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Elevated CRP levels mark metabolic and functional impairment in advanced COPD

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ABSTRACT

Background: C-reactive protein (CRP) is often used as clinical marker of acute systemic inflammation. Since low-grade inflammation is evident in chronic diseases like chronic obstructive pulmonary disease (COPD), new methods have been developed to enhance sensitivity of CRP assays in the lower range. We aimed to investigate the discriminative value of high sensitivity CRP in COPD with respect to markers of local and systemic impairment, disability and handicap.
Methods: Plasma CRP (by high-sensitivity particle-enhanced immunonephelometry), interleukin-6 (IL-6) (by ELISA), body composition (by bio-electrical impedance analysis), resting energy expenditure (REE: by ventilated hood), exercise capacity (6-minute walking distance, maximal and submaximal bicycle ergometry test), health status (by SGRQ) and lung function were determined in 102 clinically stable COPD patients (GOLD stage II-IV). The cut-off point of normal vs. elevated CRP level was 4.21 mg/l.
Results: CRP was elevated in 48 of 102 patients. In these patients, IL-6 ($p<0.001$) and REE (adjusted for fat-free mass) ($p=0.002$) were higher, while maximal ($p=0.040$) and submaximal exercise capacity ($p=0.017$) as well as 6-minute walking distance ($p=0.014$) were lower. The symptom score of the SGRQ ($p=0.003$) was lower in patients with elevated CRP as were postbronchodilator FEV_1 ($p=0.031$) and reversibility ($p=0.001$). Regression analysis also showed that, adjusted for FEV_1, age and gender, CRP was a significant predictor for body mass index ($p=0.044$) and fat mass index ($p=0.016$)
Conclusions: High sensitivity CRP is a marker for impaired energy metabolism, functional capacity and distress due to respiratory symptoms in COPD.

INTRODUCTION

Increasing evidence points towards Chronic Obstructive Pulmonary Disease (COPD) as a multi-organ systemic disease. Skeletal muscle weakness and wasting and impaired exercise performance have been well described as frequently occurring symptoms in advanced COPD. These features are poorly related to severity of the airflow limitation but appear to be linked to a systemic inflammatory response.[1] As reviewed by Gan et al, several systemic inflammatory mediators, like tumour necrosis factor-α (TNF-α), some interleukins (ILs), acute phase proteins (APP: C-reactive protein (CRP), fibrinogen, lipopolysaccharide binding protein (LBP)) and leukocytes, are increased in COPD.[2] One of the markers of systemic inflammation that is consistently shown to be slightly increased in COPD patients compared to healthy controls is CRP (reviewed in [2]). The prevalence of increased CRP in COPD has been examined in the Third National Health and Nutrition Examination Survey (NHANES III), which showed that 41% of patients with moderate COPD (FEV_1 ≥ 50%pred to 80%pred) had a CRP above 3 mg/l and 6% above 10 mg/l, while as much as 52% of patients with severe COPD (FEV_1 <50%pred) had CRP above 3 mg/l and 23% above 10 mg/l.[3]

COPD patients with slightly elevated CRP levels have not yet been characterized nor have there been studies to explore a potential role of CRP as marker for local or systemic impairments. The aim of the present study was to investigate whether an increased concentration of high sensitivity (HS) CRP is related to the degree of lung function impairment, systemic inflammation, body composition, exercise capacity, energy metabolism and quality of life in patients with advanced COPD.

MATERIALS AND METHODS

Subjects

A group of patients with clinically stable COPD, consecutively admitted to an inpatient pulmonary rehabilitation centre (Asthma Centre Hornerheide, Horn, The Netherlands), was included if they met the criteria for COPD of the American Thoracic Society (ATS).[4] Patients were excluded if suffering from concurrent diseases such as malignancies, gastrointestinal or kidney abnormalities, metabolic or endocrine diseases and inflammatory diseases since elevated CRP levels have also been described in these conditions. Twenty age-matched healthy volunteers were recruited from an advertisement in a local newspaper for baseline cytokine measurement. Prior to inclusion, the healthy controls were fully physically examined by a chest physician for lung impairment, cardiovascular disease, diabetes, or other diseases.

Pulmonary function

Forced expiratory volume in one second (FEV_1), forced vital capacity (FVC) and inspiratory vital capacity (IVC) were calculated from the flow volume curve using a spirometer (Masterlab®, Jaeger, Würzburg, Germany). The highest value of at least three measurements was used. FEV_1 was also calculated 15 min. after inhalation of a β-agonist via a metered-dose inhaler. Diffusing capacity for carbon monoxide (DL_{CO}) was determined using the single breath method (Masterlab®, Jaeger, Würzburg, Germany). Lung functional parameters were expressed as percentage of reference values.[5] For arterial blood gas analysis, blood was drawn from the brachial artery while the patients were breathing room air or using their oxygen therapy when indicated (n=16). Arterial oxygen tension (PaO_2) and carbon dioxide tension ($PaCO_2$) were analysed with a blood gas analyser (Radiometer, ABL 330, Copenhagen, Denmark).

Body composition

Body mass index (BMI) was calculated as weight divided by height^2 (kg/m^2). Fat-free mass (FFM; kg) was estimated using single frequency (50 kHz) bioelectrical impedance analysis (BIA; Xitron Technologies, San Diego, CA, USA), with subjects in supine position and calculated using the COPD-specific equation.[6] FFM index (FFMI) was calculated as FFM divided by height^2 (kg/m^2). Fat mass (FM; kg) was estimated as total body weight minus FFM. Percentage FM was calculated by dividing FM by total body weight *100%.

Energy expenditure

Resting energy expenditure (REE) was measured in the early morning (8.30 AM) by indirect calorimetry using a ventilated hood (Oxycon Beta®; Jaeger, Würzburg, Germany) after at least 10 hours of fasting. When patients were on additional oxygen during rehabilitation, the oxygen was temporarily withdrawn 30 minutes before and during measurement of REE. REE was calculated from oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) using the abbreviated Weir formula.[7] The ratio of REE and FFM was used for statistical analysis.

Blood sampling

Fasting EDTA blood was collected in the early morning (8.00-10.00 AM). Soluble tumor necrosis factor receptor 55 (sTNF-R55) and sTNF-R75 were measured in duplicate using the enzyme-linked immunosorbent assay (ELISA) protocol as previously described by Leeuwenberg et al.[8] Lower detection limits for the essays were 40 ng/ml for sTNF-R55, and 70 ng/ml for sTNF-R75. IL-6 and TNFα were determined in duplicate with a Quantikine® high sensitivity ELISA (R&D Systems, Minneapolis, USA) with lower detection of 0.039 pg/ml for IL-6 and 0.5 pg/ml for total TNFα. C-reactive protein (CRP) was assessed in duplicate by high-sensitivity particle-enhanced immunonephelometry (N Hs CRP, Dade Behring) with a lower detection limit of 0.159 mg/l.

Exercise performance

Incremental bicycle ergometry test

An incremental bicycle ergometry test was performed on an electromagnetic braked ergometer (Corival 400®, Lode, Groningen, The Netherlands). After a 2-minute resting period and 1 minute unloaded cycling, power was increased every minute by 10 Watts. None of the subjects knew the exercise load and all were encouraged to cycle at 60 revs/min until exhaustion. Of the patients who did not desaturate (PaO_2 < 88%) during the exercise tests (n=63), oxygen consumption ($\dot{V}O_2$) and carbon dioxide ($\dot{V}CO_2$) were measured and calculated from breath by breath analysis using a breathing mask (Oxycon Beta®, Jaeger, Würzburg, Germany).

Submaximal bicycle ergometry test

The patients performed a submaximal bicycle test of 2 minutes unloaded cycling, 10 minutes at 50%, followed by maximally 20 minutes at 70% of individually measured peak workload of the incremental bicycle ergometry test to assess total endurance time and net mechanical efficiency. Mechanical efficiency was calculated with the following equation:

Net mechanical efficiency [9]= (load (W) of exercise * 0.01433 (kcal/min)/ (energy expenditure during exercise (at 50% of peak workload) –REE)(kcal/min)) * 100%

Energy expenditure during exercise mentioned in the equation was calculated using the steady state values of $\dot{V}O_2$ and $\dot{V}CO_2$ and the abbreviated Weir formula.[7] A good reproducibility of this method in COPD patients was described earlier.[10]

6-minute walking distance

Exercise performance was also measured using a six-minute walk test according to a standardized protocol.[11]

Health related quality of life

Health related quality of life was measured by the St. George's Respiratory Questionnaire (SGRQ). The SGRQ consists of 3 subcategories: symptoms (distress due to respiratory symptoms), activity (disturbance of physical activity), impact (overall impact on daily life and well-being) that combine into the total score (mean of the 3 subcategories). Subscores ranged from 0-100, with a high score meaning greater impairment. A difference of 4 points in total score is considered clinically significant.[12]

Statistical analysis

The median (range) CRP level of healthy age-matched controls was 1.82 (0.16, 7.09) mg/l. The cut-off point of normal vs. elevated CRP level was determined at 4.21 mg/l, which was the 95th percentile of the CRP values of these healthy controls.
Results are presented as mean (standard deviation (SD)) for all variables that were normally distributed and as median (range) when not-normally distributed.
Differences between the groups (normal vs. elevated CRP) were analysed by the Student's T-test for independent samples and the Mann Whitney U-test when not-normally distributed. Regression analysis, adjusting for FFM (when appropriate), lung function (post-bronchodilation FEV_1 (%pred) and diffusion capacity (%pred)), age and gender, was used to establish the variables that were influences by (log-transformed) CRP (presented as regression coefficient (β) (95% confidence interval of β)).
Stepwise regression was used to select the variables that influence (log-transformed) CRP. Correlations between parameters were calculated with either Pearson or Spearman's correlation test. Data were analysed using SPSS (Statistical Package for the Social Sciences, version 11.0 for Windows, SPSS Inc., Chicago, IL, U.S.A.).
Significance was assumed at a p-value of less than 0.05.

RESULTS

Hundred-and-two (71 male/ 31 female) COPD patients (mean age: 63 (9) years) were included, of whom 22 had GOLD stage II, 37 GOLD stage III and 43 GOLD stage IV COPD. Patients were using the following pulmonary maintenance medication: 89% β_2-sympaticomimetics, 37% theophylline, 76% ipratropium bromide, 30% oral corticosteroids, 58% inhalation corticosteroids and 58% oral N-acetylcysteine.

The median value (range) of CRP in COPD patients was 3.47 (0.36, 75.60) mg/l. Twenty-six (25%) patients had a CRP level higher than 10 mg/l (21.25 (11.5, 75.6) mg/l). Patients with GOLD stage III and IV had significantly higher CRP levels than patients with GOLD stage II : II: 1.92 (0.36, 16.00); III: 4.43 (0.47, 75.60); IV: 4.90 (0.47, 65.70); both p<0.03).

CRP was elevated (higher than 4.21 mg/l) in 47% of the 102 patients (37 male/ 11 female), who had median CRP of 12.50 (4.29, 75.60) mg/l. Patients with normal CRP (34 male/ 20 female) had median CRP of 1.49 (0.36, 4.07) mg/l. **Table 1** compares BMI, body composition and lung function of patients with normal vs. elevated CRP. Age, gender, smoking history and body composition were not significantly different between the two groups. FEV_1 (post-bronchodilator) was more impaired in the patients with elevated CRP (p=0.031). In addition, patients with elevated CRP had less reversible FEV_1 than patients with normal CRP (p=0.001).

Table 1: Lung function and body composition of patients with normal vs. elevated CRP.

CRP		**Normal**		**Elevated**		**p=**
n=	(M/F)	54	(34/20)	48	(37/11)	0.122
		Mean/ median	(SD)/ range	Mean/ median	(SD)/ range	
Age	(yrs)	61.3	(10.2)	64.7	(8.0)	0.062
Smoking history*	(PY)	30.0	(0, 80)	35.0	(0, 125)	0.777
Body composition						
BMI	(kg/m^2)	21.9	(4.1)	22.8	(3.3)	0.209
FFMI	(kg/m^2)	15.8	(2.1)	16.1	(1.7)	0.363
FM	(%)	27.0	(7.8)	28.9	(6.3)	0.191
Lung function						
FEV_1 (pre)	(%pred)	35.9	(14.3)	33.1	(12.4)	0.294
FEV_1 (post)	(%pred)	39.9	(15.3)	33.8	(12.2)	0.031
FVC	(%pred)	78.0	(17.9)	75.3	(18.7)	0.472
Reversibility*	(%pred)	2.9	(-8.3, 13.0)	0.0	(-7.2, 8.7)	0.001
FEV_1/FVC	(%)	36	(9)	35	(9)	0.366
DL_{CO}	(%pred)	50.7	(19.7)	47.4	(20.2)	0.423
PaO_2	(kPa)	9.34	(1.26)	9.17	(1.15)	0.460
$PaCO_2$	(kPa)	5.49	(0.87)	5.48	(0.87)	0.538

BMI: body mass index; FFMI: fat-free mass index: FM: fat mass; FEV_1: forced expiratory volume in one second; pre: pre-bronchodilator; post: post-bronchodilator; FVC: forced vital capacity; DL_{CO}: Diffusing capacity for carbon monoxide; PaO_2: arterial oxygen tension; $PaCO_2$: carbon dioxide tension.
*: median (range) non-parametrically tested with Mann-Whitney

CRP was moderately inversely correlated with FEV_1 (post-bronchodilator) (r=-0.22, p=0.026) and reversibility (r=-0.35, p<0.001). No differences were found between patients with elevated and normal CRP in maintenance medication or long-term oxygen therapy (n=16).

Inflammatory markers are shown in **table 2.** IL-6 was increased in patients with elevated CRP (p<0.001) and correlated with CRP (r=0.59, p<0.001). In addition, sTNF-R55 was higher in patients with elevated CRP (p=0.024) and also correlated mildly with CRP (r=0.25, p=0.012), whereas TNFα and receptor 75 were not significantly different between the CRP groups. COPD patients of both groups had increased IL-6 compared to healthy controls (both p<0.005). Soluble TNF-R75 was significantly increased in patients with elevated CRP (p=0.003) and tended to be increased in patients with normal CRP (p=0.056) compared to healthy controls.

Table 2: Inflammatory markers in patients with normal and elevated CRP and in healthy controls.

		COPD				Controls	
		Normal CRP		Elevated CRP			
		median	(range)	median	(range)	median	(range)
TNFα	(pg/ml)	1.25	(0.60, 3.42)	1.31	(0.43, 3.19)	1.23	(0.74, 2.70)
sTNF-R55	(ng/ml)	0.87	(0.54, 2.51)	1.00	(0.591, 2.20) †	0.88	(0.59, 1.46)
sTNF-R75	(ng/ml)	1.42	(0.81, 3.39)	1.54	(0.84, 3.33) *	1.136	(0.68, 1.82)
IL-6	(pg/ml)	2.60	(1.00, 11.45) *	6.45	(0.90, 12.85) * †	1.762	(0.81, 6.00)

TNF- α: tumour necrosis factor alpha; sTNF-R55/75: soluble TNF-receptor 55/75; IL-6: interleukin-6
* p<0.05 vs. healthy controls
† p<0.03 vs. normal CRP

Exercise capacity was assessed with 3 tests (see **table 3**). In all 3 tests, exercise capacity was more impaired in the group of COPD patients with elevated CRP relative to patients with normal CRP (p<0.04). Log-transformed CRP was moderately inversely correlated with load/FFM (r= -0.25, p=0.012), walking distance (r=-0.27, p=0.007) and duration time (r=-0.29, p=0.010).

Resting energy expenditure (REE) adjusted for FFM was increased in patients with elevated CRP levels (elevated CRP: 31.4 (5.4) kcal/kg, normal CRP: 28.0 (4.8) kcal/kg; p=0.002) (**figure 1**). CRP was positively correlated with REE/FFM (r=0.31, p=0.002) and with REE (r=0.41, p<0.001).

Figure 2 shows the scores on health status of both groups. Patients with elevated CRP tended to have higher total score on the SGRQ (elevated CRP: 51.5 (13.9) points, normal CRP: 47.0 (10.9) points; p=0.095; difference of 4.5 points) than patients with normal CRP. A difference of 4 points is considered clinically significant.[12]. Patients with elevated CRP had a significant higher score on the symptom domain compared to patients with normal CRP (elevated CRP: 64.7 (16.5) points, normal CRP: 54.2 (17.1) points; p=0.003).

Table 3: Exercise parameters in COPD patients with normal and elevated CRP.

CRP		Normal Mean/ *median	(SD)/ (range)	Elevated Mean/ *median	(SD)/ (range)	p=
Incremental ergometry						
Workload	(Watt)	63.0	(29.3)	51.8	(24.0)	0.040
Load/FFM	(Watt/kg)	1.38	(0.59)	1.11	(0.49)	0.015
$\dot{V}O_2$	(ml/min)	996	(333)	894	(268)	0.116
$\dot{V}CO_2$	(ml/min)	989	(395)	865	(288)	0.095
RER		0.98	(0.11)	0.96	(0.09)	0.472
$\dot{V}E$	(l/min)	38.6	(13.0)	37.3	(10.3)	0.629
$\dot{V}E/\dot{V}O_2$		39.4	(7.1)	42.9	(8.6)	0.046
$\dot{V}O_2$/load*	(ml/Watt)	14.9	(8.2, 43.2)	16.2	(10.1, 27.9)	0.056
$\dot{V}CO_2$/load*	(ml/Watt)	14.7	(7.2, 35.7)	15.9	(10.6, 27.9)	0.133
Submaximal ergometry						
Duration time*	(min)	13	(3, 30)	8	(2, 30)	0.017
Efficiency*	(%)	16.2	(5.5, 25.5)	14.2	(8.1, 19.4)	0.138
6-min Walking Distance						
Distance	(m)	355	(120)	301	(96)	0.014

FFM: fat-free mass; $\dot{V}O_2$: oxygen consumption; $\dot{V}CO_2$: Carbon dioxide production; RER: respiratory exchange ratio; $\dot{V}E$: ventilation. All parameters of the incremental ergometry test are shown as peak values.
*: median (range) non-parametrically tested with Mann-Whitney

Regression analysis, adjusting for FEV_1 (post-bronchodilation), DL_{CO}, age, FFM and gender, revealed that log-transformed CRP seemed to be a significant and independent predictor for time cycled during submaximal ergometry (β=-3.105 (95% CI: -5.986, -0.224), p=0.035). Log-transformed CRP also seemed to be a significant predictor for REE (β=164 (95% CI: 91, 236), p<0.001), IL-6 (β=3.409 (95% CI: 2.308, 4.504), p<0.001), post-bronchodilation FEV_1 (β=-5.48 (95% CI: -10.30, -0.66), p<0.026) and reversibility (β=-2.52 (95% CI: -4.38, -0.66), p=0.008). In addition, log-transformed CRP seemed to be a significant predictor for the symptom score of the SGRQ (β=8.282 (95% CI: 1.345, 15.220), p=0.020). Interestingly, log-transformed CRP also seemed to be a significant predictor of BMI (β=1.376 (95% CI: 0.039, 2.713), p=0.044), which was a reflection of the relation of CRP with FMI (β=1.103 (95% CI: 0.213, 1.994), p=0.016) but not of FFMI (β=0.273 (95% CI: -0.367, 0.913), p=0.400). The observation that body composition did not differ between the groups (table 1) can be explained by the different distribution in men and women.

Stepwise regression showed that IL-6 (β =0.075 (95% CI: 0.041, 0.109), p<0.001), REE (β = 0.0006 (95% CI: 0.000, 0.001), p=0.035) and 6-minute walking distance (β = -0.0012 (95% CI: -0.002, 0.000), p=0.020) were selected as predictors of log-transformed CRP.

DISCUSSION

The aim of this study was to characterize COPD patients with elevated CRP with respect to lung function, systemic inflammation, body composition, exercise capacity, energy metabolism and health status. The main findings were that, irrespective of FEV_1, COPD patients with elevated plasma CRP concentration had increased impairment in energy metabolism, increased disability as defined by impaired exercise capacity and a more distress due to respiratory symptoms than patients with normal CRP. In addition, patients with elevated CRP had lower post-bronchodilator FEV_1 related to less reversibility in FEV_1 after inhalation of a β-agonist than patients with normal CRP.

Reports on the relation of CRP with lung function are not very consistent. Results from NHANES III excluding patients with COPD, showed an inverse relation between systemic CRP and FEV_1.[13] This was also found in the Caerphilly Prospective Heart Disease Study, including only male patients with ischaemic heart disease.[14] Although a smaller study did not find a correlation between CRP and lung function in patients with mild to severe COPD, [15], CRP seems to increase with increasing severity of COPD.[16] [3] In this study, we also found a more impaired postbronchodilator FEV_1 and reversibility in patients with elevated systemic CRP. Although the exact origin of systemic inflammation remains to be explored, lung biopsy examination clearly illustrates that local inflammation is more pronounced with worse lung function.[17] The higher level of systemic inflammation in COPD patients with low reversibility suggests a more inflammation-driven airflow limitation although no direct data of local inflammation are obtained in our study. However, at least two other studies in the stable state, have not demonstrated a direct relationship between the pulmonary and systemic compartment suggesting that pulmonary and systemic inflammation may be modulated separately.[18] Interestingly, diffusing capacity was not different between both groups, suggesting that exercise-induced intermittent hypoxemia was not different between both groups. Differences in the presence of sputum potentially pathogenic micro-organisms (PPM) could be hypothesized as another difference between both COPD groups as it has been demonstrated previously that the presence of sputum PPM in stable COPD patients has been associated with greater systemic inflammation. [19] Future studies are needed to explore the relationship between the systemic inflammatory response and the level of pulmonary inflammation.

The association between CRP and IL-6 is well established. Previously, IL-6 was identified as ‘exercise factor’, being produced by contracting muscle and subsequently released into the blood. Under normal circumstances, the IL-6 gene is rapidly activated during exercise. It has been shown that IL-6 gene transcription is mediated by glycogen content [20] and that increased IL-6 expression is associated with increased glucose uptake during exercise.[21] Therefore, IL-6 is thought to act as an energy sensor in response to exercise. When contracting muscles are low in glycogen, IL-6 is released to increase glucose uptake and induce lipolysis and gene transcription in abdominal subcutaneous fat.[21] However, it has also been shown that murine myotubes express IL-6 when exposed to oxidative stress [22] and that oral supplementation with anti-oxidants can attenuate exercise-induced plasma IL-6 in healthy humans. [23] Fisher et al have shown that supplementation of vitamin C in combination with vitamin E resulted in lower exercise-induced plasma IL-6 levels,

while no differences were found in muscle IL-6 mRNA nor in skeletal muscle IL-6 protein expression. This suggests that the release of IL-6 from the muscle was inhibited by the anti-oxidants.[23]

In COPD, several changes have been reported that can influence the above-mentioned process. First of all, in some COPD patients, decreases in oxidative enzymes involved in carbohydrate and fatty acid oxidation have been reported.[24] Furthermore, it has been shown that some COPD patients have impaired muscle glycogen content due to inactivity [25] and hypoxia [26] and have enhanced lactic acid production during cycling as compared to healthy control subjects.[27] Systemically, COPD patients also have an imbalance between oxidants and anti-oxidants in rest, but also after exercise suggestive of increased oxidative stress. [28] Moreover, Rabinovich et al have shown that COPD patients cannot adapt their muscle redox-status to training as healthy controls do.[29]

We hypothesize that these changes could disturb the normal exercise-inducted rise in IL-6 in COPD via an earlier and exacerbated induction of IL-6 at lower exercise load. IL-6 would thus be a marker for impairment of exercise metabolism. Imbalances between oxidants and anti-oxidants could independently of muscle-intrinsic changes increase the IL-6 release. Because IL-6 is a strong inducer of acute phase proteins [30], the exacerbated increase in IL-6 production of muscle could induce CRP, as is illustrated by the strong correlation of CRP and IL-6 in this study. Such an increase in CRP after exercise has in fact been shown in healthy subjects.[23] Other studies have also shown an inverse relation between CRP and exercise capacity in healthy elderly [31] as well as in COPD.[28]

The increased demand for specific amino acid to generate CRP may increase muscle protein breakdown, increasing REE [32] and inducing a vicious cycle of intrinsic muscle changes leading to decreased exercise capacity leading to more muscle impairment. CRP may thus be a marker of repetitive supra-physiological increase in IL-6 production of muscle in a subgroup of COPD patients. Prior research has also shown an association between systemic inflammation as measured by markers of the TNF-system and weight loss. [33] In this study we showed an association between CRP and BMI (when adjusted for FEV_1 (post-bronchodilation), DL_{CO}, age and gender) that could be attributed to FMI, but not to FFMI. The association between CRP and extent of obesity has previously been found in studies with non-diabetic subjects.[34] It has been proposed that inflammatory cytokines could be secreted by adipocytes and by inflammatory cells present in adipose tissue.[35] Further research is needed to further elucidate the role of different cytokines on body composition and vice versa.

The cut-off point we used in this study is not the standard cut-off point of 5 mg/l, which is often used in clinical practice. Interestingly, our cut-off point, determined as the 95^{th} percentile of our own healthy age- and gender-matched controls, is very similar to the clinical cut-off point. In addition, analysis using 3 or 5 mg/l as cut-off point provided the same results (data not shown).

High sensitivity CRP analysis already has been recommended for clinical application in cardiovascular disease detection and prevention.[36] Given the fact that cardiovascular disease is a major cause of mortality in COPD [37], combined with the facts that CRP is a predictor for acute exacerbations of COPD [38], for hospitalisation and mortality in chronic respiratory failure [39], as well as the fact that CRP seems to be a marker for impairments in exercise capacity and distress due to respiratory

symptoms as shown in this study, routine HS-CRP analysis could prove itself to be of major clinical importance in COPD. Future research is needed to assess the value of CRP as biomarker for measuring progress of the disease and for the effects of treatment of COPD.

Ethics approval: Written informed consent was obtained from all subjects, and the ethical review board of the University Hospital Maastricht approved the study.

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Competing interests: None declared

FIGURE LEGENDS

Figure 1: Resting energy expenditure (REE) adjusted for fat-free mass (FFM) is increased in patients with elevated CRP compared with patients with normal CRP. Data are presented as mean (SEM).
*: $p<0.002$

Figure 2: Patients with elevated CRP have impaired quality of life as measured with the St. George's Respiratory Questionnaire. The total score was 4.5 points higher in patients with elevated CRP. Data are presented as mean (SEM). *: $p=0.003$

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