Measure and effect of diet in chronic obstructive pulmonary disease

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Abstract

Background and objectives: Recent research has shown an association between a healthy diet and reduced chronic obstructive pulmonary disease (COPD) incidence. However, the potential role of diet in COPD prognosis is unknown. This thesis aimed to describe the characteristics of diet in COPD patients and to estimate its association with the disease evolution, in terms of pathophysiological impairment and hospitalizations. A secondary objective was to study the role of diet in asthma, as a COPD-related phenotype.

Methods: A dietary ancillary protocol was included in a well phenotyped cohort of 342 COPD patients recruited during their first admission for a COPD exacerbation in Spain. Dietary data of the last 2 years was assessed using a validated food frequency questionnaire (122 items). Levels of oxidative stress and inflammatory markers were measured in serum. Hospital admissions during follow-up were obtained from national datasets. Additionally, data from the International Study of Asthma and Allergies in Childhood (ISAAC) in Mexico was used to assess the effect of diet in childhood asthma.

Results: (i) COPD patients report an adequate intake of the main food groups and macro- and micro-nutrients according to local recommendations, excepting vitamin D; (ii) vitamin E and olive oil intakes are associated with reduced oxidative stress in COPD active smokers; (iii) intake of Ω3 and Ω6 fatty acids is related to the levels of serum inflammatory markers; (iv) cured meat intake increases the risk of COPD admission during follow-up; and (v) children adherence to a Mediterranean dietary pattern relates to reduced childhood asthma prevalence.

Conclusions: Dietary habits may modify COPD prognosis and childhood asthma. Therefore, advice on healthy diet should be considered in chronic respiratory diseases guidelines.

Resum

Antecedents i objectius: Estudis recents mostren associacions entre una dieta sana i reduccions en la incidència de malaltia pulmonar obstructiva crònica (MPOC). Tanmateix, el possible rol de la dieta en l'evolució de l'MPOC és desconegut. L'objectiu d'aquesta tesi és descriure les característiques de la dieta en pacients amb MPOC i estimar-ne l'associació amb l'evolució de la malaltia en termes d'alteracions fisiopatològiques i hospitalitzacions. Com a objectiu secundari, també es vol estudiar el paper de la dieta en l'asma, com a malaltia estretament relacionada amb l'MPOC.

Mètodes: Es va aniuar un protocol d'epidemiologia nutricional en una cohort de 342 malalts d'MPOC, ben fenotipats, reclutats a Espanya durant la seva primera hospitalització per aguditització de l'MPOC. Es va administrar un qüestionari de freqüència de consum d'aliments (122 ítems) preguntant per la dieta dels darrers 2 anys. Es van mesurar en sèrum els nivells de marcadors d'estrès oxidatiu i d'inflamació. Les hospitalitzacions durant el temps de seguiment s'obtingueren a partir de registres nacionals. Per últim, s'utilitzaren dades de l'*International Study of Asthma and Allergy in Childhood* (ISAAC) a Mèxic per a estimar l'efecte de la dieta en l'asma infantil.

Resultats: (i) El consum d'aliments i macro- i micro-nutrients fou considerat adient respecte a les recomanacions locals, exceptuant la vitamina D; (ii) la ingesta de vitamina E i oli d'oliva s'associà a menors nivells d'estrès oxidatiu en pacients fumadors actius; (iii) els nivells de ingesta d'àcids grassos Ω3 i Ω6 es va relacionar amb els nivells d'inflamació sistèmica; (iv) la ingesta d'embotits i carns curades va incrementar el risc d'hospitalització per MPOC durant el seguiment; i (v) l'adherència a un patró mediterrani d'alimentació s'associà a menor prevalença d'asma infantil.

Conclusions: Els hàbits alimentaris poden modificar l'evolució de l'MPOC i el desenvolupament d'asma infantil. Per tant, s'hauria de considerar l'inclusió de consells alimentaris en les guies clíniques per a malalties respiratòries cròniques.

Preface

This thesis consists of a compilation of scientific publications according to the procedures of the Biomedicine PhD program of the Department of Experimental and Health Sciences. The thesis includes an abstract, a general introduction, a rationale, the objectives, the results (5 original scientific papers), a discussion, and final conclusions.

Four of the scientific papers included in this thesis are based on data from the Phenotype and Course of Chronic Obstructive Pulmonary Disease (PAC-COPD) study, a Spanish multi-centric cohort study aiming to improve our understanding about the phenotypic heterogeneity of COPD and the extent to which this heterogeneity is related to its clinical course. The other manuscript is based on Mexican data from the International Study of Asthma and Allergies in Childhood (ISAAC), which is an international collaborative research project aimed to investigate asthma, rhinitis and eczema in children.

At the end of this thesis, in separate annexes, other publications regarding COPD or diet that I have co-authored during the predoctoral period are included, altogether with a list of congress contributions.

Barcelona, December 2010

Contents

1

Introduction

1.1 Chronic Obstructive Pulmonary Disease (COPD)

1.1.1 COPD definition

Chronic Obstructive Pulmonary Disease (COPD) is a complex respiratory disorder characterized by non fully reversible airway limitation. The most currently used definitions for COPD are those provided by the Global initiative for chronic Obstructive Lung Disease (GOLD) and the American Thoracic Society (ATS) and the European Respiratory Society (ERS) consensus. GOLD working definition states: “Chronic Obstructive Pulmonary Disease (COPD) is a preventable and treatable disease with some significant extrapulmonary effects that may contribute to the severity in individual patients. Its pulmonary component is characterized by airflow limitation that is not fully reversible. The airflow limitation is usually progressive and associated with an abnormal inflammatory response of the lung to noxious particles or gases” [1]. ATS/ERS consensus agreed in the following common definition: “Chronic Obstructive Pulmonary Disease (COPD) is a preventable and treatable disease state characterized by airflow limitation that is not fully reversible. The airflow limitation is usually progressive and associated with an abnormal inflammatory response of the lungs to noxious particles or gases, primarily caused by cigarette smoking. Although COPD affects the lungs, it also produces significant systemic consequences” [2]. Both definitions agree in: (i) preventability and treatability of the disease; (ii) non full reversibility of the airway obstruction; (iii) presence of extrapulmonary effects; and (iv) abnormal inflammatory response to noxious particles or gases. A spirometric definition of the disease is broadly used both in the clinical setting and for research, establishing as COPD patients those subjects with post-bronchodilator forced expiratory volume in the first second to forced vital capacity ratio (FEV_1/FVC) <0.70 [1] or ≤ 0.70 [2].

1.1.2 Burden of COPD

COPD is a very serious health problem. It was the fourth leading cause of death worldwide in 2004 [3], with approximately 3 millions of deaths (5.1% of total worldwide deaths), and projections up to 2030 show that COPD mortality will not diminish and will still be the fourth leading cause of mortality [4]. According to the Burden of Lung Disease study (BOLD) [5], COPD prevalence is high, although variable around the world, and ranges from 26.1% in Salzburg, Austria, to 11.4% in Guangzhou, China, as shown in Table 1, probably due to different smoking patterns. It is noteworthy that COPD prevalence increases with age (Figure 1). Finally, the prevalence of the disease is overall higher in men than women, although this trend is reverting in Europe and the USA, suggesting that the gender differences in deaths will probably disappear in the future, as a result of demographic changes in smoking patterns.

The morbidity and economic costs associated with COPD are high. Hospital admissions for COPD are the main contributor to COPD direct annual medical cost per patient in the USA, which in 2005 was estimated at 2700-5900US$ [7]. In 2001, the overall annual cost (excluding mortality and rehabilitation) for COPD in Europe was 38.7 billion € (4.7 billion € for ambulatory care, 2.7 billion € for drugs, 2.9 billion € for inpatient care and 28.4 billion € for lost work days) [8], and the annual cost per patient ranged 151-3912€ [9]. Overall, it is clear that COPD represent a very important threat to global economies.

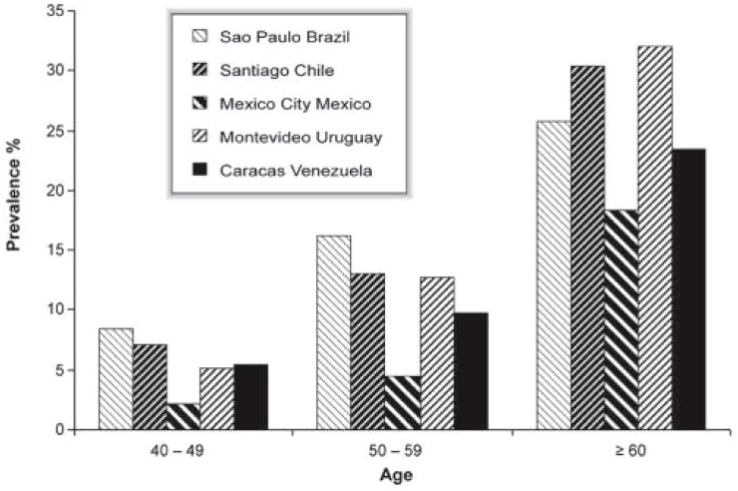

Figure 1. COPD prevalence by age in 5 Latin American cities [6].

Table 1. Estimated population prevalence of COPD according to GOLD severity stages in 12 cities participating in the BOLD study, adapted from Buist *et al* [5].

	Guangzhou China	Adana Turkey	Salzburg Austria	Cape Town South Africa	Reykjavik Iceland	Hannover Germany
n	473	806	1258	847	755	683
No obstruction	88.6% (1.5)	80.9% (1.5)	73.9% (1.3)	76.2% (1.4)	82.1% (1.4)	86.7% (1.3)
Stage I	4.2% (0.9)	8.6% (1.0)	15.5% (1.1)	4.7% (0.9)	8.9% (1.1)	7.3% (1.0)
Stage II	5.5% (1.0)	9.1% (0.9)	9.2% (0.9)	12.4% (1.1)	7.0% (0.9)	5.1% (0.9)
Stage III-IV	1.7% (0.6)	1.5% (0.4)	1.4% (0.4)	6.7% (0.9)	1.9% (0.5)	0.8% (3-8)

	Krakow Poland	Bergen Norway	Vancouver Canada	Lexington USA	Manila Philippines	Sydney Australia
n	526	658	827	508	893	541
No obstruction	77.9% (1.6)	81.2% (1.5)	80.7% (1.5)	80.4% (1.9)	86.2 (1.1)	80.8% (1.7)
Stage I	11.2% (2.3)	10.5% (1.2)	11.1% (1.2)	5.3% (1.1)	1.4% (0.6)	8.4% (1.2)
Stage II	9.0% (1.2)	7.1% (1.0)	7.3% (1.0)	10.1% (1.4)	7.5% (0.8)	9.4% (1.2)
Stage III-IV	1.9% (0.6)	1.2% (0.4)	0.9% (0.3)	4.2% (1.0)	5.0% (0.7)	1.4% (0.5)

Data shown as % (SE)

1.1.3 Risk factors of COPD

Tobacco smoke is the main risk factor for COPD. However, COPD is a complex multi-causal disease and, in terms of model of causation [10], has no single necessary or sufficient causes. This means that there are more than one events, conditions or characteristics preceding the disease without which the disease would not have occurred, and that this set of events, conditions or characteristics is not necessarily the same in all patients. Although some COPD definitions in the guidelines state that COPD is "primarily caused by cigarette smoking" [2], not all smokers develop the disease and some non-smokers present clinically significant COPD, thus smoking is not either a necessary nor sufficient cause for developing COPD. Given this fact, it can be stated that COPD is related to an interaction between genetic and environmental factors, and unfortunately, the current understanding of many of the risk factors is in many respects incomplete. Despite the efforts done to determine COPD risk factors only few of them are completely accepted. This evidences the overwhelming difficulties in unraveling COPD risk factors beyond tobacco smoke, with the two main reasons of this pitfall being the heterogeneity of the phenotype and the inherent complexity in the etiology.

Tobacco smoke
As already said, tobacco smoke is the main environmental risk factor for COPD. Approximately one-quarter of smokers [11] or even a much higher proportion of smokers [12-14] - perhaps as much as 50% - can be affected by clinically significant COPD, and this risk is dose dependent [15]. Active smoking produces premature onset of age-related lung function decline and over 40% of smokers develop chronic bronchitis [11]. Second hand smoke exposure at home and work has also been associated with a greater risk of COPD [16]. Finally, maternal smoking during pregnancy could affect negatively the lung function of offspring even in late adulthood, aggravating the cumulative effect of active cigarette consumption [17].

Genetic factors
The best known genetic factor for COPD is a severe hereditary deficiency of the serine protease α1 antitrypsin, present in 1-3% of COPD patients [18]. The low concentrations of this enzyme contribute to the premature and accelerated development of panlobular emphysema and decline in lung function, especially when combined with smoking or other exposures [18]. This

deficiency may account for a significant fraction of the early onset severe COPD patients. Several genetic association studies have been conducted based on the fact that siblings of patients with severe chronic obstructive pulmonary disease have a significant risk of airflow obstruction [19]. These studies have pointed out as potentially relevant genes those encoding: tumor necrosis factor α (TNFα) [20], transforming growth factor β (TGF-β) [21], and microsomal epoxide hydrolase 1 [22]. However, results up-to-date have been largely inconsistent. Finally, recent genome-wide association studies (GWAS) have identified new potentially relevant polymorphisms. Two polymorphisms in the α-nicotinic acetylcholine receptor 3/5 locus on chromosome 15 have shown unambiguous evidence of association with COPD after appropriate replication [23]; and a replicated GWAS of emphysema determined from chest computed tomography scans has identified genetic variants in BICD1 (related to the length of telomeres) to be associated with qualitative emphysema in COPD [24].

Other environmental factors

Although none of them is comparable to cigarette smoking, there are other environmental risk factors of COPD. Occupational exposures in COPD include dusts, chemicals, vapors, and fumes in the workplace. Several longitudinal studies have evaluated the association between COPD and occupational exposures, focusing in especially vulnerable workers such as miners as well as in general industrial workers [25]. Most of these studies reported an annual decline of 7–8ml in FEV_1 due to occupational exposures after adjustment for age and smoking [25]. The population attributable risk from occupational exposures is estimated at approximately 15% by the ATS [25]. According to the World Health Organization, exposure to biomass fuels (coal, wood, straw, animal dung, and crop residues) used to heat and cook in poorly ventilated dwellings, account for 35% of COPD cases in countries of low and middle income [26]. This exposure is particularly important in women [27]. In the other hand, outdoor air pollution, primarily from motor vehicle emissions in cities, has been associated with decrements in lung function [28].

Several additional risk factors have been proposed for COPD. However, the role of some of them is not completely clear and the evidences are limited. The most relevant of these risk factors are: infections, that may contribute to COPD pathogenesis and progression [29]; gender, as some studies have pointed out that women could be at a greater risk of COPD [30]; concomitant

asthma, as some longitudinal studies suggest that asthmatics are at a greater risk of COPD than non-asthmatics [31]; low socioeconomic status, which has been consistently associated with a higher risk of COPD although whether this is mediated through other factors such as diet or infections should be further evaluated [32]; and low levels of physical activity, which have been associated to an increased risk of smoking-related lung function decline [33], and increased risk of COPD hospital admission and mortality [34].

Finally, dietary habits have also been suggested to be implicated in the cause and prevention of COPD [35]. Unfortunately, the potential role of diet in COPD development and progression is not clear and the evidences so far are limited. Nevertheless, some foods and nutrients, such as antioxidants, have been related to lower risk of poor respiratory health and COPD, and the available information up to date will be further discussed a long this thesis.

1.1.4 Pathogenesis and pathophysiology of COPD

COPD is caused by an abnormal inflammatory response of the lung to noxious particles or gases that leads to non reversible airflow limitation and several systemic effects. The main contributors to airflow limitation are: (i) fixed narrowing of small airways; (ii) emphysema, that is a permanent, destructive enlargement of airspaces distal to the terminal bronchioles without obvious fibrosis [36]; and (iii) luminal obstruction with mucus secretions, although the relative importance of each of them is still unclear [37]. The first step in the pathogenic process is the activation of macrophages in the respiratory tract [38]. Figure 2 shows the broad range of effects produced by macrophage activation.

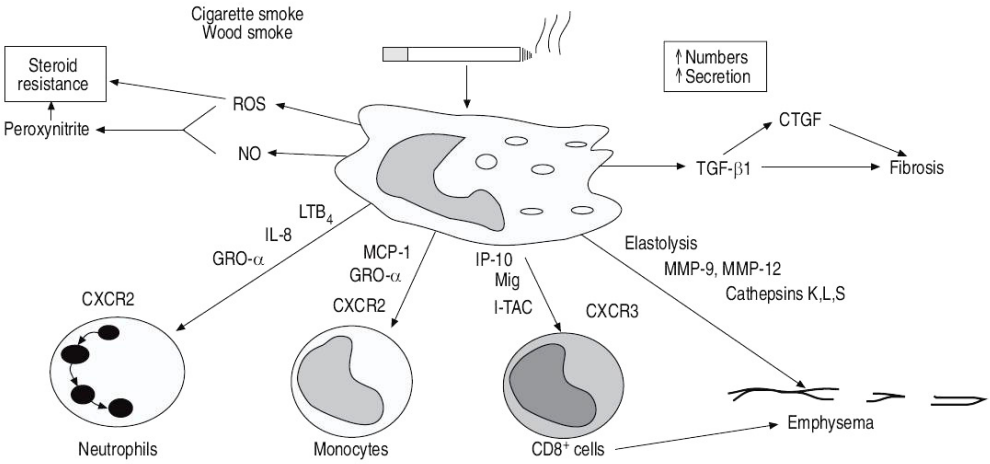

Figure 2. Pivotal role of macrophages in COPD, adapted from Barnes *et al* [37].

Activated macrophages in the respiratory tract release chemotactic factors such as interleukines (IL) and leukotrienes (LT) that recruit other inflammatory system cells including neutrophils, monocytes and $CD8^+$ lymphocytes [39]. Moreover, there is a release of enzymes including matrix metalloproteinases (MMP) and cathepsins causing elastolysis [40], and release of TGF-β and connective tissue growth factor (CTGF), that leads to fibrosis [41]. Macrophages also contribute to the increased oxidative stress burden, that causes direct injury to the lung cells, increase of proteolytic activity due to inactivation of antiproteases, mucus hypersecretion, and activation of transcription factors such as NF-κβ, leading to cytokine release and neutrophil recruitment, further increasing inflammation and oxidative stress [42,43]. This inflammatory condition, along with free radical stress, appears to extend beyond the lung in COPD patients [44], contributing to systemic manifestations of the disease [43,45]. Figure 3 represents a scheme of COPD pathogenesis, including potential therapeutic targets.

In addition to pulmonary alterations, COPD has several systemic manifestations. These manifestations may include systemic inflammation, weight loss and nutritional abnormalities, skeletal-muscle dysfunction, cardiovascular diseases, diabetes/glucose intolerance, osteoporosis and fractures, depression, and autoimmune disorders, among others [47].

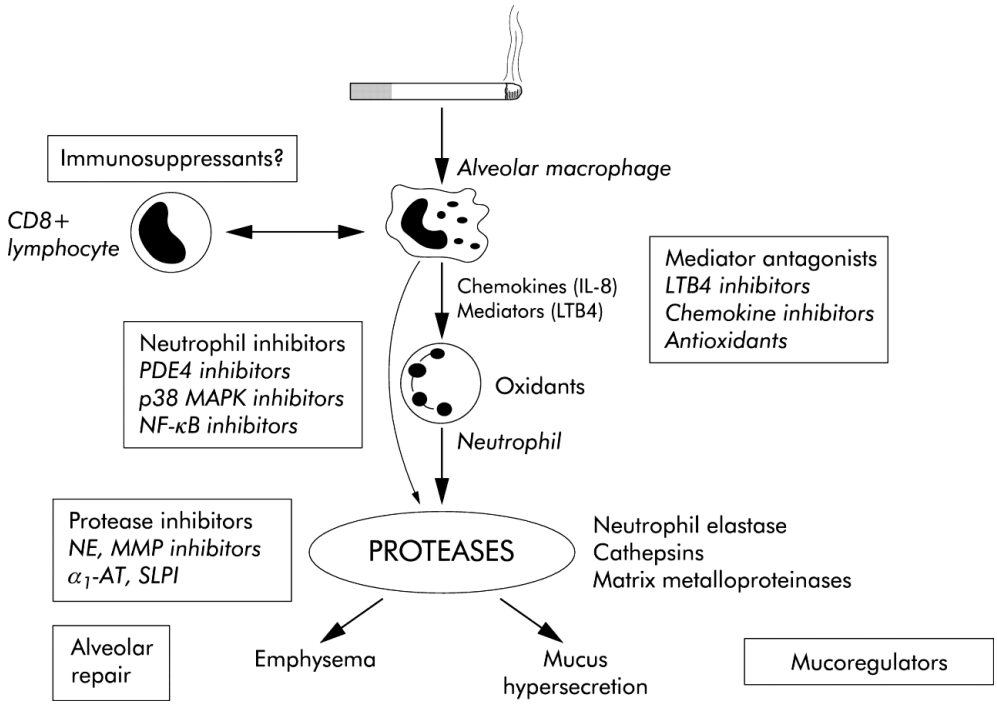

Figure 3. COPD pathogenesis and potential therapeutic targets, adapted from Barnes [46].

COPD patients have significantly higher levels of inflammatory markers (including leucocytes, fibrinogen, CRP and TNF-α) than those found in the general population, thus indicating a persistent abnormal systemic inflammation in COPD patients [48]. This persistent inflammation could play a relevant role in the disease, as other systemic manifestations of the disease such as weight loss, cachexia, osteoporosis and cardiovascular diseases have been related to systemic inflammation [48]. Several mechanisms have been proposed to explain the origin of systemic inflammation in COPD [49]. In the first one, inflamed pulmonary parenchyma, either by spill-over of pro-inflammatory molecules from the lung and/or by activation of inflammatory cells (neutrophils, monocytes, lymphocytes) during their transit through the pulmonary circulation, would cause a diffusion of the inflammation to the systemic compartment [49]. Another possibility is that systemic inflammation of COPD would be originated directly in other organs such as the skeletal muscle, the liver or the bone marrow [49]. Finally, systemic inflammation could be directly produced by cigarette smoke, which

is the main risk factor of COPD, independently of the presence or absence of COPD [49].

Unexplained weight loss, associated to muscle wasting and loss of fat free mass, is a poorly understood systemic manifestation of COPD [50] that has an specially negative impact on the prognosis of the disease [51,52] and relates to overall mortality [51]. Along with muscle wasting there is a substantial degree of skeletal muscle dysfunction that contributes significantly to limit the exercise capacity and quality of life of COPD patients [53]. Causes of this muscular dysfunction include systemic inflammation, oxidative stress, tissue hypoxia and sedentarism [53].

Given the similarities of cardiovascular inflammatory diseases and COPD [54], both diseases are usually associated, being tobacco smoking and systemic inflammation the common links between them although airflow obstruction *per se* has profound effects on cardiac function [55]. It is noteworthy that cardiovascular causes have an important impact on morbidity and mortality in COPD [56].

The prevalence of comorbid anemia in patients with COPD could range from 7.5% to 34% [57], and it has been proposed to be caused by the effects of inflammatory cytokines on erythropoiesis mechanisms [58]. In the other hand, diabetes may be associated to diminished lung function and COPD due to shared inflammatory processes and common endothelial dysfunction [59]. Other systemic manifestations of the disease may include osteoporosis, depression and autoimmune disorders [60,61].

Overall, all of these concomitant diseases potentiate the morbidity of COPD, leading to increased hospitalizations, mortality and healthcare costs, and directly affecting the health-related quality of life of COPD patients [62].

1.1.5 Natural history of COPD

COPD has a variable natural history and not all individuals follow the same course [1]. Although COPD is a progressive and non-reversible disease, stopping the exposure to noxious agents may result in some improvement in lung function and a significant slowing of the progression of the disease. In order to establish an objective classification of the severity stage of the disease, several

post-bronchodilator spirometric cut-off points have been proposed [1]:

Stage I	Mild	$FEV_1/FVC < 0.70$ $FEV_1 \geq 80\%$ predicted
Stage II	Moderate	$FEV_1/FVC < 0.70$ $50\% \leq FEV_1 < 80\%$ predicted
Stage III	Severe	$FEV_1/FVC < 0.70$ $30\% \leq FEV_1 < 50\%$ predicted
Stage IV	Very severe	$FEV_1/FVC < 0.70$ $FEV_1 < 30\%$ predicted or $FEV_1 < 50\%$ predicted plus chronic respiratory failure

However, COPD symptoms, such as breathlessness and limited exercise capacity, do not perfectly correlate with the classification of COPD severity according to spirometric values. Therefore the severity staging of the disease in the clinical setting should not be exclusively based on spirometric parameters, and the severity of symptoms should always be considered [1].

The first symptoms of the disease are usually chronic cough and sputum production, and these symptoms may precede by many years the development or airflow limitation [1]. However airflow limitation may develop without any of them. Therefore, as the impact of the particular symptoms is a key determinant of seeking medical help, the disease may be diagnosed at any severity stage. Finally, end-stage COPD is characterized by progressively worsening dyspnoea, initially during exercise, eventually during activities of daily living, and ultimately even at rest, altogether with multiple comorbidities, in line with the advanced age and the common underlying risk factor of smoking [63].

The natural history of COPD is also characterized by the so called exacerbations. Exacerbations of COPD are acute episodes of further amplification of the inflammatory response in the airways, leading to an acute worsening of symptoms, and usually requiring hospitalization [1]. However, there is not a precise and widely accepted definition of exacerbation, and a broad range of definitions has been in used in epidemiological studies [64].

Episodes of COPD exacerbation are responsible of a major part of the economic burden of the disease [65], and frequent exacerbations have been shown to be associated with poorer health outcomes [65]. Little is known regarding the mechanisms involved in exacerbations. However, bacterial or viral infection of the lungs as well as exposure to environmental pollutants may trigger them. Finally, exacerbation frequency seems to increase with disease severity, and there is evidence suggesting that exacerbations could produce a permanent reduction in lung function, which suggests that patients who experience more exacerbations could have a faster decline than those with fewer exacerbations [65].

1.1.6 Related phenotypes: Asthma

Asthma is defined by the Global Strategy for Asthma Management and Prevention as a "chronic inflammatory disorder of the airways in which many cells and cellular elements play a role. The chronic inflammation is associated with airway hyperresponsiveness that leads to recurrent episodes of wheezing, breathlessness, chest tightness, and coughing, particularly at night or in the morning. These episodes are usually associated with widespread, but variable, airflow obstruction within the lung that is often reversible either spontaneously or with treatment" [66]. Back in the 60s, Orie *et al* [67] proposed what is known as the Dutch hypothesis: that COPD and asthma were indeed manifestations of the same obstructive lung disease (OLD). Moreover, they proposed that asthma, as a form of OLD, could evolve into COPD, another form of OLD. The Dutch hypothesis, although controversial, could not be proven wrong and has been present in the scientific literature since then. In 1995 the American Thoracic Society stated that "it may be impossible to differentiate patients with asthma whose airflow obstruction does not remit completely from persons with chronic bronchitis and emphysema with partially reversible airflow obstruction and bronchial hyperresponsiveness" [68]. Finally, it is noteworthy that lung function impairment, that is a precursor of COPD, has been suggested to increase the risk of asthma both in children and adults [69], and this fact together with other available evidences has attracted attention towards the potential early origin of COPD [70].

Although both diseases present underlying complex inflammatory abnormalities, the nature of asthma's inflammation is mainly

eosinophilic and not affecting the lung parenchyma [71], whereas neutrophils are more important in COPD. This key difference may explain the different response to corticosteroid treatment in both diseases, as corticosteroids reduce eosinophil survival but prolong neutrophil survival [72]. Table 2 summarizes the main differences in the nature of the inflammation in both diseases.

Table 2. Main differences in the nature of inflammation in COPD and Asthma, adapted from Barnes [73].

Inflammation	Asthma	COPD
Inflammatory cells	Mast cells	
	Eosinophils	Neutrophils
	$CD4^+$ cells (Th2)	$CD8^+$ cells (Tc)
	Macrophages+	Macrophages++
Inflammatory mediators	LTB_4, histamine	LTB_4
	IL-4, IL-5, IL-13	TNF-α
	Eotaxin, RANTES	IL-8, GRO-α
	Oxidative stress+	Oxidative stress+++
Inflammatory effects	All airways	Peripheral airways
	AHR+++	AHR±
	Epithelial shedding	Epithelial metaplasia
	Fibrosis+	Fibrosis++
	No parenchymal involvement	Parenchymal destruction
	Mucus secretion+	Mucus secretion+++
Response to corticosteroids	+++	±

*Th2 = T-helper type 2.

At the clinical practice, the main feature that allows distinguishing asthma and COPD is the reversibility of airflow limitation in response to inhaled bronchodilators such as β-agonists, anticholinergics, methylxanthines, and corticosteroids. This criterion is commonly used to distinguish both diseases, although using it approximately 10% of COPD patients also have asthma [73]. A recent Pro/Con editorial debate on whether asthma and COPD are the same or different diseases shows that the debate is still alive [74-77].

The available evidence so far suggests that COPD cannot be sufficiently distinguished from asthma. Therefore, further research

in common risk factors and mechanisms (including diet) should be encouraged, and will probably give insight in what is known of both COPD and asthma.

1.2 Diet and COPD

1.2.1 Diet in COPD development

The role of diet in COPD development has been studied since back in the 80s, Dauber *et al* demonstrated an association between nutrition and the pathogenesis of emphysema [78]. Further epidemiological studies linked dietary factors, such as fruit consumption, with COPD development (either measured as lung function decline or self-report of a COPD diagnosis), and have been the focus of some literature reviews [79-82].

At least four studies have assessed the relation between dietary patterns and COPD onset or lung function decline [83-86]. All of them have reported that a healthy dietary pattern (i.e. high consumption of vegetables, fruits, whole-grain products and fish) may protect against impaired lung function and COPD, whereas patterns with elevated meat and French fries could have adverse effects [83-86].

Intake of fruits, vegetables and fish has been evaluated in relation to COPD or lung function [79-82]. All these food groups were suggested to have beneficial effects on either lung function or COPD development. Back in 1979, the baseline cross-sectional analysis of a cohort of 2512 middle aged Welshmen showed that lung function was 115.3ml (95% CI 30.6 to 200.0) higher for those eating five or more apples per week compared with non consumers, after adjusting for potential confounders including vitamins C and E, whilst no associations were found for citrus fruits nor fatty fish [87]. A cross-sectional analysis on 15800 subjects aged 45 to 64 years recruited in the US from 1986 through 1989, showed that fish consumption had an inverse relation to smoking-related COPD (COPD OR: 4^{th} *vs*. 1^{st} quartile=0.49; p-for-trend across quartiles=0.001) [88]. In 1995, a cross-sectional study of 6186 adults conducted in Scotland showed that the frequency of fresh fruit intake was linearly associated with higher FEV_1 (p-for-trend<0.001), similar results were found for green vegetables but not for fish [89]. Between 1994 and 1997, a cross-sectional analysis of 13651 adults participating in the MORGEN study found

that intake of fruit juice, vegetables and whole grains was positively associated with FEV_1 and inversely associated with the prevalence of COPD symptoms, although no associations were found for fish intake [90]. Further studies were unable to settle a conclusive statement of the potential role of such food groups in COPD development due to lack of results consistency [79-82]. It is noteworthy that most of them were cross-sectional. Other foods such as cured meats, soy and black tea have also been evaluated. Consumption of cured meats has been positively associated with the risk of newly diagnosed COPD in two big US cohorts [91,92]. In the other hand, soy consumption has been associated with a decreased risk of COPD and breathlessness [93]. Finally, a case-control study identified black tea as a protective factor in COPD development [94].

Increased oxidative burden plays an important role in the pathogenesis of COPD, causing direct injury to the lung cells, increase of proteolytic activity due to inactivation of anti-proteases, mucus hypersecretion, and activation of transcription factors such as NF-κβ, leading to cytokine release and neutrophil recruitment, further increasing inflammation and oxidative stress [42,43]. Therefore, it has been hypothesized that targeting oxidative stress with antioxidants or boosting endogenous levels of antioxidants is likely to have beneficial effects in COPD [95]. Vitamins C, E and A, betacarotene, carotenoids, flavonoids and selenium have been evaluated in relation to COPD onset and lung function based on their antioxidant properties [79-82]. Vitamin C, a well known water soluble antioxidant vitamin, has been the most studied nutrient and has been consistently associated with better lung function, although the relation to COPD development and symptoms has been less convincing [79-82]. The evidence regarding the role of vitamin E (known to be one of the most potent lipophilic antioxidants) and betacarotene in relation to COPD development or lung function is equivocal, even though more than ten studies have evaluated them [79-82]. Finally, other nutrients such as flavonoids (known to scavenge several free radicals) and Selenium (co-factor for glutathione peroxidasse enzyme) have not been sufficiently evaluated so far.

In the other hand, omega-3 and omega-6 polyunsaturated fatty acids (PUFA) have captured most of the interest regarding nutrients with anti- or pro-inflammatory effects [79-82]. It is known that omega-3 fatty acids mostly promote anti-inflammatory activities [96]. Studies in both healthy populations and among subjects with

cardiovascular disease have shown a negative correlation between omega-3 fatty acid intake and several pro-inflammatory biomarkers including CRP, IL-6 and TNFα [96], involved in key inflammatory processes such as NF-κβ activation and MAPK pathways (TNFα), as well as in acute phase response (IL-6). Several cross-sectional studies have evaluated the role of omega-3 PUFA in COPD development. Although the results have not been consistent, a protective effect against COPD development and loss of lung function has been suggested [79-82]. In contrast, omega-6 fatty acids are the most relevant precursors of pro-inflammatory eicosanoids, and therefore mostly mediate pro-inflammatory activities [97]. Although the evidences so far are scarce, it has been suggested that they could be a risk factor for the development of COPD [79,81].

Other nutrients such as alcohol [98], fiber [99], sodium and magnesium [82], and nitrites [91,92], have been proposed to play a role in COPD development and/or lung function decline. However, no conclusive hypotheses have been proposed so far to explain their mechanisms of action, and the number of studies involving such nutrients, if any, has been clearly insufficient. Table 3 summarizes the proposed mechanism and sources of the most relevant nutrients with potential effects on lung diseases development.

Finally, nutritional status, which is related to both dietary habits and usual physical activity, could also play a role in COPD pathogenesis. It is of note that obesity has been suggested to play a significant role in the pathogenesis of COPD contributing to persistent systemic inflammation through the production of pro-inflammatory mediators in the adipose tissue [100].

Table 3. Proposed mechanisms and sources of most relevant nutrients with potential effects on lung diseases development, adapted from Romieu [82].

Nutrient	Mechanisms	Dietary sources	Comments
Vitamin C (ascorbic acid)	Water soluble antioxidant Scavenges O_2^- Regenerates oxidized vitamin E Present in neutrophils and lymphocytes	Fruits: papaya, canteloupe, citrus fruits, strawberries Vegetables: cauliflower, broccoli, brussels sprouts, kale, sweet peppers	Humans are unable to synthesize vitamin C RDI: 75 mg/d in women, 90 mg/d in men Large doses of vitamin C are usually well tolerated (2000 mg/d). Doses above this range may result in nausea and diarrhea[3]
Vitamin E (α-tocopherol)	Lipid soluble antioxidant Reacts with peroxyl radicals to terminate membrane lipid peroxidation	Vegetable & seed oils (corn, safflower, soy bean) Eggs Green vegetables	Level of vitamin E in food best correlates with the level of unsaturated fat RDI: 15–20 mg/d In adults 200–400 mg/d is tolerated without adverse effects, with the exception of gastrointestinal upset; with 800–1200 mg/d, antiplatelet effect and bleeding may occur; over 1200 mg/d may result in adverse effects such as nausea, headache, fatigue and diarrhea[3]
β-carotene, lycopene and other carotenoids	Lipid soluble antioxidants React with peroxyl free radicals, reducing lipid peroxidation Scavenge O_2^- Growth regulation of malignant cells (via vitamin A effects)	Red, orange and yellow fruits and vegetables: sweet potato, carrots, winter squash Green vegetables	More than 600 carotenoids. Antioxidant and provitamin A activity (transformed to retinol [vitamin A] by clivage of central bond) Antioxidant activity of carotenoids may be more important than the provitamin A effects. No RDI; recommended intake is 15 mg/d Carotenoid toxicity—carotenodermia (yellowing of the skin), nausea and diarrhea[3]
Vitamin A	Lipid soluble Differentiation of epithelial cells Growth regulation of malignant cells Reproduction Vision	Liver Egg yolk Milk fat Fish oils Provitamin A carotenoids	Vitamin A status is dependent on adequate intake of proteins, calories and zinc Synthesis of RBP is zinc-dependent RDI: 4000–5000 IU (or 800–1000 RE*)/d for men and women Additional 1000 IU recommended during pregnancy and lactation. Toxicity: skin erythema, desquamation, abdominal pain, nausea. Excessive intake (over 18000 IU) in early pregnancy could lead to birth defects[4]

Flavonoids (most frequent in food: quercetin, flavone, kaempherol, rutin, hesperidin, hesperitin)	Hydro soluble Scavenging lipid peroxyl radicals Scavenger O_2^-, HO, $Fe(OH)^-_3$ Bind metal ions Inhibitor of enzymatic systems responsible for free radical production Anti-mutagenic effect	Apples, lemons, oranges Potatoes, cauliflower Tea Skin of tubers and roots Red wine	More than 3000 flavonoids have been identified in plants No RDI
n-3 fatty acids	Decreased leukotriene synthesis Inhibition of PGE_2 synthesis Growth regulation of malignant cells	Fish oils Fish and shellfish Soy and canola oil Leafy vegetables	Ratio of n-3:n-6 fatty acids in diet may be more important than absolute consumption levels No RDI Effective dose: 4 g/d for suppression of inflammation[4]
Magnesium	Co-factor in enzyme activation reactions requiring ATP Bronchodilator of airway smooth muscle Inhibits cholinergic neuromuscular transmission Stabilizes mast cells and T-lymphocytes	Nuts, legumes Cereal grains Corn, peas, carrots, parsley, spinach, lima beans Brown rice Seafood	RDI: 300–350 mg/d
Selenium	Co-factor for glutathione peroxidase, which reduces lipid and hydrogen peroxides Detoxification of heavy metals Role in DNA repair	Animal products, especially organ meats Seafood	Foods grown in selenium-poor soils may contribute to selenium deficiency in humans Vitamins C, E and A enhance selenium absorption Mercury and other heavy metals inhibit selenium absorption RDI: 55–70 μg/d RfD (daily intake without lifetime adverse effect): 0.05 mg/kg/d or 350 μg/d for a man of 70 kg[5] Toxicity: loss of hair and nails

* 1 RE= 6 μg β-carotene.
RDI = recommended daily intake; RBP = retinol binding protein; IU = international units; PGE_2 = prostaglandin E_2; ATP = adenosine triphosphate; RfD = reference dose.

1.2.2 Diet in COPD prognosis

Most of the efforts regarding the study of possible effects of diet on COPD prognosis have focused in counteracting unexplained weight loss using high-caloric nutritional support in clinical trials. It is known that nutritional status is a strong independent factor determining COPD progression [51]. However, the results of high caloric supplementation in mitigating weight loss, and improving the overall nutritional status, are not clear. A meta-analysis of the clinical trials regarding this topic up to 1998 did not identify improvements in anthropometric measures or functional exercise capacity among patients with stable COPD after caloric supplementation for more than 2 weeks [101]. A more recent review of the evidence stated that oral nutritional supplements, combined with physical training and anabolic agents, might change the prognosis of the disease in some cases [102]. However, nutritional intervention studies in COPD patients up to date have been limited by small sample sizes, short intervention periods and lack of relevant endpoints such as quality of life, body composition or exercise capacity.

The potential role of diet and dietary components, beyond high-caloric nutritional support, in COPD prognosis remains mostly unexplored. Given the proposed mechanisms of action and the available studies regarding loss of lung function, it is plausible to consider that most nutrients, foods and dietary patterns affecting COPD development could similarly affect COPD prognosis. However, the few studies evaluating loss of lung function or progression of COPD according to dietary components or supplements do not confirm this hypothesis. Intake of vitamin C, vitamin E, betacarotene, magnesium, fruit or fish was not related to decline in lung function in a cohort of 2512 middle aged Welshmen followed from 1979 to 1983 [87]. Similarly, the study of a British cohort of 2171 healthy adults followed from 1984 to 1991, found no association between average levels of fruit intake and FEV_1 decline, although changes in fresh fruit consumption levels were positively associated with changes in FEV_1 ($p=0.002$) [103]. Finally, the study of 1346 adults followed between 1991 and 2000, in the United Kingdom, found lower lung function decline amongst those with higher average vitamin C intake, although no relationships were found for intakes of vitamin A, vitamin E and magnesium [104]. Regarding supplementation studies, a small trial with 30 COPD patients daily supplemented with 400IU of vitamin E during 12 weeks showed that vitamin E supplementation does not have

any significant effect on spirometric measurements [105]. Similarly, a trial on 148 subjects with moderate-to-severe COPD and a primary component of emphysema, receiving retinoids or placebo for 6 months showed no definitive clinical benefits of retinoids on lung function, emphysema, or health-related quality of life [106]. Finally, no benefits from supplementation with alpha-tocopherol or betacarotene on COPD symptoms were found in a trial of 29133 male smokers aged 50–69 years between1985 and 1988 [107].

It is noteworthy that diet and dietary components could have an indirect impact on COPD prognosis through direct effects on comorbidities related to COPD such as anemia or diabetes, although these hypotheses remain unexplored.

1.2.3 Diet in Asthma

Given the abovementioned similarities between asthma and COPD, the reviews of the literature about diet and COPD also include the role of diet in asthma development and prognosis [79-82]. The introduction of fast food and the westernization of many worldwide dietary patterns have been proposed as one of the underlying causes behind the rise in asthma and allergy prevalence [108]. Therefore, several nutritional factors that may act on muscle constriction or inflammatory response have been hypothesized to play a role in asthma development [81]. Among them, sodium, magnesium, antioxidants, and omega-3 PUFA have been the most studied [79-82]. Studies at the food and dietary pattern levels have also been conducted, focusing in the role of fruits, vegetables, fish, and unhealthy foods such as junk food [79-82,109]. Given the fact that asthma usually appears at early ages in life, several studies have tried to assess the role of maternal food consumption during pregnancy and children's asthma [110-113]. However, these studies could not find a consistent role of dietary patterns or specific foods in children's asthma, except for fish intake. Overall, as it happens with COPD, most of the studies are cross-sectional or interventional, and their results have not been clear enough to warrant dietary recommendations for the prevention of asthma.

1.3 Methods in nutritional epidemiology

The inconsistency of some results when studying the role of diet in COPD, and other related chronic inflammatory lung disorders,

could be attributed to the methodology used for dietary assessment and the processing of the collected information. Therefore, a broader review of the most commonly used methods in nutritional epidemiology is required.

1.3.1 Methods for dietary assessment

Dietary assessment encompasses food consumption at the national level (*e.g.* food supply and production), household level, and individual level. Therefore, it is possible to monitor secular trends and geographical differences with stable and well-documented populations, as well as to assess specific nutrient intakes for a single subject. Given the complexity of analytical studies of diet and health, most studies require data at the individual level. Although nutritional epidemiology is a field with increasing interest, most methods to assess dietary intake are still imprecise and susceptible to deliberate over- or under-reporting by the subjects, incurring in measurement error and a substantial loss of power. Unfortunately, biological markers, such as urine nitrogen or serum vitamins, are insufficient to measure the broad range of nutrients that can be of interest, although they may be used as independent methods of validating dietary assessments. The most broadly used techniques designed to assess the usual diet of individuals are briefly summarized below.

Food frequency questionnaires
Food frequency questionnaires are the most used dietary assessment method in epidemiological studies. In this method, the respondent is asked to report his usual frequency of consumption of each food in a list, for a specific period of time. The questionnaire may be self-administered or administered by trained interviewers. In order to estimate the amount of each food, portion sizes are specified or asked. With this method daily intakes of each food in the questionnaire are estimated, but information such as specific characteristics of the foods or combination of foods during meals is not recorded. Their main limitation is the substantial amount of measurement error [114], given that the list of foods is limited and there are errors in the estimation of serving sizes and frequencies. In the other hand, this is a cheap and not very time-consuming method of assessing usual diet during an extended period of time, and it is the method of choice when aiming to obtain retrospective reports about past diet. Furthermore, many validated

food frequency questionnaires for different populations and uses are available in the literature.

24-hour recall

Another commonly used method for dietary assessment consists in asking to remember and report all foods consumed in the preceding 24 hours. This is conducted by a well-trained interviewer that asks probing questions, in a structured interview, designed to help the respondent remember all foods consumed throughout the day. Such interviews are usually repeated in different days of the week and different seasons in order to take into account the day to day, and seasonal, variability. An adequate training of the interviewer is very important in this method. The main strengths of this method are the easiness in recalling most of the dietary intakes, and the fact that given the burden on the respondants is low and irrespective of respondants' literacy, respondants are more likely to be representative of the study population. In the other hand, the main weaknesses of 24-hour recall are the day to day variability in the diet, so a single 24-hour recall is insufficient to characterize an individual's usual diet, and the key role of the interviewer ensuring an appropriate recall of the respondant food consumption.

Dietary records

In this method, the respondent records the characteristics and amount of each food consumed in a short period of time. The amount of each food is usually measured using a scale or household measures, and recorded at the time of each eating occasion. In order to appropriately record all information, the respondent must be trained and motivated, and an interviewer should review the records with the respondent to clarify entries and probe for forgotten foods. Therefore, this method requires both literate and motivated respondents. The main drawback of this method is that it is subject to bias both in the selection of the sample and the measurement of diet. Furthermore, the demanding task of recording all the foods consumed may alter dietary behaviors [115], and the cost of coding the collected information can be burdensome. In the other hand, this method can potentially provide accurate quantitative information on consumed foods.

1.3.2 Methods for processing dietary information

Assessing the usual food intake of a given individual may be of interest *per se*. However, many studies require information at other levels. Studies regarding the biological mechanisms behind the association of diet with specific health outcomes usually require information at the nutrient level. In the other hand, some epidemiological studies, not concerned for biological mechanisms, aim to have an overall measure of the diet, usually labeling an individual's diet as healthy or non-healthy. Overall, there are several methods for processing dietary information, and the most broadly used are summarized below.

Usual nutrient intake
Estimating the daily intake of different nutrients is a key feature of many epidemiological studies. In order to do so, food composition tables are used. Food composition tables include descriptions of most foods and the nutrient composition per 100 grams of each food. Therefore, the daily intake of each food may be transformed into the daily intake of each of its nutrients. There are very complete food composition tables, such as the provided by the U.S. Department of Agriculture [116]. However, given the regional variations on foods and their preparations, it is recommended to use specific regional tables. For example, the Centre d'Ensenyament Superior de Nutrició i Dietètica (CESNID) has published a specific food composition table including most Spanish foods [117].

Dietary patterns
Although most studies explore the effects of specific foods and/or nutrients, sometimes it is difficult to find specific associations with health outcomes given the strong correlations among them, or their small individual effects. Therefore, summarizing the whole diet of an individual in a single variable may be of great interest, and has been proposed as an interesting approach in nutritional epidemiology of chronic diseases [118]. Conceptually, dietary patterns represent a broader picture of food and nutrient consumption, and may thus be more predictive of disease risk than individual foods or nutrients [119]. Dietary patterns are constructed from the usual food intake using different statistical methods, such as factor analysis [119]. These statistical methods identify a small number of dietary patterns according to the correlations between foods. Each subject receives a factor score for each identified pattern, representing the adherence of the subject to the pattern.

Thereafter, these summary scores can be used in regression analysis to examine the relationship between the identified patterns and the risk of a given disease. It is usual to categorize the subjects according to the adherence scores and compare the risk of disease between the lowest and highest categories.

Dietary scores

Another method to summarize the whole diet of an individual is the use of dietary quality indices. These indices or scores aim to evaluate the adherence to a certain dietary pattern such as the Mediterranean dietary pattern, or the adequacy to current dietary guidelines [119]. The main difference with dietary pattern analysis is that the score is not constructed on statistical criteria. Rather than that, it is build using an *a priori* defined set of items based on available knowledge. These items may consist on: (i) positive or negative scoring given the amount of certain foods that is consumed; (ii) additions, substractions or ratios between certain foods or nutrients; or (iii) specific statements about how or when certain foods are eaten. Many scores or indices have been developed so far in order to measure specific dietary patterns. However, those regarding the Mediterranean dietary pattern are the most popular and have been extensively reviewed [120].

2

Rationale

COPD has become a global health and economic concern. Without diminishing the crucial role of anti-smoking health actions to reduce COPD development and improve COPD prognosis, the identification of other modifiable individual factors that affect this disease should be considered a priority.

Although the available evidence is not consistent enough, prior studies have shown associations between dietary factors and lung function or COPD incidence [79-82]. However, the potential role of diet in COPD progression remains unknown. Little or none research has been conducted in order to assess the potential impact of dietary factors on COPD persistent inflammation, nor other manifestations of the disease. Moreover, there is a lack of longitudinal studies evaluating the impact of diet on COPD prognosis.

Considering all these facts, identifying foods or nutrients that could potentially affect the progression of the disease would be very relevant from a public health perspective, especially given the potential benefits and low costs of medical advice. The availability of a cohort of well characterized COPD patients, with information about inflammatory and oxidative stress biomarkers, constitutes an outstanding opportunity to enhance the current knowledge about the relation between diet and COPD.

3

Objectives

3.1 General objective

This thesis aimed to give insight in the role of diet in chronic respiratory diseases (COPD and asthma). The general objective was to estimate the effects of dietary habits in pathophysiological markers and evolution of COPD.

To this effect, a specific dietary protocol was included as an ancillary study in a well phenotyped cohort of COPD patients participating in the Phenotype and Course of Chronic Obstructive Pulmonary Disease (PAC-COPD) study. Additionally, a complementary analysis regarding diet and asthma was performed in a sample of Mexican children from the International Study of Asthma and Allergies in Childhood (ISAAC).

Five specific objectives were formulated and reported in five corresponding original manuscripts. They are listed following along with a brief introduction to both PAC-COPD and ISAAC studies.

3.2 Specific objectives

The PAC-COPD study

The Phenotype and Course of Chronic Obstructive Pulmonary Disease (PAC-COPD) study aims to improve our understanding about the phenotypic heterogeneity of COPD and the extent to which this heterogeneity is related to its clinical course. This is a cohort study of 342 patients with COPD, recruited between January 2004 and March 2006 in 9 Spanish tertiary hospitals. All subjects had their COPD diagnosis confirmed (post-bronchodilator forced expiratory volume in the first second to forced vital capacity ratio (FEV_1/FVC)≤0.70) [121] in a clinically stable condition, at least 3 months after discharge. Patients have been followed for a minimum of 5 years with regard to hospital admissions and mortality, periodic

clinical assessments have been performed every 18-24 months, as well as short telephonic evaluations interspersed between them. Nine to 12 months after inclusion in the PAC-COPD study (from January 2005 to December 2007), patients were asked to participate in a telephone interview to assess dietary habits. Finally, 275 patients (80% of the PAC-COPD cohort) had available dietary information, and therefore these are the subjects included in this thesis analyses. Detailed information on PAC-COPD recruitment, methods and results has been previously published [122].

Specific objective I:

- To describe the dietary habits, and adequacy according to dietary recommendations, of a sample of COPD patients.

Dietary habits of firstly admitted Spanish COPD patients.
de Batlle J, Romieu I, Antó JM, Mendez M, Rodríguez E, Balcells E, Ferrer A, Gea J, Rodriguez-Roisin R, Garcia-Aymerich J; PAC-COPD Study Group.
Respir Med 2009;103:1904-10.

Specific objective II:

- To assess the association between dietary intake of antioxidants and oxidative stress markers in a sample of COPD patients.

Dietary modulation of oxidative stress in chronic obstructive pulmonary disease patients.
de Batlle J, Barreiro E, Romieu I, Mendez M, Gómez FP, Balcells E, Ferrer J, Orozco-Levi M, Gea J, Antó JM, Garcia-Aymerich J.
Free Radic Res 2010;44:1296-303.

Specific objective III:

- To assess the association between dietary intake of omega-3 and omega-6 fatty acids and inflammatory markers in a sample of COPD patients.

Association between Ω3 and Ω6 fatty acid intakes and serum inflammatory markers in COPD.

de Batlle J, Sauleda J, Balcells E, Gómez FP, Mendez M, Rodriguez E, Barreiro E, Ferrer J, Romieu I, Gea J, Antó JM, Garcia-Aymerich J, and the PAC-COPD Study Group.
(under review)

Specific objective IV:

- To assess the longitudinal association between dietary intake of cured meats and COPD hospitalizations in a cohort of COPD patients.

Cured meats consumption increases risk of hospitalization in COPD patients
de Batlle J, Balcells E, Gómez FP, Mendez M, Rodriguez E, Ferrer J, Romieu I, Antó JM, Garcia-Aymerich J, and the PAC-COPD Study Group.
(under review)

The Mexicali ISAAC study

The International Study of Asthma and Allergies in Childhood (ISAAC) is an epidemiological research program aimed to investigate asthma, rhinitis and eczema in children [123]. ISAAC is a collaborative research project established in 1991, involving more than 100 countries and 2 million children. In Mexicali, Mexico, 2528 children 6- to 7-year old, from all primary public and private schools, were recruited according to ISAAC methodology in 2004. Parents were asked to complete the ISAAC questionnaire on respiratory and allergic symptoms. Additionally, information regarding potential risk factors, such as diet or air pollutants exposure, was assessed in samples of these children. Up to 1476 children had available dietary information sufficient to compute a Mediterranean diet score and/or the score of their mothers during pregnancy. Therefore these are the children included in the analyses included in this thesis.

Specific objective V:

- To assess the association between the adherence to a Mediterranean dietary score of a sample of 6- to 7-year old

children and their mothers during pregnancy, and childhood asthma and allergic rhinitis.

Mediterranean diet is associated with reduced asthma and rhinitis in Mexican children.

de Batlle J, Garcia-Aymerich J, Barraza-Villarreal A, Antó JM, Romieu I.

Allergy 2008;63:1310-6.

4

Results

4.1 Paper I

Dietary habits of firstly admitted Spanish COPD patients.

de Batlle J, Romieu I, Antó JM, Mendez M, Rodríguez E, Balcells E, Ferrer A, Gea J, Rodriguez-Roisin R, Garcia-Aymerich J; PAC-COPD Study Group.

Respir Med 2009;103:1904-10.

4.2 Paper II

Dietary modulation of oxidative stress in chronic obstructive pulmonary disease patients.

de Batlle J, Barreiro E, Romieu I, Mendez M, Gómez FP, Balcells E, Ferrer J, Orozco-Levi M, Gea J, Antó JM, Garcia-Aymerich J.

Free Radic Res 2010;44:1296-303.

4.3 Paper III

Association between Ω3 and Ω6 fatty acid intakes and serum inflammatory markers in COPD.

de Batlle J, Sauleda J, Balcells E, Gómez FP, Mendez M, Rodriguez E, Barreiro E, Ferrer J, Romieu I, Gea J, Antó JM, Garcia-Aymerich J, and the PAC-COPD Study Group.

(under review)

Association between Ω3 and Ω6 fatty acid intakes and serum inflammatory markers in COPD

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Abstract

Dietary intake of polyunsaturated fatty acids, including omega-3 and omega-6, could modulate chronic obstructive pulmonary disease (COPD) persistent inflammation. We aimed to assess the relationship between dietary intake of omega-3 and omega-6 fatty acids and serum inflammatory markers in COPD.

250 clinically stable COPD patients were included. Dietary data of the last 2 years was assessed using a validated food frequency questionnaire (122 items), which provided levels of three omega-3 fatty acids: docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and α-linolenic acid (ALA); and two omega-6 fatty acids: linoleic acid (LA) and arachidonic acid (AA). Inflammatory markers (C-reactive protein (CRP), interleukin 6 (IL-6), interleukin 8 (IL-8) and tumor necrosis factor alpha (TNFα)) were measured in serum. Fatty acids and inflammatory markers were dichotomised according to their median values, and their association assessed using multivariate logistic regression.

Higher intake of ALA (an antiinflammatory omega-3 fatty acid) was associated with lower TNFα concentrations (adjusted OR=0.46; p=0.049). Higher AA intake (a proinflammatory omega-6 fatty acid) was related to higher IL-6 (OR=1.96; p=0.034) and CRP (OR=1.95; p=0.039) concentrations.

This study provides the first evidence of an association between dietary intake of omega-3 and omega-6 fatty acids and serum inflammatory markers in COPD patients.

Introduction

Chronic obstructive pulmonary disease (COPD) is a major cause of morbidity and mortality worldwide, and is expected to become the fourth leading cause of mortality by 2030 [1]. COPD is characterized by a complex chronic inflammatory condition usually associated with smoking-induced inflammation and oxidative stress [2]. This persistent inflammatory condition is located not only in the lungs [3] but also in extrapulmonary organs and tissues [4]. Levels of systemic inflammatory markers increase during COPD exacerbations [5], suggesting that inflammatory processes play a key role in COPD evolution [6]. A longitudinal population-based study has reported lower lung function decline in subjects with decreasing levels of C-reactive protein (CRP), an inflammatory marker, when compared with subjects with stable or increasing levels, suggesting that reducing the levels of circulating inflammatory markers could be an effective way of reducing lung function decline [7]. Finally, CRP serum levels have been related to mortality in COPD patients [8,9].

It has been hypothesized that dietary intake of polyunsaturated fatty acids (PUFA), including omega-3 and omega-6 fatty acids, could modulate persistent inflammation in COPD [10-12], although this hypothesis has never been tested so far. It is known that omega-3 fatty acids mostly promote antiinflammatory activities [13]. In contrast, omega-6 fatty acids are the most relevant precursors of proinflammatory eicosanoids, and therefore mostly mediate proinflammatory activities [14]. We hypothesised that COPD patients with higher omega-3 and lower omega-6 intakes would have lower levels of circulating inflammatory mediators.

Therefore, this study aims to assess the association between dietary intakes of omega-3 and omega-6 fatty acids and several serum inflammatory markers, especifically C-reactive protein (CRP), interleukin 6 (IL-6), interleukin 8 (IL-8) and tumor necrosis factor alpha (TNFα) in COPD patients, in the frame of the 'Phenotype and Course of COPD Project (PAC-COPD)' [15].

Subjects and methods

Study population

This study is a cross-sectional analysis in the 'Phenotype and Course of COPD Project (PAC-COPD)'. Briefly, the sample includes COPD patients recruited during their first hospital admission at 9 universitary hospitals in Spain between January 2004 and March 2006, with a confirmed diagnosis of COPD (post-bronchodilator forced expiratory volume in the first second to forced vital capacity ratio (FEV_1/FVC) ≤ 0.70) [16] in a clinically stable condition, at least three months after discharge. Detailed information on PAC-COPD recruitment, methods and results is available elsewhere [17]. The protocol was approved by the Ethics Committees of all the participating hospitals, and written informed consent was obtained from all the COPD patients.

Of the 342 patients included in the PAC-COPD cohort, a total of 250 had available information on dietary PUFA and serum inflammatory markers. No differences regarding socio-demographic characteristics, comorbidities, dyspnea, or lung function parameters were found between PAC-COPD patients who provided dietary information and those who did not, as previously published [18]. All epidemiological and clinical

measures as well as blood samples were obtained during clinical stability at least 3 months after recruitment.

Dietary assessment

A previously validated 122-item food frequency questionnaire (FFQ) [19] asking for dietary habits in the last 2 years was administered by trained interviewers. Reported information was converted into a daily intake frequency of each food, which was in turn converted into the daily intake in grams per day for each food. A food composition table from the U.S. Department of Agriculture [20] was used to estimate intakes of three omega-3 fatty acids: docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and α-linolenic acid (ALA); and two omega-6 fatty acids: linoleic acid (LA) and arachidonic acid (AA). Additionally, the following ratios between omega-3 and omega-6 fatty acids were computed: ALA/LA ratio, EPA/AA ratio, and DHA/AA ratio. More details about the development and validation of the questionnaire have been previously published [19]. Additionally, our group tested the reproducibility of the questionnaire when telephonically administered in a subsample of 18 subjects. Briefly, moderate to high correlations were found between the first and second questionnaire administration, and no statistically significant differences in means of intakes of most food groups, macro- and micro-nutrients were found [18].

Systemic inflammation

Blood samples were obtained after fasting overnight, and inmediatelly centrifuged at 2000-3000 rpm for 10 minutes. Serum was extracted and stored in cryotubes at -80ºC. Serum levels of high sensitivity C-reactive protein (CRP) were determined by nephelometry, and those of interleukin 6 (IL-6), interleukin 8 (IL-8) and tumor necrosis factor alpha (TNFα) by high sensitivity ELISA kit (Biosource, Camarillo, CA, USA). All analyses were performed in duplicate centrally at Hospital Universitari Son Dureta (Palma Mallorca, Spain). The lower limits of detection of these assays were 0.16 mg/L, 0.104 pg/ml, 0.10 pg/ml, and 0.09 pg/ml for CRP, IL-6, IL-8 and TNFα respectively. Intra-assay variation was always <10%, and reported values correspond to the average of the two determinations.

Clinical and Functional assessment

Information regarding socio-demographic characteristics, pharmacological treatment, respiratory symptoms, and lifestyle was obtained using a standardized epidemiological questionnaire. Nutritional status was assessed through body mass index (BMI). Post-bronchodilator spirometry (FEV_1, FVC, and FEV_1/FVC ratio), and arterial oxygen and carbon dioxide partial pressures (PaO_2, $PaCO_2$) were also measured. The Charlson index of comorbidity [21] was obtained by an expert pulmonologist from medical records and personal anamnesis and exploration. Detailed information on the methods is described elsewhere [15,17].

Statistical analysis

Socio-demographic and clinical characteristics, intakes of omega-3 and omega-6 fatty acids, and inflammatory markers were described by mean (SD), median (P25-P75) or number (%), as appropriate according to the distribution of each variable. Given their skewed distribution, inflammatory marker concentrations were dichotomised according to their median values (TNF-α: 0.238 pg/ml, IL-6: 1.004 pg/ml, IL-8: 4.296 pg/ml, and PCR: 0.37 mg/L). Levels of omega-3 and omega-6 fatty acid intakes according to

inflammatory markers categories (above or below corresponding median values) were compared using Students' t test.

The association between PUFAs intake and high levels (above the median) of inflammatory markers was estimated using logistic regresion models. In order to improve the interpretability of the results, PUFAs variables were also dichotomised at their median values (corresponding medians were: DHA: 0.42 g/day, EPA: 0.21 g/day, ALA: 1.22 g/day, LA: 11.21 g/day, AA: 0.18 g/day, ALA/LA: 0.108, EPA/AA: 1.152, DHA/AA: 2.358). Lower intakes were always used as the reference category. The following counfounders were considered and included in the final models if they were related to both the exposure and the outcome, or modified (>10% change in coefficient) the estimates for the variables of interest in each model: age, gender, BMI, FEV_1, smoking status, reported physical activity, total caloric intake, inhaled corticosteroid treatment, statin treatment, and the Charlson index of comorbidity. Finally, a multivariate model for each inflammatory marker was built including all five fatty acids and confounders. Similarly, a multivariate model for each inflammatory marker was built including all three ratios and confounders. Effect modification by smoking status was assessed by means of both stratification of final models and inclusion of interaction terms. The goodness of fit of all the models was assessed using Hosmer-Lemeshow test [22]. As a sensitivity analysis, all analyses were repeated excluding women (7% of total subjects). Data analysis was conducted using Stata 8.2 (StataCorp, College Station, TX, USA).

Results

Table 1 shows the main characteristics of the patients. Ninety-three percent of participants were males with a mean age of 68 years. Most subjects had moderate to severe COPD (distribution in COPD severity stages: 4% mild, 54% moderate, 35% severe, and 7% very severe).

In the bivariate analysis, higher intake of ALA (omega 3, anti-inflammatory) was related to lower TNFα levels (1.30 ALA g/day in the low TNFα category *versus* 1.21 g/day in the high TNFα category, p=0.03). Regarding omega 6 (pro-inflammatory), higher intakes of LA and AA were respectively associated with higher CRP and IL-6 levels (LA: 11.31 g/day in the low *vs* 12.35 g/day in the high CRP group, p=0.03; AA: 0.185 g/day in the low *vs* 0.202 g/day in the high IL-6 group, p=0.05). Remaining comparisons did not provide statistically significant differences.

Tables 2 and 3 show crude and adjusted associations between fatty acids intake and each of the inflammatory markers. Being in the higher category of ALA intake was associated with significantly lower TNFα serum concentrations (adjusted OR=0.46; p=0.049). Being in the higher category of AA intake was associated with higher serum concentrations of IL-6 (adjusted OR=1.96; p=0.034). Elevated intake of AA was associated with a higher concentration of serum CRP (adjusted OR=1.95; p=0.039). Regarding the omega3:omega6 ratios (Table 3), DHA/AA ratio was associated with TNFα serum concentrations (adjusted OR=3.02; p=0.045). No effect modification by smoking status was observed. Sensitivity analysis excluding women yielded very similar results.

Discussion

Our study with 250 clinically stable COPD patients showed for the first time that dietary intake of omega 3 and omega 6 fatty acids relates to the level of serum inflammatory markers. Specifically, high dietary intake of ALA (an antiinflammatory omega-3 fatty acid) was associated with reduced risk of high levels of serum TNFα, while dietary intake of AA (a proinflammatory omega-6 fatty acid) was related to increased risk of elevated IL-6 and CRP.

Our finding of an inverse association between ALA intake and TNFα is consistent with previous studies in both healthy populations and among subjects with cardiovascular disease, which showed a negative correlation between omega-3 fatty acid intake and several proinflammatory biomarkers including CRP, IL-6 and TNFα [13], involved in key inflammatory processes such as NF-κβ activation and MAPK pathways (TNFα), as well as in acute phase response (IL-6). Regarding omega-6 fatty acids, our results are also in agreement with the common assumption that omega-6 fatty acids may be pro-inflammatory [14], although it has been noted that the relationship between dietary omega-6 fatty acids and pro-inflammatory mediators is rather complex and not easily predictable [24]. A 25-year prospective study conducted in the Netherlands, found an increased risk of incidence of chronic lung diseases associated with LA intake (relative risk of 1.55, 95% CI=1.11-2.16), although it failed to find any associations with omega-3 fatty acids [25]. Interestingly, there are cross-sectional studies showing that omega-6 fatty acid pro-inflamatory activities occur only when the intake of omega-3 fatty acids is low [26].

Biochemical evidence about the production of pro- and antiinflammatory mediators derived from different types of PUFA supports the biological plausibility of our findings [14,24,27]. EPA and DHA, and ALA via conversion into EPA or DHA, have several ways of mediating antiinflammatory activities. One pathway involves the inhibition of AA metabolism, as these omega-3 fatty acids can compete with AA as a constituent of lipidic membrans, or directly compete as substrates for cyclooxygenases and lipooxygenases leading to synthesis of less bioactive mediators than those derived from AA [14,27]. Additionally, EPA and DHA could reduce NF-κβ DNA-binding proinflammatory activities, leading to reduced cytokine expression [24]. Alternatively, AA, and LA via conversion into AA, is the most relevant precursor of eicosanoids (prostaglandins, prostacyclins, thromboxanes and leukotrienes) which have predominantly proinflammatory activities [14,27]. Although mediators such as lipoxins and resolvins may derive from the AA cascade and have antiinflammatory activities, these are minor paths in the cascade [24,27].

The lack of associations between PUFA intake and IL-8 may be explained by the specific functions of this cytokine. IL-8 is a potent chemoattractant that is needed to recruit and activate neutrophils. IL-8 has been found to be increased in bronchoalveolar lavage of smokers with emphysema [28]. Therefore, IL-8 could be considered a local, rather than systemic, marker of inflammation and in consequence, finding an association between PUFA intake and IL-8 serum levels would have been unexpected, although it is plausible that this association could arise if measuring IL-8 levels in the airways rather than the serum.

It has been proposed that the ratios between omega-3 and omega-6 fatty acid could be more appropriate for assessing health effects than individual fatty acids levels [27]. Our study found estimates coherent with our hypotheses in 7 out of 8 of the associations between ALA/LA and EPA/AA ratios and inflammatory markers, although none of them achieved statistical significance. Results with DHA/AA ratio were in the opposite direction that would had been expected, and statistically significant for TNFα. Authors atribute the later results to a potential problem in the derivation of this nutrient from food composition tables, which is supported by the fact that DHA also provided unexpected results when assessed individually.

In our cohort, the main source of omega-3 fatty acids was fish, mainly fresh sardines and tuna, while the main sources of omega-6 fatty acids were vegetable oils, poultry, eggs and baked goods. Therefore, our findings are consistent with previous literature suggesting an association between foods rich in PUFA, such as fatty fish, and respiratory function, COPD symptoms, or the prevalence of other chronic lung diseases [10-12]. This manuscript, along with previous literature suggesting that reducing the levels of circulating inflammatory markers could be an effective way of reducing lung function decline [7], provides data about potential mechanisms for these associations.

Limitations to the current study include: (i) measurement error in the estimation of dietary fatty acids intake due to the use of a food frequency questionnaire; (ii) reduced variability in PUFA levels in our sample, as mean daily intake of PUFA was high (99% of patients had intakes above the Spanish recommendations [18]); and (iii) the lack of repeated data about inflammatory markers, that could have short-term variability [29]. All previous limitations have most likely resulted in a reduced statistical power (calculated *a posteriori* no greater than 60%), thus yielding a considerable risk of false negative results. The cross-sectional study design makes impossible to establish the direction of the observed associations. However, it seems unlikely that dietary habits would change as a result of serum inflammatory marker levels, and much more likely that the latter may have been affected by habitual diet.

In conclusion, this study provides the first evidence of an association between dietary intake of omega-3 and omega-6 fatty acids and serum inflammatory markers in COPD patients, thus providing evidence to support considering the inclusion of dietary recommendations in COPD management guidelines.

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Table 1. Description of sociodemographic and clinical data, polyunsaturated omega-3 and omega-6 fatty acids, and levels of serum inflammatory markers, in 250 COPD patients.

Male [n (%)]	234 (93.6)
Age (years) [m (SD)]	68 (8)
Primary or higher education [n (%)]	145 (58)
Active worker [n (%)]	43 (17.2)
Low socioeconomic status* [n (%)]	190 (82.25)
BMI (kg/m^2) [n (%)]	
<20	4 (1.6)
≥20 & <25	46 (18.4)
≥25 & <30	106 (42.4)
≥30	94 (37.6)
Positive skin prick test [n (%)]	30 (12)
>1 Comorbidities (Charlson index) [n (%)]	137 (54.8)
Dyspnea score (MMRC, score 0-5) median (P25-P75)	2 (2-3)
Post-bronchodilator FEV1 (% predicted) [m (SD)]	53 (16)
PaO_2 (mmHg) [m (SD)]	75 (11)
Inhaled corticosteroid treatment [n (%)]	165 (66)
Statin treatment [n (%)]	38 (15)
Current smokers [n (%)]	78 (31)
Regular physical activity (kcal/week) [m (SD)]	6708 (4976)
DIETARY INTAKE	
Daily energy intake (kcal/day) [m (SD)]	2026 (614)
Docosahexaenoic acid (DHA) (g/day) [m (SD)]	0.49 (0.28)
Eicosapentaenoic acid (EPA) (g/day) [m (SD)]	0.23 (0.13)
α-linolenic acid (ALA) (g/day) [m (SD)]	1.26 (0.39)
Linoleic acid (LA) (g/day) [m (SD)]	11.9 (4.34)
Arachidonic acid (AA) (g/day) [m (SD)]	0.2 (0.09)
Ratio ALA/LA [m (SD)]	0.11 (0.02)
Ratio EPA/AA [m (SD)]	1.22 (0.56)
Ratio DHA/AA [m (SD)]	2.53 (1.14)
SERUM INFLAMMATORY MARKERS	
Tumor necrosis factor α (TNFα) (pg/ml) [median (P25-P75)]	0.23 (0.05-1.02)
Interleukin 6 (IL-6) (pg/ml) [median (P25-P75)]	1.01 (0.55-1.96)
Interleukin 8 (IL-8) (pg/ml) [median (P25-P75)]	4.29 (3.24-5.75)
C Reactive protein (CRP) (mg/L) [median (P25-P75)]	0.37 (0.16-0.66)

* Skilled or unskilled manual workers classified as low socioeconomic status.

Table 2. Crude and adjusted associations between polyunsaturated fatty acids, and serum inflammatory markers, in a sample of 250 COPD patients.

	Tumor necrosis factor α (TNFα) > 0.238 pg/ml			
	Crude OR	p-value	Adjusted OR*	p-value
Omega 3 (anti-inflammatory):				
Docosahexaenoic acid (DHA) > 0.42 g/day	1.52	0.101	2.58	0.158
Eicosapentaenoic acid (EPA) > 0.21 g/day	1.38	0.206	0.68	0.563
α-linolenic acid (ALA) > 1.22 g/day	0.68	0.130	0.46	0.049
Omega 6 (pro-inflammatory):				
Linoleic acid (LA) > 11.21 g/day	0.94	0.800	1.56	0.264
Arachidonic acid (AA) > 0.18 g/day	1.14	0.613	0.99	0.964

	Interleukin 6 (IL-6) > 1.004 pg/ml			
	Crude OR	p-value	Adjusted OR*	p-value
Omega 3 (anti-inflammatory):				
Docosahexaenoic acid (DHA) > 0.42 g/day	0.97	0.899	0.83	0.774
Eicosapentaenoic acid (EPA) > 0.21 g/day	1.00	1.000	0.92	0.900
α-linolenic acid (ALA) > 1.22 g/day	1.07	0.800	1.24	0.577
Omega 6 (pro-inflammatory):				
Linoleic acid (LA) > 11.21 g/day	1.21	0.448	1.29	0.516
Arachidonic acid (AA) > 0.18 g/day	1.67	0.044	1.96	0.034

	Interleukin 8 (IL-8) > 4.296 pg/ml			
	Crude OR	p-value	Adjusted OR*	p-value
Omega 3 (anti-inflammatory):				
Docosahexaenoic acid (DHA) > 0.42 g/day	1.17	0.527	1.76	0.383
Eicosapentaenoic acid (EPA) > 0.21 g/day	1.14	0.613	0.58	0.405
α-linolenic acid (ALA) > 1.22 g/day	0.94	0.800	0.75	0.455
Omega 6 (pro-inflammatory):				
Linoleic acid (LA) > 11.21 g/day	1.14	0.613	1.25	0.574
Arachidonic acid (AA) > 0.18 g/day	1.47	0.130	1.56	0.154

	C-reactive protein (CRP) > 0.37 mg/l			
	Crude OR	p-value	Adjusted OR*	p-value
Omega 3 (anti-inflammatory):				
Docosahexaenoic acid (DHA) > 0.42 g/day	0.97	0.899	1.71	0.426
Eicosapentaenoic acid (EPA) > 0.21 g/day	0.88	0.613	0.40	0.181
α-linolenic acid (ALA) > 1.22 g/day	1.57	0.077	2.00	0.076
Omega 6 (pro-inflammatory):				
Linoleic acid (LA) > 11.21 g/day	1.67	0.044	1.86	0.120
Arachidonic acid (AA) > 0.18 g/day	1.67	0.044	1.95	0.039

* An adjusted model has been built for each inflammatory marker, including all five polyunsaturated fatty acids and body mass index, total caloric intake and smoking status.

Table 3. Crude and adjusted associations between polyunsaturated fatty acid intake ratios, and serum inflammatory markers, in a sample of 250 COPD patients.

	Tumor necrosis factor α (TNFα) > 0.238 pg/ml			
	Crude OR	p-value	Adjusted OR*	p-value
Ratio ALA/LA > 0.1084	0.83	0.448	0.76	0.295
Ratio EPA/AA > 1.152	1.25	0.376	0.50	0.204
Ratio DHA/AA > 2.358	1.62	0.058	3.02	0.045

	Interleukin 6 (IL-6) > 1.004 pg/ml			
	Crude OR	p-value	Adjusted OR*	p-value
Ratio ALA/LA > 0.1084	0.88	0.613	0.92	0.758
Ratio EPA/AA > 1.152	0.70	0.165	0.43	0.106
Ratio DHA/AA > 2.358	0.85	0.527	1.85	0.243

	Interleukin 8 (IL-8) > 4.296 pg/ml			
	Crude OR	p-value	Adjusted OR*	p-value
Ratio ALA/LA > 0.1084	1.07	0.800	1.04	0.881
Ratio EPA/AA > 1.152	0.85	0.527	0.66	0.407
Ratio DHA/AA > 2.358	0.91	0.704	1.32	0.579

	C-reactive protein (CRP) > 0.37 mg/l			
	Crude OR	p-value	Adjusted OR*	p-value
Ratio ALA/LA > 0.1084	0.83	0.448	0.87	0.577
Ratio EPA/AA > 1.152	0.80	0.376	0.90	0.826
Ratio DHA/AA > 2.358	0.80	0.376	0.91	0.848

* An adjusted model has been built for each inflammatory marker, including all three polyunsaturated fatty acid ratios and body mass index, total caloric intake and smoking status.

4.4 Paper IV

Cured meats consumption increases risk of hospitalization in COPD patients.

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(under review)

Cured meats consumption increases risk of readmission in COPD patients

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Abstract

Rationale: Recent studies have shown that a high dietary intake of cured meat increases the risk of COPD development. However, its potential effects on COPD evolution have not been tested.
Objective: To assess the association between dietary intake of cured meat and risk of COPD readmission in COPD patients.
Methods: 274 COPD patients were recruited during their first COPD admission between 2004 and 2006, provided information on dietary intake of cured meat during the previous 2 years, and were followed through December 31st 2007 (median follow-up 2.6 years). Associations between cured meat intake and COPD admissions were assessed using parametric regression survival-time models.
Measurements and Main Results: Mean(SD) age was 68(8) years, 93% of patients were males, 42% were current smokers, mean post-bronchodilator FEV_1 was 53(16)% predicted, and median cured meat intake was 23g/day. After adjusting for age, FEV_1, and total caloric intake, high cured meat intake (> median value) increased the risk of COPD readmission (adjusted Hazard Ratio (95% confidence interval) 2.02 (1.31 - 3.12), p=0.001).
Conclusions: High cured meat consumption increases the risk of COPD readmission in COPD patients, which suggests potential public health benefits from recommending dietary shifts to reduce intakes of these foods in COPD patients.

Introduction

Chronic obstructive pulmonary disease (COPD) is a major cause of morbidity and mortality worldwide, and is expected to become the fourth leading cause of mortality by 2030 [1]. Although cigarette smoking is the main risk factor for COPD, interest has recently increased towards the hypothesis that specific components of the diet could play a role in the development of the disease [2,3]. Fruits and vegetables have captured most of the interest given their antioxidant properties, and more recently several studies have pointed out that cured meat could have deleterious effects [4-6]. Two US prospective cohorts have shown an increased risk of COPD incidence among subjects reporting higher cured meat intake [5,6]. This effect has been attributed to the fact that nitrites, which are added as preservatives and color fixatives during cured meat production [7], could increase the nitrosative stress burden of the lung via the formation of reactive nitrogen species [8], causing damage and remodeling of the lung parenchyma [9]. A logical argument is that the lung injury should not only lead to an increased risk of chronic lung disease but also to a worse evolution. However, whether cured meat consumption modifies COPD prognosis has never been tested. The present study aims to assess the association between frequency of cured meat consumption and risk of COPD rehospitalization in a cohort of COPD patients, in the frame of the 'Phenotype and Course of COPD Project (PAC-COPD)' [10]. We hypothesized that subjects with greater intake of cured meat would be at a higher risk of COPD rehospitalization.

Subjects and methods

Study population

The PAC-COPD cohort includes subjects recruited during their first COPD hospital admission at 9 university hospitals in Spain between January 2004 and March 2006, and followed up to December 31st 2007. All measures, and the confirmation of COPD diagnosis (post-bronchodilator forced expiratory volume in the first second to forced vital capacity ratio ($FEV_1/FVC \leq 0.70$) [11] were obtained during clinical stability at least 3 months after recruitment. Details on recruitment and methods are available elsewhere [10,12]. The protocol was approved by the Ethics Committees of all participating hospitals, and written informed consent was obtained from all patients.

Measurements

At baseline, a previously validated 122-item food frequency questionnaire (FFQ) [13,14] asking for dietary habits in the last 2 years was administered by trained interviewers. Cured meat consumption was defined as the total daily consumption (grams/day) of cooked ham, Spanish cured ham, cured and other sausages, and hot dogs, based on five FFQ items.

Baseline socio-demographic characteristics, respiratory symptoms, drug treatment, and lifestyle were obtained using standardized questionnaires. Nutritional status was assessed through body mass index (BMI) and fat free mass index (FFMI). Post-bronchodilator spirometry (FEV_1, FVC, and FEV_1/FVC), arterial oxygen and carbon dioxide partial pressures (PaO_2, $PaCO_2$), carbon monoxide diffusing capacity (DLco), and serum C-reactive protein (CRP) were measured. The Charlson index of comorbidity

[15] was obtained by an expert pulmonologist from medical records and personal anamnesis and exploration.

Information on COPD rehospitalizations through December 31st 2007 was obtained from the Minimum Basic Dataset (CMBD), a national administrative database. According to the 9th revision of the International Classification of Diseases, COPD exacerbations were defined as any admission with codes 466, 480-486, 490-496, or 518.81 as the main diagnosis. Survival status was obtained for all patients from direct interviews with the patients or their relatives.

Statistical analysis

Socio-demographic, clinical, and dietary characteristics were described by mean (SD), median (P25-P75) or number (%), as appropriate. Cured meat intake was treated either as a continuous variable or dichotomised at its median value (22.68g/day). Median time to next COPD readmission was compared between cured meat intake levels using the Mann-Whitney test. Kaplan-Meier curves of time to COPD readmission were plotted according to cured meats consumption level, and compared using the log-rank test [16]. Crude and adjusted associations between cured meat intake and COPD readmission were assessed using parametric regression survival-time models, censoring subjects who died before a COPD readmission (n=2) [17]. The following counfounders were considered and included in the final model if they were related to both the exposure and the outcome, or modified (>10% change in Hazard Ratio) the estimates for the remaining variables: age, gender, BMI, FFMI, FEV_1, PCO_2, PO_2, smoking status, physical activity, inhaled corticosteroid treatment, statin treatment, Charlson index of comorbidity, and intakes of energy, fruit, vegetables and fish. Effect modification by smoking status, inhaled corticosteroid treatment, COPD severity and CRP levels was assessed by both stratification of all models and inclusion of interaction terms. Data analysis was conducted using Stata 8.2 (StataCorp, College Station, TX, USA).

Results

From the total PAC-COPD cohort (n=342), 274 patients had available information on diet. No differences regarding socio-demographic characteristics, comorbidities, dyspnea, or lung function parameters were found between patients with and without dietary information, as previously published [13]. Table 1 shows the main characteristics of the 274 COPD patients included in this study. Ninety-three percent of participants were males with a mean age of 68 years. Most subjects had moderate to severe COPD (distribution in COPD severity stages: 5% mild, 52% moderate, 37% severe, and 6% very severe). Mean cured meat intake was similar across COPD severity stages (mild: 28g/day, moderate: 26g/day, severe: 22g/day, and very severe: 30g/day). As shown in Table 2, the median follow up time was 934 days – 2.6 years– with a minimum of 250 days and a maximum of 1337 days, and 97 subjects (35%) had at least one COPD hospital readmission. Although 5% of patients died during follow-up, only 2 (1%) died before any COPD admission, thus contributing to the analysis until death.

Subjects with low cured meat intake had longer time until the first COPD readmission than subjects with high intake (median 801 days *versus* 608 days; p-value 0.002). Kaplan-Meier curves show that the time to the first COPD rehospitalization was longer in the low cured meat intake group (p=0.028) (Figure 1).

Table 3 shows that both in the crude and adjusted parametric regression survival-time models, higher cured meat intake was related to higher risk of COPD readmission (adjusted Hazard Ratio (95% confidence interval)=2.02 (1.31 - 3.12), p=0.001). Figure 2 shows that the higher the cured meat intake (as a continuos variable), the higher the risk of COPD rehospitalization. After stratification, the estimate of the association between high cured meat consumption and COPD readmission was lower in subjects treated with inhaled corticosteroids than in subjects not using this treatment (HR 1.88 *vs.* 2.56), and lower in subjects with mild and moderate COPD than in severe and very severe COPD patients (HR 1.63 *vs.* 2.29), although interaction terms were not statistically significant. Stratification according to smoking status or CRP levels showed no differences in the estimates of the association between cured meats and COPD readmission.

Discussion

We found that higher cured meat consumption increases the risk of COPD hospitalization, which could lead to changes in current management of COPD patients and a consequent improvement of disease prognosis. These results are coherent with previous studies about cured meats and COPD incidence. Jiang *et al.* reported in a cross-sectional study of 7432 subjects that frequent consumption of cured meat was associated with low lung function (FEV_1) and with an increased risk of COPD [4]. Later on, studies of two large US cohorts, one of 42915 men and the other of 71531 women, showed that cured meat consumption was associated with the risk of newly diagnosed COPD both in men and women [5,6]. The hypothesis that cured meats consumption may modify the course of COPD had not been tested before, so our findings, although relevant and promising, will benefit from replication in other COPD cohorts.

Experimental evidence about cured meat components supports the biological plausibility of our findings. There is evidence suggesting that nitrites could cause lung damage. In an experimental study, rats drinking water containing sodium nitrite over a 2 year period developed pulmonary emphysema [18], although the nitrite concentrations in the study were very high and probably not comparable to those achieved in standard human diets. Biochemical evidence shows that nitrites are pro-oxidants and can generate strong oxidizing reactive nitrogen species such as peroxynitrite ($ONOO^-$) and others [19,20]. These reactive nitrogen species are capable to produce lung damage [8], and have been suggested to play a role in the pathogenesis of COPD [9]. Our finding of a weaker effect of high cured meat consumption in subjects treated with inhaled corticosteroids supports the role of nitrites as mediators of the association between cured meats and worse prognosis, since the anti-inflammatory properties of corticosteroids could attenuate the oxidizing and pro-inflammatory effects of nitrites. Finally, it has been argued that the largest portion of the nitrite dietary intake could come from vegetables [21], via nitrate to nitrite conversion in the mouth and the stomach [22]. However, it has been shown that high nitrate intake does not cause the expected elevated gastric nitrite concentrations [23,24] nor appreciable changes in serum nitrite concentrations [25], thus enhancing the importance of cured meats as direct sources of nitrites. Another potential mechanism that could explain the deleterious effects of cured meats in COPD course involves salt, which is added during the curing process and could enhance the negative impact of cured meats *via* an increase

of the pulmonary arterial preasure. It could be specially harmful in COPD patients with concomitant pulmonary hypertension or poor haemodinamic status, where a salt excess could worsen these conditions and ultimately increase the risk of exacerbation.

There is compelling evidence that a healthy diet is a beneficial factor in improving and/or preventing multiple chronic diseases, including chronic lung diseases [2,3]. However, most influential COPD guidelines, such as that produced by the Global Initiative for Chronic Obstructive Lung Disease (GOLD) or the consensus between the American Thoracic Society and the European Respiratory Society [11,27] do not include any specific dietary recommendation to COPD patients, beyond the increase of caloric intake for the prevention of weight loss. This study adds new evidence suggesting that in addition to a possible increase in risk of COPD associated with cured meats [4-6], these foods may also increase risk of exacerbations, thus supporting the importance of considering dietary advice to COPD patients.

A limitation of the present study is the potential measurement error in the estimation of cured meat intake due to the use of a food frequency questionnaire. However, any such misclassification is likely non differential and therefore would lead to an underestimation of the effects of cured meats. Next, the presence of subjects who died prior to any COPD readmission could have produced survival bias. However, the small number of deaths before the first hospital readmission (n=2 (1%)) suggests that this bias, if present, is negligible. Finally, information on dietary changes after baseline was unavailable, although, given the current COPD management, it is unlikely that a first COPD admission could promote a reduction in cured meats consumption .

The main strengths of this study are its longitudinal design along with a very accurate characterization of the study subjects that allowed appropriate control for confounders. Importantly, the latter also included other potentially relevant food groups such as fruits, vegetables and fish. During the follow up, all hospitalizations were registered thoroughly, and only those with COPD as its main diagnosis were considered in the analysis. Finally, it is noteworthy that all subjects were recruited during their first COPD hospital admission, as we aimed to identify subjects at a similar state of the disease evolution, following the Evidence-Based Medicine recommendations for studies on prognosis [26].

In conclusion, high cured meats consumption was associated with an increase in the risk of COPD rehospitalization in COPD patients. Given the economic and health burden of COPD hospitalizations, the assessment of the effectiveness of healthy diet advice should be considered in the future.

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Table 1. Description of the main sociodemographic and clinical characteristics, and cured meat consumption, in 274 COPD patients.

Male [n (%)]	255 (93%)
Age (years) [m (SD)]	68 (8)
Primary or higher education [n (%)]	163 (59%)
Active worker [n (%)]	46 (17%)
Low socioeconomic status* [n (%)]	208 (82%)
BMI (kg/m^2) [n (%)]	
<20	6 (2%)
≥20 & <25	53 (19%)
≥25 & <30	114 (42%)
≥30	101 (37%)
FFMI (kg/m^2) [m (SD)]	19.8 (3.1)
≥2 Comorbidities (Charlson index) [n (%)]	152 (55%)
Dyspnea score (MMRC, score 0-5) [median (P25-P75)]	2 (2-3)
Post-bronchodilator FEV_1 (% predicted) [m (SD)]	53 (16)
PaO_2 (mmHg) [m (SD)]	75 (11)
DL_{CO} (% predicted) [m (SD)]	66 (21)
Current smokers [n (%)]	114 (42%)
Regular physical activity (kcal/week) [m (SD)]	6709 (5160)
Daily energy intake (kcal/day) [m (SD)]	2026 (611)
Total cured meat intake (g/day) [median (P25-P75)]	23 (11-34)
Ham (g/day) [median (P25-P75)]	8.6 (0-8.6)
Spanish cured ham (g/day) [median (P25-P75)]	8.6 (2.7-8.6)
Cured sausages (g/day) [median (P25-P75)]	2.4 (0-7.8)
Other sausages (g/day) [median (P25-P75)]	0 (0-3.2)
Hot dogs (g/day) [median (P25-P75)]	0 (0-0)

* Skilled or unskilled manual workers classified as low socioeconomic status.

Table 2. Description of the follow-up of 274 COPD patients.

Total days of follow-up [median (P25-P75)]	934 (655-1067)
Number of COPD admissions during follow up [n (%)]	
0	177 (65%)
1	34 (12%)
2	28 (10%)
≥3	35 (13%)
Days to first COPD hospital readmission only if readmission (n=97) [median (P25-P75)]	304 (141-622)
Total number of deaths [n (%)]	15 (5%)
Deaths before any COPD hospital readmission* [n (%)]	2 (1%)

* Subjects censored in the time-to-readmission analysis.

Table 3. Crude and adjusted association between high cured meat intake and COPD hospital readmission in 274 COPD patients with a median follow-up of 2.6 years.

	Crude* HR (95% CI)	p-value	Adjusted† R (95% CI)	p-value
High cured meat intake (>22.68g/day)	1.59 (1.06-2.38)	0.024	2.02 (1.31-3.12)	0.001
Age (years)	1.02 (0.99-1.04)	0.154	1.02 (1.00-1.05)	0.052
Total caloric intake (kcal/day)	1.00 (1.00-1.00)	0.892	1.00 (1.00-1.00)	0.244
FEV_1 (% predicted)	0.97 (0.96-0.99)	<0.001	0.97 (0.96-0.98)	<0.001

HR: Hazard Ratio; CI: confidence interval.

* Each line is a single model.

† The entire column is a single model.

Gender, BMI, FFMI, PCO_2, PO_2, smoking status, physical activity, inhaled corticosteroid treatment, statin treatment, the Charlson index of comorbidity, and intakes of fruit, vegetables and fish were tested as potential confounders and finally not included because they were not independently related to the outcome, nor modified estimates for the remaining variables.

Figure 1. Kaplan-Meier survival curves of time to the first COPD rehospitalization according to cured meat intake.

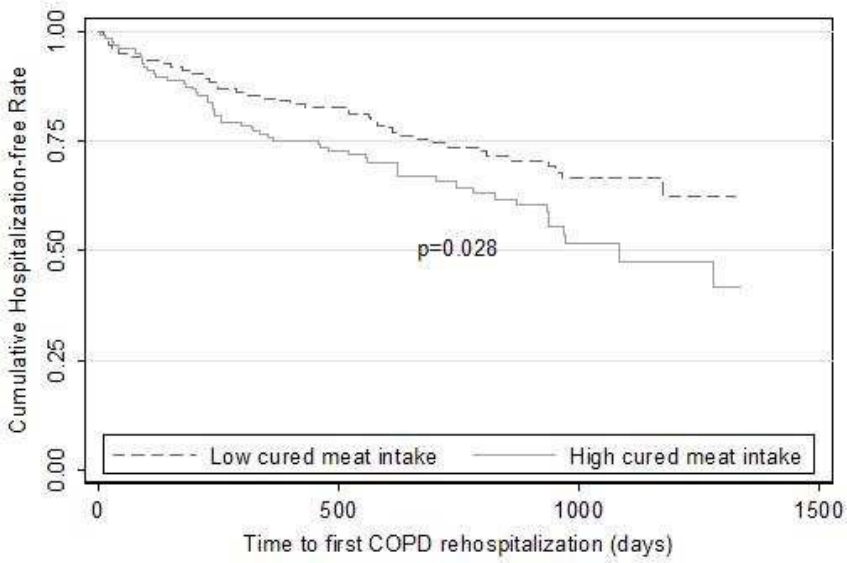
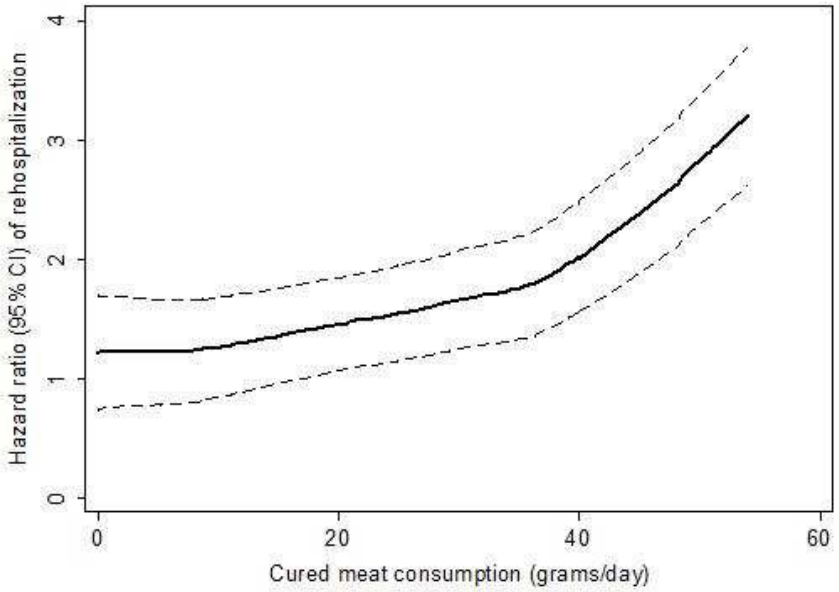


Figure 2. Hazard Ratio* (and 95% confidence intervals) of COPD rehospitalization according to cured meat intake.



* Hazard Ratio (and 95% CI) obtained from a parametric regression survival-time model with cured meat intake as a continuous variable, and adjusted for age, total caloric intake, and FEV_1. Subjects with cured meat intake above 95th percentile were excluded.

4.5 Paper V

Mediterranean diet is associated with reduced asthma and rhinitis in Mexican children.

de Batlle J, Garcia-Aymerich J, Barraza-Villarreal A, Antó JM, Romieu I.

Allergy 2008;63:1310-6.

5

Discussion

This discussion section is meant to be a global discussion, avoiding repeating what has been already discussed in each of the manuscripts conforming this doctoral thesis. Therefore, this section will build up and expand upon previous discussions on: (i) what is known about the role of diet in COPD, and especially what still remains to be known; (ii) which has been the contribution of the PAC-COPD study on the field, and which have been the main troubles and limitations that we have faced; and finally, (iii) which feasible approaches, especially from a methodological point of view, should be encouraged in order to step forward in the knowledge on the role of diet in COPD.

5.1 What is known and what is to be known about diet and COPD

In the introduction of this thesis I have given a broad view of why it is plausible that diet could play a role in both the development and prognosis of COPD, and what have been the results of the studies conducted in that sense up to date. The generation of the hypothesis of the potential role of diet in COPD has been possible thanks to the accurate understanding of COPD pathogenesis and pathophysiology. The main groups of foods and nutrients identified in this manner have been those with antioxidant and anti- or pro-inflammatory properties. Regarding antioxidants, fruits, vegetables, vitamins C, E and A, betacarotene, carotenoids, flavonoids and selenium, have been studied based on the hypothesis that these foods and nutrients could combat the oxidative insult that tobacco smoke causes in the lungs, and the abnormal perpetuation of this oxidative stress beyond smoking cessation that is characteristic of COPD. In the other hand, regarding anti- or pro-inflammatory foods and nutrients, fish and omega-3 and omega-6 PUFA have been studied based on the hypothesis that their capability to modulate inflammatory mediators synthesis could alter both the local and systemic inflammation of COPD. Additionally, alcohol has been

suggested to depress inflammatory response through an inhibition of granulocytes and alveolar macrophages [124], although this has been barely studied. Finally there is a group of foods and nutrients, not related to oxidative stress or inflammation, that have been proposed to play a role in COPD but have almost not been studied. This group includes sodium and magnesium, suggested to be implicated in the development of airway diseases through their effect on the airway smooth muscle cells; and fiber, that could modulate inflammation by slowing the absorption of glucose, decreasing lipid oxidation, or influencing the production of anti-inflammatory cytokines by the gut flora [125].

Up to date other manifestations of COPD beyond oxidative stress and inflammation, such as mucus hypersecretion, airways narrowing, colonization of the airways, and muscle wasting, have been almost completely neglected regarding a potential effect of certain foods or nutrients. For instance vitamin D is mentioned in current guidelines for the management of sarcopenia [126] and there is evidence to support the association of vitamin D and chronic lung diseases [127]. Another example could be the existing research in animal models concerning yogurt intake and lung colonization [128].

Interestingly, research regarding diet and COPD up to date has focused in dietary protective factors, while dietary risk factors have been neglected. Clear examples of this are the recent findings regarding cured meat consumption and COPD incidence. In this sense, the findings presented in the fourth paper of this thesis are very relevant.

Finally, the base role of a healthy diet in order to guarantee an optimum functioning of the whole organism seems to be forgotten. A healthy diet is a protective factor for many diseases, and especially for those that are chronic or appear on late stages of life and therefore can be concomitant to COPD [129]. Given the negative burden of comorbidities on COPD prognosis, a healthy diet could have an additional indirect effect on COPD, improving the prognosis of the disease and the quality of life of the patients.

5.2 Diet in the PAC-COPD study: Strengths and limitations

As it has been previously said, the main objectives of the PAC-COPD study were to improve our understanding of the phenotypic heterogeneity of COPD and the extent to which this heterogeneity is related to its clinical course. With the aim of being as much accurate as possible in characterizing the patients of the cohort, lifestyle factors such as usual physical activity and diet were measured. This setting was considered to be very appropriate to study the role of diet in COPD given the completeness of the data pool.

5.2.1 Strengths and right choices

The first right decision was to make an initial effort to describe, in the most accurate manner, the diet of the patients in the PAC-COPD cohort. Although this seems to be a logical first step in the study of the role of diet in COPD, we were the first to publish such kind of descriptive information. The main results that we obtained were that (i) the reported diet of the subjects of the cohort was overall appropriate according to nutritional recommendations; and (ii) there could be a generalized vitamin D dietary intake deficiency among COPD patients. This kind of descriptive approach has captured the interest of other epidemiology groups. For example, in the 2010 annual European Respiratory Society congress, a Dutch research group showed the results of the exploration of the adequacy of macro- and micro-nutrient intake in up to 1262 COPD patients entering pulmonary rehabilitation, showing very similar results and confirming vitamin D as a target for future research [130].

Another novel approach that was performed in the PAC-COPD study, in relation to the study of the role of diet in COPD, was the direct measurement of both oxidative stress and inflammatory markers in serum samples of the participating subjects. With this specific information we were able to study in depth the relationship between foods and nutrients suspected to modulate oxidative stress or inflammation in COPD and direct measures of such manifestations at the systemic level. These have been the first epidemiologic analyses directly testing the suspected biological mechanism behind most of the previously published associations between diet and lung function or COPD development. Regarding

oxidative stress, we identified a relationship between daily intake of vitamin E and low carbonyl levels (a marker of protein oxidation), and daily intake of olive oil and high levels of reduced glutathione (GSH) (an endogenous antioxidant). Regarding serum inflammatory markers, we showed that high dietary intake of α-linolenic acid (an omega-3 fatty acid) was associated with decreased levels of serum TNFα, while dietary intake of arachidonic acid (an omega-6 fatty acid) was related to increased levels of IL-6 and CRP.

Finally, it is noteworthy that we have analyzed for the first time the effect of a given food group, in this case cured meats, in the progression of the disease. Given the lack of longitudinal studies evaluating the role of foods or nutrients on the prognosis of COPD, this study is relevant in the rout-map for designing a set of dietary recommendations specifically developed in order to modify the prognosis of COPD, thus delaying hospitalizations.

5.2.2 Limitations and their potential causes

It has been somewhat disappointing that no clear associations between dietary components and key characteristics of COPD such as oxidative stress or inflammation were found in the cross-sectional studies. Although we found some specific associations, all of them in the expected directions, the overall picture of the results does not clearly support that a relationship between antioxidant intake and serum oxidative stress levels exist, nor that dietary PUFA intake can clearly modify systemic inflammation in COPD.

As discussed in each of the manuscripts, this absence of clear results can be attributed to the sum of several factors. The first of these factors is the low variability in the dietary habits of our study population. As described in the first manuscript of this thesis, the overall diet of the subjects in the cohort was appropriate according to the nutritional recommendations. Most of the study subjects followed a traditional Mediterranean diet similar to that of the general population of the same age in the study region [131]. It is noteworthy that the intake of fruits, vegetables and fish was higher than that reported in other populations of the same age as for example in the United States [132]. However, this fact may change in the future due to the westernization of the dietary patterns among younger generations. Another of the factors that could

explain the lack of clear findings is the possible measurement error in the dietary assessment. This fact will be broadly discussed in the last part of the discussion. Finally, given that the individual effect of each food and nutrient resulted to be small, the number of subjects in the PAC-COPD cohort was insufficient in order to detect most of them.

Finally, the complexity of the measurements for an appropriate phenotypic characterization of the patients, together with the requirement of those measures to be performed at clinical stability at least 3 months after discharge from their first hospital admission ever, resulted in a delay in the time-schedule of the study. This delay caused shorter available follow-up times and precluded other longitudinal analyses to be done. Moreover, the small number of deceases registered during the follow up precluded any mortality analyses. Therefore, more analyses will be performed in the future.

5.3 Lessons from Mexicali

The study conducted in Mexicali, in the frame of the International Study of Asthma and Allergies in Childhood (ISAAC), aimed to investigate asthma, rhinitis and eczema in children. As it was previously done in other ISAAC studies, children's dietary information was collected. Moreover, the mothers' diet during pregnancy was also retrospectively assessed. This setting, together with an appropriate assessment of other potentially relevant variables, allowed us to study not only the relationship between children's diet and asthma but also the relationship between mothers' diet during pregnancy and children's asthma. Several points worth of discussion can be extracted from our experience in this study and will be commented below.

The first interesting decision in our study was the use of a Mediterranean diet score. Even though our study population was not from the Mediterranean region, Mediterranean diet is considered to be one of the healthiest dietary patterns worldwide. Moreover, this decision was taken considering the following facts: (i) available dietary information (semi-quantitative FFQ) did not allow to appropriately compute the grams of each food in the questionnaire, nor consequently an accurate estimation of the amount of the different nutrients consumed; (ii) there are correlations and synergic effects and/or interactions among foods or nutrients that need to be taken into account when studying the

effects of a single food or nutrient; and (iii) the effect of a single food or nutrient on a given disease is usually very small. The use of a dietary score over-ridded all of these issues while considerably reducing the complexity of statistical methods during the analysis. The only drawback of this approach is that the specific foods or nutrients playing major roles in the studied disease are not identified.

Another interesting aspect of this study was the assessment of the mothers' diet during pregnancy, even though having the limitation of assessing diet after more than 6 years (recall bias). Exposures during pregnancy can have critical effects on fetal development, conditioning future disease susceptibility. Environmental exposures during pregnancy such as diet, microbial exposure and maternal smoking have been suggested to modify neonatal immune function, and there is emerging evidence from animal models suggesting that these factors may have epigenetic effects on immune gene expression and disease susceptibility, thus justifying exposure assessment during pregnancy in order to understand increasing trends in asthma, allergy and other immune disorders [133].

Finally, the last but not least lesson from the Mexicali study is the importance of international initiatives like the ISAAC research program. ISAAC proposed standardized methods for studies regarding a specific field (asthma, rhinitis and eczema in children). Although it could be supposed that many of the studies in a given scientific field have similar protocols, there are substantial differences that make direct comparison of the results difficult. In many complex diseases, even the definition of the disease could be different among studies. Therefore, as the epidemiological evidence on a given field is not constructed on single studies, the initiative of standardizing protocols is very positive and useful. Moreover, it provides the opportunity of easily comparing studies in different regions, or even summing up many different studies into single meta-analysis.

5.4 Stepping forward

Epidemiological studies up to date determining the possible role of diet in COPD have been inconclusive. Although the studies presented in this thesis have stepped forward in what we believe is the right direction, they have proved insufficient in order to fulfill the

objective that we embraced. The lack of consistent results when studying the role of diet in COPD, and other related chronic inflammatory lung disorders, could be attributed to several design and methodological issues. The most relevant of these issues as well as possible ways to override them will be commented below.

Study design

The two major approaches used to investigate the impact of diet on either COPD development or progression up to date have been cross-sectional and experimental studies. In cross-sectional studies dietary intake is assessed simultaneously together with lung function or respiratory symptoms, focusing in evaluating dietary factors among people with and without the disease at a single point in time. These studies aim to determine risk factors for COPD development, and can not provide information on the effect of diet on COPD prognosis. Moreover, they can not provide information on the temporal relation between dietary intake and the disease.

In the other hand, experimental studies can evaluate the effect of certain foods or nutrients on COPD prognosis. In experimental studies the patients are randomly assigned to receive a dietary intervention or placebo. This study design is likely to provide information on a true causal relation. However, the existing studies have been conducted in clinical settings on small numbers of patients, limiting the application of the results to larger populations. Moreover, most of the interventions are not representative of what is usually eaten in a healthy diet, and consist in increasing total caloric intake or giving vitamin supplementations (not in the form of actual fruits and vegetables). Therefore, most of these studies aim to determine the potential for nutritional therapy in the management of COPD, rather than evaluating the role of usual diet in COPD development or prognosis.

In order to bypass the limitations of both study designs, and make a clear step forward into the role of diet in both COPD development and progression, longitudinal studies should be encouraged. Following up cohorts of healthy adults, or even children followed up to adulthood, could be decisive in determining the risk factors for COPD development, including the possible role of diet. The inclusion of dietary assessment protocols in such studies would be highly recommended, as diet has been suggested to play a relevant role in almost all chronic diseases. Moreover, the cost of including a simple food frequency questionnaire in such studies is low, granting a good cost-results ratio. In the other hand, the

establishment of big cohorts of COPD patients, with common follow up protocols and comparable dietary assessment methods, should clarify the role of diet in COPD progression. However, such studies would require additional measures including biomarkers of inflammation and oxidative stress. Implementation of such measures is expensive, so we would recommend the inclusion of dietary measures in studies that are already measuring relevant biomarkers. Finally, research in COPD could be improved applying standardized methodologies as the ISAAC has done in asthma research. Standardizing the COPD definition, and the definition of other key aspects in COPD such as exacerbations, is important in order to make different studies comparable. This would grant the opportunity to compare or pool different data bases. Up to date, research initiatives such as BOLD [5] or PLATINO [6], are beginning to grant a substantial standardization of methodology in data collection and have resulted in the availability of more comparable COPD prevalence estimates across countries.

Dietary assessment

Having an appropriate measurement of what a given individual usually eats is very important in the non-interventional studies regarding diet and COPD. Most of them up to date used food frequency questionnaires or 24 hour recalls, and a few used direct serum measures of vitamin levels. Food frequency questionnaires have a substantial amount of measurement error [114], and serum measures are directly affected by COPD and therefore could not be representative of the actual intake of the measured factor. Hence, measurement error associated to dietary assessment could be responsible of at least a part of the null results in the study of diet and COPD.

Unfortunately, there are no other available dietary assessment methods that could be feasibly used in big epidemiological studies. Therefore, all research regarding the role of diet in COPD should take into account this inherent limitation. All protocols regarding the role of diet in big epidemiological studies should make an effort to ensure the minimum measurement error. Such efforts should include: (i) appropriate choice of FFQ or 24h recall, ensuring that the chosen tool includes most relevant foods; (ii) proper validation of the dietary assessment tool, ensuring that the chosen tool has been already validated to be used in the target population, or including a validation study in a subgroup of subjects; (iii) adequate training of interviewers; and (iv) exhaustive quality control, including a careful analysis of the collected information and

appropriate control for under- and over-reporting. Finally, increasing the number of subjects in the studies would counteract the impact of measurement error in the statistical power of the analyses thus reducing the risk of false negative results.

Intermediate outcomes

In order to fully understand the role of diet in COPD, it is necessary to understand the biological mechanisms behind any association between dietary factors and the disease. Up to date, almost no studies have tried to assess the relationship between diet and key features of COPD pathogenesis and/or pathophysiology. Examples of such features could be inflammation, oxidative stress and muscle wasting. The study of the effects of dietary factors on such features of the disease could not only help to fulfill the global understanding of the role of diet in COPD, but also help to clarify the pathophysiology of the disease, hopefully identifying new therapeutic targets.

Final remarks

1. Longitudinal cohort studies should be encouraged in order to disentangle the role of diet in COPD development and progression.

    - Such studies would benefit from standardized definitions and methodologies such as those implemented in ISAAC or BOLD studies.
    - The biological mechanisms behind associations between dietary factors and COPD may help to support findings and promote measures in further studies.
    - Methods for dietary assessment are key for the success of this research and, therefore, it is important to carefully choose the tool that will minimize measurement error in each specific study.

2. Healthy diet advice should be considered for inclusion in COPD guidelines, given the recent findings of diet effects on COPD development and prognosis, and the well-known benefits of a healthy diet in reducing risk and/or improving prognosis of many chronic diseases and conditions.

6

Conclusions

1. Moderate-to-severe COPD patients from Spain report an adequate intake of the main food groups and macro- and micro-nutrients according to recommendations, excepting vitamin D.

    - Vitamin D should be a target for future research.

2. Vitamin E and olive oil intake are associated with reduced oxidative stress levels (lower carbonyls and higher GSH, respectively) in current smoker COPD patients.

    - Other dietary antioxidants did not modify serum oxidative stress levels in COPD.
    - If these associations are confirmed in future studies, the recommendation of increasing the dietary antioxidant intake in COPD patients could be especially relevant in active smokers.

3. Dietary intake of omega-3 and omega-6 fatty acids relates to the level of serum inflammatory markers. Specifically, dietary intake of α-linolenic acid (an antiinflammatory omega-3 fatty acid) is associated with reduced risk of high levels of serum TNFα, while dietary intake of arachidonic acid (a proinflammatory omega-6 fatty acid) is related to increased risk of elevated IL-6 and CRP.

    - If these associations are confirmed in future studies, the recommendation of increasing the dietary PUFA intake in COPD patients should be considered.

4. High cured meats consumption increases the risk of COPD hospitalization in COPD patients.

- The assessment of the effectiveness of specific dietary advice such as cured meats avoidance should be considered in the future.

5. A greater adherence to a Mediterranean dietary pattern – characterized by a high intake of vegetables, fruits and nuts, legumes, fish and cereals, and a low intake of dairy products, meat and junk food and fat – is associated with having less asthma, wheezing, allergic rhinitis, current sneezing and current itchy-watery eyes, in Mexican 6- to 7-year old children.

    - Mothers' adherence to a Mediterranean dietary pattern during pregnancy was not associated to children's asthma or allergic rhinitis.

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Annexes

Other publications coauthored on COPD or diet:

Annex I
Physical activity and clinical and functional status in COPD.

Annex II
Characteristics of patients admitted for the first time for COPD exacerbation.

Annex III
Dietary intake, lung function and airway inflammation in Mexico City school children exposed to air pollutants.

Annex IV
Factors affecting the relationship between psychological status and quality of life in COPD patients.

Congress contributions:

Annex V

Annex I

Physical activity and clinical and functional status in COPD.

Garcia-Aymerich J, Serra I, Gómez FP, Farrero E, Balcells E, Rodríguez DA, de Batlle J, Gimeno E, Donaire-Gonzalez D, Orozco-Levi M, Sauleda J, Gea J, Rodriguez-Roisin R, Roca J, Agustí AG, Antó JM; Phenotype and Course of COPD Study Group.

Chest 2009;136:62-70.

Annex II

Characteristics of patients admitted for the first time for COPD exacerbation.

Balcells E, Antó JM, Gea J, Gómez FP, Rodríguez E, Marin A, Ferrer A, de Batlle J, Farrero E, Benet M, Orozco-Levi M, Ferrer J, Agustí AG, Gáldiz JB, Belda J, Garcia-Aymerich J; PAC-COPD Study Group.

Respir Med 2009;103:1293-302.

Annex III

Dietary intake, lung function and airway inflammation in Mexico City school children exposed to air pollutants.

Romieu I, Barraza-Villarreal A, Escamilla-Núñez C, Texcalac-Sangrador JL, Hernandez-Cadena L, Díaz-Sánchez D, de Batlle J, Del Rio-Navarro BE.

Respir Res 2009;10:122.

Annex IV

Factors affecting the relationship between psychological status and quality of life in COPD patients.

Balcells E, Gea J, Ferrer J, Serra I, Orozco-Levi M, de Batlle J, Rodriguez E, Benet M, Donaire-González D, Antó JM, Garcia-Aymerich J; the PAC-COPD Study Group.

Health Qual Life Outcomes 2010;8:108.

Annex V

Congress contributions:

- Oral communication: "*El patrón de dieta mediterránea reduce el riesgo de asma y rinitis en niños*". 40º Congreso Nacional de la Sociedad Española de Neumología y Aparato Respiratorio (SEPAR). Barcelona, Spain. 2007.

- Oral communication: "Mediterranean diet reduces risk of asthma and allergic rhinitis in Mexican children". 2007 European Respiratory Society Annual Congress (ERS). Stockholm, Sweden. 2007. Awards: "ERS Young Scientist Sponsorship" and participation in the special session "Meet the awardees and the Young Investigators of the Occupation and Epidemiology Assembly".

- Poster communication: "*Medida de la dieta en pacientes con EPOC incipiente*". XI Symposium Internacional sobre EPOC. Barcelona, Spain. 2008.

- Poster communication: "Dietary assessment in early COPD patients". 2008 European Respiratory Society Annual Congress (ERS). Berlin, Germany. 2008.

- Poster communication: "Dietary modulation of oxidative stress levels in COPD patients". 2009 European Respiratory Society Annual Congress (ERS). Vienna, Austria. 2009.

- Poster communication: "*Papel del consumo de ácidos grasos omega-3 y omega-6 en la inflamación sistémica de la EPOC*". XII Symposium Internacional sobre EPOC. Barcelona, Spain. 2010.

- Oral communication: "Association between Ω3 and Ω6 fatty acid dietary intakes and serum inflammatory markers in Spanish COPD patients". 2010 European Respiratory Society Annual Congress (ERS). Barcelona, Spain. 2010. Awards: "ERS Young Scientist Sponsorship".