also, possibly, malnutrition.

In severely ill patients with acute respiratory failure, necessitating mechanical ventilation, the maximal pressure generated by the diaphragm, may be one of the major determinants of weaning success, so that a reduction in the contractile properties of the respiratory muscle may be associated with prolonged mechanical ventilation [5].

Aubier et al [6] used 8 ventilated patients with hypophosphatemia to accurately demonstrate that the increase in serum P, obtained after by i.v. administration of K phosphate, was associated with a marked increase in the transdiaphragmatic pressure (Pdi) elicited by phrenic nerve stimulation.

The administration of Intravenous fructose 1.6-dishosphate (FDP) in stable malnourished COPD patients has been shown to be useful in increasing the maximal inspiratory and expiratory pressures [7].

On the contrary of critically ill patients [8], stable COPDs usually breathe quite far from the so called "fatigue threshold", so that the small (~ 10%), even though significant, increase reported, may be theoretically of minimal clinical significance [9].

The aim of this pilot physiological study was to assess in detail the changes in several components of diaphragm function, breathing pattern and neuromuscular drive, before and after P replacement, in a group of stable malnourished COPD patients with hypophoshatemia. This was carried out

in an attempt to identify possible "positive" variations in any biological signals, and clarify the possible mechanisms associated with the inotropic improvements induced by FDP, and reported by other clinical studies [6, 7].

Materials and methods

Population

Seventeen consecutive malnourished (BMI<20) patients with COPD [10] and serum hypophoshatemia (<1.5 mg/dl) were enrolled in the study, which was approved by the S. Maugeri Foundation Ethics Committee. Written informed consent was obtained from all the patients. The subjects had been in a stable clinical state for at least 6 months prior to entry into the study and had shown no recent deterioration in clinical status, spirometric values or resting blood gases.

Inclusion criteria were: BMI<20 Kg/m^2, serum level of P<1.5mg/dl, age <80 years, and clinical stability for at least 6 months.

Exclusion criteria were obstructive sleep apnea, malignancies, coronary artery disease, renal insufficiency, cardiac failure, neurological or psychiatric disorders, and an associated restrictive ventilatory deficit due to either thoracic or neuromuscular diseases. "Respiratory" medical treatment was suspended 2 days before the start of the study and included: ß2-agonists (8 patients), anticholinergic agents (2 patients), inhaled steroids (12 patients), and oral methylxanthines (3 patients). Other medication was kept unmodified throughout the study, except for loop diuretics that were, when clinical possible, suspended, since they may interfere with renal reabsorption of P [3].

The study design was double blind, randomised and placebo controlled. After initial evaluation, including Pulmonary Function Tests (PFTs), Arterial Blood Gas (ABG) analysis, body mass index, and serum phosphorous level, the patients were randomised to receive an organic phosphate (fructose 1,6-diphosphate, FDP) at the daily dose of 1.6 ml/Kg (Esafosfina, Biomedica Foscama, Ferentino. Italy) or dextrose (placebo) 5% solution 1.6 ml/Kg as a fast intravenous infusion (10-15 minutes) in a single daily administration for 7 consecutive days.

As illustrated in table 1, there was no significant difference at enrolment in any of the presented variables between the two groups.

Measurements

Resting daytime ABG was measured in blood from the radial artery taken at 10 a.m., with the patient breathing room air (ABL 550 Radiometer, Copenhagen, Denmark).

Static and dynamic lung volumes were assessed by constant-volume body plethysmography (MasterLab-Jaeger, Hochberg, Germany).

Flow was measured with a heated pneumotachograph (Screenmate Box 0586, Jaeger, Hochberg, Germany), connected to a differential pressure transducer (Validyne B 2 cm H$_2$O). Tidal volume (VT) was determined by digital integration of the flow signal. Inspiratory time (Ti), expiratory time (Te) and total breath duration (Ttot) were also obtained from the flow signal. Minute ventilation was calculated as the product of VT and respiratory rate (RR).

Changes in oesophageal pressure (Pes), as an estimate of pleural pressure, and gastric pressure (Pga), as an estimate of abdominal pressure, were measured using two balloons positioned in the middle part of the oesophagus and the stomach respectively and connected with polyethylene catheters to two differential pressure transducers (Honeywell B 250 cm H$_2$O, Freeport, Ill., USA). This allowed the transdiaphragmatic pressure (Pdi) to be calculated by subtraction of Pes from Pga. The position of the oesophageal balloon was checked using the so-called occlusion test [11]. Pressure at the airway opening was measured via a side port inserted in the mouthpiece. The subtraction of Pes from pressure at the airway opening gave the measurement of transpulmonary pressure (PL).

Dynamic lung compliance (CL) was obtained from simultaneous recordings of PL, VT and flow and calculated as the ratio of change in volume to change in PL between instants of zero flow within the same breath [12]. Pulmonary inspiratory resistance was calculated using PL, according to the

Table 1. - Main physiological variables at enrolment in the two groups of patients			
Variables	PLACEBO	P value	FDP
Age (yrs)	70.98±5.14	NS	70.41±8.80
Sex (M/F)	7/1	NS	7/1
BMI	18.77±1.39	NS	19.13±0.76
Smoking habit (Yes/No)	2/6	NS	1/7
FEV$_1$ (ml)	1021±533	NS	886±206
FEV$_1$/FVC (%)	41.3±10	NS	39.0±9
PaO$_2$ (mmHg)*	62.51±6.32	NS	65.53±8.78
PaCO$_2$ (mmHg)*	39.99±4.77	NS	41.79±8.06
pH*	7.42±0.03	NS	7.43±0.06
* breathing room air			

Neergaard-Wirtz elastic subtraction technique [13].

Dynamic intrinsic positive end-expiratory pressure (PEEPidyn) was measured as the amount of negative deflection in Pes preceding the start of inspiratory flow from which the decrease in Pga during this time interval, if present, was subtracted [14].

The pressure swings during tidal breathing (i.e. Pdi, Pes and Pga) were recorded by measuring the peak amplitude from the immediately preceding baseline.

The pressure time integral per breath of the diaphragm (PTPdib) was calculated as the area subtended by Pdi above end-expiratory baseline over Ti [15].

The PTPdi per minute (PTPdi/min) was calculated as the product of PTPdib and RR.

The product of PTPdi/min and $PaCO_2$ was also calculated as an index of the efficiency of the respiratory pump in clearing CO_2 [16].

The maximal transdiaphragmatic pressure (Pdimax) was measured using the sniff maneuver (Pdi sniff). The patients were asked to sniff maximally and quickly (less than 1 s) through the nose with the mouth closed and without using a nose-clip [17]. The ratio between tidal Pdi and maximal sniff Pdi (Pdi/Pdimax) was used to assess the force/load balance.

Respiratory muscle maximal pressures were calculated using the maximal inspiratory manoeuvre (MIP) performed at Functional Residual Capacity (FRC) and the maximal expiratory manoeuvre (MEP) performed at Total Lung Capacity (TLC), as described in detail elsewhere [18].

The tension-time index (TTdi) of the diaphragm, index of muscle endurance, was calculated as= mean Pdi/Pdimax x Ti/Ttot, where Pdi is transdiaphragmatic pressure, Pdimax is the maximal diaphragmatic pressure generated during the sniff manoeuvre, Ti is the inspiratory time and Ttot the total duration of the respiratory cycle [19].

The airway occlusion pressure after 100 ms (P0.1) was used as an estimate of neuromuscular drive [20].

Exercise performance was assessed using the 6-min walking test, modified according to McGavin et al. [21]. Verbal encouragement was given continuously throughout the test, but the patient was allowed to stop. Two practice attempts were performed on 2 days preceding the starting of the trial, and at the end of them the best result was considered for data analysis.

The patients' evaluation of dyspnea was recorded at rest using a visual analogue scale (VAS).

Protocol

All the afore-mentioned measures were recorded in both groups of patients at enrolment and during the morning after the termination of the trial.

After having swallowed the balloons the patients were allowed to rest for 15 minutes and were then asked to breathe quietly for 10–15 minutes through the mouthpiece, while wearing a nose-clip.

Every morning the breathing frequency, heart rate and systolic and dyastolic blood pressures were also monitored.

Statistical Analysis

Results are presented as mean and standard deviations (SD). Differences between those with treatments and without treatments were evaluated with two-tailed Mann-Whitney test. A p value <0.05 was chosen as the threshold of statistical significance. All of the analyses were performed using STATISTICA/W statistical package (Tulsa - OK, USA). A p<0.05 was considered statistical significant.

Results

One patient was excluded from the study, since he refused to swallow the balloons for the second time, therefore a total of sixteen patients (8 for each group) were considered for data analysis.

Table 2 shows ABG and PFTs changes during the time course of the study. No significant changes were observed after 7 days of treatment in both groups of patients.

Fig. 1 is a spirogram representing the variations in breathing pattern, before and after the treatment, in the two groups. Despite no significant changes being observed, the FDP patients increased the inspiratory time from 1.27 sec. to 1.44 at the end of the trial (p=0.08) and decreased the breathing frequency from 21.48 breath/min to 19.06 (p=0.1), while this was not the case of the placebo group.

Table 3 illustrates the mean changes in diaphragmatic function. Maximal inspiratory (MIP) and maximal expiratory (MEP) pressures, as well as maximal sniff transdiaphragmatic pressure, were close to achieving statistical significance, in the FDP group. The PTPdi/b estimate of the oxygen consumption of the diaphragm was significantly higher (p<0.05) after 7 days of treatment in the FDP group, while the PTPdi calculated over a minute (PTPdi,min) was markedly higher in the placebo group. The load/capacity balance (Pdi/Pdimax), the endurance capacity (TTdi) and the efficiency of the respiratory pump in clearing CO_2, were not affected by the treatments, even though this latter parameter was also close to achieve statistical significance (p=0.09).

Other parameters of lung mechanics were not statistically different, irrespective of the treatment (i.e. $R_{,L}$=7.54 cmH20/L/s before treatment vs 7.39 after, for the FDP group and 7.58 vs 7.03 for placebo – CL,dyn 0.09 ml/cmH20 vs 0.09 and 0.09 vs 0.09, respectively).

Concerning the clinical results, as assessed with the 6 min. walking distance and dyspnea score, no significant differences were observed.

Discussion

This pilot double-blind, placebo-controlled, randomised study was aimed to depict, in stable

Table 2. - Arterial Blood Gases (ABG) and Pulmonary Function Tests (PFTs) changes in the two groups of patients, before and after treatment

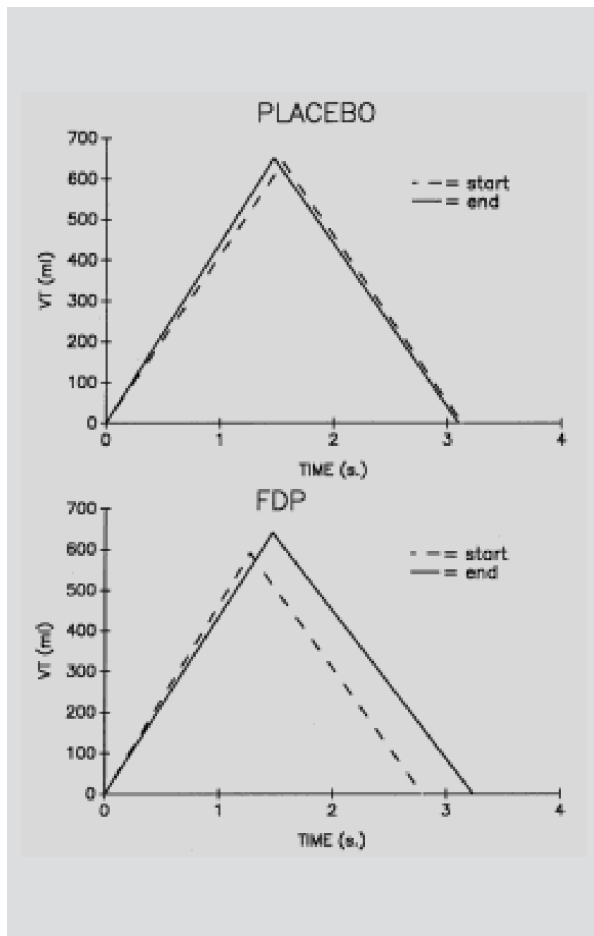| Variable	PLACEBO			FDP		
	Pre	Post	P value	Pre	Post	P value
FEV_1 (ml)	1021±533	970±464	0.66	886±206	960±278	0.49
FEV_1/FVC	41.3±10	39.6±9	0.58	39.0±9	41±5	0.75
VC (ml)	2658±1184	2515±1078	0.44	2323±389	2352±736	0.31
PaO_2 (mmHg)*	62.51±6.32	66.81±6.38	0.73	65.53±8.78	69.70±5.37	0.86
$PaCO_2$ (mmHg)*	39.99±4.77	39.41±2.76	0.84	41.79±8.06	39.95±7.44	0.45
pH*	7.42±0.03	7.40±0.4	0.36	7.43±0.06	7.42±0.03	0.77

* breathing room air

Fig. 1. - Spirogram representing the variations in breathing pattern, before and after the treatment, in the two groups of patients.

undernourished COPD patients with hypophosphatemia, the possible physiological changes on diaphragmatic function, ventilatory pattern and neuromuscular drive, induced by the administration of FDP.

Despite the fact that none of the variables analysed showed any statistically significant changes in both groups of patients, important findings were observed in the FDP group, so future clinical studies are warranted to expand our knowledge in the specific field.

We have shown that the breathing pattern is modified after 1 week of treatment, and in particular that the Ti is longer and breathing frequency lower, so that despite a significant increase in the PTPdi/b, the "total" energy expenditure of the diaphragm (PTPdi/min) was markedly reduced due to a decrease in breathing frequency. The PTPdi depends for the PTPdi/b on the time of inspiration and the tidal Pdi generated during each inspiration [15], and for the PTPdi/min, also on breathing frequency. Since Pdi swings were not affected by FDP administration, the significant increase in PTPdi/b was due to the marked increased in the contraction time of the diaphragm. This "paradoxical" rise in the diaphragm oxygen consumption per breath was clearly counterbalanced by a marked even though not statistically significant reduction in PTPdi/min, due to a decrease in breathing frequency. Interestingly enough Marchesani et al. [7] have found similar changes to ours, in breathing frequency after FDP administration (22.6 b/min before enrolment vs 20.7 after FDP), although they did not assess any physiological variables concerning the diaphragm function.

Begin and Grassino [9] showed that stable COPD patients, especially if malnourished, tend to adopt a breathing pattern characterised by a high respiratory rate and low tidal volume, the so called rapid/shallow breathing pattern.

These patients try to spare energy of respiratory muscles by decreasing their VT, keeping however their "usual" minute ventilation. As a matter of fact our patients were breathing with a Pdi/Pdimax, an index of load balance of the respiratory system similar to that described by Begin and Grassino [9].

At this point these patients have two choices if they do not want to develop alveolar hypoventilation which leads to hypercapnia. Firstly they could reduce the Pdi tidal, that is strongly dependent on the resistive and elastic workload against which they have to breathe [22]. COPD is a disease by definition "poorly reversible" to the effects of

Table 3. - Diaphragmatic function changes in the two groups of patients, before and after treatment

Variable	PLACEBO		FDP		
	Pre	Post	Pre	Post	P value
Pdi,t (cmH_2O)	7.70±2.08	7.10±1.97	6.14±2.09	6.15±1.86	0.18
PTPdi/b (cmH_2O x s)	9.29±3.44	8.29±2.28	39.0±9	41±5	0.04
PTPdi/min (cmH_2O xs/min)	157.4±24.0	155.1±24.4	155.4±43.9	141.7±30.6	0.46
Pdimax (cmH_2O)	62.38±20.1	63.82±20.8	57.46±26.1	63.26±28.1	0.31
MIP (cmH_2O)	67.13±22.6	71.29±24.6	58.18±21.8	68.6±29.0	0.12
MEP (cmH_2O)	81.88±27.7	82.53±25.1	87.58±39.3	96.±36.6	0.10
Pdi,t/Pdimax (%)	15±8	14±9	12±6	11±5	0.86
PTPdi/m $xPaCO_2$ (cmH_2Oxs/m)	6.14±1.29	5.91±1.49	6.39±1.83	5.46±1.28	0.08
TTdi	0.05±0.03	0.04±0.03	0.05±0.03	0.04±0.02	0.22
P0.1 (cmH_2O)	3.07±1.06	2.76±0.76	2.65±0.52	2.46±0.74	0.87

MIP = Maximal Inspiratory Pressure; MEP = Maximal Expiratory Pressure; Pdimax = Maximal transdiaphragmatic pressure during the sniff manoeuvre; Pdi,t = tidal transdiaphragmatic pressure; PTPdi/b = Pressure Time Product of the diaphragm for each breath; PTPdi/min = Pressure Time Product of the diaphragm per minute; Pdi/Pdimax = load/capacity index; TTdi = Tension Time Index of the diaphragm PTPdi/min x $PaCO_2$= efficiency of the respiratory pump in clearing CO_2

bronchodilators [10], so the only possible choice left is to somehow increase the maximal force generated by their respiratory muscles.

In keeping with most of the studies, we have demonstrated that P replacement was associated with an increase, close to statistical significance in both maximal inspiratory and expiratory pressure. An increase of Pdimax, MIP or MEP per se could be of poor clinical significance, since in most of the cases the absolute values of those pressure are relatively preserved [9], and they are quite far for example from the threshold of 30 cmH_2O, that is associated with possible difficulties in sustaining an unassisted breathing [5].

The administration of FDP makes our patients breathe, by the end of the trial, with a similar Pdi/Pdimax ratio compared to the enrolment, but on the other hand, since Pdimax was improved they were able to generate higher tidal Pdi, without further altering the load/balance ratio. This led to a moderate increase in tidal volume, similar to that reported [7] in a similar population (582 ml vs 629 ml), therefore minute ventilation could be maintained reducing the breathing frequency, and thus the total breathing energy expenditure, as assessed by the PTPdi/min.

The above mechanisms were also likely to responsible for the better efficiency of the respiratory pump in clearing CO_2 [16], which may be of critical importance, especially when these patients undergo an acute exacerbation of their chronic pulmonary disease.

It has been suggested that FDP administration could restore the intracellular levels of ATP and also phosphocreatine, so that it can facilitate the metabolic recovery by acting as a glycolytic intermediate [23, 24]. Since ATP is necessary for an efficient muscle contraction, the intracellular depletion of organic phosphate is very likely to be re-sponsible for the diaphragmatic weakness of these patients [6]. Indeed phosphorous depletion is also characterised by high intracellular sodium concentration with a corresponding low intracellular potassium [25].
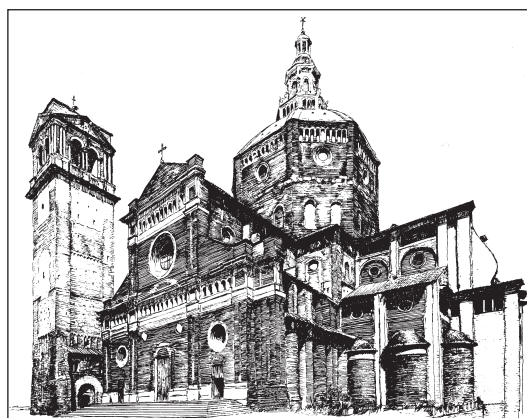
On the other hand, it has been demonstrated that FDP decreases the intracellular levels of sodium and increases those of potassium [26].

The major limitations of the study are mainly related to the lack of statistically significant results, mainly due to the small sample size that is may be not adequate to avoid β errors. This study was however designed to be a pilot, just to depict "biological signals", that may have been influenced by FDP administration, and their potential mechanisms of action. A post study analysis, based on the observed changes, calculated that a sample size of about 60 patients would allow us to achieve a statistical significance on the main variables. This is clearly not feasible to achieve, since the measurements of respiratory mechanics, is actually performed in very few Centres in the world, and indeed, the recruitment of patients may be unacceptably long, due to the invasive nature of the recordings that not all the patients would accept.

In conclusion in this pilot physiological study we have demonstrated that the administration of FDP in undernourished stable COPD patients with hypophosphatemia, is able to modify the breathing pattern. In particular the inspiratory time, was markedly increased and this induced a significant rise in the Pressure Time Product of the diaphragm per breath. However, since breathing frequency also decreased, the overall energy expenditure of the diaphragm per minute (PTPdi/min) was reduced, so that overall the diaphragmatic function improved. The increased in maximal inspiratory pressure was accompanied by a better efficiency of the respiratory pump in clearing CO_2.

References

1. Celli BR, Cote CG, Marin JM *et al.* The body-mass index, airflow obstruction, dyspnea, and exercise capacity index in chronic obstructive pulmonary disease. *N Engl J Med* 2004; 350: 1005-12.
2. Fischer J, Magid N, Kallman C *et al.* Respiratory illness and hypophosphatemia. *Chest* 1983; 83: 504-508.
3. Fiaccadori E, Coffrini E, Ronda N *et al.* Hypophosphatemia in course of chronic obstructive pulmonary disease. Prevalence, mechanisms and relationship with skeletal muscle phosphorous content. *Chest* 1990; 97: 857-868.
4. Fiaccadori E, Coffrini E, Fracchia C, Rampulla C, Montagna T, Borghetti A. Hyposphosphatemia and phosphorous depletion in respiratory and peripheral muscles of patients with respiratory failure due to COPD. *Chest* 1994; 105: 1392-1398.
5. Yang KL, Tobin MJ. A prospective study of indexes predicting the outcome of trials of weaning from mechanical ventilation. *N Engl J Med* 1991; 324: 1445-1450.
6. Aubier M, Murciano D, Leconguic Y *et al.* Effect of hypophosphatemia on diaphragmatic contractility in patients with acute respiratory failure. *N Engl J Med* 1985; 315: 420-424.
7. Marchesani F, Valerio G, Dardes N, Viglianti B, Sanguinetti CM. Effect of intravenous fructose 1, 6-diphosphate administration in malnourished chronic obstructive pulmonary disease patients with chronic respiratory failure. *Respiration* 2000; 67: 177-182.
8. Vassilakopoulos T, Zakynthinos S, Roussos C. The tension-time index and the frequency/tidal volume ratio are the major pathophysiologic determinants of weaning failure and success. *Am J Respir Crit Care Med* 1998; 158: 378-85.
9. Begin P, Grassino A. Inspiratory muscle dysfunction and chronic hypercapnia in chronic obstructive pulmonary disease. *Am Rev Resp Dis* 1991; 143: 905-911.
10. Pauwels R, Buist S, Calverley P, Jenkins C, Hurd S, on behalf of the Gold scientific committee. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease NHLBI/WHO global initiative for chronic obstructive lung disease (GOLD) workshop summary. *Am J Respir Crit Care Med* 2001; 163: 1256-1276.
11. Baydur A, Behrakis PK, Zin WA, Jaeger MJ, Milic-Emili J. A simple method for assessing the validity of the oesophageal balloon technique. *Am Rev Respir Dis* 1982; 126: 788-791.
12. Mead J, Whittenberger JL. Physical properties of human lungs measured during spontaneous respiration. *J Appl Physiol* 1953; 5: 770-796.
13. Tobin M. Monitoring respiratory mechanics in spontaneously breathing patients; in Tobin MJ (ed): Principles and Practice of Intensive Care Monitoring. New York, McGraw-Hill, 1998, pp 617-654.
14. Appendini L, Patessio A, Zanaboni S *et al.* Physiologic effects of positive end-expiratory pressure and mask pressure support during exacerbations of chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1994; 149: 1069-1076.
15. Sassoon CS, Lodia R, Rheeman CH *et al.* Inspiratory muscle work of breathing during flow-by, demand-flow, and continuous-flow systems in patients with chronic obstructive pulmonary disease. *Am Rev Respir Dis* 1992 145: 1219-1222.
16. Jubran A, Tobin MJ. Pathophysiological basis of acute respiratory distress in patients who fail a trial of weaning from mechanical ventilation. *Am J Respir Crit Care Med* 1997; 155: 906-915.
17. Laroche CM, Carroll N, Moxham J, Green M. Clinical significance of severe isolated diaphragm weakness. *Am Rev Respir Dis* 1988; 138: 862-866.
18. Black LF, Hyatt RE. Maximal respiratory pressures: normal values and relationship to age and sex. *Am Rev Respir Dis* 1969; 99: 696-702.
19. Bellemare F, Grassino A. Force reserve of the diaphragm in patients with chronic obstructive pulmonary disease. *J Appl Physiol* 1983; 55: 8-15.
20. Withelaw WA, Derenne JP, Milic-Emili J. Occlusion pressure as a measure of respiratory center output in conscious man. *Respir Physiol* 1975; 23: 181-199.
21. McGavin CR, Gupta SP, McHardy GJR. Twelve minutes walking test for assessing disability in chronic bronchitis. *BMJ* 1976; 1: 822-823.
22. Appendini L, Purro A, Patessio A *et al.* Partitioning of inspiratory muscle workload and pressure assistance in ventilator-dependent COPD patients. *Am J Respir Crit Care Med* 1996; 154: 1301-1309.
23. Hardin CD, Roberts TA. Metabolism of exogenously fructose 1, 6-biphosphate in hypoxic vascular smooth muscle. *Am J Physiol* 1994; 267: 2325-2322.
24. Juergens TM, Hardin CD. Fructose 1, 6-bisphosphate as a metabolic substrate in hog ileum smooth muscle during hypoxia. Mol Cell Biochem. 1996; 154: 83-93.
25. Montanari A, Borghi L, Curti A *et al.* Skeletal muscle cell abnormalities in acute hypophoshatemia during total parenteral nutrition. *Miner Electrolyte Metab* 1984; 10: 52-57.
26. Galzigna L. Patogenesi della componente metabolica della fatica diaframmatica e considerazioni terapeutiche. *Congress Digest* 1998; 4: 1-8.

Pavia - Piazza Duomo