

Serum leptin and tumor necrosis factor - alpha levels and maximal exercise performance in patients with chronic obstructive pulmonary disease

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ABSTRACT: Systemic inflammation plays an important role in skeletal muscle dysfunction in patients with Chronic Obstructive Pulmonary Disease (COPD). The aim of this study is to determine whether serum leptin and tumor necrosis factor - alpha (TNF- α) levels, as markers of systemic inflammation, affect the maximal exercise performance of COPD patients.

Methods: Thirty male COPD patients underwent pulmonary function and progressive exercise testing for maximal exercise and for $V_{O_2\max}$ determination. Fat Free Mass (FFM), Fat Mass (FM) and Thigh Muscle Area (TMA) were estimated by measuring skinfold thickness. Serum leptin and TNF- α levels were determined in morning blood samples.

Results: Significant correlations were found between serum leptin levels and Body Mass Index (BMI) ($r = 0.421$, $p < 0.02$), FM ($r = 0.551$, $p < 0.01$) and TNF- α ($r = 0.521$, $p < 0.001$). Exercise performance, expressed as $V_{O_2\max}$, correlated significantly with % FEV₁ ($r = 0.563$, $p < 0.001$), BMI ($r = 0.636$, $p < 0.001$), FFM ($r = 0.415$, $p < 0.02$), TMA ($r = 0.651$, $p < 0.001$), but not with serum leptin or TNF- α levels. By stepwise regression analysis TMA appeared to be a significant predictor of $V_{O_2\max}$ in COPD patients.

Conclusion: Serum leptin and TNF- α levels were poor predictors of maximal exercise capacity ($V_{O_2\max}$) in COPD patients. The best predictors of $V_{O_2\max}$ during exercise were airflow limitation (%FEV₁) and thigh muscle mass expressed by TMA.

Key Words: Maximal exercise performance, COPD, Leptin, TNF- α .

INTRODUCTION

Exercise limitation is a common complaint of patients with chronic obstructive pulmonary disease (COPD) and deteriorates their quality of life^{1,2,3}. Exercise limitation has traditionally been attributed to the increased work of breathing and the dynamic hyperinflation that result from airflow limitation⁴. Recent studies have provided evidence that COPD is a systematic disease that is associated with extrapulmonary abnormalities, such as weight loss, skeletal muscle dysfunction and signs of systemic inflammation⁵. The systemic effects of COPD are important clinical features and contribute significantly to the aggravation of exercise capacity and health status^{5,6}.

Leptin, a protein mainly secreted by adipocytes, plays an important role in body weight regulation. Increased levels of circulating leptin may contribute to anorexia and weight loss in many pathologic conditions including COPD⁷. Recent studies have provided evidence for a link between leptin and proinflammatory cytokines, as TNF- α . In animals, the administration of TNF- α or interleukin 1 (IL-1) - inflammatory cytokines with anorectic effects- resulted in a dose dependent upregulation of leptin mRNA in fat cells and an increase in circulating leptin concentrations⁸. In stable patients with emphysema leptin was found to be positively related to plasma TNF- α receptor 55⁹.

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Circulating leptin and TNF- α levels correlated with BMI and % fat in COPD patients¹⁰. In malnourished COPD patients, there was an inverse relationship between changes in body weight after 8 weeks of nutritional therapy and leptin plasma concentrations⁹. The nutritional parameters that express muscle mass, as FFM, were reported to be associated with exercise performance in COPD patients^{11,12}.

Extrapulmonary abnormalities, like systemic inflammation, weight loss and muscle dysfunction, determined the exercise capacity of COPD patients and were related with leptin and TNF- α levels. The aim of the present study is to examine whether the limitation of the exercise capacity of these patients can be related to the circulating serum leptin and TNF- α levels.

MATERIAL AND METHODS

Thirty male patients with moderate to severe COPD, defined by a FEV₁/FVC of less than 0.50, were evaluated. Patients with other potential causes of weight loss such as diabetes, thyroid dysfunction, malabsorption syndromes, alcoholism and neoplastic diseases were excluded. Patients with edema forming states, i.e. renal, liver or cardiac diseases, were also excluded in order to eliminate the effect of edema in the interpretation of body weight. At the time of the study, all the patients were stable and all received optimal medical therapy including a low dose of methylprednisolone (4-8 mg every other day) for 1-2 months (mean duration 37 \pm 12 days). Corticosteroid treatment was installed in our COPD patients during last exacerbation and tapered in small doses. None of our subjects were in long term high doses oral corticosteroid treatment. The study was approved by the Ethics Committee of the G Papanikolaou Hospital (University Hospital) of Thessaloniki. All patients gave written informed consent before inclusion.

Spirometry was performed with a computerized spirometer (Jaeger) and Forced Expiratory Volume in one second (FEV₁), Forced Vital Capacity (FVC) and the ratio FEV₁/FVC (FEV₁%) were calculated according to the ATS recommendations¹³. The results were expressed as absolute values and as percent of the predicted values.

Body height (h) was determined to the nearest 0.5 cm (Lameris WM 715, Breukelen) with the patients

standing barefoot. Body weight (Bw) was measured with a beam scale to the nearest 0.1 kg (SELA, FR-G) with subjects barefoot and with only underwear clothing. Body Mass Index (BMI) was calculated from body weight and height ($BMI = Bw/h^2$, kg/m²).

The measurement of skinfold thickness at four sites (triceps, biceps, subcapular, suprailiac) was used to assess fat mass. Total fat mass (FM) was estimated using tables and free fat mass (FFM) was obtained by the subtraction of FM from Bw¹⁴. Forceps skinfold thickness (FST) and mid-thigh circumference (MTC) were used to calculate the mid-thigh muscle circumference (MTMC). Finally, thigh muscle area (TMA) was calculated ($TMA = MTMC^2/4\pi$)¹⁵.

Exercise testing was done on a cycle ergometer (cardiopulmonary Diagnostic System Med Graphics), using the standard 1-min incremental cycle exercise protocol. Patients began with a 2-min period of unloaded pedaling at 60 cycles/min, followed by 10 Watt increments per minute. The patients were strongly encouraged to cycle to the point of discomfort or exhaustion or until an abnormal electrocardiogram (ECG) was noted (symptom-limited exercise tests). Heart Rate (HR) and cardiac rhythm were monitored with a three-lead ECG and blood pressure was measured with auscultation.

Minute ventilation and its components were measured with a pneumotachograph. The concentrations of expired O₂ and CO₂ were analyzed breath by breath with a zirconium dioxide-cell O₂ analyzer and an infrared CO₂ analyzer respectively. These measurements and the pneumotachograph flow signals were integrated electronically by a computerized system to yield 10-s averages of minute ventilation (V_E), tidal volume (V_T), respiratory rate (RR), oxygen uptake (V_{O₂}), carbon dioxide output (V_{CO₂}) and gas exchange ratio (R). HR was also measured and was used to obtain the HR reserve (HRmax/predHRmax %) and O₂ pulse (V_{O₂}/HR). Maximal O₂ pulse (O₂Pmax) was measured at the point at which O₂ pulse reached a plateau or patients were exhausted. Predicted Maximal Voluntary Ventilation (MVV) was expressed as $MVV = FEV_1 \times 35$, in units of L/min, and predicted maximal HR (HRmax) was calculated as $210 - (age \times 0.65)$ ¹⁵. Arterial hemoglobin oxygen saturation (Sa_{O₂}) was monitored non invasively with a pulse oximeter.

Table 1. Study population: anthropometric and spirometric data (mean \pm SE).

Age, years	68.7 \pm 1.06
Weight, kg	71.5 \pm 2.74
BMI, kg/m ²	26.1 \pm 0.95
FM, kg	20.9 \pm 1.53
FFM, kg	50.8 \pm 1.60
MTMC, cm	44.4 \pm 1.97
TMA, cm ²	121.7 \pm 8.39
FVC, L	2.18 \pm 0.10
FEV ₁ , L/sec	1.02 \pm 0.05
% FEV ₁	43.1 \pm 1.84
TLC, %pred	92.1 \pm 3.96

BMI: body mass index, FM: fat mass, FFM: fat free mass, MTMC: mid-thigh muscle circumference, TMA: thigh muscle area, FVC: Forced Vital Capacity, FEV₁: Forced Expiratory Volume in one second, TLC: Total Lung Capacity.

For leptin levels determination blood samples were drawn between 8-9 a.m. after fasting the previous night. The samples were stored at -70°C until analysis. Serum leptin levels were determined using a competitive enzyme immunoassay (Accucyte, Maryland USA). TNF-a levels were determined in serum using Pelikine Compact human TNF-a ELISA kits (CLB, Amsterdam). The specificity of the ELISA kit is 0-1000 pg/mL for TNF-a. In 10 normal subjects (age 63 \pm 5y) the serum leptin levels were 3.67 \pm 0.7 ng/mL and TNF-a levels were 3.11 \pm 0.45 pg/mL.

STATISTICAL ANALYSIS

Values obtained were expressed as mean \pm SD. Pearson's correlation coefficients were calculated among V_{O₂max} or maximal exercise capacity (in watts) and different metabolic and exercise parameters. Stepwise multiple regression analysis was performed to determine the best predictors of V_{O₂max}.

RESULTS

Anthropometric and lung function data of our 30 COPD patients (mean age 68,7 \pm 1.06 years) presented in Table 1. TNF-a levels were 9.58 \pm 0.56 pg/mL (range 5.65-16.7 pg/mL) and serum leptin concentration was 9.37 \pm 1.7ng/mL (range 1.17-37.4 ng/mL), increased in relation to our normal values.

There was a significant correlation between serum TNF-a and leptin levels ($r = 0.521$, $p < 0.001$). Also there were significant correlations between serum leptin levels, BMI ($r = 0.421$, $p < 0.02$) and FM ($r = 0.551$, $p < 0.01$), but no correlations were found between serum leptin levels and other nutritional parameters such as FFM, MTMC or TMA. Also no correlations were found between nutritional parameters like BMI, FM, FFM, MTMC, TMA and serum TNF-a levels.

The exercise capacity, the ventilatory and circulatory parameters during maximal exercise of the COPD patients of the study are presented in table 2. Patients with COPD achieved a work capacity (Wmax) of 42.6 \pm 5.43 watts (30.2 \pm 3.43% of predicted normal values). V_Emax was 31.3 \pm 2.05 L/min, MVV was 35.98 \pm 2.48 L/min and V_Emax/MVV was 95.8 \pm 3.87. V_{O₂max} was decreased (786 \pm 57mL/min). Maximal heart rate (HRmax) was 121.7 \pm 5 beats/min (85.7 \pm 2.3% of predicted). The oxygen pulse (V_{O₂max}/HRmax) was decreased during maximal exercise (6.47 \pm 1.17 mL/beat).

The correlations between exercise capacity (Wmax or V_{O₂max}) and ventilatory, circulatory and nutritional parameters are presented in table 3. The exercise capacity correlated significantly with %FEV₁ and oxy-

Table 2. Exercise capacity, ventilatory and circulatory parameters (mean \pm SE).

Wmax, Watts	42.57 \pm 5.43
Wmax, %pred	30.20 \pm 3.43
V _{O₂} max, mL/min	786 \pm 57
V _E max, L/min	31.30 \pm 2.05
V _E max/MVV, %	95.80 \pm 3.87
HRmax, b/min	121.70 \pm 5.00
HRmax/HRpred, %	85.7 \pm 2.30
V _{O₂} max/HRmax, mL	6.47 \pm 1.17
MVV, L/min	35.98 \pm 2.48

Wmax: work capacity, V_{O₂}max: maximal oxygen uptake, V_Emax: maximal ventilation, HRmax: maximal heart rate, V_{O₂}max/HRmax: maximal oxygen pulse, MVV: Maximal Voluntary Ventilation.

Table 3. Correlation coefficients (r) between exercise capacity (Wmax, V_{O₂}max), ventilatory, circulatory, nutritional parameters, leptin and TNF-a levels.

Work capacity	% FEV ₁	V _E max/MVV (%)	HRmax/pred (%)	V _{O₂} /HR mL	BMI Kg/m ²	FFM kg	TMA cm ²	Leptin ng/mL	TNF-a pg/mL
Wmax, watts	0.435 p<0.05	0.232 NS	0.093 NS	0.775 p<0.001	0.578 p<0.001	0.295 NS	0.623 p<0.001	-0.013 NS	0.068 NS
V _{O₂} max, mL/min	0.563 p<0.01	0.245 NS	0.09 NS	0.917 p<0.001	0.636 p<0.001	0.415 p<0.02	0.651 p<0.001	0.208 NS	0.250 NS

Wmax: work capacity, MVV: Maximal Voluntary Ventilation, V_{O₂}max: maximal oxygen uptake, V_Emax: maximal ventilation, HRmax: maximal heart rate, V_{O₂}max/HRmax: maximal oxygen pulse, BMI: body mass index, FFM: fat free mass, TMA: thigh muscle area.

gen pulse (V_{O₂}/HR) but not with V_Emax/MVV or HRmax. The exercise capacity correlated significantly better with BMI and TMA than with FFM. We found no correlations between leptin and TNF-a serum levels and exercise capacity of the COPD patients of the study.

Stepwise multiple regression analysis was performed to determine the best predictors for V_{O₂}max. It demonstrated that % FEV₁ and TMA were significant determinants of V_{O₂}max. In contrast serum leptin and TNF-a were not selected as significant predictors of V_{O₂}max. According to the above V_{O₂}max was expressed as:

$$V_{O_2\max} = 0.329 \% FEV_1 + 0.495 TMA + 211 \quad (R^2 = 0.520)$$

DISCUSSION

COPD patients stop exercise when either dyspnea or leg effort reach a severe intensity. Dyspnea-limited exercise is more frequent in patients with severe airflow limitation and V_{O₂}max is a more objective index of work during maximal exercise than dyspnea or leg effort sensation. It is open to question whether maximal ventilatory and circulatory capacities are actually reached during exercise.

In the present study the work capacity was significantly decreased in patients with severe airflow limitation. In the COPD patients of the current study the work capacity (expressed as Wmax or V_{O₂}max) related to the flow limitation (%FEV₁), but not to the dyspnoeic index (V_Emax/MVV) (table 3). Also the work capac-

ity related with the oxygen pulse ($V_{O_2\max}/HR_{\max}$), but not with the maximal heart rate ($HR_{\max}/HR_{\max\text{ pred}}$).

The absence of statistically significant correlations between exercise capacity and ventilatory ($V_{E_{\max}}/MVV$) or circulatory ($HR_{\max}/HR_{\max\text{ pred}}$) parameters in the present study suggested that most subjects stopped exercise at submaximal heart rate and ventilation. Possibly the symptoms rather than the physiologic factors limited the work capacity¹⁶. Hence it was reasonable to expect that the average patient would stop exercise when dyspnoea (mainly related to flow limitation) or the intensity of symptoms of leg effort were maximal.

Weight loss commonly occurs in patients with COPD and it is not only an epiphenomenon of the severity of the disease but also an independent death risk factor. Weight loss and muscle wasting are factors exerting several adverse effects on physical performance and respiratory muscle function, independently of the degree of airflow obstruction¹⁷⁻¹⁹. In this study patients with COPD and low BMI had diminished $V_{O_{2\max}}$ during exercise.

FFM that represents muscles mass is associated with respiratory muscles strength²⁰ and exercise performance¹¹. The patients of the study with the higher FFM had an increased $V_{O_{2\max}}$ during exercise ($r = 0.415$, $p < 0.02$). Total FFM was a significant determinant of $V_{O_{2\max}}$ in patients with COPD²¹.

The distribution of muscle mass wasting is a factor that has to be elucidated, since muscle strength is closely related to muscle mass in patients with COPD²¹. FFM of legs is a significant predictor of maximal exercise performance in patients with moderate airflow limitation²². TMA of the patients of this study reflected the muscle mass of the legs and correlated better with $V_{O_{2\max}}$ compared with FFM.

Body composition analysis by dual energy X-ray absorptiometry in COPD patients with severe airflow limitation demonstrated disproportional leg muscle wasting²¹. The distribution of muscle wasting found in COPD patients was not related to the atrophy induced by malnutrition. In malnutrition the loss of upper-limb function was equal or greater than that of the lower limbs²³. In contrast in patients with severe COPD a disproportional leg muscle wasting was found. The leg muscle wasting could be attributed to the limita-

tion of the daily activity of COPD patients and also to the limited respiratory reserve^{22,23}.

Systemic inflammation plays an important role in skeletal muscles dysfunction in COPD patients. The systemic inflammation is a characteristic feature of COPD and is demonstrated by the increased numbers of neutrophils, macrophages and T-lymphocytes, as well as by the elevated concentrations of pro-inflammatory cytokines and by the oxidative stress. In clinically stable patients, but especially during the exacerbations of the disease, increased levels of cytokines, such TNF- α and its receptors (TNFR-55, TNFR-75), interleukin -6 and -8 (IL-6, IL-8), as well as elevated levels of acute phase proteins, like C-reactive protein (CRP) and liposaccharide-binding protein (LBP) were found^{24,25}. Submaximal exercise induced an abnormal increase in plasma TNF- α and IL-6 in patients with COPD [26]. Serum TNF- α was increased in the COPD patients of the current study and also TNF- α serum levels at rest correlated statistically significantly with serum leptin concentration. Inflammatory cytokines, as TNF- α or IL-1, when administrated to animals resulted in an increase in circulating leptin concentration⁸.

Reports concerning the effect of oral corticosteroids use on leptin are contradictory. Corticosteroids in therapeutic doses were found to have a stimulating effect on leptin concentrations via the induction of insulin resistance, as glucose and insulin can induce leptin expression²⁷. On the other hand it was demonstrated that the acute administration of corticosteroids had no effects on insulin, free fatty acids or leptin concentrations compared with placebo, while prolonged administration significantly increased the fasting concentration of insulin, but not that of glucose free fatty acids and leptin²⁸. Also in stable COPD patients no effect of oral corticosteroids on serum leptin was found⁹. During an acute exacerbation of COPD the serum leptin concentration could be (in part) a result of high corticosteroid treatment, since the course of plasma leptin related with the scheme of prednisolone administration⁷. We consider that the low prolonged dose of oral methylprednisolone in the patients of the current study had insignificant effect on serum leptin. Leptin concentration in the patients of the study correlated statistically significantly with metabolic indices as BMI and FFM, but no statistically significant rela-

tion was demonstrated between serum leptin or TNF- α levels and exercise capacity.

Stepwise multiple regression analysis was performed to determine the best combination of predictors for $V_{O_{2max}}$. Only TMA and %FEV₁ appeared to be significant determinants of $V_{O_{2max}}$. Total variance in this model for $V_{O_{2max}}$ was 52%. In contrast FFM, serum levels of leptin and TNF- α were not selected as significant predictors of $V_{O_{2max}}$.

The conclusion of the present study is that serum leptin and TNF- α levels, as markers of systemic inflammation in COPD patients, are related to metabolic indices (like BMI, FM), but are very poor predictors of maximal exercise capacity (W_{max} or $V_{O_{2max}}$) in these patients. The best predictors of $V_{O_{2max}}$ are airflow limitation (%FEV₁) and leg muscle mass as expressed by TMA.

Τα επίπεδα της λεπτίνης και TNF- α και η μέγιστη ικανότητα για άσκηση σε ασθενείς με Χρόνια Αποφρακτική Πνευμονοπάθεια

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ΠΕΡΙΛΗΨΗ: Η συστηματική φλεγμονή συμμετέχει στη δυσλειτουργία των σκελετικών μυών των ασθενών με Χρόνια Αποφρακτική Πνευμονοπάθεια (ΧΑΠ). Σκοπός της μελέτης είναι να αξιολογηθεί εάν τα επίπεδα της λεπτίνης και του TNF- α του ορού, ως δείκτες φλεγμονής, επηρεάζουν τη μέγιστη ικανότητα άσκησης των ασθενών με ΧΑΠ. Μετρήθηκαν ο δείκτης μάζας σώματος (Body Mass Index, BMI), η λιπώδης μάζα (Fat Mass, FM), η ελεύθερη λίπους μάζα σώματος (Fat Free Mass, FFM), η επιφάνεια μυϊκής μάζας μηρού (Thigh Mass Area, TMA) και η μέγιστη κατανάλωση O₂ (VO_{2max}) 30 ασθενών με ΧΑΠ. Επίσης προσδιορίστηκαν τα επίπεδα λεπτίνης και TNF- α των παραπάνω ασθενών και έγινε λειτουργικός έλεγχος των πνευμόνων τους. Σημαντική συσχέτιση βρέθηκε μεταξύ των επιπέδων λεπτίνης, BMI ($r = 0.421$, $p < 0.02$), FM ($r = 0.551$, $p < 0.01$) και TNF- α ($r = 0.521$, $p < 0.001$). Η VO_{2max} σχετιζόταν σημαντικά με τον % FEV₁ ($r = 0.563$, $p < 0.001$), το BMI ($r = 0.636$, $p < 0.001$), τη FFM ($r = 0.415$, $p < 0.02$) και την TMA ($r = 0.651$, $p < 0.001$). Συμπερασματικά διαπιστώθηκε πως τα επίπεδα λεπτίνης και του TNF- α αποτελούσαν φτωχούς προγνωστικούς δείκτες της VO_{2max}. Αντίθετα τους καλύτερους προγνωστικούς δείκτες της VO_{2max} αποτελούσαν η απόφραξη (FEV₁%) και η μυϊκή μάζα του μηρού (TMA).

Λέξεις Κλειδιά: Μέγιστη ικανότητα άσκησης, ΧΑΠ, Λεπτίνη, TNF- α .

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Body height (h) was determined to the nearest 0.5 cm (Lameris WM 715, Breukelen) with the patients

standing barefoot. Body weight (Bw) was measured with a beam scale to the nearest 0.1 kg (SELA, FR-G) with subjects barefoot and with only underwear clothing. Body Mass Index (BMI) was calculated from body weight and height (BMI = Bw/h², kg/m²).

The measurement of skinfold thickness at four sites (triceps, biceps, subcapular, suprailiac) was used to assess fat mass. Total fat mass (FM) was estimated using tables and free fat mass (FFM) was obtained by the subtraction of FM from Bw¹⁴. Forceps skinfold thickness (FST) and mid-thigh circumference (MTC) were used to calculate the mid-thigh muscle circumference (MTMC). Finally, thigh muscle area (TMA) was calculated (TMA = MTMC²/4 π)¹⁵.

Exercise testing was done on a cycle ergometer (cardiopulmonary Diagnostic System Med Graphics), using the standard 1-min incremental cycle exercise protocol. Patients began with a 2-min period of unloaded pedaling at 60 cycles/min, followed by 10 Watt increments per minute. The patients were strongly encouraged to cycle to the point of discomfort or exhaustion or until an abnormal electrocardiogram (ECG) was noted (symptom-limited exercise tests). Heart Rate (HR) and cardiac rhythm were monitored with a three-lead ECG and blood pressure was measured with auscultation.

Minute ventilation and its components were measured with a pneumotachograph. The concentrations of expired O₂ and CO₂ were analyzed breath by breath with a zirconium dioxide-cell O₂ analyzer and an infrared CO₂ analyzer respectively. These measurements and the pneumotachograph flow signals were integrated electronically by a computerized system to yield 10-s averages of minute ventilation (V_E), tidal volume (V_T), respiratory rate (RR), oxygen uptake (V_{O₂}), carbon dioxide output (V_{CO₂}) and gas exchange ratio (R). HR was also measured and was used to obtain the HR reserve (HRmax/predHRmax %) and O₂ pulse (V_{O₂}/HR). Maximal O₂ pulse (O₂Pmax) was measured at the point at which O₂ pulse reached a plateau or patients were exhausted. Predicted Maximal Voluntary Ventilation (MVV) was expressed as MVV = FEV₁ x 35, in units of L/min, and predicted maximal HR (HRmax) was calculated as 210 - (age x 0.65)¹⁵. Arterial hemoglobin oxygen saturation (Sa_{O₂}) was monitored non invasively with a pulse oximeter.

Table 1. Study population: anthropometric and spirometric data (mean \pm SE).

Age, years	68.7 \pm 1.06
Weight, kg	71.5 \pm 2.74
BMI, kg/m ²	26.1 \pm 0.95
FM, kg	20.9 \pm 1.53
FFM, kg	50.8 \pm 1.60
MTMC, cm	44.4 \pm 1.97
TMA, cm ²	121.7 \pm 8.39
FVC, L	2.18 \pm 0.10
FEV ₁ , L/sec	1.02 \pm 0.05
% FEV ₁	43.1 \pm 1.84
TLC, %pred	92.1 \pm 3.96

BMI: body mass index, FM: fat mass, FFM: fat free mass, MTMC: mid-thigh muscle circumference, TMA: thigh muscle area, FVC: Forced Vital Capacity, FEV₁: Forced Expiratory Volume in one second, TLC: Total Lung Capacity.

For leptin levels determination blood samples were drawn between 8-9 a.m. after fasting the previous night. The samples were stored at -70°C until analysis. Serum leptin levels were determined using a competitive enzyme immunoassay (Accucyte, Maryland USA). TNF-a levels were determined in serum using Pelikine Compact human TNF-a ELISA kits (CLB, Amsterdam). The specificity of the ELISA kit is 0-1000 pg/mL for TNF-a. In 10 normal subjects (age 63 \pm 5y) the serum leptin levels were 3.67 \pm 0.7 ng/mL and TNF-a levels were 3.11 \pm 0.45 pg/mL.

STATISTICAL ANALYSIS

Values obtained were expressed as mean \pm SD. Pearson's correlation coefficients were calculated among V_{O₂max} or maximal exercise capacity (in watts) and different metabolic and exercise parameters. Stepwise multiple regression analysis was performed to determine the best predictors of V_{O₂max}.

RESULTS

Anthropometric and lung function data of our 30 COPD patients (mean age 68,7 \pm 1.06 years) presented in Table 1. TNF-a levels were 9.58 \pm 0.56 pg/mL (range 5.65-16.7 pg/mL) and serum leptin concentration was 9.37 \pm 1.7ng/mL (range 1.17-37.4 ng/mL), increased in relation to our normal values.

There was a significant correlation between serum TNF-a and leptin levels ($r = 0.521$, $p < 0.001$). Also there were significant correlations between serum leptin levels, BMI ($r = 0.421$, $p < 0.02$) and FM ($r = 0.551$, $p < 0.01$), but no correlations were found between serum leptin levels and other nutritional parameters such as FFM, MTMC or TMA. Also no correlations were found between nutritional parameters like BMI, FM, FFM, MTMC, TMA and serum TNF-a levels.

The exercise capacity, the ventilatory and circulatory parameters during maximal exercise of the COPD patients of the study are presented in table 2. Patients with COPD achieved a work capacity (Wmax) of 42.6 \pm 5.43 watts (30.2 \pm 3.43% of predicted normal values). V_Emax was 31.3 \pm 2.05 L/min, MVV was 35.98 \pm 2.48 L/min and V_Emax/MVV was 95.8 \pm 3.87. V_{O₂max} was decreased (786 \pm 57mL/min). Maximal heart rate (HRmax) was 121.7 \pm 5 beats/min (85.7 \pm 2.3% of predicted). The oxygen pulse (V_{O₂max}/HRmax) was decreased during maximal exercise (6.47 \pm 1.17 mL/beat).

The correlations between exercise capacity (Wmax or V_{O₂max}) and ventilatory, circulatory and nutritional parameters are presented in table 3. The exercise capacity correlated significantly with %FEV₁ and oxy-

Table 2. Exercise capacity, ventilatory and circulatory parameters (mean \pm SE).

Wmax, Watts	42.57 \pm 5.43
Wmax, %pred	30.20 \pm 3.43
V _{O₂} max, mL/min	786 \pm 57
V _E max, L/min	31.30 \pm 2.05
V _E max/MVV, %	95.80 \pm 3.87
HRmax, b/min	121.70 \pm 5.00
HRmax/ HRpred, %	85.7 \pm 2.30
V _{O₂} max/ HRmax, mL	6.47 \pm 1.17
MVV, L/min	35.98 \pm 2.48

Wmax: work capacity, V_{O₂}max: maximal oxygen uptake, V_Emax: maximal ventilation, HRmax: maximal heart rate, V_{O₂}max/HRmax: maximal oxygen pulse, MVV: Maximal Voluntary Ventilation.

Table 3. Correlation coefficients (r) between exercise capacity (Wmax, V_{O₂}max), ventilatory, circulatory, nutritional parameters, leptin and TNF-a levels.

Work capacity	% FEV ₁	V _E max/MVV (%)	HRmax/pred (%)	V _{O₂} /HR mL	BMI Kg/m ²	FFM kg	TMA cm ²	Leptin ng/mL	TNF-a pg/mL
Wmax, watts	0.435 p<0.05	0.232 NS	0.093 NS	0.775 p<0.001	0.578 p<0.001	0.295 NS	0.623 p<0.001	-0.013 NS	0.068 NS
V _{O₂} max, mL/min	0.563 p<0.01	0.245 NS	0.09 NS	0.917 p<0.001	0.636 p<0.001	0.415 p<0.02	0.651 p<0.001	0.208 NS	0.250 NS

Wmax: work capacity, MVV: Maximal Voluntary Ventilation, V_{O₂}max: maximal oxygen uptake, V_Emax: maximal ventilation, HRmax: maximal heart rate, V_{O₂}max/HRmax: maximal oxygen pulse, BMI: body mass index, FFM: fat free mass, TMA: thigh muscle area.

gen pulse (V_{O₂}/HR) but not with V_Emax/MVV or HRmax. The exercise capacity correlated significantly better with BMI and TMA than with FFM. We found no correlations between leptin and TNF-a serum levels and exercise capacity of the COPD patients of the study.

Stepwise multiple regression analysis was performed to determine the best predictors for V_{O₂}max. It demonstrated that % FEV₁ and TMA were significant determinants of V_{O₂}max. In contrast serum leptin and TNF-a were not selected as significant predictors of V_{O₂}max. According to the above V_{O₂}max was expressed as:

$$V_{O_2\max} = 0.329 \% FEV_1 + 0.495 TMA + 211 \quad (R^2 = 0.520)$$

DISCUSSION

COPD patients stop exercise when either dyspnea or leg effort reach a severe intensity. Dyspnea-limited exercise is more frequent in patients with severe airflow limitation and V_{O₂}max is a more objective index of work during maximal exercise than dyspnea or leg effort sensation. It is open to question whether maximal ventilatory and circulatory capacities are actually reached during exercise.

In the present study the work capacity was significantly decreased in patients with severe airflow limitation. In the COPD patients of the current study the work capacity (expressed as Wmax or V_{O₂}max) related to the flow limitation (%FEV₁), but not to the dyspnoeic index (V_Emax/MVV) (table 3). Also the work capac-

ity related with the oxygen pulse ($V_{O_2\max}/HR_{\max}$), but not with the maximal heart rate ($HR_{\max}/HR_{\max\text{ pred}}$).

The absence of statistically significant correlations between exercise capacity and ventilatory ($V_{E_{\max}}/MVV$) or circulatory ($HR_{\max}/HR_{\max\text{ pred}}$) parameters in the present study suggested that most subjects stopped exercise at submaximal heart rate and ventilation. Possibly the symptoms rather than the physiologic factors limited the work capacity¹⁶. Hence it was reasonable to expect that the average patient would stop exercise when dyspnoea (mainly related to flow limitation) or the intensity of symptoms of leg effort were maximal.

Weight loss commonly occurs in patients with COPD and it is not only an epiphenomenon of the severity of the disease but also an independent death risk factor. Weight loss and muscle wasting are factors exerting several adverse effects on physical performance and respiratory muscle function, independently of the degree of airflow obstruction¹⁷⁻¹⁹. In this study patients with COPD and low BMI had diminished $V_{O_{2\max}}$ during exercise.

FFM that represents muscles mass is associated with respiratory muscles strength²⁰ and exercise performance¹¹. The patients of the study with the higher FFM had an increased $V_{O_{2\max}}$ during exercise ($r = 0.415$, $p < 0.02$). Total FFM was a significant determinant of $V_{O_{2\max}}$ in patients with COPD²¹.

The distribution of muscle mass wasting is a factor that has to be elucidated, since muscle strength is closely related to muscle mass in patients with COPD²¹. FFM of legs is a significant predictor of maximal exercise performance in patients with moderate airflow limitation²². TMA of the patients of this study reflected the muscle mass of the legs and correlated better with $V_{O_{2\max}}$, compared with FFM.

Body composition analysis by dual energy X-ray absorptiometry in COPD patients with severe airflow limitation demonstrated disproportional leg muscle wasting²¹. The distribution of muscle wasting found in COPD patients was not related to the atrophy induced by malnutrition. In malnutrition the loss of upper-limb function was equal or greater than that of the lower limbs²³. In contrast in patients with severe COPD a disproportional leg muscle wasting was found. The leg muscle wasting could be attributed to the limita-

tion of the daily activity of COPD patients and also to the limited respiratory reserve^{22,23}.

Systemic inflammation plays an important role in skeletal muscles dysfunction in COPD patients. The systemic inflammation is a characteristic feature of COPD and is demonstrated by the increased numbers of neutrophils, macrophages and T-lymphocytes, as well as by the elevated concentrations of pro-inflammatory cytokines and by the oxidative stress. In clinically stable patients, but especially during the exacerbations of the disease, increased levels of cytokines, such TNF- α and its receptors (TNFR-55, TNFR-75), interleukin -6 and -8 (IL-6, IL-8), as well as elevated levels of acute phase proteins, like C-reactive protein (CRP) and liposaccharide-binding protein (LBP) were found^{24,25}. Submaximal exercise induced an abnormal increase in plasma TNF- α and IL-6 in patients with COPD [26]. Serum TNF- α was increased in the COPD patients of the current study and also TNF- α serum levels at rest correlated statistically significantly with serum leptin concentration. Inflammatory cytokines, as TNF- α or IL-1, when administrated to animals resulted in an increase in circulating leptin concentration⁸.

Reports concerning the effect of oral corticosteroids use on leptin are contradictory. Corticosteroids in therapeutic doses were found to have a stimulating effect on leptin concentrations via the induction of insulin resistance, as glucose and insulin can induce leptin expression²⁷. On the other hand it was demonstrated that the acute administration of corticosteroids had no effects on insulin, free fatty acids or leptin concentrations compared with placebo, while prolonged administration significantly increased the fasting concentration of insulin, but not that of glucose free fatty acids and leptin²⁸. Also in stable COPD patients no effect of oral corticosteroids on serum leptin was found⁹. During an acute exacerbation of COPD the serum leptin concentration could be (in part) a result of high corticosteroid treatment, since the course of plasma leptin related with the scheme of prednisolone administration⁷. We consider that the low prolonged dose of oral methylprednisolone in the patients of the current study had insignificant effect on serum leptin. Leptin concentration in the patients of the study correlated statistically significantly with metabolic indices as BMI and FFM, but no statistically significant rela-

tion was demonstrated between serum leptin or TNF- α levels and exercise capacity.

Stepwise multiple regression analysis was performed to determine the best combination of predictors for $V_{O_{2max}}$. Only TMA and %FEV₁ appeared to be significant determinants of $V_{O_{2max}}$. Total variance in this model for $V_{O_{2max}}$ was 52%. In contrast FFM, serum levels of leptin and TNF- α were not selected as significant predictors of $V_{O_{2max}}$.

The conclusion of the present study is that serum leptin and TNF- α levels, as markers of systemic inflammation in COPD patients, are related to metabolic indices (like BMI, FM), but are very poor predictors of maximal exercise capacity (W_{max} or $V_{O_{2max}}$) in these patients. The best predictors of $V_{O_{2max}}$ are air-flow limitation (%FEV₁) and leg muscle mass as expressed by TMA.

Τα επίπεδα της λεπτίνης και TNF- α και η μέγιστη ικανότητα για άσκηση σε ασθενείς με Χρόνια Αποφρακτική Πνευμονοπάθεια

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ΠΕΡΙΛΗΨΗ: Η συστηματική φλεγμονή συμμετέχει στη δυσλειτουργία των σκελετικών μυών των ασθενών με Χρόνια Αποφρακτική Πνευμονοπάθεια (ΧΑΠ). Σκοπός της μελέτης είναι να αξιολογηθεί εάν τα επίπεδα της λεπτίνης και του TNF- α του ορού, ως δείκτες φλεγμονής, επηρεάζουν τη μέγιστη ικανότητα άσκησης των ασθενών με ΧΑΠ. Μετρήθηκαν ο δείκτης μάζας σώματος (Body Mass Index, BMI), η λιπώδης μάζα (Fat Mass, FM), η ελεύθερη λίπους μάζα σώματος (Fat Free Mass, FFM), η επιφάνεια μυϊκής μάζας μηρού (Thigh Mass Area, TMA) και η μέγιστη κατανάλωση O₂ (VO_{2max}) 30 ασθενών με ΧΑΠ. Επίσης προσδιορίστηκαν τα επίπεδα λεπτίνης και TNF- α των παραπάνω ασθενών και έγινε λειτουργικός έλεγχος των πνευμόνων τους. Σημαντική συσχέτιση βρέθηκε μεταξύ των επιπέδων λεπτίνης, BMI ($r = 0.421$, $p < 0.02$), FM ($r = 0.551$, $p < 0.01$) και TNF- α ($r = 0.521$, $p < 0.001$). Η VO_{2max} σχετιζόταν σημαντικά με τον % FEV₁ ($r = 0.563$, $p < 0.001$), το BMI ($r = 0.636$, $p < 0.001$), τη FFM ($r = 0.415$, $p < 0.02$) και την TMA ($r = 0.651$, $p < 0.001$). Συμπερασματικά διαπιστώθηκε πως τα επίπεδα λεπτίνης και του TNF- α αποτελούσαν φτωχούς προγνωστικούς δείκτες της VO_{2max}. Αντίθετα τους καλύτερους προγνωστικούς δείκτες της VO_{2max} αποτελούσαν η απόφραξη (FEV₁%) και η μυϊκή μάζα του μηρού (TMA).

Λέξεις Κλειδιά: Μέγιστη ικανότητα άσκησης, ΧΑΠ, Λεπτίνη, TNF- α .

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