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ORIGINAL RESEARCH

Fat-free mass change after nutritional rehabilitation in weight losing COPD: role of insulin, C-reactive protein and tissue hypoxia

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Background: Fat-free mass (FFM) depletion marks the imbalance between tissue protein synthesis and breakdown in chronic obstructive pulmonary disease (COPD). To date, the role of essential amino acid supplementation (EAAs) in FFM repletion has not been fully acknowledged. A pilot study was undertaken in patients attending pulmonary rehabilitation.

Methods: 28 COPD patients with dynamic weight loss > 5% over the last 6 months were randomized to receive EAAs embedded in a 12-week rehabilitation program (EAAs group n = 14), or to the same program without supplementation (C group n = 14). Primary outcome measures were changes in body weight and FFM, using dual X-ray absorptiometry (DEXA).

Results: At the 12th week, a body weight increment occurred in 92% and 15% of patients in the EAAs and C group, respectively, with an average increase of 3.8 ± 2.6 kg ($P = 0.0002$) and –0.1 ± 1.1 kg ($P = 0.81$), respectively. A FFM increment occurred in 69% and 15% of EAAs and C patients, respectively, with an average increase of 1.5 ± 2.6 kg ($P = 0.05$) and –0.1 ± 2.3 kg ($P = 0.94$), respectively. In the EAAs group, FFM change was significantly related to fasting insulin (r^2 0.68, $P < 0.0005$), C-reactive protein (C-RP) ($r^2 = 0.46$, $P < 0.01$), and oxygen extraction tension (PaO_{2x}) ($r^2 = 0.46$, $P < 0.01$) at end of treatment. These three variables were highly correlated in both groups ($r > 0.7$, $P < 0.005$ in all tests).

Conclusions: Changes in FFM promoted by EAAs are related to cellular energy and tissue oxygen availability in depleted COPD. Insulin, C-RP, and PaO_{2x} must be regarded as clinical markers of an amino acid-stimulated signaling to FFM accretion.

Keywords: COPD, pulmonary rehabilitation, branched chain amino acids, insulin, systemic inflammation

Introduction

Cachexia, defined as weight loss with disproportional fat-free mass wasting, occurs in about one-third of patients with severe chronic obstructive pulmonary disease (COPD) eligible for pulmonary rehabilitation,[1] and represents a cause of increased mortality independent of ventilatory limitation.[2]

Despite the fact that fat-free mass (FFM) depletion is recognized as a marker of the imbalance between tissue protein synthesis and breakdown in chronic wasting syndrome, there is insufficient knowledge about the determinants of the switch to catabolic intermediary metabolism during the course of the disease. Previous studies have documented a fall in muscle protein synthesis in depleted patients with emphysema,[3] and a high protein turnover in post-absorptive state in stable COPD patients.[4] Further studies[5–8] have also indicated that an elevated inflammatory state has consequences for protein need and intermediary metabolism, putting forward the

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idea that a hypoxic-mediated and cytokine-mediated wasting occurs in COPD patients, who involuntary lose weight.

These findings would entail making accretion or maintenance of FFM a rehabilitation strategy to amplify reconditioning effects of physical training and to improve physical performance in depleted COPD patients. As similar strategies are not fully acknowledged yet, future studies capable of either addressing the issue of prevention of pulmonary cachexia, or shedding light on its pathogenesis are advisable. Metabolic studies[9] have documented that high physiologic concentrations of branched chain amino acids, particularly leucine, exert a bi-directional modulation on insulin signaling pathways that promote protein synthesis and gluconeogenesis, while impairing glucose transport and glycogen synthesis. To date the idea of a cross-talk between amino acid and insulin signaling to glucose and protein metabolism remains largely speculative in depleted COPD patients. Thus, the present study has two aims. First, to evaluate the change in body weight and FFM after an essential amino acid supplementation (EAAs) intervention embedded in a rehabilitation program in weight-losing COPD patients. Second, to explore the mechanisms that modulate FFM accretion in response to ingestion of amino acids (ie, the factors that control protein turnover in tissues). We hypothesized a relationship between the supposed anabolic response to EAAs, ie, the change in FFM, and fasting insulin plasma levels, C-reactive protein (C-RP), and tissue oxygen availability assessed by oxygen extraction tension (PaO_{2x}). The latter is a noninvasive measurement, which summarizes the degree of compensation among arterial oxygen tension, blood oxygen carrying capacity and affinity.[10] In addition, the mutual interrelationships between insulin plasma levels, C-RP and PaO_{2x} were investigated before and after 12 weeks of nutrition and/or rehabilitation interventions.

Methods

Participants

Twenty-eight patients with severe COPD according to American Thoracic Society (ATS) criteria,[11] and experiencing a dynamic weight loss (>5% of body weight) over the previous 6 months were consecutively recruited at the start of an inpatient pulmonary rehabilitation program while clinically stable at the Pulmonary Rehabilitation Unit of S Maugeri Foundation I.R.C.C.S (Montescano-Italy). Exclusion criteria included: any malignancy, gastrointestinal disorders, severe endocrine disorders or recent surgery, recent (ie, within the last 4 weeks) respiratory tract infection, and abnormal fluid balance as manifested by the presence of edema or regular use of diuretics. The medical ethics committee of the Scientific Institute of Montescano approved the study design and a written informed consent was obtained prior to the study.

Pulmonary rehabilitation

All the eligible patients were randomly allocated to one of the following interventions: A) EAAs embedded in a 4-week inpatient rehabilitation program, followed by EAAs embedded in an 8-week outpatient rehabilitation program (2 sessions a day of 30 minutes unloaded bicycle training) (EAAs group), and B) a 4-week inpatient rehabilitation program, followed by an 8-week outpatient rehabilitation program (2 sessions a day of 30 minutes unloaded bicycle training) (C group). All measurements were performed during the first 5 days after admission to the hospital and in addition after 12 ± 2 weeks of intervention. The response to nutrition and/or rehabilitation interventions was evaluated as body weight change, and FFM change reached at the end of nutritional rehabilitation. The essential and nonessential amino acid mixture (Aminotrofic®, Erre Kappa Euroterapici, Milan, Italy) contains high and balanced branched chain amino acid concentrations (see Table 1). The dosage was 4 g, given twice a day, as two liquid supplements of 200 mL each. The total protein content of the supplement together with that of the consumed regular meals was more than enough to ensure the amount of protein needed for the optimal protein synthesis that is recommended for repletion of malnourished subjects (1.5 to 1.7 g protein/kg body weight/day).

Resting energy expenditure (REE) and dietary intake were assessed at baseline and end of treatment. REE was measured by an open circuit indirect calorimetry system using

Table 1 Essential amino acid composition

L-Leucine	1250 mg
L-Lysine	650 mg
L-Isoleucine	625 mg
L-Valine	625 mg
L-Threonine	350 mg
L-Cysteine	150 mg
L-Histidine	150 mg
L-Phenylalanine	100 mg
L-Methionine	50 mg
L-Tyrosine	30 mg
L-Tryptophan	20 mg
B6 vitamin	0.15 mg
B1 vitamin	0.15 mg

a ventilated hood. Measurements were started in the early morning (08:00 hours). After an overnight fasting, patients had a period of at least 30 minutes of bed rest prior to the measurements during which subjects were comfortably lying on a bed in a supine position. After stabilization, REE was recorded during a period of 30 minutes and calculated from oxygen consumption and carbon dioxide production using the abbreviated Weir formula:

$$REE\ (kcal/h) = (3.9 \times VO_2 + 1.1 \times VCO_2) \times 1.44/24.$$

Dietary intake was assessed using a 3-day food diary, by recording everything the patients ate or drank for 3 consecutive days, using household measurements to quantify items. Physical activity performed which lasted >10 minutes was also recorded. Apparent discrepancies were checked with the subject. The information was coded for computer nutrient analysis by the same trained dietician. The nutrient database was derived from the IEO food composition database (BDA).[12] At end of treatment, a dietician reviewed the food diary to ascertain maintenance of actual content and total caloric value.

The inpatient rehabilitation program consisted of general physical training, with particular attention to exercise in relation to daily activities.[13] Five days a week a combined endurance and strength exercise training was followed, consisting of 30 minutes of submaximal cycle ergometry, and 30 minutes of treadmill walking, and one session of unsupported arm exercise training. The submaximal cycle ergometry was set at 50% of peak workload and increased during the training progression under the supervision of an exercise therapist. The outpatient program was a 5-day week, self-reported home program.

Body composition

Body height and weight were measured to calculate body mass index. Possible disturbances in body composition were assessed by dual-energy X-ray absorptiometry (DEXA), which is a direct method of assessing bone mineral content and the soft tissue surrounding the bone: FFM and fat mass (FM). Each patient lying in supine position on a scan table for approximately 15 minutes was scanned by DPX-L Bone Densitometry (Lunar Radiation Corp., Madison, WI, USA). Total and subregional FM and FFM were derived according to the computer algorithm (Lunar software version 1.3; Madison, WI, USA) provided by the manufacturer. The FFM index (FFMI) was calculated as FFM to height-squared ratio. According to previous studies[14] a low FFMI was classified as a FFMI $\leq$ 15 kg/m^2 (women) and $\leq$ 16 kg/m^2 (men).

Lung function, and performance status

Lung function testing included spirometry FEV_1 and FVC, thoracic gas volumes, and carbon monoxide (CO) transfer factor (TL_{CO}), (Masterlab; Jaeger, Wurzurg, Germany). Predicted equations utilized were those of Quanjer et al.[15] Arterial blood samples were gently drawn from the brachial artery using a dedicated pre-heparinized blood sampler, while patients were seated and breathing room air. Oxygen breathing was temporarily withdrawn for at least 30 minutes in patients under oxygen therapy. Immediately after sample collection, mixing, and removal of the first drops of blood, the acid-base and oxygen status were analyzed. Arterial negative logarithm of hydrogen ion concentration (pH) and arterial carbon dioxide tensions ($PaCO_2$), together with arterial oxygen tension (PaO_2) and the oximetry parameters were measured using a blood gas analyzer (ABL Model 700 System Radiometer, Copenhagen, Denmark). The following oximetry parameters were directly measured: 1) concentration of total hemoglobin in the blood (tHb), which includes all types of hemoglobin ie, oxy- (O_2Hb), deoxy- (HHb), carboxy- (COHb), and meta-hemoglobin (MetHb); 2) arterial oxygen saturation (sO_2) (ie, the ratio between the concentration of O_2Hb and HHb + O_2Hb); 3) fraction of oxyhemoglobin (FO_2Hb) (ie, the ratio between the concentration of O_2Hb and tHb); 4) fraction of carboxyhemoglobin (FCOHb) (ie, the ratio between concentration of COHb and tHb); 5) fraction of metahemoglobin (FMetHb) (ie, the ratio between concentration of MetHb and tHb); 6) total arterial oxygen content (ctO_2) (ie, the sum of the concentration of hemoglobin-bound oxygen and the concentration of physically dissolved oxygen). The concentration of effective hemoglobin (ceHb), ie, the concentration of total hemoglobin (ctHb) corrected for fractions of carboxyhemoglobin (FCOHb) and metahemoglobin (FMetHb), which are unable to bind oxygen reversibly, was calculated as a measurement of hemoglobin oxygen binding capacity. Both oxygen tension at half saturation (50%) of blood (P_{50}), and oxygen extraction tension of arterial blood (PaO_{2x}), were calculated by extrapolation along the shape of the oxygen binding curve on the log PaO_2-ctO_2 diagram, by a dedicated computer program.[16] The shape of the curve was modeled on the basis of the PaO_2 and the hemoglobin oxygen saturation fraction measured in the arterial blood, using a hyperbolic tangent function.[10] Performance status of the patients was assessed by body-mass index (B), the degree of airflow obstruction (O) and dyspnea (D), and exercise capacity (E) (BODE index).[17]

Blood sampling

Fasting blood samples were collected, early in the morning (08:00 to 10:00 hours). Plasma glucose concentration was determined by glucose oxidative methods (Dimension clinical chemistry system, Dade Behring), using a glucose auto analyzer. Plasma insulin (95% intra-assay coefficient of variation $\leq$ 29.1 μUI/mL) was determined by commercial radio-immuno assay (Immulite 2000, Siemens). C-reactive protein (CRP) was measured in duplicate by high sensitivity turbidimetric immuno-assay (PETIA) (Dade Behring), with a lower detection limit of 0.5 mg/L.

Statistical analysis

Comparisons between groups were carried out by one-factor ANOVA or Chi-square test if the examined variable was, respectively, continuous or categorical. To test for the effect of EAA supplementation on body weight and FFM, we used a two-factor ANOVA (factors: treatment and time, with repeated measurements in the latter), adjusting for sex. Univariable and multivariable linear regression analysis was used to assess the association between insulin, C-RP blood level and PaO_{2x}, (the explanatory variables) and the change in FFM after nutritional intervention (the response variable). Multivariable model building was carried out by a forward selection procedure with 0.50 significance level for entry of variables into the model (Proc. Reg, SAS/STAT–SAS Institute Inc., Cary, NC, USA). Person's correlation coefficient was used to assess the association between continuous variables. All tests were two-tailed and a $P < 0.05$ was considered statistically significant. Descriptive statistics are given as mean (±SD) or as a percentage value for continuous, and respectively categorical variables.

Results

Figure 1 details the flow diagram for patients' allocation to the intervention groups.

The majority of patients (96%) were on maintenance medication consisting of inhaled β_2-agonists, and inhaled corticosteroids therapy; none of them took systemic steroids at the time of the study.

Baseline anthropometrics, smoking habitus, performance status and lung function data in the EAAs and C groups are reported in Table 2. The two groups were comparable for age, smoking habit and performance status as assessed

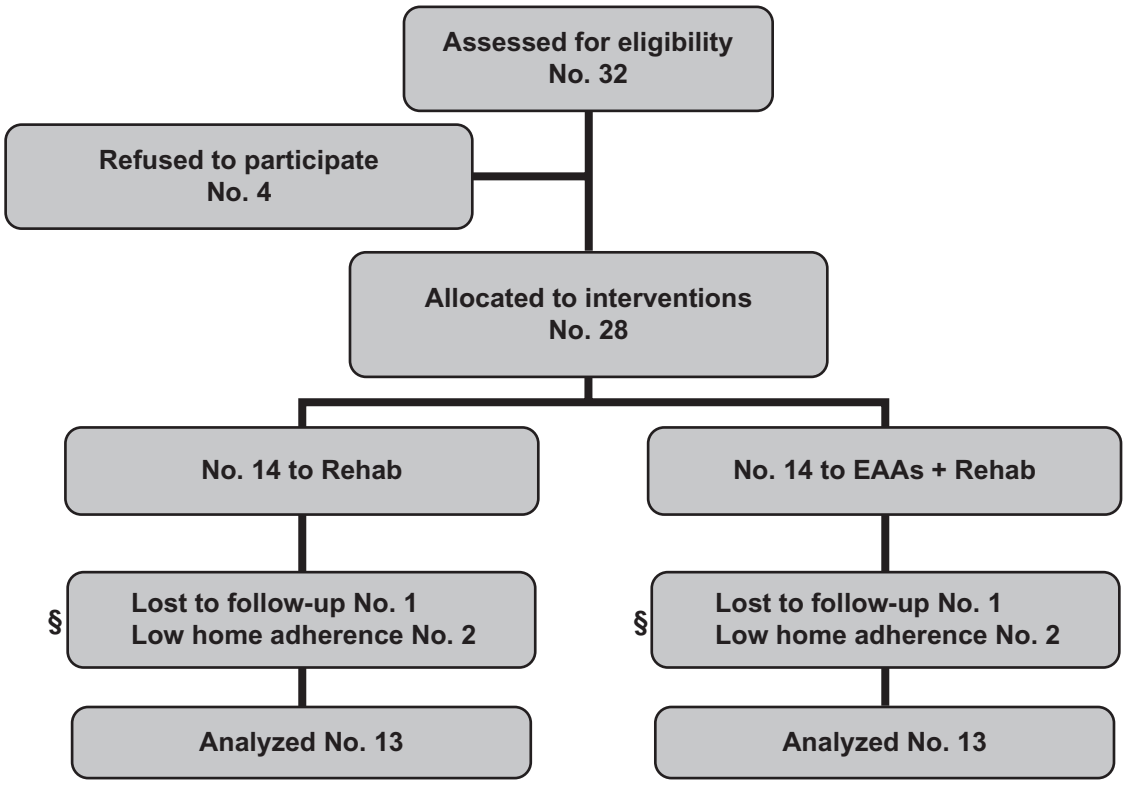

Figure 1 The flow diagram for patients' allocation to EAAs + rehabilitation and rehabilitation interventions.
[§]Number of patients who did not exercise on a regular basis and/or spontaneously reduced the dose of EAA to 4 g daily.
Abbreviation: EAAs, essential amino acid supplementation.

by the four domains of the BODE index, and pulmonary functional impairment. A higher number of females were allocated to the C group. The frequency of patients with long term oxygen therapy was evenly distributed between the groups.

Table 3 reports baseline body composition, dietary intake, resting energy expenditure, oxygen status of arterial blood, and laboratory parameters in both groups. All these variables were comparable in the two groups. In both groups weight loss in the previous 6 months occurred due to hypermetabolism, despite a normal baseline dietary intake, and resulted in FFM depletion in 77% of patients. The average C-RP mg/L was increased over normal values, while insulin and glucose plasma levels measured in the fasting state were within the normal range in all the patients. In addition, the serum concentrations of creatinine within the normal range indicated a normal kidney function in all the patients.

At the 12th week, the change in dietary intake was 34 ± 92 kcal/24 h in the EAAs group, and 43 ± 87 kcal/24 h in the C group ($P = 0.80$); change in resting energy expenditure as a percentage of Harris-Benedict prediction equation, was –19% ± 17% in the EAAs group, and –12% ± 15% in the C group ($P = 0.28$). The change in insulin, C-RP and PaO_{2x} was, respectively, –0.9 ± 4.4 μUI/mL ($P = 0.49$), 0.2 ± 3.7 mg/L ($P = 0.83$) and –0.3 ± 2.7 mmHg (0.73) in the EAAs group. In the C groups these changes were, respectively, –0.6 ± 2.1 μUI/mL ($P = 0.33$), 1.0 ± 8.7 mg/L ($P = 0.71$) and 0.9 ± 2.7 mmHg ($P = 0.26$). The change in glucose blood level was –0.7 ± 12.7 mmol/L ($P = 0.85$) and 4.1 ± 16.4 mmol/L ($P = 0.39$) in the EAAs and C group, respectively.

A body weight increment occurred in 12 out of 13 patients (92%) in the EAAs group, and in 2 out of 13 (15%) in the C group. Body weight increased by 3.8 ± 2.6 kg in the EAAs group ($P = 0.0002$) and –0.1 ± 1.1 kg in the C group ($P = 0.81$). The interaction test between the treatment and time factors ($P = 0.0001$) further supported the significance of the different response in the two groups. An FFM increment occurred in 9 out of 13 patients (69%) in the EAAs group, and in 2 out of 13 (15%) in the C group. FFM increased by 1.5 ± 2.6 kg in the EAAs group ($P = 0.05$) and –0.1 ± 2.3 kg in the C group ($P = 0.94$). The interaction test between the treatment and time factors was borderline significant ($P = 0.05$).

In order to explore the mechanisms that modulate FFM accretion in response to ingestion of amino acids, we assessed the association between the change in FFM and 12th-week insulin plasma level, C-RP, and PaO_{2x}, in the EAAs group.

Table 2 Baseline characteristics of the 28 weight-losing patients with COPD: anthropometrics, smoking habitus, oxygen therapy, performance status and lung function data

Characteristics		EAAs group		C group		*P* value
Started/Completed	No		14/13		14/13	
Age	years	73.1	(6.0)	70.1	(5.8)	0.29
Gender	M/F	12	2	8	6	†0.09
Smokers	Current/Ex	10	4	9	5	†0.32
Pack-years	No	71.9	(28.5)	47.4	(29.8)	0.12
LTOT	Y/N	3	11	3	11	0.25
BMI	kg/m^2	19.9	(2.8)	21.0	(3.8)	0.35
FEV_1	% pred.	41.3	(14.1)	43.6	(14.42)	0.89
MMRC-D	points	3.2	(0.8)	3.1	(0.7)	0.69
6MWD	m	224.4	(153.9)	259.5	(120.5)	0.51
BODE	points	6.6	(2.4)	6.2	(1.9)	0.82
VC	% pred	70.7	(13.2)	79.7	(8.7)	0.09
FEV_1/VC	%	40.2	(14.1)	47.3	(13.8)	0.89
TLC	% pred	119	(10.8)	110.8	(23.4)	0.70
RV	% pred	165.4	(29.3)	178.4	(60.1)	0.35
TL_{CO}	% pred	40.5	(14.1)	43.3	(16.9)	0.63

Note: Baseline statistics are given as means and (SD). †by χ^2 test.

Abbreviations: BMI, body mass, index; BODE, degree of airflow obstruction, dyspnea and exercise capacity index; COPD, chronic obstructive pulmonary disease; Pack-years, no. cigarettes/day/20 years of smoking; LTOT, long-term oxygen therapy; FEV_1, forced expiratory volume in one second; MMRC-D, scores on the Modified Medical Research Council dyspnea scale; 6MWD, distance walked in 6 minutes; VC, slow vital capacity; FEV_1/VC, Tiffeneau index; TLC, total lung capacity; RV residual volume; TL_{CO}, CO transfer factor.

Table 3 Baseline characteristics in 28 weight-losing patients with COPD: body composition, dietary intake, resting energy expenditure, oxygen status, and laboratory parameters*

Characteristics		EAAS group		C group		*P* value
Started/Completed	No		14/13		14/13	
Body weight	kg	55.7	(8.4)	57.8	(9.0)	0.59
FFM	kg	42.7	(6.1)	39.5	(4.8)	0.24
FM	kg	11.8	(6.6)	14.8	(6.1)	0.36
FFMI	kg/m^2	15.1	(2.0)	15.3	(2.0)	0.89
Dietary intake	kcal/24 h	1897	(406)	1816	(308)	0.57
REE	kcal/24 h	1319	(305)	1282	(229)	0.73
REE_HB	% pred	146	(23)	137	(15)	0.25
$PaCO_2$	mmHg	41.5	(6.9)	42.4	(6.7)	0.56
PaO_2	mmHg	61.5	(9.8)	58.3	(6.8)	0.46
P_{50}	mmHg	24.4	(4.2)	23.6	(1.9)	0.60
PaO_{2x}	mmHg	31.0	(2.7)	30.2	(2.4)	0.44
tHb	g/dL	13.7	(1.9)	14.2	(2.2)	0.86
Fasting glucose	mmol/L	5.0	(0.6)	4.9	(0.7)	0.83
Fasting insulin	μUI/ml	5.5	(4.7)	5.2	(4.8)	0.89
Albumin	g/dL	3.4	(0.7)	3.5	(0.5)	0.62
Pre-albumin	mg/dL	21.9	(5.6)	26.6	(6.2)	0.09
Creatinine	mg/dL	0.9	(0.2)	0.9	(0.1)	0.59
C-RP	mg/L	9.6	(7.1)	12.0	(7.2)	0.30

Note: Values are given as means and (SD).
Abbreviations: FFM, fat-free mass; FM, fat mass; FFMI, fat free mass index; REE, resting energy expenditure; REE_HB, resting energy expenditure as a percentage of Harris-Benedict prediction equation; $PaCO_2$, arterial carbon dioxide tension; PaO_2, arterial oxygen tension; tHb, total hemoglobin; P_{50}, oxygen half saturation tension; PaO_{2x}, oxygen extraction tension; C-RP, C-reactive protein.

Univariable linear regression results are shown in Figure 2. To investigate a possible direct effect of glucose blood level on FFM change, we also included in this figure a plot showing the relationship between change in FFM and 12th-week glucose level. A substantial linear association was found between the change in FFM and insulin plasma levels ($r^2 = 0.68$, $P < 0.001$ – Figure 2, panel A), while a moderate association was found between the change in FFM and both C-RP ($r^2 = 0.46$, $P < 0.01$ – Figure 2, panel B) and PaO_{2x} ($r^2 = -0.46$, $P < 0.01$ – Figure 2, panel C). On the contrary, there was no association change in FFM and fasting glucose plasma levels ($r^2 = 0.04$, $P = 0.50$ – Figure 2, panel D). As expected, insulin, C-RP, and PaO_{2x} at the 12th week were mutually highly correlated ($r > 0.7$, $P < 0.005$ in all tests) (see Table 4).

The multivariable linear regression model included insulin and C-RP, with p values 0.019 and 0.45, respectively. This model explained 70% of the variance of the change in FFM after the rehabilitation program with amino acid supplementation. These results suggest that the predictive information of C-RP is already "contained" in that of the insulin variable, and that PaO_{2x} does not provide any additive information to this two-variable model.

In the C group there was no significant association between change in FFM and 12th week insulin (r^2: 0.0003, $P = 0.95$ – Figure 3, panel A), C-RP (r^2: 0.10, $P = 0.39$ – Figure 3, panel B), and PaO_{2x} (r^2: 0.06, $P = 0.42$ – Figure 3, panel C). Furthermore, no association was found between change in FFM and fasting glucose plasma levels ($r^2 = 0.004$, $P = 0.98$ – Figure 3, panel D). As found in the EAAs group, insulin, C-RP and PaO_{2x} at the 12th week were mutually highly correlated ($r > 0.77$, $P < 0.005$ in all tests), (see Table 4).

Discussion

Results of this study suggest the potential for EAAs associated with physical training to halt or even reverse lean body mass depletion in hypoxemic COPD patients, who experience dynamic weight loss. Furthermore, the study provides several novel insights on the pathophysiological mechanisms that modulate FFM accretion in this disease condition.

First, the positive association between 12th-week FFM change and insulin plasma levels suggests a specific modulation of insulin actions by the amino acid mixture on signaling to protein metabolism in weight losing COPD patients. Second, the significant association between FFM change

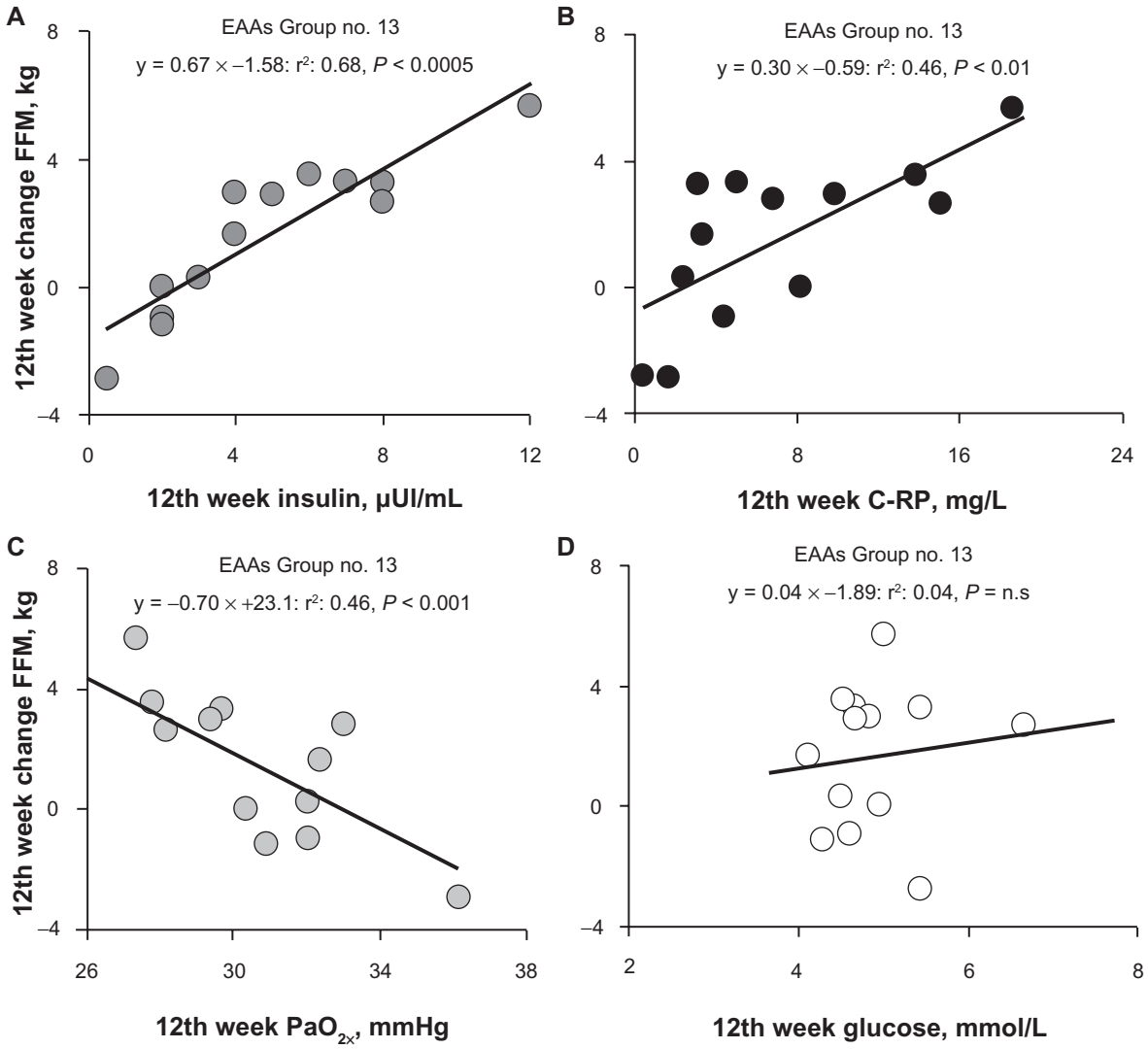

Figure 2 Panel A: 12th-week change FFM, as assessed by DEXA, plotted against 12th-week insulin plasma levels, µUl/mL. Panel B: 12th-week change FFM, plotted against 12th-week C-RP, mg/L. Panel C: 12th-week change FFM, plotted against 12th-week PaO_{2x}, mmHg in 13 patients allocated to EAAs group. Panel D: 12th-week change FFM, plotted against 12th-week glucose plasma levels, mmol/L.

Abbreviations: C-RP, C-reactive protein; DEXA, dual X-ray absorptiometry EAAs, essential amino acid supplementation; FFM, fat-free mass; PaO_{2x}, oxygen extraction tension.

Table 4 Correlations between insulin, C-RP, and PaO_{2x} in EAAs and C groups

Group		Baseline measurements			Group
		Insulin	**C-RP**	**PaO_{2x}**	
E	Insulin		0.12 (0.73)	–0.39 (0.22)	C
A	C-RP	0.03 (0.92)		0.56 (0.08)	O
A	PaO_{2x}	–0.34 (0.27)	–0.31 (0.32)		N
S					T
		12th-week measurements			
E	Insulin		0.77 (<0.005)	–0.87 (<0.0005)	C
A	C-RP	0.71 (<0.01)		–0.89 (<0.0001)	O
A	PaO_{2x}	–0.80 (<0.001)	–0.79 (<0.005)		N
S					T

Note: Values are correlation coefficients and *P* values given in parentheses.

Abbreviations: C-RP, C-reactive protein; PaO_{2x}, oxygen extraction tension; EAAs, essential amino acid supplementation.

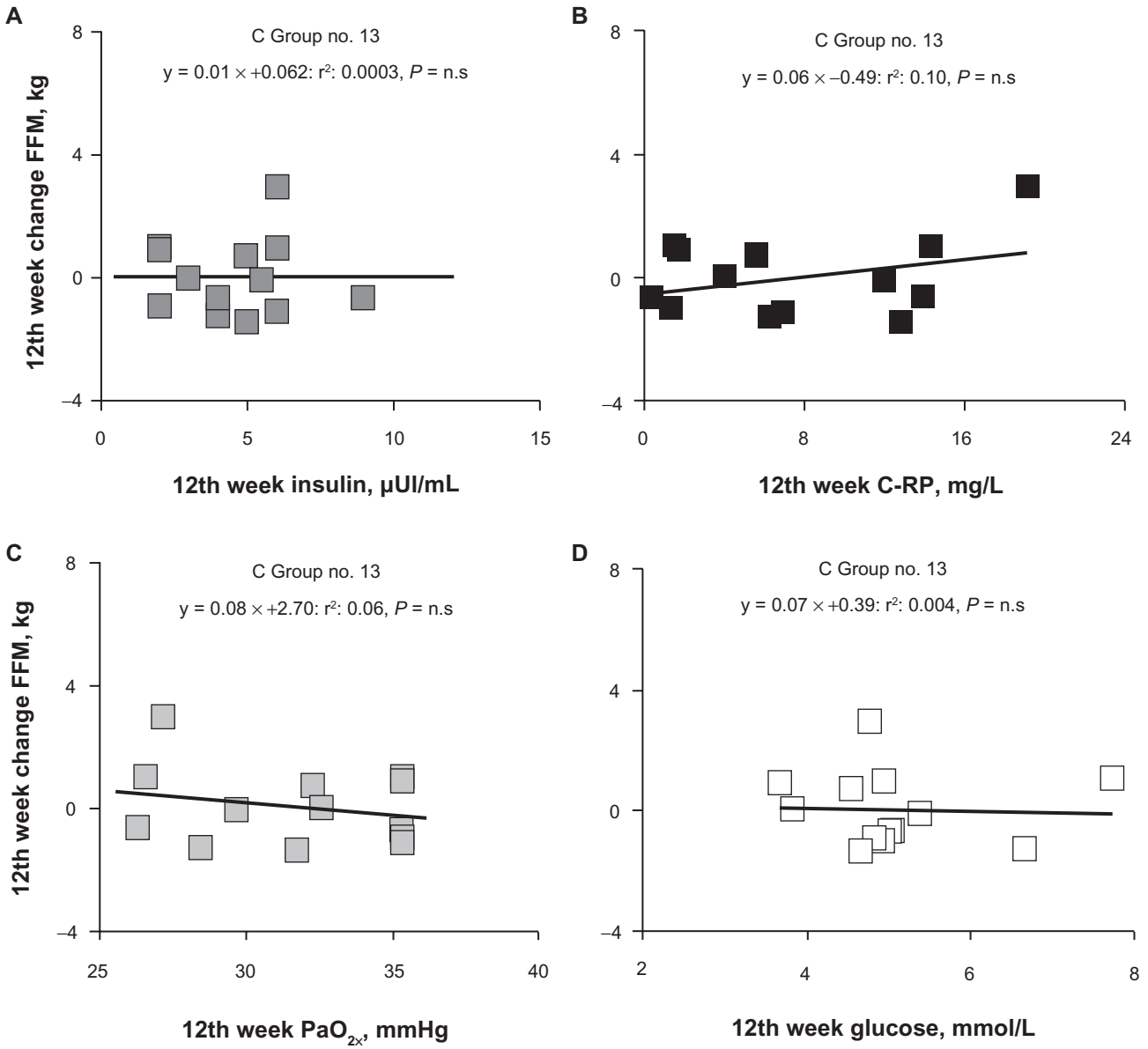

Figure 3 Panel A: 12th-week change FFM, as assessed by DEXA, plotted against 12th-week insulin plasma levels, µUl/mL. Panel B: 12th-week change FFM, plotted against 12th-week C-RP, mg/L. Panel C: 12th-week change FFM, plotted against 12th-week PaO_{2x}, mmHg 13 patients allocated to C Group. Panel D: 12th-week change FFM, plotted against 12th-week glucose plasma levels, mmol/L.
$P > 0.38$ in all tests.
Abbreviations: DEXA, dual X-ray absorptiometry EAAs, essential amino acid supplementation; FFM, fat-free mass; PaO_{2x}, oxygen extraction tension.

and C-RP blood levels allows some speculation on the involvement of C-RP synthesis pathway in the scenario of downregulation of insulin actions by the amino acid mixture on signaling to glucose metabolism. Third, the mutual relationship between tissue oxygen availability, insulin plasma levels, and C-RP sheds light on the interaction between tissue hypoxia, energy metabolism, and substrate utilization after rehabilitation interventions.

The anabolic effect on FFM after nutritional rehabilitation is consistent with previous metabolic studies on ingestion of proteins, or infusion of mixed amino acids conducted in normal human subjects,[18,19] and normal-weight COPD patients.[20] While increased availability of supplemented amino acids or increased cellular level of ATP resulting from their metabolism is supposed to account for this anabolic effect, the notion that branched chain amino acids specifically induce protein synthesis suggests a direct action on signaling to several metabolic pathways.

Several studies have consistently documented lower plasma concentration of branched chain amino acids and reduction in glutamate status in COPD patients compared to control subjects.[5,7,8,21,22] These differences have been

specifically associated with weight loss and decreased muscle mass, and respectively with reduced muscle glutathione levels, and enhanced glycolysis during ischemic and hypoxic conditions.[8,23] To date however, any cross-talk between amino acid and insulin signals as determinant of the switch to FFM accretion remains largely speculative in depleted patients with COPD.

Reportedly, high physiologic concentrations of branched-chain amino acids, particularly leucine, activate intermediates important in the initiation of protein synthesis, including p70 S6 kinase and PHAS-I, in synergy with insulin. Concurrently, amino acids inhibit early steps of insulin action critical for glucose transport and inhibition of gluconeogenesis, including decreased insulin-stimulated tyrosine phosphorylation of IRS-1 and IRS-2, and a marked inhibition of insulin-stimulated phosphatidylinositol 3-kinase.[9] The significant relationship between 12th-week FFM change and 12th-week insulin plasma levels in this study extends this hypothesis to COPD patients, and provides us with a clinical model to explore the amino acid accretion into proteins in relation to insulin signaling pathways. The clinical rehabilitation setting of the study prevents the ability of using sophisticated techniques to ascertain to which extent glucose uptake mechanisms (ie, membrane transport and phosphorylation), and their metabolic regulation may contribute to the amino acid anabolic effect. Nevertheless, the finding that change in FFM bears a strong relationship to insulin plasma levels, and no relationship to glucose plasma levels, further supports the hypothesis of a direct association between insulin and amino acid accretion into proteins. These results seem to be in line with experimental evidence obtained in the ovine fetus that insulin promotes amino acid utilization and oxidation, independent of its simultaneous effects on glucose metabolism.[24]

At first, we were puzzled by the observation that higher FFM gain was associated with higher C-RP blood levels; however, the notion that branched chain amino acids down-regulate insulin actions on glucose metabolism in vivo, specifically blunting glycogen synthesis as well as stimulating gluconeogenesis, provides us with a line of reasoning to explain our findings. The association between CRP and IL-6 release induced by exercising muscle is well established.[25,26] Furthermore, several lines of evidence raise the intriguing possibility that muscle-derived IL-6 has a hormone-like glucoregulatory role, signaling critical low levels of glycogen content,[27] and stimulating hepatic glucose output. Looking at the correlation between 12th-week C-RP and 12th-week changes in FFM reported in Figure 2B, one may speculate that increased C-RP blood levels in patients showing higher gain of FFM is a marker of IL-6 overproduction in response to impaired insulin action on signaling to glycogen synthesis induced by amino acid supplementation. Alternatively, C-RP may directly contribute to impairment of insulin signaling pathways that promote glucose transport and glycogen synthesis,[28] while stimulating endogenous glucose production.[29] Along this line of reasoning, wasting of lean body mass, commonly present in patients with COPD, must be regarded as a complex process involving changes in the control of glucose, as well as of intermediary metabolism that is modulated by nutritional signaling through a variety of different pathways. Among these, hypoxia seems to play a pivotal role in the regulation of metabolic demand of lean body mass.[8,23] The mutual association between insulin plasma levels, C-RP, and PaO_{2x} suggests an interplay between tissue oxygen availability and sensing and signaling mechanisms to substrate utilization after nutritional rehabilitation.

Limitations of the study

While measuring the change of FFM by DEXA, a well validated technique for evaluation of body composition,[30,31] we would rather with this study unravel pathophysiological mechanisms elicited by EAAs under condition of wasting than specifically address the issue of response to nutritional supplementation in COPD. Nevertheless, the prospective changes of body weight and FFM we obtained are far beyond the reported within-day variability of whole body weight.[32] In comparison to pretrial information provided by randomized controlled trials about the improvements of anthropometric measurements by any caloric supplementation lasting >2 weeks[33] this result is clinically meaningful. However, there are some limitations to this study.

First, it must be stressed that the number of patients allocated to each group was too low to rely on for definitive conclusions about the potential benefit of EAAs. Thus, our preliminary results should be interpreted with caution, as the small number of patients studied may have emphasized the positive effect of treatment on body weight and FFM. Second, the assessor was not blinded to patient allocation, which could have led to bias in evaluating the effect of treatment. The higher number of females in the C group although not statistically significant likely represents a randomization bias. Third, it was apparent that about 14% of the participants had some difficulties in adhering to the home-based nutrition-rehabilitation intervention. Thus, support, motivation, and a realistic request for commitment should to be considered for future recruitment. Last, although the inability to weigh the

role of EAAs independent of standardized metabolic load of training may be viewed as a confounding factor in the evaluation of potential benefits of the study, it was unethical to withhold pulmonary rehabilitation in patients who had been proved to benefit of this treatment option.

In summary, the potential for supplemented circulating amino acids in the regulation of insulin mediated signaling to protein and glucose metabolism reconciles the bedside with bench investigations. These results provide us with a rationale for use of EAAs as a valuable adjunct to exercise training programs aimed at stabilizing or even reversing the negative effects of lean body mass wasting in COPD patients.

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Disclosures

The authors report no conflict of interest in this work.

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