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Epidemiological Study of Diet, Obesity and Asthma in the French EGEA Study

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Par

Mme. Zhen LI

**Epidemiological study of diet, obesity and asthma
in the French EGEA study
(Alimentation, obésité et asthme dans l'étude EGEA)**

Co-directrices de thèse : Mme. Raphaëlle VARRASO et Mme. Rachel NADIF

Thèse présentée et soutenue à Villejuif, le 21 avril 2017

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RESUMÉ

L'objectif principal de la thèse était d'étudier les effets de l'alimentation et de l'obésité sur l'asthme et son évolution, en prenant en compte des facteurs de médiations spécifiques.

La première partie de la thèse visait à étudier le rôle de l'alimentation dans l'asthme, en prenant en compte l'indice de masse corporelle (IMC) comme un médiateur potentiel, et en évaluant l'effet modificateur du tabac dans ces associations. **Ce travail a d'abord porté** sur le rôle de la consommation de charcuterie, récemment classée cancérigène. Parmi 971 adultes de l'étude des facteurs génétiques et environnementaux de l'asthme (EGEA), nous avons montré qu'une consommation élevée de charcuterie (au moins 4 fois par semaine) était associée de façon directe à l'aggravation des symptômes de l'asthme, et que seulement 14% de l'association entre la consommation de charcuterie et l'asthme était expliqué par l'IMC (effet indirect). **Ce travail a ensuite porté** sur le rôle de la qualité globale de l'alimentation, évaluée par le score alimentaire Alternate Healthy Eating Index 2010. Parmi 969 participants d'EGEA, nous avons montré qu'une alimentation de qualité était associée de façon directe à une amélioration des symptômes de l'asthme chez les non-fumeurs. L'effet indirect lié à l'IMC n'était pas significatif.

La deuxième partie de la thèse visait à mieux comprendre l'association entre l'obésité et l'activité de l'asthme, en prenant en compte la leptine, une adipokine pro-inflammatoire, comme un médiateur potentiel. Parmi 331 adultes d'EGEA avec un asthme actif à l'inclusion, les analyses ont montré que différentes mesures élevées de la composition corporelle étaient associées à une activité persistante de l'asthme, avec un effet indirect très fort de la leptine dans ces associations.

Mots-clés : Epidémiologie, charcuterie, qualité de l'alimentation, asthme, obésité, analyses de médiation

ABSTRACT

The general aim of the thesis was to study the role of diet and obesity in asthma, while accounting for potential mediators in these analyses.

The first part of the thesis aimed to investigate the role of dietary factors in asthma at a "macro-level", considering body mass index (BMI) as a potential mediator, and to evaluate effect modification by smoking. We **first focused on** processed meat intake, a recent carcinogen. Among 971 participants from the Epidemiological study on the Genetics and Environment of Asthma, bronchial hyperresponsiveness and atopy (EGEA), analysis showed that high processed meat intake (at least 4 servings/week) was associated with worsening asthma symptoms over time, through a direct effect and to a lesser extent an effect mediated by BMI. We **then focused on** the overall diet quality assessed by the Alternate Healthy Eating Index 2010. Among 969 participants from the EGEA study, the analyses showed that a greater adherence to a better diet quality was associated with improved asthma symptoms over time in never smokers only and was not mediated through BMI.

The second part of the thesis aimed to better understand the association between obesity and asthma at a "micro-level", considering leptin, an inflammatory biological marker related to obesity, as a mediator. Including 331 participants from the EGEA study with current asthma at baseline, analysis showed that high body adiposity estimated by different measures was associated with persistent asthma activity, likely mediated by leptin.

Keywords: Epidemiology, processed meat, diet quality, asthma, obesity, mediation analysis

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ABBREVIATIONS

ACOS: Asthma–COPD Overlap Syndrome
ADII: Adapted Dietary Inflammatory Index
AHEI: Alternate Healthy Eating Index
AHEI-2010: Alternate Healthy Eating Index 2010
AHR: Airway Hyperresponsiveness
ATP III: American Adult Treatment Panel III guidelines
ATS: American Thoracic Society
aMED: alternate Mediterranean diet
BD: Bronchodilator
BIA: Bioelectrical Impedance Analysis
BMI: Body Mass Index
BMRC: British Medical Research Council
CDC: Centers for Disease Control and Prevention
CDE: Controlled Direct Effect
CFA: Confirmatory Factor Analysis
CHD: Coronary Heart Disease
COPD: Chronic Obstructive Pulmonary Disease
CVD: Cardiovascular Disease
DAG: Directed Acyclic Graph
DASH: Dietary Approaches to Stop Hypertension
DE: Direct Effect
DEXA: Dual-Energy X-ray Absorptiometry
DII: Dietary Inflammatory Index
DQI-I: Dietary Quality Index-International
DR: Diet Record
E3N: Epidemiological prospective cohort study among women of the MGEN
EGEA: Epidemiological study on the Genetics and Environment of Asthma
ECRHS: European Community Respiratory Health Survey
EPIC: European Prospective Investigation on Cancer
FeNO: Fractional exhaled Nitric Oxide

FFQ: Food-Frequency Questionnaire
FEV₁: Forced Expiratory Volume in 1 second
FRAP: Ferric Reducing Antioxidant Power
FVC: Forced Vital Capacity
FVS: Food Variety Score
GINA: Global Initiative for Asthma
GWAS: Genome-Wide Association Study
HDL: High-Density Lipoprotein cholesterol
HEI: Healthy Eating Index
HFI: Healthy Food Index
hs-CRP: high-sensitivity C-Reactive Protein
IARC: International Agency for Research on Cancer
ICS: Inhaled Corticosteroids
IDF: International Diabetes Federation
IDE: Indirect Effect
IgE: Immunoglobulin E
IL: Interleukin
ISAAC: International Study of Asthma and Allergies in Childhood
IUATLD: International Union against Tuberculosis and Lung Disease
Kcal/day: kilocalories per day
LABA: Long-Acting β 2-Agonists
LCA: Latent Classes Analysis
MDS: Mediterranean Diet Score
METs: Metabolic Equivalents
MGEN: Mutuelle Générale de l'Éducation Nationale
NDE: Natural Direct Effect
NHANES: National Health and Nutrition Examination Survey
NHS: Nurses' Health Study
NIE: Natural Indirect Effect
NO: Nitric Oxide
PM: Particulate Matter
PCA: Principal Component Analysis
PLS: Partial Least-Squares Regression

PNNS-GS: Programme National Nutrition Santé-Guideline Score
PUFAs: Polyunsaturated Fatty Acids
RCTs: Randomized Controlled Trials
ROS: Reactive Oxygen Species
RNS: Reactive Nitrogen Species
RRR: Reduced Rank Regression
SABA: Short-Acting β 2-Agonists
SES: Socio-Economic Status
SU.VI.MAX: SUplémentation en VIamines et Minéraux AntioXydants
TAC: Total Antioxidant Capacity
TE: Total Effect
 T_H2 : T-Helper type 2 lymphocytes
TNF- α : Tumor necrosis factor- α
TRAP: Total Radical-Trapping Antioxidant Parameter
UK: United Kingdom
US: United States
WHO: World Health Organization

TABLE OF CONTENTS

INTRODUCTION	11
STATE OF THE ART	14
1 Overview of asthma	14
1.1 Clinical aspects.....	14
1.2 Epidemiological aspects.....	18
1.2.1 Prevalence.....	18
1.2.2 Assessment of asthma in epidemiological studies.....	19
1.2.3 Risk factors	22
2 Diet and asthma.....	27
2.1 Diet in nutritional epidemiology	27
2.1.1 Tools to assess dietary intakes.....	27
2.1.2 Approaches to analyzing dietary data.....	30
2.1.3 Diet and major chronic diseases: a brief overview	38
2.2 Associations between diet and asthma.....	47
2.2.1 Potential mechanisms	47
2.2.2 Windows of exposure in the diet–asthma association	50
2.2.3 Association between individual foods/nutrients and asthma in adults ...	51
2.2.4 Association between dietary patterns and asthma in adults	53
3 Obesity and asthma	64
3.1 Tools to assess body composition	64
3.1.1 Reference methods	64
3.1.2 Methods most used in epidemiological studies	66
3.2 Association between obesity and asthma.....	72
3.2.1 Obesity as a likely risk factor of asthma.....	72
3.2.2 The obese–asthma phenotype	73
3.2.3 Potential mechanisms	74
4 Methodological challenges in epidemiological research on nutritional factors and asthma	79
4.1 Complexity of the association between nutritional factors and asthma.....	79
4.1.1 Obesity: a potential mediator in the diet–asthma association.....	79
4.1.2 Role of smoking in the diet–asthma association.....	81
4.1.3 Biological markers: potential mediators in the obesity–asthma association	82
4.2 Application of mediation analyses in epidemiology	82
4.2.1 Traditional approach.....	83
4.2.2 Counterfactual approach.....	84
OBJECTIVES	91
MATERIALS AND METHODS.....	93
5 The EGEA study	92
5.1 Study design and follow up.....	92
5.2 Data collection	95
5.3 Data used in this thesis.....	97

5.3.1 Dietary data.....	97
5.3.2 Data on body composition	98
5.3.3 Data related to respiratory phenotypes	98
5.3.4 Biological data.....	99
5.3.5 Other variables.....	100
6 Statistical analysis	100
RESULTS AND DISCUSSION	102
7 Processed meat intake and change in asthma symptoms	102
8 Overall diet quality and change in asthma symptoms.....	120
9 Leptin, body adiposity, and asthma activity.....	137
GENERAL CONCLUSION	148
GENERAL DISCUSSION	149
10 Methodological aspects	149
10.1 Analyzing approaches in nutritional research.....	149
10.2 Assessment of the disease status in asthma.....	150
10.3 Causal inference in nutritional epidemiological studies	152
11 Public health implications	154
11.1 Reducing processed meat intake	154
11.2 Improving diet quality and preventing smoking	155
11.3 Preventing obesity	156
12 Limitations	157
13 Perspectives.....	158
13.1 Aspects in “macro” epidemiology	158
13.1.1 Dietary pattern reflecting dietary biological potential and asthma outcomes.....	158
13.1.2 Dietary factors and asthma phenotypes.....	158
13.1.3 Analyses using repeated assessment of diet and multiple measures of body composition	159
13.1.4 Trajectory of body shape and asthma outcomes	159
13.2 Aspects in “micro” epidemiology	160
13.2.1 Biological markers	160
13.2.2 Epigenetic factors.....	161
13.2.3 Microbiota	161
REFERENCES	163

INTRODUCTION

Asthma is one of the most common chronic diseases of the airways in the world, affecting more than 300 million individuals [1]. During the past half century, a remarkable global increase in asthma prevalence has been observed. While the prevalence of asthma remains high in industrialized countries, new epidemic of asthma has been observed in developing countries [1]. In this context, it has been suggested that changes in environmental and lifestyle factors during the urbanization and industrialization of the societies may play a role in the global epidemic of asthma [2]. As asthma is not curable and has a strong clinical heterogeneity and phenotypic variability over time, it is essential to study not only factors related to the primary prevention of asthma (i.e. incidence), but also factors related to the secondary prevention (i.e. recurrence and progression).

Several remarkable changes in diet have been observed in modern lifestyle, in particular decreased intake of fresh vegetables and fruit, increased intake of prepared and processed foods, and in general, decreased overall diet quality. For decades, the role of these dietary factors has been intensively studied in relation with cardiovascular disease, cancer, and type 2 diabetes, and more recently in relation with chronic respiratory diseases, including asthma. Several hypotheses have been raised regarding potential mechanisms of the association between dietary factors and asthma. However, previous findings on the association between dietary factors and asthma remain inconclusive, due to several conceptual and methodological challenges, such as, the likely mediating role of obesity in the diet–asthma association. Moreover, while previous research has mostly focused on the potential protective effect of specific foods or nutrients with anti-oxidant potential, such as fruit and vegetables, very few studies have been conducted regarding the potential deleterious effect of diet in asthma or regarding the effect of overall diet in asthma.

Contrary to dietary factors, obesity – a condition of high body adiposity – has been associated with a greater risk of asthma incidence, especially in women, and also with more severe asthma, more active asthma, worse asthma control, and reduced response to standard therapy [3]. More recently, it has been proposed that asthma in the obese may represent a unique phenotype [3]. Although several hypotheses have been proposed regarding potential mechanisms between obesity and asthma incidence

and activity [3], due to several methodological challenges in investigating these hypotheses in epidemiological studies, the underlying mechanisms are still poorly understood.

In the past decades, methodological advances in epidemiological research have provided useful tools to studying the complex association between nutritional factors and asthma. Mediation analysis in the counterfactual framework [4–6] may be a useful tool to address several methodological challenges in studies regarding nutritional factors and asthma, both at a “macro-level” to take into account complex interrelationships within nutritional factors, and at a “micro-level” to better understand potential biological mechanisms of the association.

The general aim of the thesis is to study the role of diet and obesity in asthma among adults, while accounting for potential mediators in the analyses. Potential mediators have been selected at two levels: first, at a “macro-level” to take into account complex interrelationships within nutritional factors in the diet–asthma association, and then, at a “micro-level” to better understand potential biological mechanisms of the association between body adiposity and asthma activity.

The first chapter of the thesis starts with an overview of clinical and epidemiological aspects of asthma. Then epidemiological aspects of diet and a review of the literature regarding the association between diet and asthma are presented, followed by different tools for the assessment of body composition and a review of the literature regarding the association between obesity and asthma. Lastly, several methodological challenges in epidemiological research regarding the role of nutritional factors in asthma are described.

The second chapter presents the general and specific aims of the thesis.

The third chapter presents the study population among which the analyses were performed, the main variables, and the statistical methods used.

The fourth chapter presents and discusses the results of the thesis, firstly regarding the association between dietary factors, including processed meat intake and overall diet quality with change in asthma symptoms, taking into body mass index (BMI) as a potential mediator, and then regarding the role of leptin in the association between body adiposity and asthma activity.

The fifth chapter presents the general conclusion of the thesis.

The last chapter discusses the implications of the thesis for future research regarding methodological challenges and public health, the main limitations of the performed analyses, and finally several perspectives from the thesis.

STATE OF THE ART

This chapter of the thesis includes four sections.

The first section presents an overview of asthma, including clinical and epidemiological aspects.

The second section presents first a summary of approaches that are available in nutritional epidemiology to assessing dietary intakes and to analyzing dietary data. Then, a brief review of current evidence on the diet–health association is presented, as the role of diet in health, protective or deleterious, was first formulated in relation with major chronic diseases including cardiovascular disease, cancer, type 2 diabetes, and COPD. Finally, the associations between diet and asthma are reviewed, first regarding potential mechanisms, then regarding windows of dietary exposure, and lastly regarding the role of diet – individual foods and nutrients or dietary patterns – in asthma.

The third section presents the tools for the assessment of body composition and a review of the associations between obesity and asthma.

In the last section, several methodological challenges due to the complexity of the association between nutritional factors and asthma in epidemiological studies are discussed, and then, statistical approaches that can help to address these issues are presented.

1 Overview of asthma

In this section, first a brief summary on clinical aspects of asthma is presented, then epidemiological aspects, including prevalence, assessment of asthma in epidemiological studies, and risk factors, are introduced.

1.1 Clinical aspects

Definition

The definition of asthma is evolving. While asthma was considered as a single disease for decades, recent studies have consistently shown that asthma is likely a complex entity involving multiple phenotypes or consistent grouping of characteristics, promoting a new definition of asthma [7]. According to the latest report of Global Initiative for Asthma (GINA), asthma is a heterogeneous disease, usually characterized by chronic airway inflammation. It is defined by the history of respiratory symptoms variable over time and in intensity, including wheeze, shortness of breath, chest tightness and cough, and variable expiratory airflow limitation [8].

Clinical manifestation and diagnosis

In GINA 2016 guideline, the latest recommendations regarding clinical processes and considerations of asthma diagnosis are presented in detail [8]. In this section, two important features that are essential for the diagnosis of the disease [8] – the variability of symptoms and of expiratory airflow limitation – are briefly presented.

The typical respiratory symptoms of asthma (i.e. wheeze, shortness of breath, chest tightness and cough) vary over time and in intensity. Worse symptoms may occur at night or in the early morning. Moreover, the symptoms can be triggered by several factors (i.e. bronchial hyperreactivity), including certain allergens, as well as viral infections, physical exercise, certain medication (e.g. aspirin), changes in weather, laughter, and irritants such as smoke.

Also, expiratory airflow limitation, from completely normal to severely obstructed lung function, can be observed for the same patient. Airway airflow limitation is usually assessed by a reduced ratio of forced expiratory volume in 1 second (FEV_1) to forced vital capacity (FVC) (this ratio is normally > 0.75 – 0.80 in healthy adults and > 0.90 in children). The variability of airway airflow limitation can

be assessed by several tests, among which the most used one is the positive bronchodilator (BD) reversibility test (after inhaling a bronchodilator, FEV₁ increases by > 12% and > 200 mL from baseline in adults, and by > 12% of predicted FEV₁ in children) [8].

Due to the great variability of symptoms and the heterogeneity of the disease (discussed at the end of this section), the diagnosis of asthma may be difficult in some patients [8]. For instance, some individuals, smokers and older adults in particular, may have clinical features of both asthma and chronic obstructive pulmonary disease (COPD), which were once considered as two distinct diseases but now recognized as heterogeneous and often overlapping conditions [9]. Under this circumstance, the notion “asthma–COPD overlap syndrome” (ACOS) has been proposed [9]. Further studies are needed to better understand the character, underlying mechanisms and treatment of ACOS in clinical practice.

Disease management

As asthma cannot be cured, the long-term goals of asthma management include good symptom control, as well as risk reduction in future exacerbations, fixed airflow limitation and side effects of treatment [8].

In addition to prevention and treatment of modifiable risk factors, **medications**, including controller medications, reliever medications, and add-on therapies, also play an important role in symptom control and risk reduction [8]. Controller medications, typically including low-dose inhaled corticosteroids (ICS) or low-/high-dose ICS plus long-acting beta2-agonist (LABA), are used as daily maintenance treatment to reduce airway inflammation, to control symptoms, and to reduce future risk of exacerbations. Reliever medications, typically including inhaled short-acting beta2-agonist (SABA), are used to relieve asthma symptoms in a short time, together with controller medications in most cases. For patients with severe asthma, multiple add-on therapies may be considered, including omalizumab (anti-immunoglobulin E), tiotropium (long-acting muscarinic antagonist), mepolizumab (anti-interleukin-5 treatment), and low-dose oral corticosteroids.

A continuous cycle of “*assess* → (*adjust*) *treatment* → *review response* → *assess*” has been proposed in GINA 2016 [8] for a control-based asthma management (i.e. management mainly based on symptom control). After the diagnosis and assessment (“*assess*”), an initial treatment is given to patients (“*treatment*”), and then

several regular visits are needed to review the response to treatment, including symptoms, exacerbations, side-effects, patient satisfaction and lung function (“*review response*”). Again, the control of the disease is assessed, and then the treatment is to be adjusted (stepped down when well controlled and stepped up when not).

Besides the medical treatment received from health care providers, previous research has highlighted the importance of self-management of patients and non-pharmacological interventions in the long-term management of asthma. The GINA 2016 guide has recommended several **non-pharmacological interventions**, among which several have been classified at the A evidence level (i.e. evidence obtained from at least one properly designed randomized controlled trial), including cessation of smoking in patients with asthma, avoidance of passive smoking exposure to children with asthma, engagement in regular physical activity and prevention of exercise-induced bronchoconstriction, avoidance of occupational exposures, avoidance of nonsteroidal anti-inflammatory drugs (NSAIDs, such as aspirin) if there is a history of previous reactions, and avoidance of indoor allergens. It is also recommended to be engaged in regular physical activity and to choose a healthy diet rich in fruits and vegetables for their general health benefits. [8].

Heterogeneity of asthma

Current evidence has consistently suggested that, rather than a single disease, asthma is more likely a collection of heterogeneous phenotypes sharing overlapping syndromes [10]. The heterogeneity of asthma has been observed at different levels, such as clinical manifestation of the disease (e.g. age of onset, allergy, severity), inflammatory phenotypes (e.g. eosinophils and/or neutrophils), and genetics [10]. Many phenotypes have been identified and overlap with each other[7,10] (Figure 1).

The most ancient and common distinction of asthma phenotypes is defined **according to several clinical features** of the disease, such as the age of onset and allergy/atopy status (i.e. early- or late-onset of asthma, and allergic or non-allergic asthma). While individuals with early-onset often have allergic symptoms, those with late-onset are often non-allergic, and lack atopy [7,10,11]. Moreover, asthma patients are likely to have various airway inflammatory profiles. Although airway eosinophil inflammation (i.e. high sputum eosinophils) often present in patients with allergic symptoms and predict better response to corticosteroids treatment – main controller medication in asthma treatment, other airway inflammatory profiles, including

neutrophil, mixed, and paucigranulocytic inflammation, are also observed, mainly among patients without atopy [7,10,11]. In addition, several particular phenotypes have also been identified, most of which are late-onset, such as obesity-related asthma, occupational asthma, and smoking-related asthma [7,10,11].

More recently, it was proposed that asthma phenotypes could also be identified **according to the molecular and physiological pathways** (e.g cytokines). Two phenotypes – type 2 high and type 2 low asthma – have been proposed according to inflammatory status which involves cytokines mainly produced by T-helper 2 cells, including Interleukin-4 (IL-4), IL-5, and IL-13 (i.e. type 2 inflammation) [12]. Type 2 high asthma was characterized by high IL-5 and IL-13 in lung tissue, increased blood and airway eosinophils, airway hyperresponsiveness, and had greater lung function improvements after ICS treatment [12]. However, type 2 low asthma, which lacks these characteristics and is likely to overlap other particular phenotypes (e.g. obesity-related asthma and smoking-related asthma), remains to be better understood.

In addition to these “candidate” approaches [11] based either on expert guidelines or on several specified features, **statistical approaches**, such as clustering, have been recently applied in epidemiological studies to better understand the heterogeneous nature of asthma [13]. For instance, in a cohort of more than 726 patients, using data from 34 core variables (compressed from 628 initial variables), five clinical asthma phenotypes were identified [14]: two phenotypes were early-onset atopic asthma, mild or moderate; one was late-onset non-atopic asthma among obese women; two were with severe airflow obstruction and bronchodilator responsiveness but differed in sex, age of onset, atopic status, baseline lung function and the response to bronchodilators. In a study applying latent transition analysis to longitudinal data followed over ten years, seven phenotypes were identified and showed high temporal stability [15]. More recently, genetic differences according to phenotypes have also been observed [16].

Overall, even though still not fully identified, heterogeneity of asthma has been widely observed and increasingly recognized. Better understanding the phenotypic heterogeneity of asthma may provide new insight into personalized asthma treatment.

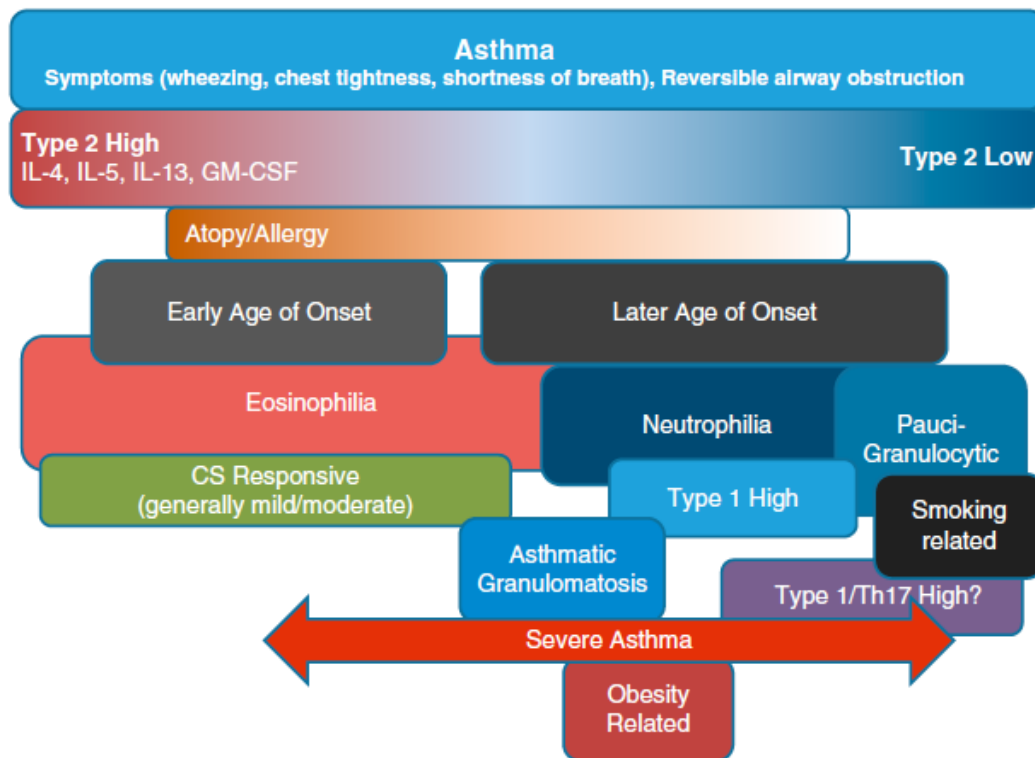


Figure 1 An example of current asthma phenotypes as they relate to inflammatory type (type-2 high or type 2 low) and other variables. Reproduced from Gaithier *et al.* Am J Respir Crit Care Med. 2015 [7].

1.2 Epidemiological aspects

1.2.1 Prevalence

Affecting more than 300 million individuals, asthma is one of the most common chronic diseases of the airways worldwide [1]. The prevalence of asthma varies across different countries. According to the World Health Survey including 178,215 adults from 70 countries in 2002-2003, the global prevalence of doctor-diagnosed asthma in adults were 4.3%, ranging from 0.2% in China to 21% in Australia [17]. According to the International Study of Asthma and Allergies in Childhood (ISAAC) including 798,685 children aged 13–14 years from 97 countries and 388,811 children aged 6-7 years from 61 countries, the prevalence of current wheeze (in the past 12 months) ranged from 0.8% in Tibet (China) to 32.6% in Wellington (New Zealand) among the 13–14 year olds, and from 2.4% in Jodhpur (India) to 37.6% in Costa Rica among the 6–7 year olds [18]. In France, according to the ESPS survey (Enquête sur la Santé et la Protection Sociale) conducted in 2006, the prevalence of ever asthma was 10.2% [19].

Since half a century, a remarkable global increase in asthma prevalence has been observed [20]. However, from 1993 to 2003, according to the ISAAC study, the prevalence of asthma changed little and even declined in a few high-prevalence countries, such as the US, Australia, and England, while increasing prevalence was observed in many developing countries in Africa and Latin America, which previously had low prevalence [21]. Since 2003, according to the latest data from the Centers for Disease Control and Prevention in the US, current asthma prevalence has newly increased in the US [22]. In France, the prevalence of ever asthma in 2006 has nearly doubled compared to the prevalence in 1998 (5.8%) [19]. Generally, asthma prevalence stays high in westernized/industrialized countries and increases in developing countries during urbanization and industrialization.

1.2.2 Assessment of asthma in epidemiological studies

In epidemiological studies – when the population is large – the questionnaire is the preferred approach to identifying individuals with asthma [23]. Standardized questionnaires on respiratory diseases were developed as early as in the 1950s by the British Medical Research Council (BMRC) to investigate the prevalence and several risk factors of chronic bronchitis and chronic airway obstruction [24]. In the following years, several questionnaires were developed based on the BMRC questionnaire, including the National Heart and Lung Institute (NHLI) questionnaire for respiratory disease, which was proposed in 1978 by the American Thoracic Society (ATS) and was recommended for assessing COPD in epidemiological studies [25]. In the 1980s, the International Union against Tuberculosis and Lung Disease (IUATLD) developed a new questionnaire with particular interest on asthma identification [26]. In the 1990s, based on the IUATLD questionnaire, two standard questionnaires focusing on asthma and asthma-like symptoms have been developed – by the European Community Respiratory Health Survey (ECRHS) for adults [27] and by the ISAAC for children [28]. Based on these questionnaires, a number of definitions were proposed to evaluate asthma in epidemiological studies. A brief introduction of several common used ones is presented in this section.

Asthma (yes/no)

Like other chronic diseases, the dichotomous measure (yes/no) of asthma is the most widely used in epidemiological studies to assess incidence or prevalence [29]. Asthma

status has been mostly collected using self-report questionnaires. While doctor diagnosed asthma has been considered as the most reliable, studies have shown that asthma is often under-diagnosed in the population [30]. Today, in large epidemiological studies, including the ISAAC study [18] among children and the ECRHS study [31] among adults, the definition of asthma is based on symptoms. Taking the ECRHS study for example, asthma was assessed through questions on attack of shortness of breath, attack of asthma, and use of asthma medications in the past 12 months [31]. Using dichotomous definition of asthma in epidemiological studies provides important information on the prevalence of the disease and allows us to track the global trend in the prevalence. However, in studies investigating risk factors for asthma, several methodological limitations have been raised.

Using classical longitudinal approach to studying risk factors for incidence of asthma, individuals with the disease at baseline are excluded and new cases during the follow-up are counted. However, as mentioned previously, the distinction of individuals with and without asthma is not always easy [32]. Like any other chronic disease, asthma may develop over a long period of time with preclinical status difficult to define. Moreover, the variability of asthma makes the distinction even more difficult. Due to the low incidence and inconsistent definition of asthma, using a dichotomous asthma definition might lead to misclassification and biased estimates of the investigated association. In this context, the asthma symptom score [33,34] has been proposed to study risk factors for asthma in longitudinal studies.

Asthma symptom score

The asthma symptom score has been proposed by Pekkanen *et al.* as a continuous measure of asthma, for studying risk factors for the disease in longitudinal studies [33]. The original version of the score [33] includes eight questions regarding the symptoms in the past 12 months: 1) wheezing or whistling in the chest and had breathless when wheezing sound present; 2) woken up with a feeling of chest tightness; 3) attack of shortness of breath at rest; 4) attack of shortness of breath after exercise; 5) woken by attack of shortness of breath; 6) ever asthma; 7) attacks of asthma; and 8) medication of asthma. Soon later, Sunyer *et al.* proposed an up-dated, simplified version, suggesting that including five of the eight questions (questions 1–5) showed almost the same internal consistency as the original score [34].

This asthma score has been applied to study several risk factors, including total [35] and traffic-related air pollution [36], smoking [37], socio-economic status [38], and the use of cleaning products [39]. Compared to self-report ever asthma or bronchial hyperresponsiveness, the asthma symptom score was shown to have increased specificity and positive predictive values [33]. It has also been shown that the asthma symptoms score at baseline can well predict incidence of asthma, asthma attacks, use of asthma medication, bronchial reactivity, and not COPD markers (decline in FEV₁ and airway obstruction) at follow-up [34]. The changes in the score overtime can either reflect asthma incidence, or capture the temporal variability of the disease among individuals with asthma [34]. Being a multiple categorical measure, the asthma symptoms score showed greater statistical power than other operational asthma definition in investigating risk factors for asthma. The use of the asthma symptoms score is likely to be relevant in epidemiological studies on both the primary and the secondary prevention of asthma.

Assessment among individuals with asthma

Multiple measures have been proposed among individuals with asthma to assess various aspects of the disease. Besides heterogeneous phenotypes of asthma defined to better understand and treat the disease (previously discussed in section 1.1), multiple measures among individuals with asthma have been proposed to assess the management of asthma and to study risk factors for the secondary prevention of asthma.

Regarding asthma activity and asthma control, measures on both short term and middle term have been proposed. In addition to the measures based on one or a few features, such as the frequency of attacks or exacerbations of asthma during a given time (from several weeks to one year), several measures based on multiple features have been developed. **The asthma control test (ACT)** [40], which include five questions on its influence on daily life, asthma symptoms (shortness of breath, woken by symptoms), the use of emergency medication use, and self-evaluated control during the past 4 weeks, is a common measure of asthma control for a short term. Similarly, the **Juniper Asthma Control Questionnaire (ACQ)** [41] has been proposed to measure asthma activity/control during the past week through seven questions, including five asthma symptoms during the past week (woken at night by symptoms, wake in the mornings with symptoms, limitation of daily activities,

shortness of breath, and wheeze), FEV₁% predicted, and daily use of short-acting bronchodilator. **Current asthma**, defined according to status of the disease during the past 12 months, is commonly employed to describe activity of the disease in a middle term. Different definitions of current asthma have been applied [29]. According to the definition in ECRHS II, current asthma is defined among individuals reporting the following events in the previous 12 months [42]: attacks of shortness of breath at rest with wheezing in the chest or asthma attacks, respiratory symptoms (wheeze, nocturnal chest tightness, attacks of breathlessness following activity, at rest or at night time, asthma attacks) or any use of medication because of breathing problems. In addition, **Asthma Quality of Life Questionnaire (AQLQ)** [43], a measure of quality of life in patients with asthma, has been developed to assess the impairment of quality of life due to the disease.

Assessing asthma activity and/or control, these measures are particularly relevant to investigating risk factors that potentially contribute to the secondary prevention of the disease in epidemiological studies.

1.2.3 Risk factors

The etiology of asthma remains not fully understood. Despite the likely heterogeneity of asthma, most of the previous studies have investigated the risk factors without further identification of different phenotypes. It is currently considered that asthma is a consequence of complicated interplay of multiple factors, including genetic and epigenetic, environmental, lifestyle/behavior, as well as social risk factor. Main risk factors identified in the literature are briefly reviewed here.

Genetic and epigenetic factors

It is widely recognized that family history for asthma increases the risk of asthma, due to both genetic and shared environmental factors among family members [44]. With the development and extensive use of genome wide association studies (GWAS) approach in recent years, up to date, several **genes** associated with asthma development or progression have been identified. Including 10,365 doctor-diagnosed asthma cases and 16,110 healthy controls, the GABRIEL Consortium (large-scale GWAS of Asthma) reported associations between asthma and several single-nucleotide polymorphisms (SNPs) among both early-onset asthma (< 16 years old) and later-onset (\geq 16 years old) asthma [45]: *IL1RL1/IL18R1* gene on chromosome

2q, *HLA-DQ* on chromosome 6p21, *IL33* on chromosome 9p24, *SMAD3* on chromosome 15q22, and *IL2RB* on chromosome 22q12, and the *ORMDL3* and *GSDMB* genes on chromosome 17q21 among early-onset asthma. The role of genetic factors in asthma development and progression is undeniable. However, probably due to the heterogeneity of asthma and the complex underlying pathological processes, it was estimated, in a large-scale GWAS study, that genetic factors accounted for only a small fraction of variance in asthma prevalence [46]. Given the potential important role of environmental factors in asthma, which may interact with susceptibility genes and promote disease progression, studies regarding gene-environment interactions, including epigenetic studies, have been a new focus on risk factors in asthma.

Epigenetic studies, which investigate the heritable changes without underlying DNA sequence alterations, reveal the influence of environmental factors on asthma, and provide new insight into the development of asthma [47]. Common epigenetic processes include DNA methylation, histone modification, and non-coding RNA induced gene silencing, which reflect the effect of gene-environment interaction. Due to its robustness, user-friendliness, and quantitative nature, DNA methylation is the most used method for studying the association between epigenetic factors and asthma. Previous research suggested that epigenetic changes may play an important role in the immune regulation such as the differentiation and activation of T lymphocyte cells [48]. In a recent case-control study including 194 children (97 with atopic persistent asthma and 97 healthy controls), different methylation levels have been observed in 81 regions, including *IL13*, *RUNX3*, and specific genes relevant to T lymphocytes [49]. Given that epigenetic changes are reversible, these findings provide an exciting future for asthma prevention and intervention.

Environmental factors

The development and manifestation of asthma are affected by several environmental factors, including living environment (e.g. in a farm or not), occupational and domestic exposure to cleaning products, as well as outdoor and indoor air pollution [50].

Regarding **living environment**, on one hand, exposures to **allergens** in the environment produced by pets (cats and dogs in particular), mice, pests (e.g. mites and cockroaches), plants (pollen) and fungi are important risk factors for the exacerbation of allergic asthma. On the other hand, previous research has consistently

shown that growing up on a farm with livestock is associated with decreased risk of developing asthma in the later life [51]. According to the “hygiene hypothesis” proposed by David Strachan in 1989 [52], the lack of exposure to environmental microbes might link to increasing prevalence of allergic diseases. Recent high-throughput sequencing technologies, such as whole genome sequencing (WGS), has enabled identification of **individual microbiota** structure (e.g. species of bacteria) and functional potential (e.g., vitamin synthesis genes) [53], giving important clues to study the role of microbiota in the pathophysiology of the host. In line with the “hygiene hypothesis”, recent findings using such technologies showed an important role of environmental microbes in gut and airway microbiota colonization in human, which can further influence the maturation of innate immunity in early life [54,55]. Studies investigating the association between gut/airway microbiota and asthma are likely to provide new insight for the prevention and management of asthma.

Today **occupation-related asthma** has been estimated as high as 15% in the adult-onset asthma and asthma is the leading occupational respiratory disease [56]. The use of cleaning products, including cleaning sprays, has been linked to increased risk of asthma incidence and control, among cleaners and healthcare workers in particular [57–59]. Besides occupational exposures, domestic exposure to household cleaning sprays has also been associated with an increased risk of asthma incidence [60] and a poor control of asthma in adults [61]. Given the wide use and the variety of cleaning sprays, further studies are needed to better understand the association between different types of sprays and asthma.

It is well established that acute exposure of **ambient (outdoor) air pollution** is associated with asthma exacerbations among both children and adults [62]. Besides, prenatal and childhood long-term exposure to outdoor air pollution, traffic-related air pollution in particular, has been associated with higher risk of asthma incidence, persistence and exacerbation in children [63]. In a current meta-analysis using data from birth cohorts [64], increased risk of asthma has been observed in children highly exposed to black carbon and particulate matter < 2.5µm in diameter (PM_{2.5}). Among adults, a prospective cohort study [65] has linked a high exposure to high PM_{2.5} and to nitrogen dioxide (NO₂) with incident wheezing and PM_{2.5} with adult-onset asthma and wheezing in women. Recently, another cohort study [66] has reported an increased risk of ACOS incidence among individuals who exposed to higher levels of PM_{2.5} and

ozone (O₃). In general, more data are needed to clarify the role of air pollution in asthma incidence among adults.

Regarding **indoor air pollution**, exposure to secondhand smoke is an important link to wheezing incidence in children and asthma severity in both children and adults [67]. However, its association with asthma incidence remains unknown in both children and adults. Particulate matter (PM), mainly from smoking, sweeping, and stove use, and nitrogen dioxide, mainly from unvented gas appliances (e.g. stoves and furnaces), are principal compositions of indoor air pollution [68]. Due to the difference in source, composition, and concentration between indoor and outdoor PM, the role of indoor PM in asthma cannot be extrapolated from the findings on the outdoor air pollution. Although results from a few studies suggested that indoor PM and NO₂ are associated with increased asthma symptoms in children, more studies are needed [69].

Lifestyle/behavior factors

The increased asthma prevalence worldwide has been linked to the modification in lifestyle factors in modern life, including increasing obesity, changing dietary habits (less fruit/vegetables, more prepared or processed foods), and insufficient physical activity [2]. Overwhelming evidence has suggested that **obesity** is a likely risk factor for asthma incidence and worse control in both children and adults [3]. Regarding **dietary factors**, several hypotheses have been proposed based on previous findings on other chronic diseases. However, these hypotheses were mostly investigated using cross-sectional data, and the results were inconsistent, among adults in particular. Details regarding the hypotheses and current evidence on the role of obesity and dietary factors in asthma are presented in sections 2.2 and 3.2. Regarding **physical activity**, whereas intense physical activity can be a trigger of asthma symptoms, insufficient physical activity may be associated with asthma due to increased inflammatory status [70]. However, probably due to several methodological challenges, such as difficulties in assessing physical activity, and potential reverse causation (i.e. asthma may limit patients' practice of physical activity), the effect of physical activity on asthma remains inconclusive [71,72].

Cigarette smoking has been considered as a major source of oxidant and has been associated with increased oxidative stress and airway inflammation, which are two main processes involved in asthma development [73]. The role of cigarette

smoking in asthma was once a debate, mainly due to inconsistent findings among adults regarding asthma incidence [74]. However, current evidence has suggested that smoking is a likely risk factor for both asthma incidence and asthma control [74,75]. Moreover, being part of lifestyle, cigarette smoking interacts with other lifestyle factors (e.g. dietary factors), and thus, could be a disease modifier in the association between these factors and asthma (further discussed in section 4.1.2).

Social factors

It is well known that many lifestyle or behavior “choices” (e.g., physical activity level, smoking and alcohol use, diet) do not represent a truly individual choice. Rather, they are the result of a combination of several factors in one’s social environment [76]. Therefore, complex interrelationships occurred when integrating the role of social factors in the study of lifestyle determinants of asthma. However, the role of **socio-economic status** (SES) itself in asthma as a risk factor has been insufficiently discussed. The association between SES and asthma incidence is still inconclusive [38,77]. However, a low SES has been associated with worse asthma control in both adults and children [78]. Given that measuring SES is still a challenge in epidemiology, most current studies use educational level or job category estimates as proxy for SES. Further studies evaluating SES through both individual and geographical indices may help to better understand the role of SES in asthma [50].

2 Diet and asthma

Nutritional epidemiology is one of the youngest disciplines in epidemiology. This may be partially due to the difficulties in measuring diet, which is known for its nature of complexity and variability. Over the past decades, a large number of observational studies have attempted to elucidate the role of diet in health and chronic diseases [79], such as cardiovascular disease, cancer, type 2 diabetes, obesity and more recently healthy aging. As several of the mechanisms underlying the diet–health association may also be involved in respiratory diseases (e.g. potential beneficial effect of antioxidant intakes), recently, these “diet–health” hypotheses have been enlarged into respiratory research, with regard to COPD and asthma in particular.

This section presents first a summary of approaches that are available in nutritional epidemiology to assessing dietary intakes and to analyzing dietary data, then a brief review of current evidence on the diet–health association, and finally the association between diet and asthma in epidemiological studies.

2.1 Diet in nutritional epidemiology

2.1.1 Tools to assess dietary intakes

The most common tools for dietary assessment include 24-hour recall, diet record, and food frequency questionnaires (FFQs).

Both the 24-hour recall and the diet record methods are short-term assessments. The **24-hour recall** method collects detailed information on all foods and beverages consumed by a participant in the past 24 hours through the recall of the participant [80]. This method is usually conducted by a trained or certified interviewer, and is most widely used to estimate mean intakes in a population. With the **diet record** method, participants are trained and asked to record in detail (by weighing or estimating) all foods and beverage consumed by oneself or another person (e.g., a child) on one or more days [80]. Unlike the 24-hour recall method, the diet record method records the data when the foods are consumed, and thus do not rely on participants’ memory. Diet records during multiple days over seasons (typically 7-day diet records over seasons) can provide direct and accurate quantification of food intakes, and is commonly used to validate other tools for assessing dietary intakes [81]. However, such multiple assessment is infrequently used in large-scale

epidemiological studies, as they are expensive, time-consuming, and cause high burden of participants [82].

In epidemiological studies regarding risk factors for chronic diseases, the average long-term diet is usually more appropriate than short-term diet, and ranking of individual intakes is more important than absolute intakes. In this context, the **food frequency questionnaire (FFQ)** method has been proposed as a less expensive tool to assess the long term-diet. As oppose to those short-term assessment, the FFQ collects the frequency of food intakes in terms of a specified portion size over a specific period (typically several months or a year). Calibration studies are needed for evaluating reproducibility and validity of food frequency questionnaires [83]. Ideally, repeated FFQs during follow-up can reduce measurement error and better represent long-term diet [84].

As all methods above collecting data through reporting process cannot avoid the under-reporting bias, several **biomarkers** of nutrients in blood or other tissues have been proposed as an objective assessment of dietary intakes. Similar to other assessment of dietary intakes, a dietary biomarker needs to reflect the cumulative effect of diet over a long period rather than the fluctuation of intakes for a short time [85]. Furthermore, as a validate measure of a nutrient/food, it is essentially required a good correlation between the biomarker and the nutrients/foods supposed to measure. However, modest correlations between most biomarkers and dietary intakes have been observed due to the bioavailability (the portion that is digested and absorbed *in vivo*) of nutrients/foods and the homeostatic mechanisms controlling the concentration of nutrients in body tissues and fluids [85]. Up to date, only a few biomarkers have been proposed to assess intakes of nutrients (e.g., carotenoids and vitamin D) and no biomarkers are available for foods [85].

The **strengths and limitations** of different dietary assessment measures are presented in Table 1. In summary, despite several dietary assessment are available nowadays, it remains a challenge to measure dietary intakes precisely, especially in large epidemiological studies. Balancing strengths and limitations of existing methods, validated FFQs offer an inexpensive tool to assess usual (long-term) diet and have been the main tool for collecting dietary data in epidemiological studies.

Table 1 Strengths and limitations of different dietary assessment measures (adapted from Nutritional epidemiology, Walter Willet [86])

Method	Strengths	Limitations
24-hour recall	<ul style="list-style-type: none"> • Most widely used to estimate mean intakes of a population 	<ul style="list-style-type: none"> • Not representative of usual dietary intakes in case of insufficient number of recalls • Inaccuracy of portion size reporting due to conceptualization and memory errors • Under- or over-reporting of foods (e.g. alcohol and fruits) • Trained or certified interviewer needed
Diet record	<ul style="list-style-type: none"> • Weighed diet record can provide direct and accurate quantification of food intakes (not relying on participants' memory) • Most commonly used as a reference method for validating FFQs. 	<ul style="list-style-type: none"> • Modifying of diet habit by the record itself • Not representative of usual dietary intakes without repeated recording over seasons • Underreporting bias, especially among the obese • Literate, trained, highly motivated participants needed • High burden for participants
Food frequency questionnaire (FFQs)	<ul style="list-style-type: none"> • Estimating average long-term dietary intakes • Relatively inexpensive • Low burden of participants • Feasible in large epidemiological studies 	<ul style="list-style-type: none"> • Need to be culture and population specific (calibration needed) • Underreporting bias, especially among the obese
Biomarkers of nutrients in blood or other tissues	<ul style="list-style-type: none"> • Increased reliability and objectivity (not relying on participants' memory) 	<ul style="list-style-type: none"> • Complex relationship with intakes due to physiological processes <i>in vivo</i> • Lack of sensitive biomarkers for many nutrients • Invasive • Costly

2.1.2 Approaches to analyzing dietary data

Over the last decades, two main approaches have been proposed to study the diet–health relationship: first, the “traditional” approach through individual foods/food groups or nutrients, and more recently, a second approach through dietary patterns. In the following sections, these two approaches are briefly reviewed.

2.1.2.1 Approach through foods and nutrients

More than two centuries ago, James Lind discovered that fresh citrus fruit could cure scurvy, which has been explained by vitamin C deficiency later [87]. Afterward, deficit in several nutrients has been found related to several diseases. For instance, deficiency of thiamine (vitamin B₁) is related to Beriberi, and deficiency of iodine is associated with the thyroid goiter [88]. Such studies introduced the traditional approach to studying diet–health associations – the approach through specific foods and nutrients. Nowadays, the use of this approach has been largely expanded and commonly used in public health to study risk factors for major chronic diseases, including cardiovascular disease, cancer, diabetes, and chronic respiratory diseases [88].

Nutrients contained in foods can be roughly represented by macronutrients such as carbohydrates, proteins, fats, and micronutrients such as vitamins and minerals. The approach through nutrients has been broadened to constituent chemicals that include not only nutrients, but also additives, contaminants, and chemicals formed in the cooking or processing of foods. To compute the total intake of a nutrient/constituent chemical from foods for an individual, usually a food composition table is needed in addition to data on food intakes. Assuming that nutrients of a specific food are approximately constant, food composition tables provide detailed information on nutrients in commonly consumed foods. Several food composition tables have been developed worldwide (<http://www.fao.org/infoods/infoods/tables-and-databases/>). In France, commonly used food composition tables are as follow: the ANSES/CIQUAL French food composition table (the version 2013 has been widely used, and a new version has been proposed in 2016, <https://pro.anses.fr/TableCIQUAL/>), the one from the OQALI (Observatoire de la qualité de l'alimentation, <http://www.oqali.fr/Donnees-publiques>), and also the one from the Vitamin and Antioxidant Element Supplementation (SUplémentation en Vitamines et Minéraux AntioXydants, SU.VI.MAX) study [89]. Alternatively, dietary

intakes can be described and studied through foods (e.g. soybean) or food groups (e.g. vegetables, fruits), which can be calculated by using tools assessing dietary intakes and summing intakes of several related foods.

With regard to the approach through nutrients, several issues need to be carefully addressed, or biased results can be reported [88]. First, to assess nutrient intakes of individual properly, the food composition table should be complete, accurate and up-to-date. Although a few biomarkers have been proposed as an objective assessment for several nutrients, most of them remain unable to assess through biomarkers. Second, as the wide use of nutritional supplements [90], accounting for intakes from supplements is essential for studies regarding nutrients, particularly when the supplement is the main source of the nutrient (e.g. folic acid). Also, it is worth noting that nutrient composition cannot fully represent foods due to unmeasured nutrients and potential interactions of single nutrient.

While the approach through nutrients may be more relevant to potential biological process involved in a disease, the approach through foods/food groups is more relevant to dietary recommendations. Dietary recommendations can be made on the intake of specific foods even when the potential biological process and the responsible factors remain unknown. Employing both nutrients and foods in analysis may offer more information regarding the investigated association: hypotheses with nutrients should be examined by replicating the results with several major foods contributing to this nutrient; and associations observed with specific foods can lead to further studies on nutrients to examine the potential biological process [88].

However, it remains extremely difficult to study the separate effect of individual foods/nutrients. Since foods are consumed in a form of complex combination (i.e. dishes and meals), foods and nutrients can interact with each other and influence their bioavailability and absorption. Moreover, due to cultural factors and individual behavioral patterns, several foods/nutrients are highly correlated. For instance, negative correlation has been observed between vegetable and chips intake. Results from studies without taking into account such correlation can be misleading [81]. To address these issues, dietary patterns analysis has been proposed as an alternate approach [91].

2.1.2.2 Approach through dietary patterns

Dietary pattern analysis has been proposed to take into account the interrelations among foods/nutrients and to study the combined and overall effect of diet. Three methods have been proposed in the literature to derive dietary patterns: the “*a priori*” approach, the “*a posteriori*” approach, and the methods “in between” (Figure 2). In this section, these three approaches are briefly reviewed.

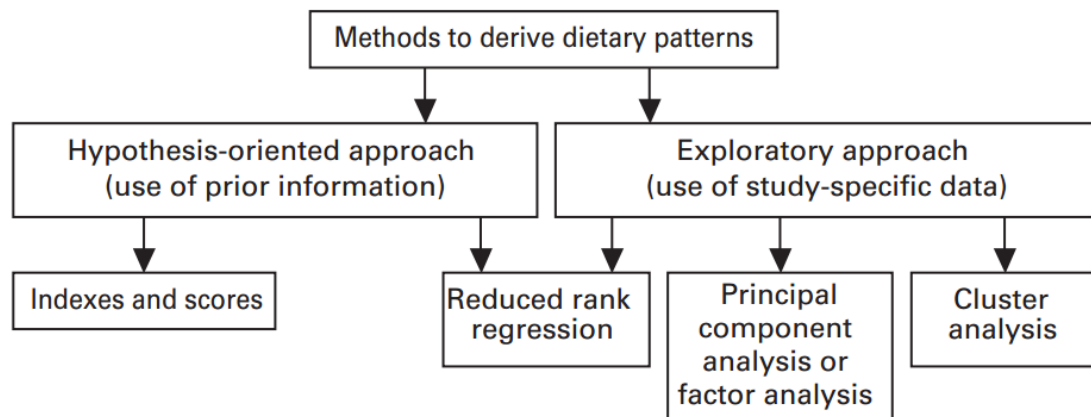


Figure 2 Approaches to define dietary patterns in observational studies (reproduced from Schulze *et al.* Br J Nutr 2006 [92]).

A priori approach – diet scores

The *a priori* approach is represented by various diet scores that have been developed based on established knowledge in nutritional epidemiology. *A priori* scores have been constructed either 1) based on nutrients/foods with specific biological properties, or 2) based on recommended foods or nutrients for disease prevention. Since our knowledge on nutritional epidemiology is rapidly increasing, it is essential to take into account the latest evidence for these hypothesis-driven diet scores. For this reason, diet scores are often revised and several versions have been proposed.

Several diet scores have been developed based on biological properties of dietary factors, which may contribute to certain pathophysiological processes *in vivo*, including antioxidant defense and inflammation. For example, the Total Antioxidant Capacity (TAC) [93–96] has been proposed to assess the combined effect of antioxidants present in foods. Several versions of the Dietary Inflammatory Index (DII) [97–100] have been proposed to assess the inflammatory potential of diet.

Another bunch of diet scores have been developed based on current evidence on foods or nutrients in nutritional epidemiology. Several of these scores were designed to assess the adherence to a specific type of diet, like the Mediterranean diet score (MDS) [101], or to nutritional guidelines, like the American Healthy Eating Index (HEI) [102] and the French Programme National Nutrition Santé-Guideline Score (PNNS-GS) [103]; and several were designed to reflect a healthy eating pattern that was likely beneficial in disease preventing, such as the Dietary Approach to Stop Hypertension (DASH) [104], the Alternate Healthy Eating Index (AHEI) [105], and Dietary Quality Index-International (DQI-I) [106]. This kind of scores is usually considered as a measure of overall healthy eating. In the following paragraphs, the most used diet scores are presented and discussed, including the Mediterranean diet scores, the Dietary Approach to Stop Hypertension, and the Healthy Eating Indexes and the Alternate Healthy Eating Indexes.

The **Mediterranean diet score (MDS)** is the first and the most famous diet score. The original score was proposed by Trichopoulou *et al.* in 1995 [101] to assess the relation between the traditional Mediterranean diet and longevity in the Greek population. Scoring based on the gender specific median value of intakes, higher Mediterranean diet scores corresponds to higher adherence to the traditional Mediterranean dietary pattern. To take into account new findings in nutritional epidemiology, this score has been updated several times. First it was modified by adding the fish intake as a component [107], and then the alternate Mediterranean score (aMED) has been proposed by Fung *et al.* with multiple modifications [108]: excluding the dairy products, including nuts, and restricting cereals to only whole-grains and meat to only red and processed meat. Components of aMED are presented in Table 2. The adherence to the Mediterranean diet has been associated with longevity [101,107], lower mortality [109], and lower risk of obesity [110], cardiovascular disease [111], cancer [112], and diabetes [113].

The **Dietary Approach to Stop Hypertension (DASH)**, a diet rich in fruits, vegetables, low-fat dairy foods, and low in saturated and total fat, has been shown to be able to reduce blood pressure [104]. Among several versions of score based on the DASH diet, the most used DASH score is an 8-item version [114] proposed by Fung and colleagues. For each of the items, a score ranging from one to five was allocated based on the rank of intakes in the study population (from lowest to highest quintile).

A greater adherence to the DASH diet has been associated with lower mortality as well as lower risk of cardiovascular disease (CVD), type 2 diabetes and cancer [115]. Hence, the DASH score is now considered as a measure of overall diet-quality.

The **Healthy Eating Index (HEI)** and its variant versions are also commonly used in epidemiological studies. The original Healthy Eating Index was developed based on the 1995 US Dietary Guidelines by the US Department of Agriculture [102]. Higher scores represent better adherence to the guideline. However, the original HEI has been weakly associated with major chronic diseases (cardiovascular disease, cancer, or nondramatic death) [116,117], probably because diet components that have inverse effect on health-related outcomes are not distinguished, such as whole grains and refined cereals, red/processed meat and fish, as well as saturated and poly-unsaturated fatty acids. To overcome these issues and to better predict the reduction of risk of chronic diseases, McCullough *et al.* proposed the **Alternate Healthy Eating Index (AHEI)** [105], with higher scores representing a healthier diet. The HEI has been revised twice to reflect the new American Dietary Guidelines 2005 and 2010, leading to the HEI-2005 [118] and the **HEI-2010** [119]. Also, the **AHEI-2010** – the updated version of the AHEI, has been proposed to take into account new findings in nutritional epidemiology. Both HEI-2010 and AHEI-2010 have been associated with major chronic diseases, including CVD, cancer and type 2 diabetes [120]. Current evidence on the AHEI-2010 and major chronic diseases are reviewed in section 2.1.3.

Although all these diet scores emphasize high intakes of vegetables, fruits, and whole grains, with different initiative objective, these scores differ in some ways. The components and scoring criteria of these scores are presented and compared in Table 2. Specific to the Mediterranean diet, the Mediterranean diet scores (MDS and aMED) may not be ideal for assessing diet outside the Mediterranean population, even though the scoring is based on median intakes of the study population. For instance, the adherence to a modified Mediterranean diet was strongly associated with lower mortality in Greece and Spain whereas no such association was observed in the Netherlands and Germany [121]. To adapt the cultural and geographical specificity of diet, some modified versions have been applied in different populations; however, comparability of the results across populations is problematic. With regard to the DASH score, as it is calculated with quintiles-based intakes of each item, the results

across different populations are difficult to compare. Moreover, the DASH scores were more often associated with higher energy intake than the HEI-2010 and the AHEI-2010 [122]. The HEI-2010 may need further improvement because the scoring does not include sugar-sweetened beverages and red/processed meat, which have been related to higher risk of multiple chronic diseases. The AHEI-2010 is the latest diet score proposed in the literature to reflect the overall healthy eating. Being associated with multiple health outcomes (reviewed in section 2.1.3), the AHEI-2010 is a promising tool for today's research on diet with chronic diseases and health outcomes in different populations.

A posteriori approach – data-driven dietary patterns

The *a posteriori* approach derives dietary patterns from study-specific dietary data using statistical methods. Among several proposed methods, the most used statistical procedures are principal component analysis (PCA) and cluster analysis [91].

As stated previously, food intakes have correlations as several foods are frequently consumed together. Examining the correlation matrix of foods/food groups, **PCA** aims to aggregate food variables and identify uncorrelated components (factors) that explain most of the variance of the data. A continuous score of each factor is then derived for each individual and can be used instead of original food variables in analysis to study the association between dietary patterns and outcomes of interest. However, components derived in a study are probably different from those derived in another study, due to potential differences in dietary habits across populations and in available dietary data across studies. The reproducibility of the results may also be limited by several choices made regarding the statistical model when applying PCA, including the group of food items, the number of factors to identify, the method of rotation and the labeling of the components [91].

Different from principal component analysis, **cluster analysis** mainly aims to group individuals having similar food choices. In cluster analysis, observations of individuals are positioned in a multi-dimensional space in which each food variable constitutes an axis/dimension; those with small distance between each other are considered to have similar food choice. Individuals who were grouped into the same cluster (i.e. group) have more similar dietary habits to each other than to those in other groups. After the clustering procedure, dietary profiles need to be compared across clusters to interpret and label the cluster. Two main limitations of the cluster

approach are 1) different clusters can be derived using different algorithms for computing distance, which may limit the reproducibility of the study, and 2) for individuals in the same group, no more individual difference in dietary profiles is taken into account.

Table 2 Comparison of four common used diet scores.

	aMed [108]	HEI-2010 [119]	AHEI-2010 [120]	DASH [114]
Score range	0–9	0–100	0–110	8–40
Number of components	9	12	11	8
Scoring criteria	1 or 0 point for each item according to sex-specific median intake.	0–5 or 0–10 point for each item based on energy-standardized intakes.	0–10 point for each item based on recommended values or deciles	1–5 point for each item according to sex-specific intake quintiles
Components recommended with high intakes*(range of score)	<ul style="list-style-type: none"> • Total vegetables (0/1) • Total fruit (0/1) • Legumes (0/1) • Nuts (0/1) • Whole grains (0/1) • Fish (0/1) • Monosaturated to saturated fat ratio (0/1) 	<ul style="list-style-type: none"> • Total fruit (0–5) • Whole fruit (0–5) • Total vegetables (0–5) • Greens and beans (0–5) • Whole grains (0–10) • Dairy (0–10) • Total protein foods (0–5) • Seafood and plant protein (0–5) • Unsaturated to saturated fat ratio (0–10) 	<ul style="list-style-type: none"> • Vegetables without potatoes (0–10) • Fruit (whole fruit only) (0–10) • Whole grains (0–10) • Nuts and legumes (0–10) • Long-chain (n-3) fats (0–10) • Polyunsaturated fatty acids (0–10) 	<ul style="list-style-type: none"> • Total fruit (1–5) • Total vegetables without potatoes (1–5) • Whole grains (1–5) • Low-fat dairy products (1–5) • Nuts, seeds, legumes (1–5)
Components recommended with moderate intakes*(range of score)	<ul style="list-style-type: none"> • Alcohol (0/1) 		<ul style="list-style-type: none"> • Alcohol (0–10) 	
Components recommended with low intakes*(range of score)	<ul style="list-style-type: none"> • Red/processed meat (0/1) 	<ul style="list-style-type: none"> • Refined grains (0–10) • Sodium (0–10) • Calories from solid fats, alcohol, and added sugars (0–20) 	<ul style="list-style-type: none"> • Trans fat (0–10) • Red/processed meat (0–10) • Sugar-sweetened beverages (0–10) • Sodium (0–10) 	<ul style="list-style-type: none"> • Red/processed meat (1–5) • Sugar-sweetened beverages (1–5) • Sodium (1–5)

*For components recommended with high intakes, higher scores represent higher intakes; for components recommended with moderate intakes, higher scores represent moderate intakes; for components recommended with low intakes, higher scores represent lower intakes.

Approach “in between”

To combine established knowledge with statistical methods in dietary patterns identification, several methods that lie between the *a priori* and *a posteriori* approaches have been proposed, some of which define dietary patterns as a latent variable.

Structural equation modeling (SEM), such as confirmatory factor analysis (CFA) and latent class analysis, has been proposed to identify latent variables associated with dietary intakes [123]. Such methods have several advantages: 1) established knowledge can be adapted into SEM, such as the omission of a certain food group in the loading of a specific factor (dietary pattern); 2) the retained factors can be correlated; and 3) controlling for confounders are allowed [124]. Traditionally, CFA has mainly been applied to verify dietary patterns derived using other methods, such as PCA. However, recently two studies favored the direct use of CFA (alone), instead of PCA, for the identification of dietary patterns in epidemiologic studies [125,126]. Latent class analysis has been proposed as an alternative methods to cluster analysis, without assuming the same error variance for all outcomes [124]. More recently, latent transition models, an extension of latent class analysis, have been used in longitudinal studies to define dietary patterns at different time points and to estimate probabilities of changing patterns over time [127].

Several **“in between” methods without latent variables** have also been proposed. Reduced rank regression (RRR) [128] determines correlation of foods by identifying the factors (patterns) that explain most of the variance in disease-specific biomarkers or key nutrients as response variables. Thus, it allows taking into account knowledge about hypothesized biological pathways. This method may be of particular interest in studies on a disease for which multiple dietary risk factors has been identified (e.g. coronary heart disease) [92]. Other methods, such as partial least-squares regression (PLS) [128] or the treelet transform (TT) method [129], have also been proposed but are still little used.

2.1.3 Diet and major chronic diseases: a brief overview

According to the WHO, major chronic diseases include cardiovascular disease (CVD), cancer, diabetes, and chronic obstructive pulmonary disease (COPD). Representing more than 60% of all deaths, they are the leading causes of mortality worldwide [130]. A large number of studies have shown that diet has significant effects, protective or deleterious, on CVD, cancer and type 2 diabetes, depending on the biological profiles of foods and quantity of intakes [79]. More recently, increasing interest has been raised on the association between diet and chronic respiratory disease, including COPD and asthma.

In this section, major protective and deleterious dietary factors for major chronic diseases are briefly reviewed; after that, more discussion is given to a recent carcinogen – processed meat, and then to the latest healthy diet score proposed in the literature – the AHEI-2010.

2.1.3.1 Major protective and deleterious dietary factors for major chronic diseases

Protective dietary factors

Dietary items that have potential benefit on long-term health mainly include vegetables and fruits, whole grains, polyunsaturated fatty acids (PUFA), nuts and legumes, and a moderate intake of alcohol.

Vegetables and fruits are important source of antioxidant vitamins, including vitamin A, C, E, as well as fiber and folic acid. A large number of studies have shown the health benefits of greater consumption of fruits and vegetables, including lower mortality [131], and lower risk of CVD [132], diabetes [133], some cancers [134], and COPD [135,136]. Hence, high intake of fruits and vegetables are suggested by most of the dietary recommendations.

Whole grains, which contain much higher B vitamins, vitamin E, and fiber compared to refined cereals [137], have risen increasing interest in nutritional epidemiology over the past years. A growing number of studies have shown that eating whole grains rather than refined grains can reduce total cholesterol, triglycerides, and insulin level [79]; and higher intake of whole grains has been related with lower risk of CVDs [138], diabetes [139], colorectal cancer [140], and COPD [141,142].

It has been shown that low total fat diet has no beneficial effect, and that **different types of fat** intake have distinct effects on human health. High intake of *trans* fatty acids and saturated fats and low intake of polyunsaturated fatty acids (PUFAs) increase the concentration of low-density lipoprotein (LDL) cholesterol, which leads to development of atheromatous plaques and finally cardiovascular disease like myocardial infarction [143]. Omega-3 PUFA intake has been widely recommended because of its association with reduced mortality and reduced risk of CVD [144,145]. Although there was concern, mainly based on animal studies [146], about the potential pro-inflammatory effect of high ratio of omega-6 to omega-3 PUFA intakes, recent epidemiological findings in human have suggested that higher omega-6 intakes can also reduce the risk of CVD [147]. For this reason, the American Heart Association recommends a PUFA intake (including both omega-3 and omega-6 PUFA) of up to 10% of total energy. The negative association between higher intakes of PUFA and type 2 diabetes has also been reported [148]. Regarding cancer, despite a large amount of longitudinal studies and RCT, there is now no clear evidence linking any specific type of fat with cancer. Regarding COPD, a recent prospective study observed no potential benefit from high PUFA intakes [149].

Nuts and legumes are rich in PUFAs, as well as other beneficial compounds, including protein, fiber, and a variety of vitamins and minerals. Previous studies have consistently shown a reverse association between intakes of nuts and legumes and risk of CVD, especially for coronary heart disease [150]. Limited evidence has shown the benefit of high intake of nuts and legumes on other major chronic diseases including cancer, diabetes, and COPD [151,152].

Alcohol consumption may have different effects on health depending on the quantity of intake. High alcohol intake can have many adverse effects, including increased risk of certain cancers and alcoholism, whereas moderate alcohol intake has been associated with lower mortality and lower risk of coronary heart disease (CHD) [153], dementia [154], and type 2 diabetes [155]. Little data has been reported regarding the association between alcohol intake and COPD.

Deleterious dietary factors

Dietary items that have potential deleterious effect on long-term health mainly include the well-known *trans* fatty acids, , as well as red/processed meat, sodium and sugar sweetened beverages.

Trans fatty acids mostly come from products containing partially hydrogenated oils, including baked goods, margarine, and deep-fried foods. Intake of *trans* fatty acids, even of small amount, increases significantly total and LDL cholesterol. Strong evidence has been found regarding the association of *trans* fatty intake with increased mortality [156] and risk of coronary heart disease [157].

Processed meat, which is high in nitrite, is recently classified as carcinogenic to humans, based on evidence from a large number of studies, by the International Agency for Research on Cancer (IARC) [158]. Positive associations have been reported of processed meat intake with all-cause mortality and other major chronic diseases, including CVD, type 2 diabetes, and COPD [142,159]. Similar findings have been reported with total red meat intake, but no association has been observed for unprocessed red meat in several studies [158–160]. The association between processed meat intake and chronic diseases are further discussed in the next section (2.1.3.2).

While **salt** is indispensable in human diet, excess salt intake increases both the amount of fluid in body and the volume of blood in vessels. High intake of salt can significantly increase risk of hypertension and has also been associated with higher risk of stroke and CHD [161].

Sugar-sweetened beverages, which contain a large amount of added sugars, such as sucrose or fructose, have little nutritional value. A number of epidemiological studies have shown that intake of sugar-sweetened beverages are related to low diet quality, and higher risk of obesity [162], type 2 diabetes [163], and cardiovascular disease [164].

2.1.3.2 Processed meat intake and chronic diseases

According to the WHO, processed meat refers to meat that has been transformed through salting, curing, fermentation, smoking, or other processes to enhance flavor or to improve preservation, such as hot dogs, ham, and sausages. With sufficient evidence on the relation between processed meat intake and increased risk of cancers, the WHO classified processed meat as carcinogenic to humans in 2015 [158]. Overwhelming evidence has been observed for colorectal cancer, but associations have also been seen for stomach and lung cancer [158,165]. The carcinogenic effect of processed meat intake could be at least partly explained by several carcinogenic chemicals that are formed during meat processing, including

N-nitroso-compounds (NOC), heterocyclic aromatic amines (HAA), and polycyclic aromatic hydrocarbons (PAH) [158].

Interestingly, the adverse effect of processed meat intake on health is likely more than only carcinogenic. A prospective study conducted among over half a million participants in the US [166] shown that high processed meat intake was associated with increased mortality and increased risk of total, cancer, and CVD. Findings regarding several chronic diseases have also been largely reported. One meta-analysis [167] including five large prospective cohorts has showed positive association between processed meat intake and stroke in populations from the US, Sweden and Japan. Using data from five prospective cohort studies and one case-control study conducted in the US, UK, Spain and Italy, another meta-analysis [159] showed that processed meat consumption was associated with higher risk of incidence of coronary heart disease. Results from a large prospective cohort among Swedish men [168] showed a significant association of processed meat consumption with higher risk of heart failure and higher mortality from heart failure. Also, the positive association with type 2 diabetes has been consistently suggested, according to a meta-analysis using data from seven prospective cohorts [159] conducted in the US, as well as three large prospective studies published later in populations from Sweden and the US [169,170,160]. With regard to COPD, seven studies [171–177] conducted in the US, UK, China, Spain and Sweden showed significant positive association of processed meat intake with decreased lung function, and increased COPD prevalence, incidence and exacerbations. In addition, processed meat intake has been positively associated with hypertension [178] and obesity [179].

Despite different eating habit across countries, processed meat intake has been consistently associated with major chronic diseases. Several hypotheses with regard to potential mechanisms have been proposed. First, processed meat intake may be related to oxidative and nitrosative stress, which has been associated with development of several diseases, including cardiovascular disease, diabetes and COPD [180–183]. Nitrite, added in meat processing depending on different regulations across countries, can be transformed into nitroso derivatives by bacteria in the mouth and the digestive gastrointestinal tract, and react with reactive oxygen species to form nitrogen reactive species – one important source of oxidative and nitrosative stress [184]. A positive association between processed meat intake and

concentration of biomarkers of oxidative stress has been reported [185]. Second, processed meat intake may be associated with systemic inflammation, a pathophysiological process involved in many chronic diseases [186]. Third, high contents of salt and saturated fat in processed meat may also contribute to the adverse effect of processed meat intake. Last, given that processed meat intake is strongly associated with obesity, an important risk factor for many chronic diseases, the associations may be partly mediated through obesity.

In summary, based on studies performed in different countries, where both the process and the cultural habits regarding processed meat intake differ, current evidence suggests adverse effect of processed meat intake on multiple health outcomes.

Table 3 Association between processed meat intake and health outcomes

Outcome	Association with processed meat intake	Level of evidence	Comment
Cancer	+	Classified as carcinogenic by WHO	
Mortality	+	Large prospective studies	Association observed for total, cancer, and CVD mortality.
Stroke	+	Meta-analysis, ≥ 5 prospective studies	
Coronary heart disease	+	Meta-analysis, ≥ 5 prospective studies	
Heart Failure	+	One large prospective study	
Type 2 diabetes	+	Meta-analysis, ≥ 5 prospective studies	
COPD	+	Large prospective studies	Associations reported for COPD incidence and readmission
Hypertension	+	Cross sectional study	
Obesity and higher BMI	+	Meta-analysis	

+: Positive association.

2.1.3.3 The overall healthy eating (the AHEI-2010) and chronic diseases

Findings regarding protective and deleterious foods/nutrients have contributed to the construction of diet scores, which allow to studying the combined effect of foods/nutrients. The latest diet score reflecting healthy eating proposed in the literature is the AHEI-2010. Up to now, eight large prospective studies have concluded that a greater adherence to a diet reflecting by higher AHEI-2010 scores was associated with lower all-cause, cardiovascular and cancer mortality [122,187–193]. Fifteen studies have been published on the relation between the AHEI-2010 with the risk of major chronic diseases or related health outcomes [120,194–207] (see Table 4).

A greater adherence to a healthy diet as defined by the AHEI-2010, has been associated to a lower risk of cardiovascular disease, including acute myocardial infarction, coronary heart disease, and stroke, based on evidence from several studies from more than 30 different countries [120,194–196]. According to studies conducted in the US and in Australia, the healthy diet has also been associated with lower risk of lung cancer, prostate cancer and urothelial cell carcinoma [197–199]. Regarding diabetes, three large cohorts conducted in the US have reported an association between the healthy diet and a lower risk of type 2 and the first gestational diabetes [200–202]. In the US, the healthy diet has also been associated with a lower risk of COPD [203], a lower risk of hypertension [207], and with healthy aging [205].

The healthy diet was also associated with a lower risk of obesity [206], and with favorable profiles of several biomarkers of the brain-adipose axis [208,209]. Like for processed meat, it is likely that the association between healthy eating and chronic diseases is partly mediated through obesity. The potential intermediate role of obesity in the association between the AHEI-2010 diet score and disease merits further investigation.

Table 4 Studies on the relation between the Alternate Healthy Eating Index 2010 (AHEI-2010) and major chronic diseases or related health outcomes

Reference	Outcome	Population	Study design	Results	Comments
<i>Multiple major chronic diseases</i>					
Chiuvè <i>et al.</i> J Nutr 2012 [120]	Incidence of major chronic disease (MCD), including CVD, CHD, stroke, diabetes, cancer	71,495 women and 41,029 men in the US	Prospective cohort	RR (95%CI) comparing the highest to the lowest quintile of AHEI-2010: MCD: 0.81 (0.77, 0.85) CVD: 0.76 (0.71, 0.81) CHD: 0.69 (0.62, 0.76) Stroke: 0.80 (0.71, 0.91) Diabetes: 0.67 (0.61, 0.74) Cancer: 0.94 (0.89, 0.98).	
<i>Cardiovascular disease</i>					
Fung <i>et al.</i> Am J Clin Nutr 2016 [194]	Coronary artery disease	207,491 adults in the US	Prospective cohort	RR comparing top to bottom deciles of AHEI-2010: 0.85 (95%CI lower than one, exact value not provided)	The association between the AHEI-2010 and coronary artery disease was studied to compare with the results using a food quality score.
Neelakantan <i>et al.</i> J Nutr 2016 [195]	Acute myocardial infarction	751 cases and 1,443 controls in Singapore Chinese	Case-control	OR (95%CI) comparing the highest to the lowest quartile of AHEI-2010: 0.62 (0.47, 0.81)	
O'Donnell <i>et al.</i> Lancet 2016 [196]	Stroke	13,447 cases and 13,472 controls adults from 32 countries	Case-control	OR (95%CI) comparing the highest to the lowest tertile of AHEI-2010: 0.60 (0.53, 0.67)	
<i>Cancer</i>					
Anic <i>et al.</i> Eur J Clin Nutr 2015 [197]	Incidence of lung cancer	460,770 adults in the US	Prospective cohort	HR (95%CI) comparing the highest to the lowest quintile of AHEI-2010: 0.86 (0.80–0.92)	

Bosire <i>et al.</i> Am J Epidemiol 2013 [198]	Incidence of total prostate cancer	293,464 adults in the US	Prospective cohort	HR (95%CI) comparing the highest to the lowest quintile of AHEI-2010: 0.93 (0.88–0.99)	
Dugué <i>et al.</i> Int J Cancer 2016 [199]	Urothelial cell carcinoma (UCC)	4,1514 adults in Australia	Prospective cohort	HR (95%CI) per 1 SD decrease in score: Superficial UCC: 1.17 (1.01–1.34). Not significant for all cases and for invasive UCC.	
Diabetes					
Jacobs <i>et al.</i> Diabetologia 2014 [200]	Incidence of type 2 diabetes	89,185 adults in the US	Prospective cohort	HR (95%CI) comparing the highest to the lowest quintile of AHEI-2010: In men: 0.88 (0.81, 0.96) In women: 0.88 (0.80, 0.97)	
Cespedes <i>et al.</i> Am J Epidemiol 2016 [201]	Incidence of type 2 diabetes	101,504 postmenopausal women in the US	Prospective cohort	HR (95%CI) per 1 SD increase in score: 0.87 (0.85–0.89)	Adjusting for baseline BMI attenuated but did not eliminate the association
Zhang <i>et al.</i> BMJ 2014 [202]	Incidence of 1st gestational diabetes	20,136 adults in the US	Prospective cohort	HR (95%CI) comparing the highest to the lowest quintile of AHEI-2010: 0.75 (0.59–0.94)	
COPD					
Varraso <i>et al.</i> BMJ 2015 [203]	Incidence of COPD and asthma	73,228 women and 47,026 men in the US	Prospective cohort	HR (95%CI) comparing the highest to the lowest quintile of AHEI-2010: COPD: 0.67 (0.53 to 0.85), Asthma: 1.04 (0.90 to 1.21).	Results after adjusting for BMI
Shang <i>et al.</i> J Nutr Health Aging 2016 [204]	Abdominal aortic calcification	262 older adults in Australia	Prospective cohort	OR (95%CI) comparing the highest to the lowest tertile of AHEI-2010: 0.38 (0.20, 0.70)	
Healthy aging					
Samieri <i>et al.</i> Ann Intern Med 2013 [205]	Healthy aging	10,670 women in the US	Prospective cohort	OR (95%CI) comparing the highest to the lowest tertile of	Healthy aging was defined as no major chronic diseases or

					AHEI-2010: 1.34 (1.09, 1.66)	major impairments in cognitive or physical function or mental health.
<i>Obesity</i>						
Boggs <i>et al.</i> J Nutr 2013 [206]	Incidence of obesity	19,885 African women in the US (BMI 18.5–29.9)	Prospective cohort	HR (95%CI) comparing the highest to the lowest quintile of AHEI-2010: 0.76 (0.58, 0.98)		Component contributing the most to the association: red/processed meat (deleterious), whole grain (protective)
<i>Hypertension</i>						
Li <i>et al.</i> Hypertension 2016 [207]	Hypertension	3,818 women with gestational diabetes history in the US	Prospective cohort	HR (95%CI) comparing the highest to the lowest quartile of AHEI-2010: 0.76 (0.61–0.94)		

2.2 Associations between diet and asthma

As introduced in the previous section, diet is a likely risk factor for multiple chronic diseases, including cardiovascular disease, cancers, type 2 diabetes, and more recently COPD. Given the parallel increase of asthma and industrialization worldwide, it has been suggested that the increased asthma prevalence may be also, at least partly, related to modification in diet (i.e., decreased intake of fruit and vegetables and increased intake of “Westernized” processed foods) [2,210]. Several potential mechanisms have been proposed with regard to the diet–asthma association, and the diet–asthma association has raised particular interest in recent years.

In this section, first, the potential mechanisms underlying the diet–asthma association are presented; then, different windows of exposure of and their effects on the diet–asthma association are discussed; last, current evidence on the association between diet in adults (individual food/nutrients and dietary patterns) and asthma are reviewed.

2.2.1 Potential mechanisms

Based on previous research on the association between diet and major chronic diseases, it has been first suggested that the antioxidant/oxidant and the anti-inflammatory/pro-inflammatory potential of diet are likely to contribute to the development and/or the progression of asthma. More recently, several other potential mechanisms have also been proposed, including vitamin D deficiency, epigenetic factors, and the role of microbiota.

Antioxidant and oxidant potential of diet

Normally, the toxicity of oxidants/reactive oxygen species (ROS, such as superoxide anion, hydroxyl radical, hypohalite radical, hydrogen peroxide, generated through pathophysiological processes or exposure to external oxidants) and the intracellular and extracellular antioxidant defense systems are balanced *in vivo* [211]. The imbalance between oxidant exposure and antioxidant defense in favor of oxidants was defined as oxidative stress [211]. Whereas low-level oxidative stress can be balanced by the antioxidant defense, high-level oxidative stress, inducing the production of many pro-inflammatory factors, overwhelms antioxidant defense and leads to cellular apoptosis or necrosis and pulmonary dysfunction [212]. Oxidative stress has been

associated with many chronic diseases, as well as the development and progression of asthma [213].

In parallel, although nitric oxide (NO) plays an important role in the homeostasis of the lung (e.g. the dilatation of the pulmonary blood vessels) and other organs, excessive NO derived from the inducible NO synthase reacts with superoxide anion produces reactive nitrogen species (RNS, such as peroxynitrite) [180], which act together with ROS and cause nitrosative stress [180]. The potential role of nitrosative stress has been highlighted in airway inflammation and lung diseases [214].

Several environmental or behavior factors may alter the oxidant-antioxidant and nitrosant–antinitrosant balance, including cigarette smoking and air pollution as a source of oxidant/nitrosant, and diet as a source of both antioxidant and oxidant/nitrosant [180]. Several nutrients, including vitamin C, vitamin E, carotenoids, and selenium, are known for their antioxidant potential [211], and may provide important defense to oxidant and nitrosant exposures [215]. Fruit and vegetables, in which these antioxidant nutrients present abundantly, may be protective against asthma.

More recently, another face of diet – the oxidant/nitrosant potential – has risen increasing interest. High intake of processed meat, which is high in nitrite, has been associated with higher concentration of biomarkers of oxidative stress and nitrosative stress [185]. As a deleterious factor for many chronic diseases (as previously reviewed), the association between processed meat and asthma needs further investigation.

Anti-inflammatory and pro-inflammatory potential of diet

As a chronic inflammatory disease, asthma could be affected by dietary factors that have anti-inflammatory or pro-inflammatory potential. In particular, higher omega-3 PUFA intake has been consistently associated with lower circulating inflammatory biomarkers, showing its anti-inflammatory potential. The protective effect of omega-3 PUFA on asthma has been observed in several studies (see section 2.2.3). On the other side, red and processed meat have been reported as having a pro-inflammatory potential [186]. In this context, processed meat intake might be of particular interest in association with asthma.

Effects of Vitamin D

The level of vitamin D in human body is determined by two main sources: dietary intakes (e.g., fortified milk) and the synthesis by skin upon the exposure to UVB radiation from sunlight. The modern lifestyle (most time indoor) has been associated with vitamin D insufficiency worldwide.

Besides its key role in the absorption of calcium, it was recently suggested that vitamin D is also involved in immunomodulation by its effect on different immune cells [216]. In particular, vitamin D can affect the activities of T-helper 2 (T_H2) cells, which plays a main role in regulating the type 2 inflammation in asthma (type 2 high asthma in particular) [216]. Also, the association between vitamin D and the activities of T_H17 and regulatory T cells (Treg) has been reported [216]. Moreover, vitamin D may have a protective effect on airway remodeling by inhibiting the epithelial–mesenchymal transition [217].

Epigenetic mechanisms

Recently, it was proposed that diet might also play a role in asthma through epigenetic mechanisms, including DNA methylation. Several nutrients, including folic acid, choline, vitamin B₁₂, vitamin B₂, and vitamin B₆ are dietary methyl donors and are involved in the methylation process [218]. Results from studies using animal models have suggested that a maternal diet high in methyl donors (including folic acid, choline, and vitamin B₁₂) during pregnancy resulted in increased IgE and eosinophil and airway responsiveness in the exposed offspring [219]. Different intake of these nutrients might lead to differences in DNA methylation and ultimately have an effect on asthma [220].

Microbiota

As discussed previously in section 1.2.3, gut and lung microbiota may be important for the development of immune systems in early life. Diet in both childhood and adulthood has been associated with the structure of gut microbiota. Among infants, given the sterility of gastrointestinal and respiratory tract at birth, early life events, including the mode of delivery (through vaginal birth or caesarean section), breastfeeding, and introduction of solid foods, play an important role in the colonization of gut microbiota and can have long-term effects on microbiota structure in later life [221,222]. Among adults, it has been shown that different long-term dietary patterns

were associated with different gut microbiota structure [223]. In children, the specific gut microbiota accompanied by a reduction of short-chain fatty acids (SCFAs) was associated with a higher risk of incident asthma [224]. Higher levels of SCFAs, produced by bacteria in the gut during fermentation of insoluble fiber from dietary plant matter, were recently associated with the Mediterranean diet [225], which include a high intake of fruits, vegetables, and legumes (i.e. high fiber content foods) .

Compared with gut microbiota, much less is known with regard to the association between diet and lung microbiota. Since microbes at the oropharynx are the main source of lung microbiota [226], it is therefore possible that diet can also affect lung microbiota colonization. Moreover, it is suggested that gut microbiota may also influence immune responses far beyond the local environment, such as in lung [227,228]. Future studies in this emerging area, with a better understanding this gut-lung axis [227,228], are likely to provide new insight regarding the diet–asthma association.

2.2.2 Windows of exposure in the diet–asthma association

Dietary habits evolve throughout life and may thus influence health in different ways and by different mechanisms, according to the window of exposure. Worldwide sufficient data has suggested the importance of maternal diet during pregnancy on the health of offspring, supporting the theory of Developmental Origins of Health and Disease (DOHaD) [229]. While maternal diet during pregnancy may have long-term effects on the development and health of offspring, dietary habits during childhood and through adulthood may also have shorter-term effects. As a result, the diet–asthma association may differ according to the windows of exposure, including prenatal period (through the maternal diet), childhood, and adulthood.

Maternal diet during the pregnancy is likely to be associated with asthma incidence of offspring. Several cohort studies have linked higher intakes of vitamin E, zinc, PUFAs, as well as fruits and vegetables to lower risk of asthma incidence in children [210,220,230]. No association has been found for vitamin C, vitamin A and several methyl donor nutrients (folic acid, B₁₂, B₂, and B₆) [210,220,230]. Regarding dietary patterns during the pregnancy, inconsistent results have been reported, and a recent meta-analysis showed no association between dietary patterns during the pregnancy and asthma [231].

With regard to **diet in childhood**, a large body of evidence has shown the beneficial effect of fruits and vegetables on asthma [210,220,230]. Associations of vitamins A and E, zinc, vitamin D and PUFAs with asthma have also been reported by several cross-sectional studies, whereas no association has been found for vitamin C and vitamin A [210,220,230]. Data from cross-sectional studies have suggested a probable link between dietary patterns in childhood and asthma, while further prospective cohorts are needed [231].

With regard to the association between dietary factors in adulthood and asthma, results are reviewed in the following sections.

2.2.3 Association between individual foods/nutrients and asthma in adults

Based on potential mechanisms proposed regarding the diet–asthma association, several foods and nutrients have been studied in association with asthma-related outcomes in adults. While the potential protective effect of foods and nutrients with antioxidant potential has been intensively studied, other foods/nutrients, including the PUFAs, processed meat, vitamin D, and methyl donor nutrients, have also been investigated in a few studies. Several nice reviews have previously been published [210,218,230,232,233]. In this section, a brief presentation for each type of food/nutrients is given regarding their association with prevalent asthma (i.e. from cross-sectional or case-control data), asthma incidence, and asthma control.

Antioxidant nutrients, including vitamin C, vitamin E, vitamin A and selenium, as well as fruits and vegetables have been inversely associated with prevalent asthma in cross-sectional and case-control studies [210,230,233]. However, regarding asthma incidence, only a limited number of longitudinal studies have been conducted. While previous research consistently showed significant associations for fruits and vegetables, inconsistent results have been reported for vitamin E, and no association has been observed for vitamin C [210,230,233]. Regarding asthma control, a RCT showed that participants administrated with a high fruit and vegetable diet for 3 months had a decreased risk of asthma exacerbation, compared with those having a low fruit and vegetable diet [234]. RCTs have showed no beneficial effect on asthma control with supplemental use of vitamin C, vitamin E, selenium [210,230,233] among patients with asthma.

PUFAs or fish intake (high in PUFAs) have been associated with prevalent asthma in cross-sectional or case-control studies [210,230,233]. With regard to

incidence of asthma, very few studies investigated the potential effect of PUFAs or fish intake, with inconsistent findings [149,235–237]. While three studies, one in Europe [236] and two in the US [149,237], showed no association, one longitudinal study in the US reported significant inverse associations between dietary intake of PUFAs and incidence of asthma during 20 years of follow-up [237]. Overall, a meta-analysis including three of these studies [235–237] showed no significant effect of PUFAs on the incidence of asthma [238]. Findings from a RCT suggested that PUFAs or fish intake might not be beneficial for asthma control [239].

The effect of foods with pro-inflammatory potential has scarcely been studied. Only two studies in the US reported no association between **processed meat** intake and incidence of asthma [172,173]. However, it is worth noting that BMI, a potential mediator in the investigated association, was adjusted in the analysis, and biased results may be reported (the role of BMI as a mediator in the diet–asthma association is further discussed in section 4.1.1). No study regarding the association between processed meat intake and asthma control has been conducted.

Only a few studies have investigated the association between **vitamin D** and asthma outcomes in adults. A large cross-sectional study (n = 6,857) in the US [240] reported that lower serum vitamin D concentration were inversely associated with current asthma. However, a prospective study in Danish adults [241] reported no association between serum vitamin D concentration and incidence of asthma. Among patients with asthma, inconsistent results have been reported in a few small RCTs regarding the role of vitamin D supplementation on asthma control in adults [216].

Recently, few studies have investigated role of **methyl donor nutrients**, including folic acid, choline, vitamin B₁₂, and vitamin B₆, in asthma among adults. Very limited data are available regarding both asthma incidence and control, and inconsistent results were reported [218,220].

Taken together, high intake of fruits and vegetables are likely beneficial for both primary and secondary prevention of asthma, whereas insufficient evidence support the antioxidant nutrient supplemental use for preventing asthma. In line with this finding, the last Global Initiative for Asthma Guidelines encourage patients with asthma to consume a diet high in fruits and vegetables for its general health benefits [8]. Despite significant observations in cross-sectional and case-control studies, RCTs have suggested that vitamin C, vitamin E, selenium, PUFAs supplements and fish

intake are unlikely beneficial for primary or secondary prevention of asthma. Moreover, it is urged that further prospective and interventional studies be conducted to better understand the role of deleterious dietary factors, vitamin D and methyl donor nutrients in asthma among adults.

2.2.4 Association between dietary patterns and asthma in adults

To date, seventeen studies have investigated the association between dietary patterns in adulthood and asthma-related outcomes, including prevalent asthma using cross-sectional or case-control data, asthma incidence, and asthma control. First, studies using *a priori* approach with different diet scores are presented. Then, studies using *a posteriori* approach are reviewed and summarized.

2.2.4.1 Diet scores and asthma

Only five studies have investigated the association between different diet scores and asthma outcomes in adults (see Table 5).

Prevalent asthma

In a small case-control study in Australia, including 99 cases and 61 healthy controls, Wood *et al.* reported that participants with ever asthma had higher DII scores (more pro-inflammatory diets) than healthy controls, and that the DII was negatively associated with FEV₁ [242]. This study has taken into account important potential confounders in the analyses such as age, sex, and smoking status. However, several other potential confounders such as total energy intake and educational level have not been adjusted for. Furthermore, BMI was not taken into account in the analyses.

Asthma incidence

A prospective cohort including 73,228 women and 47,026 men in the US reported no association between the AHEI-2010 diet score and incidence of asthma, during a follow-up time of 14–16 years [203]. In this large epidemiological study, the researchers adjusted for many potential confounders, including physical activity, total energy intake, active and passive smoking, pack years of smoking, menopausal status, race, physician visits, US region, and spouse's highest educational level. However, BMI was included in the model as a potential confounder, which raised several methodological concerns (further discussed in section 4.1.1).

Asthma control

The other three studies investigated the association between diet scores and asthma control among patients with the disease. One cross-sectional study in Portugal (n = 174) showed that a higher “alternate Mediterranean Diet” (aMED) score was associated with better asthma control [243]. The analyses were adjusted for age, sex, education, inhaled corticosteroids and total energy intake. However, BMI was not taken into account in the analyses. A small RCT (n = 38) conducted in New Zealand reported no improvement in asthma control over 12 weeks for the interventional groups encouraged to adopt a Mediterranean diet [244], but perhaps due to the small sample size and the short follow-up time. And recently, using a covariate-adaptive randomization method (accounting for age, sex, race, smoking status, DASH score and the Asthma Control Questionnaire (ACQ) score), a pilot RCT performed in the US [245] reported that adults with persistent asthma had better asthma control (greater improvement measured by ACQ score) after a 6-month of healthy-eating intervention based on the DASH diet.

Overall, evidence is lacking regarding the role of diet scores in the primary prevention of asthma (incidence). Regarding the role of diet scores in the secondary prevention of asthma (progression of the disease), inconsistent results have been reported, by a very limited number of studies. Further prospective studies are needed.

Table 5 Studies investigating the association between diet scores and asthma outcomes in adults

Reference	Population	Study design	Diet score	Outcome	Results	Potential confounders
<i>Prevalent asthma</i>						
Wood <i>et al.</i> Clin Exp Allergy 2015 [242]	99 cases and 61 controls in Australia	Case-control	DII (based on 25 of 45 possible food items [98])	Ever asthma (cases defined by doctor diagnosed asthma)	Significant association between the DII and ever asthma: OR = 1.7 (1.03– 2.14) for one unit increase	The analyses were adjusted for age, sex, smoking status and BMI.
<i>Incidence of asthma</i>						
Varraso <i>et al.</i> BMJ 2015 [203]	73,228 women and 47,026 men in the US	Prospective cohort	AHEI-2010 [120]	Incidence of asthma (self reported)	Follow-up time: 14–16 years. No association between AHEI-2010 and incidence of asthma.	The analyses were adjusted for physical activity, BMI, total energy intake, active and passive smoking, pack- years of smoking, (pack- years) ² , menopausal status, race, physician visits, US region, and spouse's highest educational level.
<i>Asthma control</i>						
Barros <i>et al.</i> Allergy 2008 [243]	174 adults with asthma in Portugal	Cross-sectional	aMED score (based on the aMed score developed by Fung <i>et al.</i> [108] except for alcohol)	Asthma control (measured by symptoms, FEV ₁ and exhaled NO)	Significant inverse association between aMED and uncontrolled asthma: OR = 0.22 (0.05– 0.85), <i>P</i> -trend = 0.028.	The analyses were adjusted for age, sex, education, inhaled corticosteroids and total energy intake.
Sexton <i>et al.</i> J Asthma 2013 [244]	38 adults with symptomatic asthma in New	Open-label RCT (12 weeks). High intervention group: intensive initial advice and 41h sessions with dietitian; low-	Mediterranean score (0 to 5 for never, rare, frequent, very frequent, weekly and daily consumption of non-refined cereals, fruits, vegetables,	Asthma control (measured by Juniper Asthma Control	No association between Mediterranean score and Asthma Control Questionnaire score.	Differences between control and intervention groups and percentage changes were adjusted for age and sex.

	Zealand	intervention group: less intensive advice and 2h with a dietitian; control group: no intervention.	legumes, olive oil, fish and potatoes; reverse scaled scores for red meat and products, poultry and full fat dairy products)	Questionnaire)		
Ma <i>et al.</i> Eur Respir J 2016 [245]	90 adults with uncontrolled asthma and low-quality diet in the US	Pilot RCT (6 months). Intervention group: receive dietician-delivered behavioral intervention to achieve and maintain a DASH diet; control group: no intervention.	DASH diet (7–12 servings of fruits/vegetables, 2–4servings of low-fat/fat-free dairy products, total fat grams at 27% of estimated caloric needs, and ≤ 2300 mg of sodium)	Asthma control (measured by Juniper Asthma Control Questionnaire)	Moderate difference of Asthma Control Questionnaire (ACQ) score between intervention and control groups at 6 months: -0.2 (-0.5, 0.1).	A covariate-adaptive randomization method (age, sex, race, smoking status, DASH score and the ACQ score) was applied.

2.2.4.2 Data-driven dietary patterns and asthma

Twelve studies have investigated the association between data-driven dietary patterns and diverse asthma-related outcomes, among which two studies [246,247] investigated multiple outcomes. Findings of these studies are presented in Table 6 according to the outcomes investigated (i.e. prevalent asthma, incidence of asthma, or asthma control). Among these studies, most have used PCA to derive dietary patterns. Other methods including factor analysis [248], CFA [126], and latent trait models [249] have also been employed in several studies.

Prevalent asthma

Seven studies [246,247,250–253,249] have investigated the association between dietary patterns and ever asthma. Four studies [249,250,252,253], conducted in the Netherlands, Australia, China, and Portugal, have reported significant associations between at least one dietary pattern and ever asthma, and the other three studies [246,247,251], one conducted in France, one in the UK, and one in three different European countries, reported no association. The study in Portugal applied latent variable models to derive dietary patterns, and the other six studies applied PCA. In these seven studies the analyses were adjusted for several potential confounders, and six of them further adjusted for BMI.

Incidence of asthma

Using PCA, all five large prospective studies, which were conducted in Singapore, the US, or France, reported no association between dietary patterns and incident asthma [126,246,254–256]. Using CFA, Bédard *et al.* reported a positive association between the "Western" pattern derived and adult-onset asthma in 30,589 French women [126]. In these five studies the analyses were adjusted for several potential confounders and three of them further adjusted for BMI. In the study performed by Bédard *et al.* the analyses were stratified on BMI at baseline to overcome this issue.

Asthma control/asthma activity

The association between dietary patterns and asthma control was investigated in four studies [246–249]. A cross-sectional study in France [246], including 992 women with current asthma, reported that frequent asthma attack was positively associated with the "Western" pattern and inversely associated with the "Nuts and wine" pattern.

A cross-sectional study in the UK [247], including 559 individuals with asthma, showed no association. A cross-sectional study in Portugal [249], including 32,644 participants (5.3% participants had ever asthma), reported a positive association between the "high fat, sugar and salt" pattern and current severe asthma, and a negative association between the "fish, fruit and vegetables" pattern and current medicated asthma. Another cross-sectional study in Australia [248], including 158 pregnant women with asthma, reported a positive association between a "High fat/sugar/takeaway" pattern derived by factor analysis and uncontrolled asthma. In all these studies, the analyses were adjusted for several potential confounders and further adjusted for BMI.

Due to various eating habits across populations, very different dietary patterns have been derived by studies, which makes it not easy to compare the results across studies. Lv *et al.* have performed a meta-analysis [231] on the association between dietary patterns and ever asthma in adults, including one prospective study [246] and five cross-sectional/case control studies [247,250–253]. Grouping all dietary patterns into three categories – healthy, unhealthy, and neutral – according to constituent foods of each pattern, this study suggested no association between dietary patterns and ever asthma.

Overall, current evidence from prospective studies do not support any association between dietary patterns and incidence of asthma, and the effect of dietary patterns on asthma control among patients with asthma merits further investigation in prospective studies.

Even though dietary intake appears to have little (if any) role as a cause in asthma, the role of diet as a disease modifier has more promise. Taken together, the literature is still inconclusive, probably due to the complexity of both the exposure and outcome and, consequently, of their association. Indeed, published results are very heterogeneous regarding the handling of potential confounders in the diet–asthma association, and mostly, regarding the role of BMI (probably a mediator and not a confounder). A clearer understanding of potential confounders and/or mediators in the diet–asthma association is warranted to guide future research.

Table 6 Studies investigating the association between data-driven dietary patterns and asthma outcomes in adults

Reference	Population	Study design	Dietary patterns	Outcome	Results	Potential confounders
<i>Prevalent asthma</i>						
Varraso <i>et al.</i> Eur Respir J 2009 [246]	54,672 women in France	Cross-sectional	Derived using PCA: 1) Prudent 2) Western 3) Nuts and wine	– Ever asthma (self-reported physician-diagnosis) – Current asthma (past 12 months)	– No significant association for both outcomes.	The analyses were adjusted for age, total energy intake, BMI, physical activity, smoking status, menopausal status, education and dietary supplementation.
Bakolis <i>et al.</i> Allergy 2010 [247]	599 cases and 854 controls in the UK	Case-control	Derived using PCA: 1) Prudent 2) Vegetables and fruit 3) Western 4) Vegetarian 5) Traditional (vegetables, red meat and offal)	– Ever asthma (attack, symptom, or asthma medication in the past 12 months)	– No significant association.	The analyses were adjusted for all other dietary patterns, age, sex, BMI, social class, housing tenure, employment status, whether a single parent, smoking, passive smoke exposure at home, total energy intake, ethnicity, number of siblings, paracetamol and supplement use.
Mckeever <i>et al.</i> Am J Clin Nutr 2010 [250]	12,648 adults in the Netherlands	Cross-sectional	Derived using PCA: 1) Cosmopolitan (vegetables, fish, chicken) 2) Traditional (meat, potatoes) 3) Refined foods	– Ever asthma (self-reported physician-diagnosis)	– Positive association between the "Cosmopolitan" pattern and ever asthma. Highest vs. lowest quintile: OR (95%CI) = 1.4 (1.0 to 1.9)	The analyses were adjusted for age, sex, smoking status, pack-years of smoking, educational level, total energy intake, and location.

Hooper <i>et al.</i> Br J Nutr 2010 [251]	1,174 adults from Germany, UK and Norway	Cross-sectional	Derived using PCA: 1) Meat and potato 2) Fish, fruit and vegetables	– Current asthma (attack, symptom, or asthma medication in the past 12 months) – Asthma symptom score (0–5)	– Overall, no association for both outcomes. – Heterogeneity across countries for the association between the "meat and potato" pattern and the asthma symptom score: negative association in Germany, and positive associations in UK and Norway.	The analyses were adjusted for age, sex, social class, smoking status, physical activity, BMI and quintiles of total energy intake.
Rosenkranz <i>et al.</i> Nutr J 2012 [252]	156,035 adults in Australia	Cross-sectional	Derived using PCA. In men: 1) Meats/cheese 2) Fruits/vegetables 3) Poultry/seafood 4) Grains/alcohol In women: 1) Meats 2) Fruits/vegetables 3) Poultry/seafood 4) Cereal/alcohol 5) Brown bread/cheese	– Ever asthma (self-reported physician-diagnosis)	– Positive association between the "Meat-cheese" pattern and ever asthma in men. Highest vs. lowest quintile: OR (95% CI) = 1.18 (1.08 to 1.28). – Negative association between the "Cheese-brown bread" pattern and ever asthma in women. Highest vs. lowest quintile: OR (95% CI) = 0.88 (0.82 to 0.94)	The analyses were adjusted for categories of age, education, physical activity, BMI, and smoking status.
Shi <i>et al.</i> PLoS One 2012 [253]	1,486 adults in China	Cross-sectional	Derived using PCA: 1) Traditional (rice, wheat flour, and vegetable) 2) Macho (meat and alcohol) 3) Sweet tooth (cake, milk, and yoghurt) 4) Vegetable rich	– Ever asthma (self-reported physician-diagnosis)	– Positive association between the "Traditional" pattern and ever asthma. OR (95% CI) = 2.25 (1.45 to 3.51) for 1 SD increased.	The analyses were adjusted for age, sex, alcohol drinking, income, overweight/obesity, smoking status, total energy intake, manual job, monosodium glutamate intake, and the other dietary patterns.

Barros <i>et al.</i> Clin Exp Allergy 2015 [249]	32,644 adults in Portugal	Cross- sectional	Derived using latent variable models: 1) Dairy and fruit 2) Soup and starchy foods 3) High fat, sugar and salt 4) Fish, fruit and vegetables 5) Sugary and fatty foods	– Ever asthma – Current asthma (having asthma symptoms within previous year) – Asthma diagnosed in the previous year (self reported)	– Positive association between the "high fat, sugar and salt" pattern and ever asthma. OR (95%CI) = 1.13 (1.03 to 1.24) for 1 SD increased. – Negative association between the "fish, fruit and vegetables" pattern and current asthma. OR (95% CI) = 0.84 (0.73–0.98) for 1 SD increased. – No association between dietary patterns and asthma diagnosed in the previous year.	The analyses were adjusted for age, gender, education, family income, proxy reporting information, smoking, BMI, and physical activity.
<i>Incidence of asthma</i>						
Butler <i>et al.</i> Am J Respir Crit Care Med 2006 [254]	52,325 Chinese in Singapore	Prospective	Derived using PCA: 1) Vegetable-fruit-soy 2) Meat-dim sum	– Incidence of asthma (self-reported physician-diagnosis)	Follow-up time: 2–12 years. – No association between dietary patterns and incidence of asthma.	The analyses were adjusted for age, total energy intake, sex, dialect group, smoking status, age at starting to smoke, cigarettes per day, passive tobacco smoke exposure, and educational level.
Varraso <i>et al.</i> Thorax 2007 [255]	42,917 men in the US	Prospective	Derived using PCA: 1) Prudent 2) Western	– Incidence of asthma (self-reported physician-diagnosis or asthma treatment)	Mean follow-up time: 12 years. – No association between dietary patterns and incidence of asthma.	The analyses were adjusted for age, smoking, pack-years, (pack-years) ² , race/ethnicity, physician visits, US region, BMI, physical activity, multivitamin use, and total energy intake.
Varraso <i>et al.</i> Am J Clin	72,043 women in the US	Prospective	Derived sing PCA: 1) Prudent 2) Western	– Incidence of asthma (self-reported physician-diagnosis or	Mean follow-up time: 16 years. – No association between dietary patterns and incidence of asthma	The analyses were adjusted for age, smoking status, pack-years, (pack-

Nutri 2007 [256]				asthma treatment)		years) ² , exposure to secondhand tobacco smoke, menopausal status, race/ethnicity, spouse's educational attainment, physician visits, US region, BMI, physical activity, multivitamin use, and total energy intake.
Varraso <i>et al.</i> Eur Respir J 2009 [246]	54,672 women in France	Prospective	Derived using PCA: 1) Prudent 2) Western 3) Nuts and wine	– Incidence of asthma (self-reported physician-diagnosis during the follow-up)	Mean follow-up time: 10 years. – No significant association between dietary patterns and incidence of asthma.	The analyses were adjusted for age, caloric intake, BMI, smoking, physical activity, menopausal status, education and multivitamin use.
Bédard <i>et al.</i> J Nutr 2011 [126]	30,589 women in France	Prospective	Derived using PCA and CFA: 1) Prudent 2) Western 3) Aperitif	– Incidence of asthma (self-reported asthma attacks)	Mean follow-up time: 12 years. – Positive association between the "Western" pattern derived using CFA and incidence of asthma. Highest vs. lowest quintile: OR (95%CI) = 1.30 (1.02 to 1.67). – No association between dietary patterns derived using PCA and incidence of asthma.	The analyses were adjusted for age, menopausal status, years of education, total energy intake, having parents who were farmers, and physical activity. Analyses were stratified on BMI at baseline.
<i>Asthma control</i>						
Varraso <i>et al.</i> Eur Respir J 2009 [246]	992 women with current asthma in France	Cross-sectional	Derived using PCA: 1) Prudent 2) Western 3) Nuts and wine	– Frequent asthma attacks (≥ 1 /week, among participants with asthma)	– Positive association between the "Western" pattern and frequent asthma attacks. Highest vs. lowest quintile: OR (95%CI) = 1.79 (1.11 to 3.73). – Negative association between the "Nuts and wine" pattern and	The analyses were adjusted for age, caloric intake, BMI, smoking, physical activity, menopausal status, education, and multivitamin use.

					frequent asthma attacks. Highest vs. lowest quintile: OR (95%CI) = 0.65 (0.31 to 0.96)	
Bakolis <i>et al.</i> Allergy 2010 [247]	599 asthma cases in the UK	Cross-sectional	Derived using PCA: 1) Prudent 2) Vegetables and fruit 3) Western 4) Vegetarian 5) Traditional (vegetables, red meat and offal)	– Asthma severity (frequency of waking at night because of asthma symptoms and quality of life)	– No significant association.	The analyses were adjusted for all other dietary patterns, age, sex, BMI, social class, housing tenure, employment status, whether a single parent, smoking, passive smoke exposure at home, total energy intake, ethnicity, number of siblings, paracetamol and supplement use.
Barros <i>et al.</i> Clin Exp Allergy 2015 [249]	32,644 adults in Portugal	Cross-sectional	Derived using latent variable models: 1) Dairy and fruit 2) Soup and starchy foods 3) High fat, sugar and salt 4) Fish, fruit and vegetables 5) Sugary and fatty foods	– Current medicated asthma (use of asthma medication within previous year) – Current severe asthma (emergency visit because of asthma within previous year)	– Positive association between the "high fat, sugar and salt" pattern and current severe asthma. OR (95% CI) = 1.23 (1.03 to 1.48) for 1 SD increased. – Negative association between the "fish, fruit and vegetables" pattern and current medicated asthma. OR (95%CI) = 0.84 (0.72–0.98) for 1 SD increased	The analyses were adjusted for age, gender, education, family income, proxy reporting information, smoking, BMI, and physical activity.
Grieger <i>et al.</i> Public Health Nutr 2016 [248]	158 pregnant women with asthma in Australia	Cross-sectional	Derived using factor analysis: 1) High protein/fruit 2) High fat/sugar/takeaway 3) Vegetarian-type	– Asthma control (GINA 2012)	– Positive association between the "High fat/sugar/takeaway" pattern and uncontrolled asthma. OR (95%CI) = 1.54 (1.07 to 2.23) for 1 SD increased.	The analyses were adjusted for age BMI, smoking status, socio-economic status, parity, and ethnicity.

3 Obesity and asthma

The word obesity is basically used to refer to the status of having excess body fat, a result of energy intake-expenditure imbalance. According to the WHO, more than 1.9 billion adults (39%) were overweight in the world in 2014, out of whom over 600 million (13%) were obese [257]. In children under the age of 5, 41 million were overweight or obese in 2014 [257]. The epidemic of obesity worldwide has been associated with the global burden of multiple chronic diseases [257,258], including asthma [3,259]. Obesity, which is preventable, has raised a critical issue in public health.

Although obesity is commonly defined using the body mass index (BMI) in practice, different measures of body composition have been proposed with pros and cons of each. In this chapter, first the tools to assess body composition are presented, and then, the link between obesity and asthma is reviewed.

3.1 Tools to assess body composition

In obesity research, body composition is traditionally divided into fat mass and fat-free mass (two-compartment model). Although multi-compartment models have been recently developed, which provide more measures including total body water, bone mineral, protein, fat, and other measures, the two-compartment model is still simple and efficient in obesity-related research [260]. Thus, in this section, discussion is focused on the two-compartment model.

Several tools have been proposed to assess body composition. These tools are of different interests depending on the objective of the study. First, a brief presentation is given to several methods that are frequently used to validate or calibrate anthropometric measures of obesity, referred to as “reference methods”; then, a brief overview is given to several methods that are most used in epidemiological studies.

3.1.1 Reference methods

Densitometry

The hydrodensitometry, also called underwater or hydrostatic weighing, is considered as the gold-standard method for measuring body composition [260]. Participants’ weights are accurately measured in air and underwater. Using measures of body

weight, body volume (calculated using two measures of body weight), and residual lung volume, body fat percentage can be calculated using well-established formulae [261]. However, this method requires active cooperation of participants, which is particularly problematic for children, and participants with reduced mobility [260].

To overcome the limitations mentioned above, a similar method, the air-displacement plethysmography (ADP) has been proposed. Using this method, participants sit in a testing chamber with minimal clothing, such as a swimsuit. Body volume and density are calculated by air rather than water displacement [260]. The ADP provides an alternative method to the hydrodensitometry, however, this method is not commonly used among obese participants, due to the unwillingness of participants for swimsuit dressing [262].

Hydrometry

The hydrometry, also referred to as dilution method or total body water method, uses water labeled with isotopes, particularly deuterium ($^2\text{H}_2\text{O}$), tritium ($^3\text{H}_2\text{O}$), and oxygen-18 (H_2^{18}O), to measure total body water [260]. Since the water proportion in fat free mass (FFM) is relatively stable in healthy individuals, FFM and body fat are estimated based on the dilution principle. However, the application of this method is limited in individuals without weight loss, hydration or disease status that may change the water proportion in FFM [262].

Whole-body potassium counting

Whole-body potassium counting is a classic reference method based on the naturally occurring radioactivity potassium isotope ^{40}K in human tissues which represents total-body potassium (TBK) [260]. Since ^{40}K accounts for 0.0118% of TBK, and TBK is evenly distributed in intracellular components of FFM, FFM can be estimated with the formula: $\text{FFM} = \text{TBK}/66.3$, where 66.3 is the TBK/FFM ratio in mmol/kg. Then one can derive total body fat by the difference between body weight and FFM. The precision of the whole-body potassium counting is more accurate for measuring FFM and adiposity than densitometry. But the interpretation of results is complicated through different sex and ethnic groups, because of different rates of TBK change with age in different sex and ethnic groups. Also, the equipment for whole-body potassium counting is special and costly.

Dual-energy x-ray absorptiometry (DXA)

X-ray attenuates differentially when passing through different body tissues, such as bone, fat tissue, and fat-free tissue. Using two x-ray beams with different energy, dual-energy x-ray absorptiometry (DXA) provides estimates of bone mineral density, fat-free mass, and fat mass calculated from established algorithms. DXA has been widely used as a gold standard for measuring bone mineral density. Now it is also proposed as a reference method for measuring body composition [260].

Previous studies have intensively shown the precision of DXA measurements of body composition compared with other reference methods [260,262]. The short-term and long-term reproducibility of this technique also suggests the benefit of DXA. However, it is not suitable for pregnant women, and different models of equipment, analyze methods, and software may yield different results.

Imaging methods (CT and MRI)

Imaging methods such as computed tomography (CT) and magnetic resonance imaging (MRI) are becoming ever more popular because of their precision. They both provide high-resolution cross-sectional scans and thus enable accurate body composition assessment [260]. One additional advantage of MRI is that it does not expose subjects to ionizing radiation, which makes it suitable for children and pregnant women. They may serve the purpose of calibrating or validating for other simple methods. However, their application to obese individuals may cause problem. For CT technology, measurement for large body sizes induces high radiation exposure. MRI is not suitable for large body sizes but is applicable for whole body assessment for normal weight and moderate overweight individuals.

Summary

It is essential for obesity research to have accurate assessment of body composition [260,262]. These reference methods of body composition provide accurate estimations, however, due to some practical limitations, they are mostly used in small-scaled studies to validate anthropometric measures only.

3.1.2 Methods most used in epidemiological studies

Body mass index

Body mass index (BMI), calculated as weight (kg)/height (cm)², is the most used measure of body composition in epidemiological studies. Although BMI is a measure of both fat and lean body mass according its definition, evidence from a large number of studies has shown the strong correlation between BMI and body fat [260,263,264]. Moreover, BMI is also highly correlated with concentrations of adipokines secreted by adipocytes, including leptin and adiponectin [265,266]. Thus, BMI is a simple, valid, widely used indicator of body adiposity among adults. Defined by the WHO, overweight, in adults, is the status of having a BMI greater than or equal to 25 kg/cm², and obesity is the status of having a BMI greater than or equal to 30 kg/cm² [257].

However, as an indirect measure of nutrition status and adiposity, there are several limitations when using BMI alone as a measure of body composition. First, different body compositions/adiposity status may exist for a given BMI, according to age, sex, and ethnicity. For the same BMI, higher body fat percentages have been observed among older adults compared with younger ones, among women compared with men, and among Asians compared with Whites [260]. Second, BMI is not capable of reflecting body fat distribution, whereas abdominal obesity – a key component of the metabolic syndrome – has been associated with chronic diseases independent of overall obesity [267,268].

Waist circumference and waist-to-hip ratio

It is well known that body fat distribution, rather than body fat amount solely, is associated with multiple health-related outcomes. Since decades, two shapes of body have been distinguished: an “apple shape” of body with excess body fat accumulated near abdomen, and a “pear shape” with excess body fat accumulated at hips and thighs. Abdominal obesity, represented by the “apple shape”, has been considered as an independent risk factor for multiple health-related outcomes and included as an component of the metabolic syndrome .

Waist circumference and waist-to-hip ratio are commonly used to measure abdominal obesity in epidemiological studies. Detailed protocols are provided for the measurement of waist circumference, including the measurement site, the tightness and type of tape, the posture, the phase of respiration, and the abdominal tension of participants [269,270]. Four sites for measuring waist circumference have been proposed. According to the WHO STEPwise approach to Surveillance (STEPS) [269], the measurement of waist circumference is made at the approximate midpoint

between the lowest rib margin and the iliac crest. According to the US National Institutes of Health, the measurement is made at the level of the iliac crest or the umbilicus [270]. And several studies have made the measurement at the point of the minimal waist [271]. The measure of hip circumference is typically made at the widest part of the buttocks [269]. Although waist circumference measurement site differs across studies, Ross *et al.* reviewed more than 100 studies and concluded that difference in waist circumference measurement site did not affect the association of waist circumference with mortality and risk of CVD and diabetes [271].

Waist circumference is shown to be an excellent measure of abdominal obesity [272], and is positively associated with higher mortality, and higher risk of cancer, cardiovascular disease, and type 2 diabetes [267]. Several cutoff points of waist circumference have been proposed. For instance, universal cutoffs, which were 102 cm for men and 88 cm for women, were proposed by American Adult Treatment Panel III guidelines (ATP III) [273]. According to the International Diabetes Federation (IDF), sex and ethnicity specific cutoffs of waist circumference have been used to define central obesity – a component of metabolic syndrome, with the highest value (94 cm) for European men and lowest value (80 cm) for South Asian and Chinese women [268]. However, it remains controversial about the use of these cutoffs, as the association between waist circumference, risk of metabolic syndrome and cardiovascular disease appears to be linear [260].

Compared with waist circumference, **waist-to-hip ratio** has been shown a less effective measure of central obesity. Clasey *et al.* [272] have compared three measures of central obesity, including waist circumference, waist-to-hip ratio, and DXA, with the reference method single-slice CT scan among women and men. In this study, waist circumference was more strongly associated with total abdominal fat and abdominal visceral fat measured by CT when compared with waist-to-hip ratio, and DXA did not offer a significant advantage. Kamel *et al.* [274] reported that among non-obese men, waist circumference, waist-to-hip ratio, and DXA were associated similarly with intra-abdominal fat measured by MRI, and among non-obese women, stronger association was observed for DXA, followed by waist circumference, compared with waist-to-hip ratio. Moreover, combining two anthropometric measures, waist-to hip ratio may introduce more measurement error, and is more complicated to interpret [260].

Skinfolds

Skinfold thickness is a measure of subcutaneous fat at predetermined sites, including triceps, biceps, the subscapular region, abdomen, and thigh, using a special caliper according to standard measurement guidelines [260]. It is used, among both children and adults, either in raw values to represent peripheral body fatness, or in prediction equations to estimate total body fat [260].

Using population-specific equations, a few studies reported that skinfold thickness could well estimate total body fat [275,276]. However, inconsistent results have been reported regarding the association between skinfold thickness and cardiovascular disease [277–279], while the association between total body fat and cardiovascular disease has been well established using BMI and waist circumference [267]. The following reasons may account for these findings [260]. First, even when using standard measurement techniques, skinfold measurement has greater measurement error than other anthropometric measures, such as weight, height, and body circumference. Second, skinfold thickness cannot reflect visceral adiposity. And third, skinfold thickness measured at different sites might reflect different fat distributions.

Bioelectrical Impedance Analysis (BIA)

Although BMI and waist circumference are considered as good indicators of body fatness, such anthropometric measures do not differentiate body fat mass and fat free mass. The bioelectrical impedance analysis provides an alternative method. Based on the fact that different body tissues have different resistances, by measuring the impedance or resistance of the whole body to a small electrical current, the bioelectrical impedance analysis (BIA) estimates total body water, fat-free mass, and fat mass through prediction equations [260].

Several prediction equations have been developed, most of which were limited by small sample size [280]. Using data from 1,829 participants in the US, Sun *et al.* developed a set of sex-specific, white and black race-combined prediction equations, which provide excellent precision to predict total body water measured by dilution, and fat free mass estimated with a model based on densitometry, isotope dilution, and dual-energy X-ray absorptiometry. However, among obese individuals, the BIA tends to underestimate the fat free mass, limiting the application of the BIA [281].

Since the BIA is easy to use, relatively inexpensive, and not burdensome for participants, it has been applied in a few large epidemiological studies [282,283]. However, inconsistent results have been reported regarding whether or not BIA-derived measures are better associated with obesity-related diseases than BMI. Using data of more than 12,000 participants from the American NHAHES III study, body fat percentage derived from BIA and BMI were associated similarly with markers of cardiovascular disease, including blood pressure, blood glucose, high-density lipoprotein cholesterol (HDL), and triglycerides [282]. In a Swedish cohort including more than 27,000 participants, body fat percentage derived from BIA was more strongly associated with total mortality than BMI [283].

Body silhouettes

Body silhouette is determined by skeleton framework, lean mass and fat mass, as well as fat distribution. Since the skeleton framework is relatively fixed, body silhouettes are also proposed as a simple and validate measure to assess body composition [284].

Several studies have shown the validity and reliability of body silhouettes for measuring body composition. Tehard *et al.* examined the correlation of self-reported body silhouettes with technician-measured BMI among 91,815 women from the French E3N study, and reported that with a coefficient of correlation of 0.78, self-reported body silhouettes may be a proxy of BMI in large-epidemiological studies [284]. Similar findings were reported in an African population [285]. More recently, a pilot study [286], including 200 boys and girls of mixed ethnicity, showed that prediction models using silhouettes generated from photo images can well predict the fat mass index, fat free mass index, and fat percentage measures by DXA (the adjusted coefficients of determination R^2 were 0.86, 0.95, and 0.75 for boys and 0.90, 0.89, and 0.69 for girls, respectively).

Considering the simplicity and high cost-effectiveness of this method, self-reported body silhouettes have been used to measure age-specific body composition, especially in large epidemiological studies. In the E3N cohort study, it was reported that an increase in body silhouette between menarche and adulthood was associated with higher risk of adult-onset asthma [287]. A case-control study in Mexico showed an increased risk of breast cancer among women who had an increase in body silhouette over time [288]. However, given that self-reported silhouettes may be less precise due to underestimation and to recall bias, several innovative measures, such as

silhouette recorded by a smartphone camera and application may be a promising tool for measuring body composition in epidemiological studies [289].

Summary

BMI and waist circumference provide a simple, valid measure of overall and abdominal adiposity respectively in epidemiological studies. Bioelectrical impedance analysis provides a valid, relatively inexpensive method to assess body fat and fat free mass. Body silhouettes appear to be an alternate valid, cost-effective tool to assess body fat. Given different biological emphasis of these measures, analysis combining different measures of body composition may add supplemental information to the prediction for disease risks and help to understand the potential underlying mechanisms.

3.2 Association between obesity and asthma

Previous studies have shown complex association between obesity and asthma. The observed obesity–asthma association was once linked with the potential weight gain due to inactivity and the side effects of systemic corticosteroids in asthma. However, in the past two decades, a large body of evidence has suggested that obesity is a likely risk factor for asthma [3,259], and that obese asthma patients present a distinct phenotype of asthma [14].

In this chapter, first, current evidence suggesting that obesity is a likely risk factor for asthma is reviewed, then, the obese–asthma phenotype is presented, and finally, potential mechanisms of the obesity–asthma association are discussed.

3.2.1 Obesity as a likely risk factor of asthma

Since more than two decades, a large number of cross-sectional studies have reported the association between obesity and asthma prevalence [290]. However, the role of obesity as a risk factor remained unclear until evidence from longitudinal studies was found. In 1999, Camargo *et al.* first reported, in a large prospective cohort study in the US, that obesity was associated with increased risk of incident asthma among women [291]. Since then, studies in different countries, conducted among both men and women, have reported the association between obesity and asthma incidence. Beuther *et al.* performed a meta-analysis of 7 prospective cohort studies [292], involving more than 300,000 individual in total. They reported that overweight and obese individuals, both men and women, had increased risk of asthma incidence, with a dose-response effect for the association between BMI and asthma incidence.

Similarly, a number of studies have reported a positive association between obesity and asthma control. Novosad *et al.* reviewed 11 studies of obesity as a risk factor for asthma control, among which nine studies reported a significant positive association between obesity and worse asthma control [293]. Two other studies reported non-significant associations, probably due in part to differences in baseline characteristics including asthma control and the proportion of obese patients [293].

The obesity–asthma association has been further strengthened by consistent findings showing that weight reduction in obese patients with asthma, through bariatric surgery or not, was associated with improvement in multiple asthma-related outcomes [294]. The improvements include asthma control [295], exacerbations [296], response to treatment [297], lung function [297], and airway responsiveness [297]. Taken together, all current

evidence suggest that obesity is as a risk factor for both asthma incidence and control [3,290,293,294,298,299].

Although BMI is the most used valid measure of body adiposity, it fails to reflect the fat distribution. Given the likely link between abdominal obesity and systemic inflammation, only a few studies have investigated the association between abdominal obesity, defined using waist circumference or waist-to-hip ratio, and asthma-related outcomes [300–307]. A study including 4,060 Australians reported that BMI, waist circumference, and waist-to-hip ratio were associated with non-atopic asthma in men and in women [304]. A study including 88,304 US women reported that, even among women with a normal BMI, central obesity (waist circumference ≥ 88 cm) was positively associated with prevalence of current asthma [305]. The only one prospective study, including 23,245 participants in Norway, has reported that central obesity (defined as waist circumference ≥ 88 cm in women and ≥ 102 cm in men) was associated with increased risk of incident asthma, and the results remained significant in women after adjusting for BMI [306]. Regarding asthma control/asthma activity, little data have been published using measures other than BMI. A cross-sectional study including 124 women reported that central obesity was associated with worse asthma control [308]. Few studies have investigated the association between body adiposity and asthma-related outcomes using other measures of body composition in adults.

Overall, the magnitude of the obesity–asthma association may differ according to several characteristics of the study population. Although the obesity–asthma association has been observed among men and women, the association between obesity and asthma incidence appeared to be more consistent in women than in men [292]. Also, the association is more consistent among non-atopic individual than among atopic individuals [3,259]. In addition, it was recently suggested that obesity is more likely a risk factor for late-onset asthma, whereas for early-onset asthma, it is likely a comorbidity that can alter the disease [309].

3.2.2 The obese–asthma phenotype

It is widely recognized that asthma is a heterogeneous entity with different phenotypes. Using clustering methods, several studies have found that obesity was an important determinant of asthma phenotype, playing a more significant role than other commonly assessed clinical, physiologic, or inflammatory variables, leading to the conclusion that asthma in the obese may represent a unique phenotype [10,14,293]. This “obese–asthma” phenotype is mainly characterized by late-onset asthma, poor asthma control, altered airway inflammation, and

poor response to asthma medications, and improved asthma control with weight reduction [259,14,309].

Altered airway inflammation has been highlighted in the obese–asthma phenotype. While sputum eosinophil is a typical airway inflammatory marker in asthma, it has been negatively associated with BMI in patients with severe asthma [310]. These findings are also in line with the fact that obese patients had reduced response to inhaled corticosteroid or the combination of inhaled corticosteroid/long-acting bronchodilator [311], treatments decreasing mainly eosinophilic inflammation, compared with patients with normal weight. Higher sputum neutrophil percentage [312] and blood neutrophils levels [313] were observed among obese patients with asthma as compared with lean patients with asthma, particularly among women. However, no significant change in airway eosinophilic or neutrophilic inflammation has been observed for patient who had better asthma control after weight reduction [314,315]. Instead, current evidence has suggested that obesity-related low-grade systemic inflammation may be a potential link between obesity and asthma (see section 4.2.1).

More recently, it has been suggested that two sub-phenotypes may be identified within the obese–asthma phenotype [309,316,317]: 1) one with earlier onset, high IgE, and poor asthma control, complicated/worsened by obesity, and 2) the other with later-onset, non-atopic, more common in women, developed due to obesity.

3.2.3 Potential mechanisms

Several potential mechanisms have been proposed with regard to obesity as a risk factor for asthma. According to current evidence, the mechanism of obesity-related systemic inflammation provides a likely link between obesity and asthma; this mechanism is discussed in the first section. Other mechanisms have also been proposed including comorbidities, common genetic factors, obesity-related changes in lung mechanics and function, effect of sex hormones, and effect of microbiota; they are briefly reviewed in the second section.

3.2.3.1 Obesity-related systemic inflammation

Adipose tissue is shown to be not only an organ for triacylglycerol storage but also an endocrine organ that secretes various compounds involved in metabolic and inflammation processes [265]. In obese individuals, hypertrophic adipocytes release increased pro-inflammatory adipokines such as leptin, and decreased anti-inflammatory adiponectin [318]. Moreover, adipose tissue, visceral fat in particular, is infiltrated with CD8⁺ T-lymphocytes

and activated macrophages, producing increased pro-inflammatory cytokines, such as Interleukin-6 (IL-6), IL-8, tumor necrosis factor- α (TNF- α), and monocyte chemoattractant protein 1 (MCP-1) [266]. These compounds act complexly in many pathological processes, including inflammation process, immune regulation, and the response to oxidative stress, which are associated with insulin resistance, diabetes, cardiovascular disease [319], and more recently asthma development [320]. Among various compounds released by adipose tissue in obese individuals that may contribute to local and systemic inflammation, leptin and adiponectin are mainly secreted by adipose tissue, regulating both metabolic and inflammation processes. Hence, great interest has been shown in the association of these two biomarkers with asthma.

Leptin, expressed by the *ob* gene of adipocytes, is remarkably increased in obese individuals and highly correlated with measures of obesity [265,321]. Besides balancing energy intake through a direct effect on the hypothalamus, leptin also acts as a pro-inflammatory factor, by enhancing the phagocytosis, activation, and proliferation of macrophages, and inducing the production of pro-inflammatory cytokines, including TNF- α , IL-6, as well as reactive oxygen species (ROS) [266,318]. Furthermore, leptin administration to wild-type mice resulted in increase airway inflammation induced by O₃ [322].

Current evidence from epidemiological studies has suggested a potential link between leptin and prevalent asthma [323]. Mai *et al.* reported higher leptin concentrations in overweight children with current asthma than those without the disease [324]. Similarly, Guler *et al.* have observed an association between higher leptin concentrations in obese boys with allergic asthma as compared to controls [325]. Sood *et al.* observed higher serum leptin concentrations in women with current asthma as compared to women who never had asthma [326]. Sideleva *et al.* observed higher expression of leptin in visceral adipose tissue among obese women with late-onset, T_H-2 low asthma as compared to obese women without the disease [315]. Recently, a meta-analysis showed that higher leptin concentrations were associated with asthma prevalence among both adults and children [327]. A few studies have also investigated the association between leptin and asthma control, severity or lung function using cross-sectional data. Among children, Tanju *et al.* observed that higher serum leptin concentrations were associated with more severe asthma in 65 children [328]. Huang *et al.* observed a negative association between serum leptin concentration and the FEV₁/FVC ratio in 74 obese adolescents with asthma [329]. In contrast, Kattan *et al.* reported no association between serum leptin and asthma control in 368 adolescents with moderate to severe asthma

[330]. Among adults, Tsaroucha *et al.* observed higher serum leptin concentrations in women with severe asthma compared with those with mild-to-moderate asthma [331]. Leivo-Korpela *et al.* reported in 35 women that higher serum leptin concentrations were associated with lower lung function and more asthma symptoms [332].

Overall, these cross-sectional data have suggested a potential role of leptin in the obesity–asthma association. However, it remains unclear whether leptin lies in the causal pathway between obesity and these asthma outcomes, as causal inference cannot be made using cross-sectional data, which are likely to be affected by potential reverse causation (i.e. the increased leptin concentration is a result rather than a cause of the disease status). Further studies with longitudinal data and larger study sample are needed, among adults in particular.

In contrast to leptin, decreased concentration of **adiponectin** has been observed in case of obesity [318]. Adiponectin inhibits the phagocytosis of macrophages, suppresses the production of pro-inflammatory cytokines (e.g. IL-6, TNF- α) and the transcription factor NF- κ B, and increases the production of anti-inflammatory cytokines, such as IL-10 and IL-1 receptor antagonist [266]. Adiponectin is mainly secreted by adipocytes, but the secretion of adiponectin by other cells has also been observed [333]. Adiponectin and its receptors, such as AdipoR1 or AdipoR2, are expressed on airway epithelial cells in the lung [315]. In mice it has been shown that adiponectin may decrease allergic airway inflammation and airway hyperresponsiveness [334,335]. In human, the evidence for the adiponectin–asthma association remains moderate, with discrepant findings [336]. Several studies using human data have suggested a potential link between lower adiponectin level and prevalent asthma [337–340] whereas a few others reported no significant association [341,342]. The meta-analysis performed by Zhang *et al.* showed that lower adiponectin level was associated with prevalent asthma in adults, but not in children [327].

Based on evidence from animal models and epidemiological studies, obesity-related inflammation is a likely mediator in the association between obesity and asthma, and leptin might play a key role. However, to avoid potential reverse causation, prospective data are needed to further clarify the role of leptin in the association between body adiposity and asthma incidence and activity. Studies employing different measures of adiposity, including BMI and waist circumference, may provide additional information to the obesity–asthma association. Some new analyzing approach, such as mediation analysis that can decompose the total effect into a direct effect and an indirect effect mediated by a certain mediator, may help to provide new insight to this mechanism.

3.2.3.2 Other potential mechanisms

Changes in lung mechanics and function

Several significant changes in lung mechanics and function have been observed in obese individuals. Most obviously, reduced lung volumes in breathing, including reduced expiratory reserve volume (ERV) and functional residual capacity (FRC) [343], have been observed. These changes may be caused by fatty infiltration of respiratory muscles, altered elastic properties of the chest wall, and increased pulmonary blood volume [298]. Decreased FEV₁ and FVC have also been observed in obese individuals, but FEV₁/FVC ratio appeared to be less affected by obesity [299]. These mechanical changes in obese individuals may result in reduced peripheral airway diameter, increased airway resistance, and thus could potentially increase the risk of the airway hyperresponsiveness (AHR). However, inconsistent findings have been reported with regard to the association between obesity and AHR [344,345].

Genetic factors

Several studies have shown some common genetic predispositions to both obesity and asthma. Results from twin studies indicated that obesity and asthma might have shared genetic pathways [346,347]. Using data from five cohort studies, González *et al.* reported that a common genomic inversion at 16p11.2 was related to the joint susceptibility to asthma and obesity [348]. Polymorphisms of several genes have also been linked with both obesity and asthma [349]. More recently, the Chitinase 3-like 1 gene, whose protein product is known as Chi311 or YKL-40, is suggested to play a central role in Th2 immune response and lie in the common pathway of obesity and allergic asthma [350,351].

Effect of sex hormones

Although the obesity–asthma association has been observed among men and women, the association appeared to be more consistent among women than among men [292]. Female hormones, estrogen in particular, may be involved in multiple steps in allergic sensitization and play a significant role in allergic disease including asthma [352]. Sex differences in asthma have been widely recognized. Most remarkably, higher prevalence and severity of asthma have been observed among boys than girls before puberty, whereas the sex–asthma association is reversed among adults [353]. Although estrogen is mainly synthesized in ovary, adipose tissue is also an important organ that produces estrogen [265]. Increased

estrogen level has been observed among obese individuals, particularly among men, girls before menarche, and postmenopausal women [354]. Hence, it is plausible that sex hormones may contribute to, at least in part, the association between obesity and asthma.

Effect of microbiota

As previously discussed (in section 1.2.3), modifications in both gut and lung microbiota may be associated with allergy and asthma through different pathways [54]. A link between obesity and gut microbiota has been found in animal models, and recently, also in human individuals [355]. Reduced diversity of gut bacteria has been observed in obese individuals compared to lean ones [356,357]. Moreover, decreased ratio of Firmicutes to Bacteroidetes has been observed among those with weight loss through dietary intervention [358]. Similarly, the reduction of several species of Firmicutes has been observed among individuals with weight loss after surgical intervention [359]. Several studies using animal models have suggested that changing gut microbiota can also contribute to weight gain and obesity, but less evidence are available in humans [360].

Comorbidities

In addition to the mechanisms discussed above, it was also suggested that the obesity–asthma association could in part be explained by comorbidities of obesity, including gastro-esophageal reflux disease, sleep disordered breathing, insulin resistance, diabetes, hyperlipidemia, and hypertension [3]. Each of these comorbidities may increase the oxidative stress, and might in turn contribute to the development or the worsening of asthma [212].

4 Methodological challenges in epidemiological research on nutritional factors and asthma

In this section, first, the methodological challenges related to the complexity of the association between nutritional factors and asthma are discussed, and then, mediation analysis, a statistical approach that can help address these methodological issues, is introduced.

4.1 Complexity of the association between nutritional factors and asthma

Several challenges manifest themselves when investigating the association between nutritional factors and asthma. At the macro-level, the diet–asthma association is complicated by the high correlation within isolate food intakes (as previously discussed in section 2.1.2). Moreover, a number of potential confounders manifest themselves in the diet–asthma association, such as age, sex, educational level, and total energy intake. In addition, being part of lifestyle, diet is highly associated with obesity and other lifestyle factors, including physical activity and smoking. These lifestyle factors may act in a more complicated way rather than simply as confounders in the diet–asthma association. At the micro-level, the mechanisms of the association between nutritional factors and asthma merits investigation. Unlike the diet–asthma association, the obesity–asthma association is now well established with sufficient evidence regarding a temporal and potentially causal association. In this specific context, research on potential pathophysiological mechanisms may help to better understand the obesity–asthma association at the micro-level, and provide potential target interventions.

This section is organized in three parts. First, the likely role of obesity as a mediator in the diet–asthma association is discussed. Then, the complex role of smoking in the diet–asthma association is presented. Lastly, methodological issues regarding the mediating role of biological markers in the obesity–asthma association are discussed.

4.1.1 Obesity: a potential mediator in the diet–asthma association

Obesity has been studied intensively as an exposure, outcome, or confounder in epidemiological studies. Despite complex interrelationships within nutritional factors, obesity (or BMI) was usually considered as a confounder in the diet–health association. However, during the past decades, with the development of causal theories and application of directed

acyclic graph (DAG) in epidemiology, more and more researchers have recognized that obesity may be a potential mediator that link several factors, including diet and physical activity in particular, to chronic diseases [186,361].

The established evidence that links diet to obesity and that links obesity to asthma can illustrate the role of obesity as a potential mediator in the diet–asthma association. Although obesity can result from multiple factors, including genetic predispositions, certain disease status (e.g. hypothyroidism), or medication use (e.g. corticosteroids and antidepressants), it is most often a result of unhealthy lifestyle, including excessive dietary energy intakes and insufficient physical activity [362]. It has been shown that individuals with higher intake of red and processed meat [179], sugar-sweetened drinks [162], or lower overall diet quality [206] have higher risk of obesity. These findings, suggesting an association between diet and obesity, are represented as the arrow “a” in Figure 3. With regard to the obesity–asthma association, as reviewed in section 3.2, a large number of studies have shown that obesity is a likely risk factor for asthma. This obesity–asthma association is represented as the arrow “b” in Figure 3. As a result, obviously, obesity is a potential mediator in the association between diet and asthma.

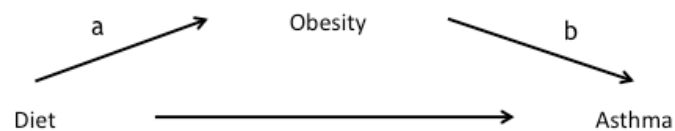


Figure 3 Simplified mediation model of the diet–asthma association taking into obesity as a mediator.

Most of the studies investigating the diet–asthma association have included BMI as a confounder in the analysis. Such analysis, adjusting for a mediator, cannot assess the true association between diet and asthma and may provide misleading conclusions [363,364]. Being aware that obesity may be a mediator in the diet–asthma association, several studies provided results with and, in addition, without adjustment for BMI [186,361]. However, this multiple-adjusting approach has critical methodological limitations [365]. Some novel analyzing approaches, such as the counterfactual approach, provide a new tool to face the above issues in mediation analysis (further discussed in section 4.2).

4.1.2 Role of smoking in the diet–asthma association

Besides interrelations within nutritional factors, cigarette smoking may also complicate the diet-asthma association.

The fact that smokers and non-smokers have different dietary habits has been widely reported. Compared with non-smokers, smokers are more likely to have an unhealthy dietary habit, including lower intake of fruit and vegetables in particular, and also higher intake of sugar, refined cereals, processed food, and alcohol [366–372]. A meta-analysis including 51 studies from 15 countries showed that smokers had remarkable higher intakes of saturated fat (+9%), cholesterol (+11%), alcohol (+78%), and lower intakes of fiber (–12%), vitamin C (–17%), vitamin E (–11%) and β -carotene (–12%) than nonsmokers [373]. Due to these correlations between smoking and dietary intakes, smoking may be an important confounder in the association between several dietary factors and asthma.

Moreover, for the same dietary intakes of antioxidants vitamins, lower circulating concentrations of related biomarkers have been observed among smokers compared with non-smokers. Margetts *et al.* reported that, for the same dietary intake, smokers had lower plasma concentration of β -carotene when compared to non-smokers, and that plasma and dietary intake of β -carotene were highly correlated among non-smokers, but not among smokers [366]. Similarly, Dietrich *et al.* reported that, independently of dietary intakes, smokers had lower concentrations of six plasma antioxidants: γ -tocopherol, total ascorbic acid, β -carotene, β -cryptoxanthin, lutein, and zeaxanthin [374]. These findings may be due to an increased depletion of antioxidants in smokers, as smoking is a known important source of oxidants [375]. However, these findings may be also due to the altered bioavailability (the portion that food is digested and absorbed *in vivo*) of foods and nutrients related to smoking [376]. Given the potential difference in bioavailability related to smoking, the effect of dietary intakes on asthma may be modified by smoking habits.

Smoking also has complex association with body weight. Current-smokers are often reported to have lower BMI, whereas smoking cessation is related to weight gain and overweight [377]. Since obesity is a likely mediator in the diet–asthma association (as discussed in the previous section), smoking can also alter the diet–asthma association through its effect on BMI. In addition, the diet–asthma association needs to be carefully discussed according to smoking due to the potential overlap between asthma and COPD. As cigarette smoking is the major risk factor for COPD [378], the diet–asthma association in current smokers may be biased by this overlapping condition (e.g., smokers with asthma or asthma-

like symptoms are more likely to have the asthma–COPD overlap condition as compared to non smokers).

In summary, when investigating the associations between nutritional factors and asthma, it is important to investigate the role of smoking as a potential effect modifier, and to discuss the potential asthma–COPD overlap.

4.1.3 Biological markers: potential mediators in the obesity–asthma association

As discussed previously (section 3.2.3), leptin may be a mediator in the association of obesity with asthma and asthma-related outcomes. However, such hypothesis is difficult to study with traditional statistical approaches.

To identify the underlying biological mechanisms, animal studies can provide important data to generate hypotheses. Nevertheless, animal models can never replicate the pathophysiological process of human bodies, and thus cannot completely reveal the mechanisms of human diseases. In epidemiological studies, current analyzing approaches have contributed significantly to the identification of risk factors for many diseases, while leaving a “black box” with regard to the potential mechanisms (Figure 4). Once again, the recent counterfactual approach of mediation analysis may provide a potential tool to open the black box [379].



Figure 4 Black box in the association between exposure and disease in epidemiological studies.

4.2 Application of mediation analyses in epidemiology

It is very often in epidemiology that we face the following question: how the risk factor affects the disease? The question represents the urge to understand the rationale of the association. Rather than only investigating the association between an exposure and a disease (an outcome), mediation analysis hypothesizes that the exposure affects a third variable that in turn contributes to the development of the disease. The overall effect of the exposure on the disease (outcome) is called total effect, the effect passing through the mediator is indirect effect, and the effect unexplained by the mediator is direct effect.

Mediation analysis has two main interests in epidemiological studies. First, at the “macro-level”, it allows us to correctly take into account the mediators (e.g. BMI) in the

model to assess the association between the exposure (e.g. diet) and the outcome (e.g. asthma). Second, at both a “macro” and a “micro” level, by decomposing the total effect into direct effect and indirect effect through a certain mediator, it allows us to statistically test a potential mechanism (e.g. effect through a specific biomarker) of the observed association (e.g. obesity–asthma association).

In the next sections, after a brief presentation of the traditional approach of mediation analysis, mediation analysis in the counterfactual framework – a novel approach – is introduced.

4.2.1 Traditional approach

The traditional approach of mediation analysis was proposed by Baron and Kenny in 1986 [380]. This approach involves three steps. First, the association between the outcome (Y) and the exposure (X) is modeled, without taking into account the mediator (M) (unadjusted model in Figure 5, equation 1). Second, the association between the mediator and the exposure is modeled (adjusted model, equation 2). Third, the association between the outcome (Y) and the exposure (X) is modeled, adjusting for the mediator (M) (adjusted model, equation 3).

$$\text{Equation 1: } Y = \beta_1 + cX + \varepsilon_1$$

$$\text{Equation 2: } M = \beta_3 + aX + \varepsilon_3$$

$$\text{Equation 3: } Y = \beta_2 + c'X + bM + \varepsilon_2$$

The results are interpreted as follows. The coefficient of X in equation 1, c , is interpreted as the “total effect” of the exposure on the outcome. The coefficient of X in equation 3 (adjusted model), c' , is then interpreted as the “direct effect” of the exposure on the outcome, or called the effect independent of the mediator. Several methods have been proposed to calculate the “indirect effect” mediated through the mediator [365]. The most used ones include the difference of coefficients approach, calculated as $c-c'$, and the product of coefficients approach (also known as the Sobel approach), calculated as $a \times b$ [365]. To test the significance of the indirect effect, the difference or the product of the coefficients is divided by its standard error, and the ratio is then compared to the normal distribution.

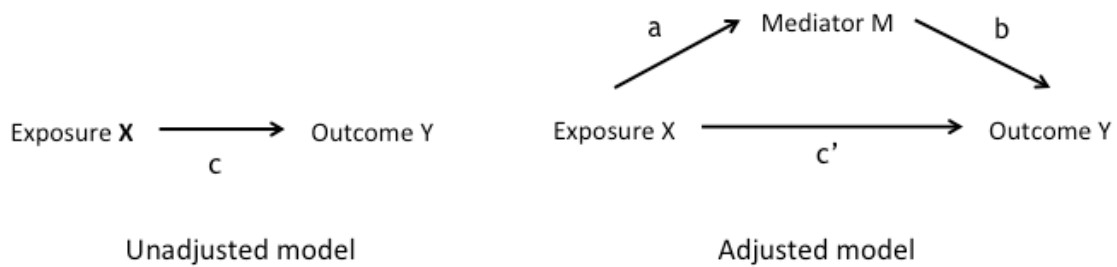


Figure 5 Models in the traditional approach of mediation analysis.

This classical mediation approach has been widely used. However, it has several important limitations. First, the estimation for total effect and direct effect is shown to have low power [381]. Second, as this approach is applicable only to linear models without interactions, it fails to estimate the mediation in other common situations, such as logit and probit models, and interactions between exposure and mediator [382].

4.2.2 Counterfactual approach

This section first introduces the concept of causal inference in the counterfactual framework, and then, mediation analysis in the counterfactual framework. Two practical models that have been used in the thesis are presented in detail.

4.2.2.1 Causal inference in the counterfactual framework

The counterfactual theory was proposed for causal inference as early as 1748 by the philosopher David Hume. “We may define a cause to be an object followed by another, and where all the objects, similar to the first, are followed by objects similar to the second. Or, in other words, where, if the first object had not been, the second never had existed.” [383] Then in 1973, David Lewis has further developed this theory employing possible world semantics for counterfactuals [384]. These theories represent the empirical concept for causal inference: we observe and compare the outcome when the event A is present to the outcome when A is absent; if a difference is observed, we conclude that A is a cause, although it is likely not the only one, of the outcome; otherwise A is not a cause of the outcome.

In the real world, only one of these two conditions – A is present or not – can be observed. As a result, the causal effect cannot be assessed at the individual level. In contrast, causal effect at the population level, also referred to as average causal effect, could be estimated within the counterfactual framework. Several definitions of causal effect in the counterfactual framework [385] are introduced as follows.

The **counterfactual outcome** $Y(a)$ is defined as the potential outcome that we would have observed, which may be contrary to the outcome that we observe in real, when the exposure were set at level a . The **average causal effect** of A on Y can then be defined as the expected difference when A were set to a and a^* : $E(Ya) - E(Ya^*)$. More generally, when a set of confounders C present, the **conditional average causal effect** is defined and denoted as $E[Ya - Ya^*|C]$. Here, it is important to note the difference between this definition using counterfactual outcomes and the usual notation for estimating the **association** between A and Y, $E[Y|A = a] - E[Y|A = a^*]$, where Y denotes the observed outcome in the condition that the exposure is a or a^* . While the association is defined by a difference of risk between two subsets of the population classified by observed values of the exposure (see Figure 6), the causal effect is defined by a difference of risk in the same subset (which represents the entire population) under two potential exposure values [385].

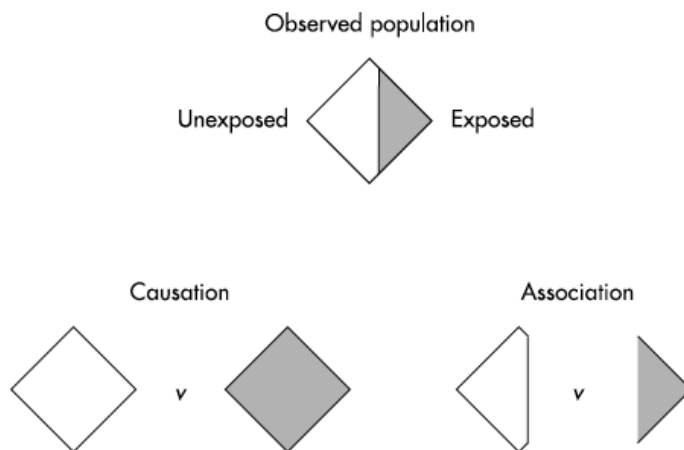


Figure 6 Definition of causation (causal effect) and association in an observed population (reproduced from Hernan et al. *J Epidemiol Community Health* 2004 [385])

A classic example of the implementation of counterfactual theories in medical research is the design of the randomized controlled trials (RCTs). In the ideal situation, both groups (assuming two groups) in the RCT represent the same population, and thus are exchangeable, which means that group 1 would have the same outcome as group 2, if they received the intervention given to group 2. Under this circumstance, the observed outcome $E[Y|A = a]$ equals to the counterfactual outcome $E(Ya)$. Therefore the difference of the expected outcome of the two groups is considered as causal effect [385]. Although being considered as the “gold standard” for causal inference, estimates in RCTs can also be biased due to some common situations, including the lost to follow-up (as the outcome is no more representative

for the investigated population), the non-compliance of the participants (as it leads to a misclassification of the exposure), and the unblinding (as multiple factors, rather than the assigned exposure only, can contribute to the outcome). Indeed, similar assumptions and analytical methods are required for causal inference in RCTs and in observational studies [385].

In epidemiological studies, several methods have been proposed to estimate the causal effect, including inverse probability weighting, standardization, and G-estimation [386]. Several statistical models have been developed based on these methods, such as marginal structural models (MSM), parametric g-formula, and structural nested models respectively [386].

4.2.2.2 Mediation analysis in the counterfactual framework

The counterfactual theory has not been applied to mediation analysis until 2001, when Pearl proposed formulated definitions of total, direct and indirect effects and articulated the conditions/assumptions for identification and causal interpretations of the effects [4].

The **total effect (TE)** can be defined as the expected change of the outcome Y in total for a change in the exposure from level a^* to level a : $TE = E(Y_a) - E(Y_{a^*})$. It has been illustrated that in all situations, including non-linear models and models with exposure-mediator interaction, the total effect can be decomposed into the natural direct effect and the natural indirect effect.

The **natural direct effect (NDE)** denotes the expected change of Y if the exposure were set at level a versus level a^* when, for each individual, the mediator were kept at the level it would have taken in the absence of the exposure: $NDE = E(Y_{a, Ma^*}) - E(Y_{a^*, Ma^*})$.

The **natural indirect effect (NIE)** denotes the expected change of Y if the exposure were set at level a , but the mediator were changed from the level it would take if the exposure were a^* to the level it would take if the exposure were a : $NIE = E(Y_{a, Ma}) - E(Y_{a, Ma^*})$.

The **controlled direct effect (CDE)** can be defined as the expected change of Y if the exposure were set at level a versus level a^* when the mediator were kept at a given level m : $CDE = E(Y_{a, m}) - E(Y_{a^*, m})$. Whereas the natural effects help investigating the potentials mechanisms of the association, the CDE is of more interest in the policy evaluation (which have set the mediator at a specific level) [4]. As a result, in the following part of the thesis, we focus on the natural effects only.

Four assumptions need to be made when identifying the effects using the counterfactual approach to mediation analysis [4]: 1) no unmeasured confounders for the associations between the exposure and the outcome; 2) no unmeasured confounders for the associations between the mediator and the outcome; 3) no unmeasured confounders for the associations between the exposure and the mediator; 4) no unmeasured confounders for the associations between the mediator and the outcome that are affected by the exposure. The causal inference would be questionable when these assumptions are not met.

Compared with the Baron and Kenny approach, the mediation analysis in the counterfactual framework provides precise mathematical definitions of the effects, which can be applied to non-linear models and models with exposure-mediator interactions, and allows a more precise discussion on the assumptions of the analysis [5,6]. However, the mediation analysis in the counterfactual framework has not been widely applied in epidemiology until very recent development of statistical programming [5,6]. For instance, Valeri and VanderWeele have proposed SAS and SPSS macros to implement mediation analysis through a two-step regression approach [6], and Lange *et al.* introduced an approach based on MSM allowing almost all data types [5]. As these two methods have been employed in the thesis, essential concepts and the procedure for their implementation are detailed below.

The two-stage regression model proposed by Valeri and VanderWeele [6]

In 2009, a two-stage regression model has been proposed to estimate the effects in mediation analysis by VanderWeele and Vansteelandt [382]. Soon after, Valeri and VanderWeele have extended this work in allowing more types of variables in models and providing SAS and SPSS macros [6].

In this method, the following two regression models are employed, where g denotes the link function, A denotes the exposure, M denotes the mediator, C denotes the confounders, and the term θ_{3am} denotes the exposure-mediator interaction.

$$\text{Stage 1: } g(E[M|A = a, C = c]) = \beta_0 + \beta_1 a + \beta_2' c$$

$$\text{Stage 2: } g(E[Y|A = a, M = m, C = c]) = \theta_0 + \theta_1 a + \theta_2 m + \theta_3 a m + \theta_4' c$$

Under the no-unmeasured confounders assumptions stated before, the direct and indirect effects for several combinations of models (linking functions) can be identified using the coefficients in the two regression models above. For instance, for a study with dichotomous exposure, continuous mediator and dichotomous outcome, the odds ratio for natural direct effect is given by:

$$\exp[\{\theta_1 + \theta_3(\beta_0 + \beta_1 a^* + \beta_2' c + \theta_2 \sigma^2)\}(a - a^*) + 0.5\theta_3^2 \sigma^2 (a^2 - a^{*2})],$$

and the natural indirect effect is given by:

$$\exp[(\theta_2\beta_1 + \theta_3\beta_1a)(a - a^*)].$$

SAS and SPSS macros have been developed to facilitate the implementation of this method [6]. Options are given to name the variables, to specify the types of regression for the mediator and the outcome, and to specify whether an exposure-mediator interaction is present or not. The following types of variables are allowed in the macro: binary or continuous exposures, binary or continuous mediators, and binary, continuous, or count outcomes. With regard to stage-1 model, a linear or logistic regression can be used, and for the stage-2 model, linear, logistic, Poisson or negative binomial regressions are allowed. The confidence intervals can be calculated either through a delta method or a bootstrapp procedure.

The marginal structural model proposed by Lange et al. [5]

Lange *et al.* have proposed to estimate the natural causal effects through MSM in 2012. Briefly, for an exposure of two levels – a and a^* , the expected outcome is denoted as:

$$g(E[Y_{a,Ma^*}]) = c_0 + c_1a + c_2a^* + c_3aa^* + c_4C,$$

where g denotes the link function for the outcome, c_3aa^* denotes the interaction between the exposure and the mediator, and C denotes the confounders. The coefficients for the natural direct effect is $c_1(a - a^*) + c_3 a(a - a^*)$, for the natural indirect effect is $c_2(a - a^*) + c_3 a(a - a^*)$, and for the total effect is the sum of the two measures. The demonstration of the model has been described in detail elsewhere [5] and would not be discussed here.

In practice, taking an example with a dichotomous exposure and a dichotomous outcome, this method can be implemented in the following steps:

1) Model the association between the exposure (as a dependent variable) and the confounders (as independent variables) using the original data set.

2) Model the association of the mediator (as a dependent variable) with the exposure and confounders (as independent variables) using the original data set.

3) Create a new data set by repeating each observation twice including a new variable A^* , which is each time set to one of the two possible values or the exposure A .

4) Apply steps 1 and 2 to the new data set, and by using predicted probabilities from the models with A and A^* , compute weights as $W_i^c = \frac{P(M = M_i | A = A_i^*, C = C_i)}{P(M = M_i | A = A_i, C = C_i)}$, where C denotes all the confounders.

5) Apply the following weighted logistic regression model to estimate the association between the outcome and the exposure: $logit [E[Y_{a,Ma^*}]] = c_0 + c_1a + c_2a^* + c_3aa^* + c_4C$.

When no exposure-mediator interaction presents, the odds ratio of the natural direct effect is estimated by $\exp[c_1(a - a^*)]$, and the natural indirect effect by $\exp[c_2(a - a^*)]$. Confidence intervals can be obtained either through generalized estimating equations (normally by default in software) or a bootstrap procedure.

This method holds the same assumptions as other mediation analysis in the counterfactual framework do [5]. Similar to the two-stage regression model proposed by Valeri and VanderWeele, this method allows non-linear models and the interaction between the exposure and the mediator. The most attractive advantage of this method is that it can be applied to almost any combination of variable types and can be implemented in any statistical software allowing weighted modeling. Moreover, this method has been expanded to analysis including multiple mediators [387], which may be of particular interest to studies aiming to test several potential mechanisms simultaneously. However, this method has several limitations. In particular, it may not be ideal for the study with a continuous mediator, as unstable weights are more likely to be produced in this case [5].

Other practical options in the counterfactual framework

Since the two methods introduced above (and used in the thesis), other methods have been proposed in the meantime for implementing mediation analysis in the counterfactual framework. For instance, Imai *et al.* provided an R package that can accommodate various type of variables [388]. Besides, Nguyen *et al.* provided a guideline using inverse odds ratio weighting, with accompanying Stata sample code [389].

All these practical methods, available for different types of the studying variables and diverse software, are likely a soft introduction of implementing the counterfactual approach to mediation analysis in epidemiology.

OBJECTIVES

The **general objective** of the thesis is to study the role of diet and obesity in asthma, while accounting for potential mediators in these analyses. Potential mediators have been selected at two levels: first, at a “macro-level” to take into account complex interrelationships within nutritional factors in the diet–asthma association, and then, at a “micro-level” to better understand potential biological mechanisms of the association between body adiposity and asthma activity.

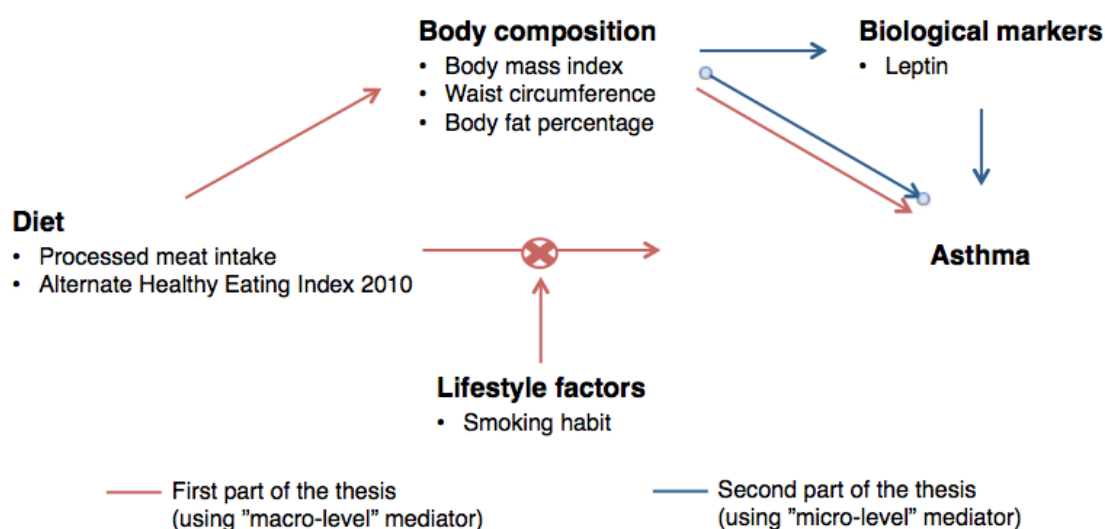


Figure 7 Specific objectives of the thesis

The first part (represented as red arrows in Figure 7) of the thesis aims to investigate the role of dietary factors in asthma at a “macro-level” using mediation analysis. The focus is first on a food item recently identified as an independent risk factor for major chronic diseases – processed meat, and then, on the overall effect of diet assessed by the latest diet score regarding healthy eating and diet quality – the AHEI-2010. It is plausible that BMI lies in the causal pathway between diet and asthma, and that the magnitude of this association may depend on complex interactions with other lifestyle factors such as smoking. Thus, the first part of the thesis has two specific objectives:

- i. to study the association between processed meat intake and change in asthma symptoms over time considering BMI as a potential mediator, and to evaluate effect modification by smoking.

ii. to study the association between the AHEI-2010 diet score and change in asthma symptoms over time considering BMI as a potential mediator, and to evaluate effect modification by smoking.

The second part (represented as blue arrows in Figure 7) of the thesis aims to better understand the association between obesity and asthma activity at a “micro-level” using mediation analysis. Specifically, the third objective of the thesis is to specifically address the potential role of leptin, an inflammatory biological maker related to obesity, as a mediator in the longitudinal association between body adiposity and persistent asthma activity.

MATERIALS AND METHODS

All the analyses conducted in this thesis were performed using data from the French Epidemiological study on the Genetics and Environment of Asthma, bronchial hyperresponsiveness and atopy (EGEA). First the EGEA study is presented, with a description of the study design, the follow-up, and the data collection. Then data used in the thesis are detailed, including data related to diet, body composition, respiratory phenotypes, and biological markers. Finally, statistical methods used in different analyses are presented.

5 The EGEA study

5.1 Study design and follow up

The EGEA study is a longitudinal study that was designed to study the genetic and environmental factors as well as their interactions in asthma and asthma-related respiratory phenotypes (bronchial hyperresponsiveness and atopy), and to clarify the heterogeneity of the disease [390–392]. Including three surveys followed-up over 20 years (<https://egeanet.vjf.inserm.fr/index.php/fr/>), the EGEA study has collected detailed data for respiratory research from detailed questionnaires, clinical examination, and a high quality biological collection (i.e. biological, genetic, and epigenetic data) (Figure 8).

Ethical approval was obtained from the relevant institutional review board committees (Cochin Port-Royal Hospital and Necker-Enfants Malades Hospital, Paris. n° 01-07-07, 04-05-03, 04-11-13 and 04-11-18, DGS “Direction Générale de la Santé” n°2002/0106 and n°910048). Written informed consent was obtained from participants.






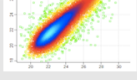
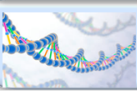

	EGEA1 (1992-1995)	EGEA2 (2003-2007)	EGEA3 (2011-2013)
 EGEA Étude épidémiologique des facteurs Génétiques et Environnementaux de l'Asthme, Hyperactivité bronchique et fatigue			
 Population	2047 subjects aged 7-70 years (388 cases with asthma, 1244 first degree of cases and 415 controls)	1601 subjects	1557 subjects
 Detailed questionnaire	Interview-based questionnaire on health and environment	Interview-based questionnaire on health and environment	Self-completed questionnaire on health and environment
 Clinical examination	Lung function tests with methacholine challenge, Skin prick tests to 11 allergens	Lung function tests with methacholine challenge, FeNO, Skin prick tests to 12 allergens	
 Biological data	total IgE, specific IgE to 160 allergens*, white blood cell counts	Total IgE, specific IgE to 160 allergens*, white blood cell counts, several cytokines	
 Genetic and epigenetic data	GWAS data (Illumina 610 Quad), methylome data (methylC-capture sequencing)*	Methylome data* GWAS data (Illumina 610 Quad)	
 Biological collection (about 66 000 stored samples)	Serum, plasma, buffy coat, DNA	Serum, plasma, buffy coat, DNA, RNA, lymphoblastoid cell lines, exhaled breath condensate	

Figure 8 General presentation of the EGEA study.

EGEA1 (First survey)

The first survey (EGEA1) of the study was conducted between 1992 and 1995 in five cities (Paris, Lyon, Grenoble, Marseille, and Montpellier) in France. Proband (asthmatics and controls) were defined as individuals aged 7 to 65, born in France, with parents born in France, and living in one of the five French cities. Self-administered questionnaires were distributed in chest clinical centers. Cases with asthma were defined by responding positively to the four following questions. For children (< 16 years old), the questionnaires were answered by their parents.

Q1. Have you ever had attacks of breathlessness at rest with wheezing? (BMRC/ATS/ECRHS questionnaires)

Q2. Have you ever had asthma attacks? (BMRC questionnaires)

If yes,

Q2a. Was this diagnosis confirmed by a physician? (ATS questionnaire)

Q2b. Have you had an asthma attack in the last 12 months? (ECRHS questionnaire)

First-degree family members of the asthma cases (spouse, children, and siblings of the adult cases, and parents and siblings of the children cases) were invited to participate in the survey.

Controls matched for age, month of exam, and city were recruited in surgery hospitals for children, and from electoral rolls or surgery hospitals or check-up centers for adults.

In total, 2,047 participants were included at EGEA1, among which 388 were asthma cases (217 adults), 1,244 first-degree relatives or spouse of the cases (308 with asthma, 917 adults), and 415 controls (309 adults).

EGEA2 (Second survey)

Between 2003 and 2007, the participants of EGEA1 were contacted for the second survey (EGEA2). Out of 2,002 participants alive, 92% (n = 1,845) completed a short self-administered questionnaire. In addition, 58 new participants, which were family members of asthma cases defined at EGEA1, accepted to participate in the survey and completed the questionnaires. Among all these participants, 1,601 (77% of the initial participants of EGEA1 and 58 new participants) had a complete medical examination, including spirometry, skin prick test, and blood sampling. At EGEA2, “asthma ever” was defined by either being recruited as a case or responding positively to Q1 or Q2 listed above. According to this definition, 715 out of 1,601 participants had ever asthma.

At EGEA2, detailed data were collected on respiratory symptoms, treatment (for asthma cases), quality of life, physiological and biological profile (spirometry, skin prick test, body composition, and various biological markers), environmental/occupational exposures, as well as lifestyle factors (dietary data, physical activity, and smoking) and hormonal-related events.

EGEA3 (Third survey)

Between 2011 and 2013, a follow-up study of EGEA1 and EGEA2 (n = 2,120 participants) was performed (EGEA3). Among the 2,120 participants, 87 died between EGEA1 and September 2011, 344 were lost to follow-up and 64 refused to participate. Overall, 1,558 filled out the self-completed questionnaire (response rate = 79.1%). Detailed data were collected regarding respiratory symptoms, treatment (for asthma cases), quality of life, environmental and occupational exposures, as well as physical activity, smoking, and hormonal-related events.

5.2 Data collection

Questionnaires

The questionnaires used in the three surveys of the EGEA study are based on international standardized recommendations with adapted questions mostly from the European Community Respiratory Health Survey (ECRHS), but also from the British Medical Research Council (BMRC), and the American Thoracic Society (ATS) and the Division of Lung Diseases [393].

At EGEA1, during the recruitment of participants, a short self-questionnaire was used to collect data on asthma, as well as other respiratory symptoms (dyspnea, cough, sputum and nocturnal symptoms in the past 12 months). This short questionnaire also included questions about cigarette smoking and family status. At the time of the examination, the adult participants at EGEA1 (> 16 years) answered a detailed questionnaire including respiratory and allergic symptoms, personal allergic and disease history (in both early childhood and adulthood), family history, active and passive smoking, living environment, educational level and professional experience. Participants with asthma also answered additional questions about the disease, such as the age of the first asthma attack, frequency of attacks, triggering factors and hospitalization history due to asthma. When the participant was younger than 16 years old, the questionnaire was answered by a parent.

At EGEA2, the long questionnaire was built on the same model as the detailed questionnaire used at EGEA1, with additional questions on some new research hypotheses. For example, asthma treatment (inhaled or not) during the last 12 months was asked in detail, two questionnaires were included to assess the quality of life, a Short Form-36 (SF-36) generic questionnaire [394] for all of the participants, and the standardized Asthma Quality of Life Questionnaire [43] for those with asthma. Participants were also asked in detail about their professional experience, with particular interest in certain occupations such as health workers, laboratory staff or cleaners. A food frequency questionnaire was added, as well as questions regarding lifetime body shapes and physical activity. Several questions also covered the contact with domestic or farm animals and household cleaning tasks.

At EGEA3, two self-questionnaires were used (one for participants with asthma and one for participants without asthma) to collect data on respiratory health and related environmental factors. Additionally, the EGEA3 questionnaire included questions on domestic exposures to cleaning products and on residential history since 1990.

Clinical examination

During the complementary examinations at EGEA1 and EGEA2, lung function tests with spirometry and methacholine challenge were performed by trained research technicians with similar equipment (Biomedin Srl, Padua, Italy; Spirometer Masterscreen, Jaeger at EGEA1 and SpiroDyn'R, Dyn'R at EGEA2) across centers according to the ATS/ERS guidelines [395]. Standardized operating procedures were implemented, including calibration of all equipment before each measurement, and quality control visits were performed to ensure correct protocols by study coordinators. All measurements were corrected for body temperature, pressure, and saturation. The best of 5 forced expirations (FEV₁ and FVC) was selected, according to the ATS/ERS guidelines [395]. A methacholine challenge (maximum cumulative dose, 4 mg) was then performed among those without medical contraindications (excluding subjects with a cardiac history) and with FEV₁ of 80% of predicted value or greater. The bronchial hyperreactivity test was carried out according to the protocol of the ECRHS [396].

Skin prick tests (SPT) were performed by trained professionals among participants over 4 years old for 11 allergens at EGEA1 and 12 at EGEA2, including domestic allergens (cat, *Dermatophagoides pteronyssinus*, *Blatella germanica*), fungi (*Cladosporium herbarum*, *Alternaria tenuis*, *Aspergillus*) and pollen (olive, birch, timothy grass, *Parietaria judaica*, ambrosia, and cypress at EGEA2 only). Negative (uncoated) and positive (histamine) SPT controls were assessed. SPT with a mean wheal diameter of 3mm or more was considered as positive.

Data on body composition were also collected during the examination. Related details are presented later in the section “Data on body composition”.

Biological sample collection

The EGEA study maintained a high quality of samples and data through a professional quality management system (QM-system) with an ISO 9001 certification since 2006. All work processes during handling and storage of biological samples are based on standard operating procedures. At EGEA1, a first biological collection was established including DNA, serum and plasma. At EGEA2, this biological collection was extended, including lymphoblastoids cell lines, DNA, RNA, serum, plasma, erythrocytes and exhaled breath condensate (EBC) collected with an RTube™ (Respiratory Research Inc., Charlottesville, VA, USA) under standardized procedures [397].

5.3 Data used in this thesis

Since diet, body composition, and leptin were measured at EGEA2 only, we used data collected at EGEA2 as baseline. The evolution of respiratory phenotypes was therefore evaluated between EGEA2 and EGEA3.

5.3.1 Dietary data

Dietary data were collected at EGEA2 using a 118-item self-administered food frequency questionnaire (FFQ), which is based on a validate French questionnaire [398]. Participants were asked to indicate their frequency of consumption of all food items by checking one of eight given categories, including “never or < 1 time/month”, “1–3 times/month”, “1 time/week”, “2–4 times/week”, “5–6 times/week”, “1 time/day” 2–3 times/day”, and “≥ 4 times/day” (see Figure 9), for a standard portion size of each item. For example, if a participant eats one portion of meat (beef, lamb, veal, or pork) 3 times per week, the category “2–4 times/week” should be checked.

	Jamais ou < 1 fois par mois	1-3 fois par mois	1 fois par sem	2-4 fois par sem	5-6 fois par sem	1 fois par jour	2-3 fois par jour	4 ou + fois par jour
<u>Exemple 1</u> : Un morceau de viande (bœuf, agneau, veau, porc) <i>Si vous mangez de la viande 3 fois par semaine</i>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<u>Exemple 2</u> : Des prunes ou des cerises <i>Si l'été dernier pendant la saison des cerises, vous en avez mangé une fois par semaine</i>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Figure 9 Example of the food frequency questionnaire used in the EGEA study.

The questionnaire was organized in 10 sections, including diary products (14 items), cereals (11 items), fruits (11 items), vegetables (24 items), eggs/meat/processed meat (8 items), fish (6 items), prepared or homemade foods (10 items), fats and seasoning (11 items), drinks (13 items), and appetizers/desserts/sweets (10 items). Data on dietary supplements (daily, occasionally, never) have also been assessed.

Standard portion sizes were listed for each food. The selected frequency category for each food item was converted to a daily intake. For example, a response of “1 serving/week” was converted to 0.14 servings/d. Through the use of the French food-composition data from the Vitamin and Antioxidant Element Supplementation (SU.VI.MAX) survey [89], the average daily intake of nutrients was calculated by multiplying the frequency of consumption of each item by its nutrient content per serving and totaling the nutrient intake for all food items.

Out of the 1,601 participants from EGEA2, 35 adolescents (less than 16 years-old) were firstly excluded (no FFQ in adolescents). Among adults (n = 1,566), we excluded 109 participants who did not fill the FFQ. Participants who left more than 4 items blank on the diet questionnaire were excluded (n = 60). For participants with 1–3 blank items (n = 198 with 1 blank item, 94 with 2 blank items, and 34 with 3 blank items), each missing value was replaced by the median of the food item in the remaining population. We also excluded participants with an implausibly high (> 4,200 kcal/d for men; > 3,500 kcal/d for women) or low total (< 800 energy intake kcal/d for men; < 500 kcal/d for women) [125]. Dietary data were available for 1,236 adults.

5.3.2 Data on body composition

Data on body composition were measured by trained health technicians at EGEA2.

Body weight was measured in kilograms, and height was measured to the nearest centimeter. **Body mass index (BMI)** was calculated as weight/height² and expressed in kg/m². **Waist circumference** was measured at the midway between the lowest rib margin and the most prominent part of the iliac crest according to standard instructions; the measurements were rounded to the nearest centimeter [399].

Bioelectrical Impedance Analysis (BIA) was performed only among participants examined in Paris and Grenoble. The resistance to a small electrical current (800 μA, 50 kHz) was measured to estimate body composition. According to the prediction equation given by Sun *et al.* [400] (see below), **body fat** and **fat free mass (FFM)** were estimated using data including resistance, sex, weight, and height.

For men: $FFM = -10.68 + 0.65 \text{ height}^2/\text{resistance} + 0.26 \text{ weight} + 0.02 \text{ resistance}$

For women: $FFM = -9.53 + 0.69 \text{ height}^2/\text{resistance} + 0.17 \text{ weight} + 0.02 \text{ resistance}$

FFM is expressed in kg, height²/resistance in cm²/Ω, and resistance in Ω.

Body fat was computed as the difference between body weight and FFM, and **body fat percentage** was calculated as the ratio of body fat to weight.

Data on BMI were available for 1420 adults, waist circumference for 1369 adults, and body fat percentage for 626 adults.

5.3.3 Data related to respiratory phenotypes

Ever asthma

Ever asthma was defined by either being recruited as an asthma case or responding positively to Q1 or Q2 at EGEA1 or EGEA2 (as previously introduced in section 5.1). Ever asthma has been considered in the statistical model as a covariate to take into account the case-control design of the EGEA study in this thesis.

Current asthma/asthma activity

Among participants with asthma, current asthma was defined by having had 1) asthma attacks or attacks of shortness of breath with wheezing at rest, or 2) respiratory symptoms (wheezing, nocturnal tightness in the chest, attack of shortness of breath following strenuous activity, at rest, or at night), or 3) inhaled and/or oral medication because of breathing problems during the previous 12 months [401]. At EGEA2 and EGEA3, the same definition of current asthma was applied.

Participants with current asthma at both EGEA2 and EGEA3 were considered to have **persistent asthma activity**, and those with current asthma only at EGEA2 were considered to have **remitted asthma activity**.

Asthma symptom score

The asthma symptom score was proposed by Pekkanen *et al.* [33,34] to better study risk factors for asthma in longitudinal studies. Ranging from 0 to 5, the score is based on the number of respiratory symptoms during the past 12 months reported in the questionnaire: 1) breathless while wheezing, 2) woken up with chest tightness, 3) attack of shortness of breath at rest, 4) attack of shortness of breath after exercise, and 5) woken by attack of shortness of breath. The asthma symptom score was calculated among participants with and without asthma, at EGEA2 and EGEA3.

The change in asthma symptom score is calculated as $EGEA3\ score - EGEA2\ score$. The asthma symptom score detects the change in asthma symptoms over time, reflecting either asthma incidence, or captures the temporal variability of the disease among participants with asthma - remission, relapse or persistence [33]. Change in asthma symptom score was categorized as “improved” if $EGEA3\ score - EGEA2\ score < 0$, “stable” if $EGEA3\ score - EGEA2\ score = 0$, and “worsening” if $EGEA3\ score - EGEA2\ score > 0$. These categorical variables were used in this thesis.

5.3.4 Biological data

Among participants at EGEA2, biological data were collected among the participants who had current asthma and available data on lung function, eosinophils and neutrophils (n =

428), and among their age-and-sex matched group randomly selected from the participants who had never asthma (n = 558).

Serum leptin concentration (ng/ml) was measured at EGEA2 using the Luminex xMAG® technique in a BioPlex system (Bio-Plex Pro human inflammation cytokine 9-plex assay, Bio-Rad Laboratories, Hercules, California, United States) at the IMIM (Instituto Hospital del Mar de Investigaciones Médicas) in Barcelona, Spain. An aliquot of the same human serum pool was run by duplicate in each plate. No value above or below the limit of detection was observed.

Data on leptin were available for 828 adults, among which 428 had current asthma and 400 never had asthma.

5.3.5 Other variables

Baseline potential confounders, including **age**, **sex**, **smoking status** (never, former or current smoking), **educational level**, and **physical activity**, were assessed at EGEA2. Educational level was categorized into 2 classes according to whether the participants had higher education (university or equivalent). Leisure-time physical activity was assessed in metabolic equivalents per week (METs/w) taking into account a variety of intensive activities (such as jogging, aerobic, swimming, cycling, tennis, soccer, digging in the garden), moderate activities (such as cleaning, walking, golf, slight gardening), and light activities (such as reading, watching TV, office work, driving, eating). Average physical activity was estimated by summing the product of average activating active time per week and the metabolic equivalent value of each activity. **Atopy** (yes/no) was defined by positive result of the skin prick test at EGEA2 (12 allergens, SPT+, wheal diameter ≥ 3 mm).

6 Statistical analysis

All analyses were performed using SAS version 9.3 (SAS Institute Inc., Cary, North Carolina). Directed acyclic graphs (DAGs) were used to present the studying association. Classical statistical tests, including ANOVA, t-test, Mantel-Haenszel and Pearson chi-square test, were used for descriptive analyses. Two methods of mediation analysis in the counterfactual framework, a marginal structural model approach [5] and a two-step regression approach [6], were employed to disentangle the direct and indirect effects in this thesis. Implementation details are presented in the next chapter for each specific objective.

We assumed the following conditions were satisfied for the application of mediation analysis [4–6]: no unmeasured confounders for the associations between: 1) the exposure and the outcome, 2) the potential mediator and the outcome, 3) the exposure and the potential mediator, as well as 4) no mediator-outcome confounders affected by the exposure.

RESULTS AND DISCUSSION

Main results of the thesis are presented in the following sections, first regarding the association between processed meat intake and change in asthma symptoms, then regarding the association between overall diet quality and change in asthma symptoms, and finally regarding the role of leptin in the association between body adiposity and asthma activity.

7 Processed meat intake and change in asthma symptoms

This work has been presented as oral communication at the European Respiratory Society (ERS) international congress in Amsterdam in September 2015, and led to the publication of a scientific article in the journal *Thorax* with worldwide media coverage.

Introduction

The deleterious health effects of high processed meat intake have been increasingly observed, first on cancer and major chronic diseases [165], and recently on lung health, including lung cancer [165,176,402], decreased lung function [171,175], and COPD symptoms [171,176], exacerbations [174], and incidence [172,173]. As processed meat is known for its high-nitrite content [403] which may lead to nitrosative stress-related airway inflammation – one of the several physiological processes involved in asthma [214], it is reasonable to posit that processed meat intake is an independent risk factor for asthma. However, the association between processed meat intake and asthma remains unclear.

Given that previous studies showed weak association between dietary factors and adult-onset asthma incidence [210], the asthma symptom score, which has been proposed to study risk factors for asthma in longitudinal studies [33,34], might be particularly relevant to studying the role of dietary factors as an etiological risk factor (targeting the primary prevention) and as a disease modifier (targeting the secondary prevention) [404].

In the current study, we aimed to investigate the association between processed meat intake and the change in asthma symptom score considering BMI as a potential mediator, and to evaluate effect modification by smoking.

Methods

Study sample

Out of the 1,236 participants from EGEA2 with valid dietary data, 10 participants with missing data for the asthma symptom score were further excluded. Among the 1,226 remaining, 185 (15%) were lost to follow-up. Compared with participants lost to follow-up (n = 185), participants followed at EGEA3 (n = 1,041) were less likely to be men or smokers and were similar regarding age, BMI, educational level, physical activity, and asthma status (Table 7). After further excluding 70 participants with missing data for the asthma symptom score assessed at EGEA3 out of the 1041, the final study sample included 971 participants (Figure 10).

Among adults, excluded participants (n = 595) were similar compared with participants included in analysis (n = 971) regarding age, BMI, physical activity, sex, smoking and asthma status, but they were more likely to have a lower educational level (Table 8).

Table 7 Comparison of characteristics between participants followed at EGEA3 (n = 1,041) and participants lost to follow-up (n = 185)

	Participants		P Value
	Followed at EGEA3 (n = 1,041)	Lost to follow-up (n = 185)	
Age, y	43 ± 16	41 ± 17	0.07
Men, n (%)	508 (49)	117 (63)	< 0.001
BMI, kg/m ²	24.4 ± 4.2	24.8 ± 4.5	0.38
Smoking (ever), n (%)	514 (49)	107 (58)	0.04
Higher education*, n (%)	519 (50)	83 (45)	0.20
Leisure time physical activity, METs/week	45 ± 17	47 ± 18	0.11
Ever asthmatic at EGEA2, n (%)	439 (42)	90 (49)	0.10
Processed meat intake	3.2 ± 2.9	3.2 ± 2.1	0.83

Data are presented as mean ± SDs, or number of subjects (%).
 *Higher education: college education or equivalent.
 BMI: body mass index; METs/w: metabolic equivalents per week.

Table 8 Comparison of characteristics between included (n = 971) and excluded (n = 595) adult participants

	Participants		P Value
	Included (n = 971)	Excluded (n = 595)	
Age, y	43 ± 16	43 ± 17	0.47
Men, n (%)	476 (49)	297 (50)	0.73
BMI, kg/m ²	24.4 ± 4.2	24.7 ± 4.5	0.27
Smoking (ever), n (%)	470 (49)	313 (53)	0.08
Higher education*, n (%)	494 (51)	220 (43)	0.002
Leisure time physical activity, METs/week	45 ± 17	45 ± 18	0.87
Ever asthmatic at EGEA2, n (%)	408 (42)	272 (46)	0.15

Data are presented as mean ± SDs, or number of subjects (%).
 *Higher education: college education or equivalent.
 BMI: body mass index; METs/w: metabolic equivalents per week.

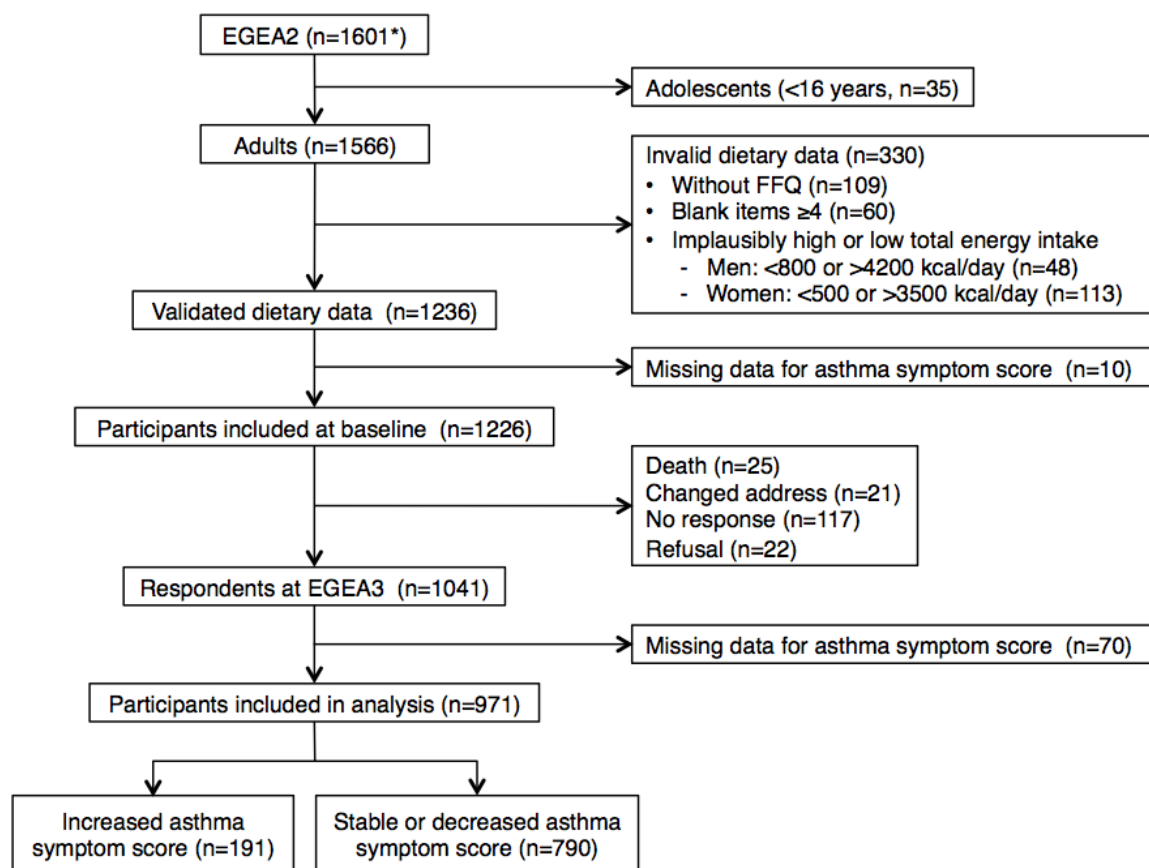


Figure 10 Flowchart for inclusion of participants. *Among the 1,601 participants, 715 (45%) had ever asthma. FFQ, food frequency questionnaire.

Dietary intakes

The processed meat group included 3 items: ham (2 slices/portion), sausage (1/portion) and dried sausage (2 slices/portion). According to the distribution of processed meat intake, exposure was categorized in 3 classes: < 1 serving/week (lowest, 19%), 1–3.9 servings/week (medium, 48%) and ≥ 4 servings/week (highest, 33%). The group with the lowest intake was considered as “non-exposed” and was taken as the reference group.

In the EGEA study, two dietary patterns were previously identified using confirmatory factor analysis (CFA) on 46 food groups: 1) a Prudent pattern characterized by a high intake of vegetables, fruits, oils, whole grains cereals and fish, and 2) a Western pattern loaded by a high consumption of prepared meal, French fries, processed meat, condiments, alcohol, beer/cider, sandwiches and potatoes [125]. In the current analysis, we derived a “modified Western pattern” and a Prudent pattern using CFA on 45 food groups (all food groups except processed meat,

Table 9). To account for the remaining effect of the overall diet in the processed meat–asthma association, the individual scores obtained from CFA for the “modified Western pattern” and the Prudent pattern were used as potential confounders in the analyses.

Asthma symptom score

Change in asthma symptom score was categorized to better interpret the results. To obtain sufficient statistical power of analysis, a dichotomized variable was used: “stable or improved” if $EGEA3 \text{ score} - EGEA2 \text{ score} \leq 0$, and “worsening” if $EGEA3 \text{ score} - EGEA2 \text{ score} > 0$.

Body mass index (BMI)

BMI (kg/m^2) at EGEA2 was categorized into 5 classes: < 22.5 , $22.5\text{--}24.9$, $25\text{--}27.4$, $27.5\text{--}29.9$, and ≥ 30 .

Statistical analysis

To study the potential modifying effect of smoking habit on the processed meat–asthma association, we formally tested the interaction between processed meat intake and smoking habit, and we reported no significant interaction (P value for interaction = 0.60).

Including a interaction (i.e. product) term in the model, we formally tested interactions of processed meat intake with asthma status (due to the case-control design of the EGEA study), and with sex (since processed meat intake is usually higher in men than in women [405,406]). No statistical interaction was observed (P values for interaction were 0.50 and 0.50, respectively).

We used a DAG to represent our mediation model (Figure 11). Due to the case-control design of the EGEA study, the study population was enriched with participants with asthma as compared with the general population. To take into account this study design, ever asthma was considered as a covariate in analysis. Besides, age (continuous), sex, smoking status (never/ever), educational level (low/high), leisure-time physical activity (expressed in metabolic equivalents/week, continuous), dietary patterns, total daily energy intake were also considered as potential confounders. In addition, stratified analyses according to sex, smoking, and asthma status were performed.

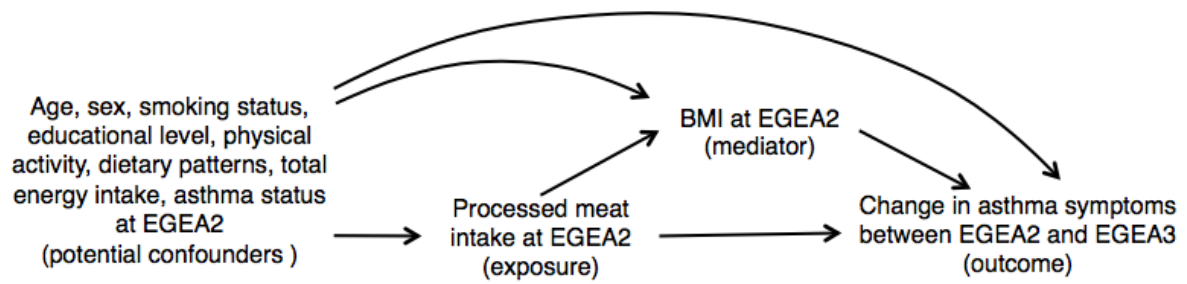


Figure 11 Direct acyclic graph of the proposed mediation model.

Table 9 Factor-loading matrix for the major factors (dietary patterns) using confirmatory factor analysis in EGEA2 study (n = 1,236)

	Factor 1 (Prudent pattern)	Factor 2 (Modified Western pattern)
Fruity vegetables	<u>0.62</u>	0.20
Leafy vegetables	<u>0.59</u>	
Other vegetables	<u>0.58</u>	
Fruits with flavonoids	<u>0.55</u>	
Root vegetables	<u>0.54</u>	<u>0.32</u>
Fruits with β -carotene	<u>0.48</u>	
Citrus fruits	<u>0.42</u>	
Cruciferous vegetables	<u>0.40</u>	
Olive oil	<u>0.35</u>	
Other oil	<u>0.32</u>	
White fish	0.26	
Dried Legumes	0.27	0.25
Whole grains products	0.23	
Blue fish	0.23	0.23
Tea	0.20	
Other fruits	0.20	
Prepared meal		<u>0.54</u>
Condiments		<u>0.45</u>
French fries	-0.28	<u>0.45</u>
Pods and peas		<u>0.33</u>
Alcohol		<u>0.31</u>
Potatoes		<u>0.30</u>
Beer / cider		<u>0.30</u>
Sandwiches	-0.28	0.28
Snack	-0.23	0.27
Cakes		0.26
Soda	-0.28	0.27
Shellfish		0.21
Nuts and seeds		0.21

45 food groups (all food groups except processed meat) were included in analysis. Factor loadings represent the correlation between factor scores and intakes of food groups. The food groups with factor loadings < 0.20 for both factors were excluded. Absolute values < 0.20 were not listed in the table for simplicity; those with loadings of 0.30 or greater are underlined.

Several methods with practical implementation have recently been proposed to disentangle the natural direct and indirect effects in the counterfactual framework [5,6,388]. Implementation details in this study are presented in the following paragraphs. As previously described [5,6] (in the chapter materials and methods), several conditions are required to apply such methods, and we assumed that they were satisfied.

As the main analysis, we applied the marginal structural model proposed by Lange and colleagues [5], which has been described as a simple approach and can be implemented in standard software for almost any variable type. The marginal structural model is implemented in several steps as previously described in detail [5]. Briefly, we first created a new data set by repeating each observation three times including a new variable A^* , which was each time set to one of the three possible categories of the processed meat intake (exposure, denoted by A). Next, a generalized linear regression model was applied to the new dataset to estimate the association between BMI (mediator, denoted by M) and processed meat intake, first using the original variable A , and then the new variable A^* . Using predicted probabilities from the models with A and A^* , the individual stabilized weights were calculated as $W_i^c = P(M = M_i | A = A_i^*, C = C_i) / P(M = M_i | A = A_i, C = C_i)$, where C denoted all the confounders. Finally, the following weighted logistic regression model was applied to estimate the association between the change in asthma symptom score and processed meat intake: $\text{logit} [E[Y_{a,M_{a^*}}]] = c_0 + c_1a + c_2a^* + c_3C$. The odds ratio of the natural direct effect was estimated by $\exp[c_1(a - a^*)]$, and the natural indirect effect by $\exp[c_2(a - a^*)]$.

To evaluate how much of the total effect was due to the effect of BMI, we calculated the “proportion explained” by BMI as $(OR_{TE} - OR_{NDE}) / (OR_{TE} - 1)$, where OR_{TE} and OR_{NDE} were respectively the odds ratio of total effect and natural direct effect [407]. The total effect was calculated as $OR_{NDE} \times OR_{NIE}$, where OR_{NIE} denoted the natural indirect odds ratio.

As a sensibility analysis, we replicated our results using another method of mediation analysis in the counterfactual framework, a 2-stage regression model, which provides estimation of total effect and standard errors using bootstrapped samples [6]. For the 2-stage regression model, a macro statement [6] was applied. Processed meat intake was regrouped into two classes: < 1 serving/week and ≥ 1 serving/week, because a three-class exposure was not supported in the SAS macro. The following linear regression model was implemented in the first stage to assess the association between BMI and processed meat intake: $E(M | A = a, M = m, C = c) = \beta_0 + \beta_1a + \beta_2'c$; and in the second stage, the following

logistic regression model was implemented to assess the association between the change in asthma symptom score and processed meat intake: $\text{logit}[P(Y = 1|A = a, M = m, C = c)] = \theta_0 + \theta_1 a + \theta_2 m + \theta_3' c$. Confidence intervals (95%) were estimated from 1,000 bootstrapped samples. As the 2-stage regression approach was performed using the exposure as a two-classes variable, we also applied the marginal structural model using processed meat intake as a dichotomous variable.

Results

Description of population

The study sample comprised 971 participants. The mean age was 43 years old and 49% were men. At EGEA2, 42% participants had ever asthma, 51% were never smokers, 35% were overweight and 9% were obese. The median processed meat intake was 2.5 (Q1–Q3: 1.5 - 4.5) servings/week.

Baseline characteristics of the participants according to processed meat intake are presented in Table 10. Compared with participants with the lowest intake (< 1 serving/week) of processed meat, participants with the highest intake (≥ 4 serving/week) were younger ($P < 0.001$), more likely to be men ($P < 0.001$) and smokers ($P = 0.04$), to report a higher total energy intake ($P < 0.001$), and to have ever asthma ($P = 0.03$); as expected, they also consumed more sodium ($P < 0.001$) and saturated fat ($P < 0.001$). Besides, participants who had a higher intake of processed meat were more likely to be in a higher BMI category ($P = 0.01$). Additionally, baseline characteristics of the participants according to change in asthma symptoms are presented in Table 11.

Processed meat intake and change in asthma symptom score

At EGEA3, after a mean follow-up time of seven years (ranging from five to nine years), 513 participants (53%) reported the same asthma symptom score as EGEA2 (stable), 191 (20%) reported more symptoms (worsening), and 267 (27%) had fewer symptoms (improved). The proportion of participants with worsening asthma symptoms was 14%, 20% and 22% respectively among those who ate < 1, 1–3.9 and ≥ 4 servings/week of processed meat (P for trend = 0.04).

Results of mediation analyses using the marginal structural model are presented in Table 12. Among all the participants, after adjustments for age, sex, smoking, educational level, physical activity, dietary patterns, total energy intake, and asthma status, we observed a

positive direct effect of processed meat intake on worsening asthma symptoms: the odds ratio (OR) was 1.76 (95% CI: 1.01, 3.06) when comparing the highest to the lowest intake. We also reported a positive indirect effect mediated by BMI: the adjusted OR was 1.07 (95% CI: 1.01, 1.14) when comparing the highest to the lowest intake (Figure 12). The percentage mediated by BMI of the association was 14%. When we performed stratified analyses according to sex, smoking, and asthma status, results were similar in all subgroups (OR > 1), although the power was limited (Table 12). Analyses using another method of mediation analysis in the counterfactual framework (the 2-stage regression model) yielded similar results to those obtained through the marginal structural model (Table 13, Table 14).

Table 10 Characteristics for participants at baseline (EGEA2) according to processed meat intake (n = 971)

	Processed meat intake			P Value
	< 1 s/w (n = 181)	1–3.9 s/w (n = 462)	≥ 4 s/w (n = 328)	
Age, y	45 ± 16	44 ± 16	40 ± 16	< 0.001
Men, n (%)	60 (33)	221 (48)	195 (59)	< 0.001
BMI, kg/m ²	23.7 ± 3.8	24.5 ± 4.1	24.6 ± 4.4	0.07
BMI, kg/m ² , n (%)				0.01
< 22.5	85 (47)	172 (37)	131 (40)	
22.5–24.9	40 (22)	128 (28)	71 (22)	
25–27.4	34 (19)	77 (17)	54 (16)	
27.5–29.9	13 (7)	44 (9)	32 (10)	
≥30	9 (5)	41 (9)	40 (12)	
Smoking (ever), n (%)	82 (45)	209 (45)	179 (55)	0.03
Higher education, n (%)	87 (48)	254 (55)	153 (47)	0.06
Total energy intake, kcal/day	2,187 ± 593	2,357 ± 606	2,657 ± 650	< 0.001
Leisure-time physical activity, METs/w	44 ± 16	45 ± 17	45 ± 17	0.72
Ever asthma at EGEA2, n (%)	68 (38)	184 (40)	156 (48)	0.04
Asthma symptom score at EGEA2, n (%)				0.11
0	86 (47)	231 (50)	142 (43)	
1	39 (22)	116 (25)	70 (21)	
2 - 5	56 (31)	115 (25)	116 (36)	
Modified Western pattern*	-1.5 ± 3.3	-0.5 ± 3.3	1.6 ± 4.4	< 0.001
Prudent pattern*	0.5 ± 2.4	0 ± 1.9	-0.2 ± 2.4	0.002
Foods/nutrients intake				
Processed meat, serving/day	0.7 ± 0.4	2.3 ± 0.7	6.2 ± 3.5	< 0.001
Fruits, serving/day	3.9 ± 2.8	3.7 ± 2.8	3.6 ± 3.0	0.47
Vegetables, serving/day	3.9 ± 2.1	3.7 ± 2.0	3.8 ± 2.2	0.83
Whole grain cereals, serving/day	0.6 ± 1.0	0.5 ± 0.8	0.4 ± 0.8	0.07
Fish, serving/day	0.4 ± 0.3	0.4 ± 0.3	0.5 ± 0.4	0.18
Vitamin C, mg/day	252 ± 136	240 ± 124	248 ± 133	0.50
Sodium, g/day	3.2 ± 1.1	3.5 ± 1.1	4.3 ± 1.2	< 0.001
Total fat, g/day	88 ± 27	100 ± 29	115 ± 33	< 0.001
Saturated fat, g/day	33 ± 10	38 ± 12	45 ± 13	< 0.001

Data are expressed as mean ± SDs, or number of subjects (%). Overall *P* values were calculated from ANOVA for continuous variables, from Mantel-Haenszel chi-square test for ordinal categorized variables (BMI classes and asthma symptom score), and from Pearson chi-square test for other categorized variables.

*Modified Western pattern, Prudent pattern: identified using confirmatory factor analysis (CFA). Mean individual scores obtained from CFA were shown.

s/w, serving/week; METs/w, metabolic equivalents per week.

Table 11 Characteristics for participants at baseline (EGEA2) according to change in asthma symptoms (n = 971)

	Change in asthma symptoms		P Value
	Stable or improved (n = 780)	Worsening (n = 191)	
Age, y	42 ± 16	45 ± 16	0.04
Men, n (%)	388 (50)	88 (46)	0.36
BMI, kg/m ²	24.2 ± 3.9	25.0 ± 5.0	0.001
BMI, kg/m ² , n (%)			0.001
< 22.5	323 (41)	65 (34)	
22.5–24.9	191 (25)	48 (25)	
25–27.4	141 (18)	24 (13)	
27.5–29.9	66 (8)	23 (12)	
≥ 30	59 (8)	31 (16)	
Smoking (ever), n (%)	406 (52)	93 (51)	0.39
Higher education, n (%)	410 (53)	84 (44)	0.03
Total energy intake, kcal/day	2,418 ± 638	2,464 ± 662	0.38
Leisure-time physical activity, METs/w	45 ± 17	45 ± 17	0.79
Ever asthma at EGEA2, n (%)	310 (40)	98 (51)	0.004
Asthma symptom score at EGEA2, n (%)			0.02
0	364 (47)	95 (50)	
1	180 (23)	45 (24)	
2 - 5	236 (30)	51 (26)	
Modified Western pattern*	0.0 ± 3.8	0.2 ± 4.0	0.46
Prudent pattern*	0.0 ± 2.2	0.2 ± 2.3	0.47
Foods/nutrients intake			
Fruits, serving/day	3.7 ± 2.9	3.8 ± 2.8	0.46
Vegetables, serving/day	3.7 ± 2.0	3.9 ± 2.2	0.37
Whole grain cereals, serving/day	0.5 ± 0.9	0.4 ± 0.8	0.12
Fish, serving/day	0.4 ± 0.3	0.4 ± 0.3	0.28
Vitamin C, mg/day	242 ± 127	259 ± 138	0.11
Sodium, g/day	3.7 ± 1.2	3.8 ± 1.3	0.26
Total fat, g/day	102 ± 32	105 ± 32	0.24
Saturated fat, g/day	39 ± 13	41 ± 13	0.23

Data are expressed as mean ± SDs, or number of subjects (%). Overall P values were calculated from ANOVA for continuous variables, from Mantel-Haenszel chi-square test for ordinal categorized variables (BMI classes and asthma symptom score), and from Pearson chi-square test for other categorized variables.

*Modified Western pattern, Prudent pattern: identified using confirmatory factor analysis (CFA). Mean individual scores obtained from CFA were shown.

s/w, serving/week; METs/w, metabolic equivalents per week.

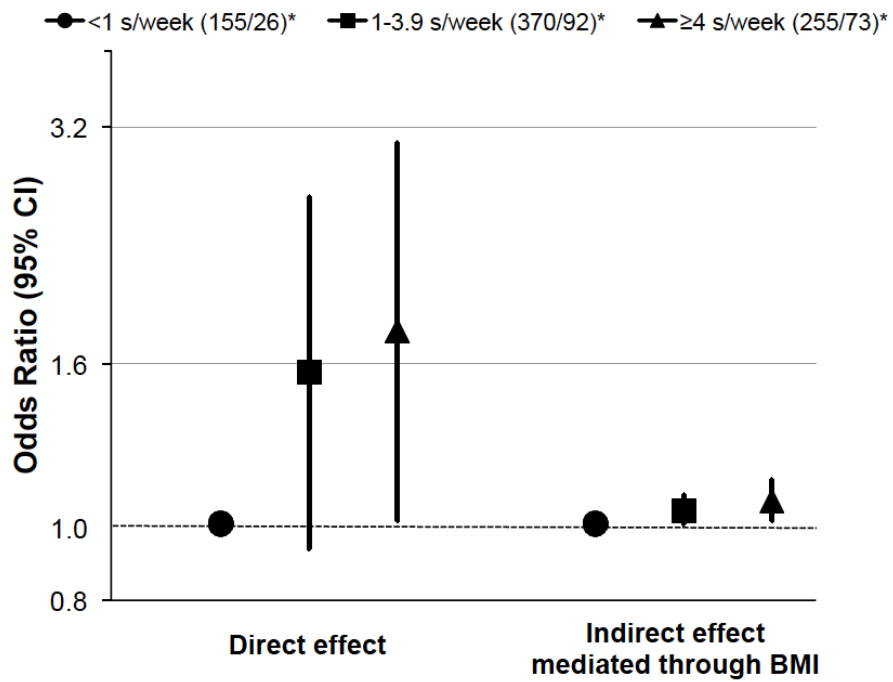


Figure 12 Direct and indirect effects of processed meat intake on worsening asthma symptoms between EGEA2 and 3, using the marginal structural model (n = 971). We adjusted for age, sex, smoking status, educational level, leisure-time physical activity, modified Western and Prudent patterns, total energy intake, and asthma status at EGEA2. *Numbers of participants (stable or improved/worsening). s/week, serving/week.

Table 12 Direct and indirect effects of processed meat intake on worsening asthma symptoms between EGEA 2 and 3, using the marginal structural model (n = 971)

	No. stable or improved/ worsening			Direct effect* OR (95%CI)			Indirect effect* OR (95%CI)		
	< 1 s/w	1–3.9 s/w	≥ 4 s/w	< 1 s/w	1–3.9 s/w	≥ 4 s/w	< 1 s/w	1–3.9 s/w	≥ 4 s/w
All participants	155/26	370/92	255/73	1.00	1.56 (0.93, 2.61)	1.76 (1.01, 3.06)	1.00	1.04 (1.00, 1.09)	1.07 (1.01, 1.14)
Subgroups									
Sex									
Men	51/9	184/37	153/42	1.00	1.04 (0.43, 2.50)	1.31 (0.54, 3.20)	1.00	1.04 (0.96, 1.12)	1.06 (0.96, 1.17)
Women	104/17	186/55	102/31	1.00	1.97 (1.03, 3.77)	2.00 (0.96, 4.16)	1.00	1.05 (1.00, 1.11)	1.09 (1.00, 1.18)
Smoking									
Never	87/12	206/45	113/36	1.00	1.61 (0.73, 3.54)	2.47 (1.03, 5.92)	1.00	1.08 (0.98, 1.19)	1.11 (0.99, 1.24)
Ever	68/14	162/47	142/37	1.00	1.52 (0.76, 3.06)	1.25 (0.60, 2.61)	1.00	1.02 (0.99, 1.04)	1.05 (0.98, 1.11)
Asthma									
Ever	57/11	135/49	118/38	1.00	2.21 (1.00, 4.88)	2.16 (0.93, 5.04)	1.00	1.08 (1.00, 1.16)	1.13 (1.01, 1.27)
No	98/15	235/43	137/35	1.00	1.27 (0.64, 2.49)	1.63 (0.80, 3.33)	1.00	1.02 (0.97, 1.08)	1.04 (0.97, 1.11)

*For all participants, we adjusted for age, sex, smoking status, educational level, leisure-time physical activity, modified Western and Prudent patterns, total energy intake, and asthma status at EGEA2; for subgroup analyses, we adjusted for all potential confounders except the stratifying variable.

P values for interaction between processed meat intake with sex, smoking and asthma were 0.50, 0.60 and 0.50 respectively.

s/w, serving/week.

Table 13 Total, direct and indirect effects of dichotomous processed meat intake on worsening asthma symptoms between EGEA 2 and 3, using the 2-stage regression model (n = 971)

	No. stable or improved/ worse		Total effect*	Direct Effect*	Indirect effect*
	< 1 s/w	≥ 1 s/w	OR (95% CI)	OR (95% CI)	OR (95% CI)
All participants	155/26	625/165	1.80 (1.08, 2.95)	1.70 (1.02, 2.76)	1.06 (1.01, 1.14)
Subgroups					
Sex					
Men	51/9	337/79	1.70 (0.67, 4.36)	1.59 (0.62, 4.23)	1.07 (0.97, 1.22)
Women	104/17	288/86	2.06 (1.08, 3.95)	1.94 (1.01, 3.68)	1.06 (1.00, 1.16)
Smoking					
Never	87/12	319/81	2.24 (1.04, 4.77)	1.97 (0.92, 4.17)	1.14 (1.02, 1.32)
Ever	68/14	304/84	1.76 (0.80, 3.85)	1.73 (0.80, 3.70)	1.02 (0.97, 1.09)
Asthma					
Ever	57/11	253/87	2.50 (1.09, 5.63)	2.22 (0.96, 5.06)	1.13 (1.01, 1.32)
No	98/15	372/78	1.61 (0.80, 3.07)	1.57 (0.80, 3.04)	1.03 (0.96, 1.11)

*For all participants, we adjusted for age, sex, smoking status, educational level, leisure-time physical activity, modified western and prudent patterns, total energy intake, and asthma status at EGEA2; for subgroup analyses, we adjusted for all potential confounders except the stratifying variable. Reference: < 1 serving/week. Estimates and confidence intervals (CIs) obtained using 1,000 bootstrapped samples.

s/w, serving/week.

Table 14 Direct and indirect effects of dichotomous processed meat intake on worsening asthma symptoms between EGEA 2 and 3, using the marginal structural model (n = 971)

	No. Stable or improved/worse		Direct effect*	Indirect effect*
	< 1 s/w	≥ 1 s/w	OR (95% CI)	OR (95% CI)
All participants	155/26	625/165	1.63 (1.00, 2.67)	1.05 (1.01, 1.11)
Subgroups				
Sex				
Men	51/9	337/79	1.15 (0.50, 2.62)	1.05 (0.96, 1.14)
Women	104/17	288/86	1.99 (1.07, 3.70)	1.06 (1.00, 1.13)
Smoking				
Never	87/12	319/81	1.87 (0.88, 3.97)	1.09 (0.99, 1.21)
Ever	68/14	304/84	1.43 (0.74, 2.79)	1.03 (0.99, 1.07)
Asthma				
Ever	57/11	253/87	2.09 (0.97, 4.47)	1.10 (1.01, 1.20)
No	98/15	372/78	1.41 (0.71, 2.66)	1.03 (0.97, 1.09)

*For all participants, we adjusted for age, sex, smoking status, educational level, leisure-time physical activity, modified western and prudent patterns, total energy intake, and asthma status at EGEA2; for subgroup analyses, we adjusted for all potential confounders except the stratifying variable. Reference: < 1 serving/week. Estimates and confidence intervals (CIs) obtained using 1,000 bootstrapped samples.

s/w, serving/week.

Discussion

In this prospective study of adults, using mediation analysis in the counterfactual framework, we reported a positive association between high processed meat intake (≥ 4 serving/week) and worsening asthma symptoms over time. While the indirect effect mediated through BMI accounted for only 14% of this association, the direct effect explained a greater proportion, suggesting a deleterious role of processed meat independent of BMI.

Diet is highly influenced by culture as well as geographical, environmental, and socioeconomic factors. In this study, processed meat food group included three typical French processed meats – ham, sausages, and dried sausage. The mean intakes of processed meats in the EGEA study were similar to those reported by the French National Individual Survey on Food Consumption 2006–2007 [406]. The EGEA results are in agreement with previous researches indicating a harmful association between processed meat intake and different measures of lung health in several countries, where the types and preparation of processed meats may be different [171,174–176,402]. However, two large prospective American studies [172,173], with different types of processed meat (mainly bacon, hot dogs and sausages), reported that processed meat intake may increase the risk of newly diagnosed COPD, but not the risk of incident asthma, after adjustment for BMI and other potential confounders. This different finding may result from the differences either in the role of BMI taken into account in the association (confounder *vs.* mediator) or in the assessment of respiratory phenotypes (incidence *vs.* change in asthma symptoms).

In the present study, BMI was taken into account as a mediator rather than a confounder. Previous studies have consistently suggested a dose-dependent association between BMI and the risk of asthma incidence and control [3,292,408], indicating that obesity is a likely mediator in the diet–asthma association. However, the adjustment for BMI has been widely used when investigating the role of nutritional factors in obesity-related diseases. The traditional BMI-adjusted method could over adjust the association and lead to biased results. Mediation analysis in the counterfactual framework allows taking into account BMI correctly in such association. Moreover, it provides a quantitative measure of the proportion mediated through a given mediator, which could help to better understand the observed association. To the best of our knowledge, this is the first application of newly developed methods for mediation analysis to estimate the association between processed meat intake and asthma.

Accounting for BMI as a mediator, we reported an overall positive association between high processed meat intake and worsening asthma symptoms. The indirect effect mediated through BMI accounted for only 14% of the total effect, supporting the hypothesis that the processed meat–asthma association is related to other mechanisms. Several potential mechanisms were proposed by previous studies involving biological markers. First, processed meats are rich in nitrite, which may lead to nitrosative stress [214] and oxidative stress [409] related lung damage and asthma. Second, the positive relation between processed meat intake and C-reactive protein [186] indicated that processed meat might increase the systemic inflammation, which may have an influence on asthma. In addition, the high content of salt and saturated fat in processed meat might also contribute in part to the association with asthma, though existing evidence has been mainly for childhood-onset asthma [20]. Further studies need to be carried out to improve our understanding regarding potential mechanistic processes.

Besides the BMI issue (mediator *vs.* confounder), we have used, unlike the previous studies [172,173], an asthma symptom score instead of incident doctor-diagnosed adult-onset asthma. Studies on incidence assume that the disease is a true dichotomous entity, which might be inappropriate in asthma (as previously discussed in the state of the art). Without making the dichotomous assumption of the asthma status, the asthma symptom score captures temporal variability of asthma and allows assessment of the disease progression in longitudinal studies [33], and it is a more powerful tool to investigate risk factors for asthma. In participants with asthma, worsening symptoms capture relapse or exacerbation of the disease; in participants without asthma, worsening symptoms coincide with the asthma incidence and activity. While the asthma symptom score was developed and validated in a general population [401], our results were obtained in a population among which 42% had ever asthma. Although previous research has suggested that diet could be a modifier on asthma control rather than an etiological risk factor for incidence of asthma [404], we did not observe interaction between processed meat intake and asthma status at EGEA2 on the association with worsening symptoms. The stratified analysis according to asthma status at EGEA2 showed similar effects in participants with ever asthma. Further studies are needed to clarify potential difference related to asthma status in the effect of processed meat intake on worsening asthma symptoms.

Because people consume complex meals rather than isolated food/nutrients, which may interact with each other, the association between processed meat intake itself and the asthma

symptom score is difficult to assess and may be partly due to the confounding by other food groups [210]. Therefore we adjusted the associations for scores of dietary patterns. The total, direct, and indirect effects remained after adjustment for these diet scores. We acknowledge that findings might not be generalizable to populations with completely different diet habits, such as infrequent processed meat intake and very different food consumptions. The association between processed meat intake and the asthma symptom score may also be due, in part, to a residual confounding by cigarette smoking [75]. To minimize this possibility, we performed a stratified analysis according to smoking status and yielded comparable results in never smokers, although we faced a statistical power issue. Additionally, since processed meat intake has been suggested as a risk factor for COPD [172,173], the potential overlap between COPD and asthma might contribute to the association between high processed meat intake and asthma. Nevertheless, the overlap between COPD and asthma is less likely in the current study because: 1) comparable results were obtained among never smokers, 2) the EGEA study was initiated as a case-control study on asthma with cases recruited in hospital, with both childhood asthma and adult-onset asthma [391,392], and 3) the use of the asthma symptom score reduces the possibility of this overlap [34]. We acknowledge that our definition of “non-exposed” group (< 1 serving/week) was not ideal. Due to the limited number of non-eaters of processed meat in the EGEA study, we faced a statistical power issue in subgroup analyses. We acknowledge that 15% of the participants were lost to follow-up. However, most of the characteristics were similar among included and lost to follow-up participants, excepted for sex and smoking (as expected, more men and more smokers were lost to follow-up). We also acknowledge that deterministic approach was used to deal with missing data for asthma symptom score (7% participants). Similar to any other observational study, although we were able to adjust for many potential confounders due to the extended information available in our study, the validity of the results relies on the assumption of no unmeasured confounding [410]. Besides, we acknowledge that the recall bias could exist as data from questionnaires were used.

In summary, correctly accounting for BMI as a potential mediator in the diet–asthma association, higher processed meat intake was associated with worsening asthma symptoms over time, through not only an indirect effect mediated by BMI but also a greater direct effect. This research extends the deleterious effect of processed meat in health, and the effect of diet on asthma in adults, and provides a novel analyzing approach regarding the role of BMI in the diet–asthma association.

8 Overall diet quality and change in asthma symptoms

This work has been presented as oral communication at the American Thoracic Society (ATS) congress in San Francisco in May 2016, and led to the publication of a scientific article that is currently in press in the British Journal of Nutrition.

Introduction

The modification of dietary habits (i.e., decreased intake of fruits/vegetables and increased intake of “westernized” ultra-processed foods) has been proposed as a plausible explanation of the increase in asthma prevalence [210].

Traditionally, studies on specific foods or nutrients have been conducted. Nevertheless, foods are consumed in a form of complex combination that can interact with each other and influence their bioavailability and absorption. The potential confounding by other food groups is difficult to be totally ruled out through statistical adjustment on dietary patterns. Therefore, studying effects of diet quality, which can take into account correlations and interactions among foods and nutrients, has been proposed as an alternate approach [91]. Regarding the effect of diet quality on asthma, previous research showed inconclusive results and few prospective studies have been conducted. The Alternate Healthy Eating Index 2010 (AHEI-2010) [120], summarizing 11 diet components, is the latest evidence-based diet score reflecting the overall diet quality. Studying diet–asthma association using the AHEI-2010 diet score could provide some insight into the synergic effects of foods and nutrients on asthma.

In the present study, we aimed to investigate the association between diet quality estimated through the AHEI-2010 diet score and change of asthma symptoms considering BMI as a potential mediator, and to evaluate effect modification by smoking.

Methods

Study sample

Out of the 1,236 participants from EGEA2 with valid dietary data, we further excluded participants with missing data for the asthma symptom score at EGEA2 ($n = 10$), or for smoking status ($n = 2$). The final baseline population included 1,224 participants. During the follow-up, 25 participants died, 21 changed address, 117 did not respond at EGEA3, and 22 refused to participate EGEA3. Finally, 1,039 participants responded to the EGEA3 questionnaire. Compared with respondents at EGEA3 ($n = 1,039$), participants lost to follow-up ($n = 185$) were more likely to be men ($P < 0.001$), had lower AHEI-2010 scores (mean

score: 50 vs. 52, respectively; $P = 0.03$), and had similar age, BMI, educational level, physical activity, and asthma status ($P > 0.05$) (Table 15). After further excluding 70 participants with missing data for the asthma symptom score assessed at EGEA3, the final study sample included 969 participants. Compared with included participants ($n = 969$), the excluded adults ($n = 597$) had similar age, BMI, physical activity, sex, smoking and asthma status ($P > 0.05$), but they less often had higher education (43% vs. 51%, respectively; $P = 0.002$) (Table 16).

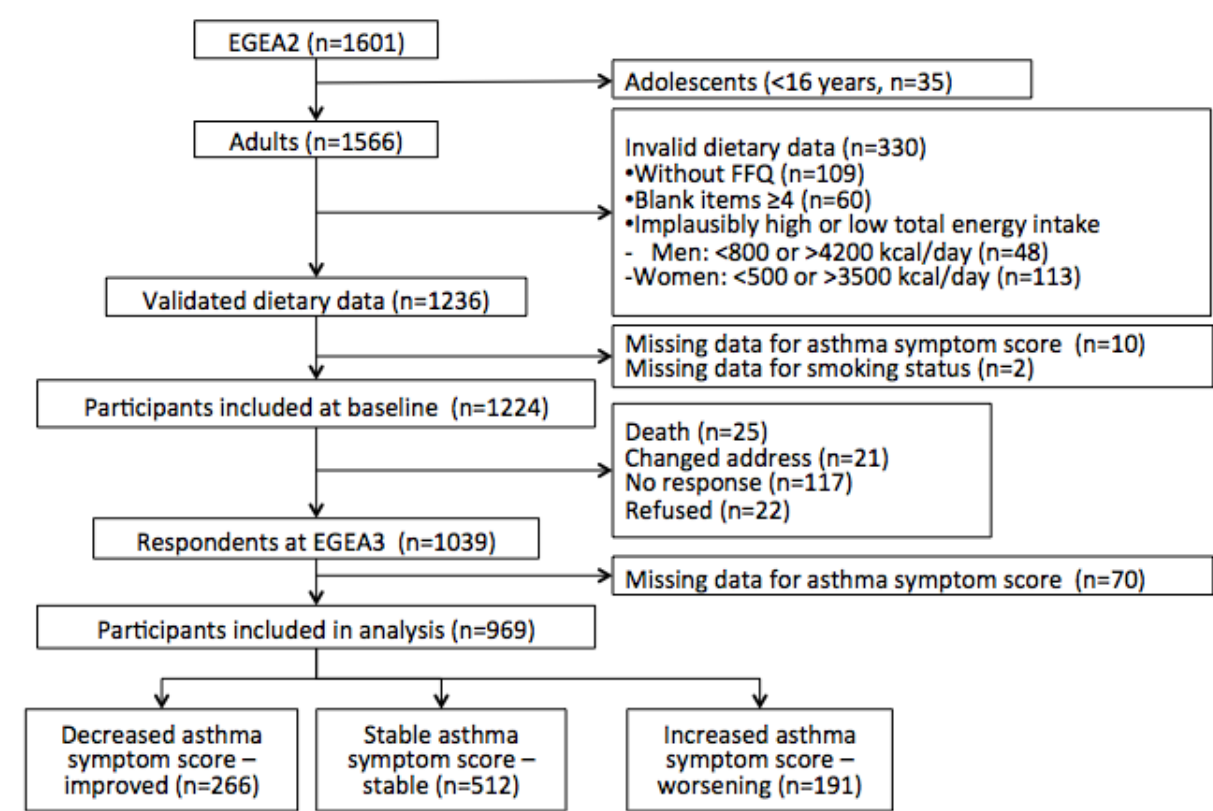


Figure 13 Flowchart for participant inclusion. EGEA, Epidemiological study on the Genetics and Environment of Asthma.

Table 15 Comparison of characteristics between participants followed at EGEA3 (n = 1,039) and participants lost to follow-up (n = 185)

	Participants		P
	Followed at EGEA3 (n = 1,039)	Lost to follow-up (n = 185)	
Age, y	43 ± 16	41 ± 17	0.08
Men, n (%)	507 (49)	117 (63)	< 0.001
BMI, kg/m ²	24.4 ± 4.2	24.8 ± 4.5	0.39
Smoking, n (%)			0.06
Never	525 (50)	78 (42)	
Former	289 (28)	54 (29)	
Current	225 (22)	53 (29)	
Higher education*, n (%)	518 (50)	83 (45)	0.20
Leisure time physical activity, METs/week	45 ± 17	47 ± 18	0.11
Ever asthmatic at EGEA2, n (%)	439 (42)	90 (49)	0.11
AHEI-2010	52 ± 9	50 ± 9	0.03

Data are presented as mean ± SDs, or number of subjects (%). BMI: body mass index; METs/w: metabolic equivalents per week.

*Higher education: college education or equivalent.

Table 16 Comparison of characteristics between included (n = 969) and excluded (n = 597) adult participants

	Participants		P
	Included (n = 969)	Excluded (n = 597)	
Age, y	43 ± 16	43 ± 17	0.42
Men, n (%)	475 (49)	298 (50)	0.84
BMI, kg/m ²	24.4 ± 4.2	24.7 ± 4.5	0.27
Smoking, n (%)			0.15
Never	499 (51)	276 (47)	
Former	259 (27)	163 (28)	
Current	211 (22)	150 (25)	
Higher education*, n (%)	493 (51)	221 (43)	0.002
Leisure time physical activity, METs/week	45 ± 17	45 ± 18	0.87
Ever asthmatic at EGEA2, n (%)	408 (42)	272 (46)	0.18
AHEI-2010	52 ± 9	51 ± 9	0.29

Data are presented as mean ± SDs, or number of subjects (%). BMI: body mass index; METs/w: metabolic equivalents per week.

*Higher education: college education or equivalent.

Dietary intakes

The scoring criteria of the Alternate Healthy Eating Index 2010 was described in detail elsewhere [120]. Briefly, the AHEI-2010 score includes 11 components. A high AHEI-2010 score denotes high intake of vegetables, fruits, whole grains, nuts and legumes, long-chain Ω -3 fatty acids and polyunsaturated fatty acids; moderate intake of alcohol; and low intake of sugar-sweetened drinks and fruit juice, red/processed meat, *trans* fat, and sodium. For each component, a minimal score of 0 and a maximal score of 10 was allocated based on its intake, and intermediate values scored proportionally. The total AHEI-2010 score sums scores of all the 11 components, ranging from 0 to 110; a higher score represents a healthier diet.

In the EGEA study, we calculated a modified AHEI-2010 including all the items except for *trans* fat, which is not available in the French food composition table from the SU.VI.MAX study. Besides, cereal fiber intake was calculated instead of whole grain intake, because only intakes of whole wheat bread and of brown rice have been recorded as whole grains in the EGEA2 study. An intake of 15 g cereal fiber/day was considered as ideal based on the scoring criteria of the Alternate Healthy Eating Index (AHEI) [35]. Foods and nutrients included in the scoring of each component of the AHEI-2010 are presented in Table 17. Therefore, the highest value for the AHEI-2010 diet score in the EGEA study was 100 (rather than 110). The AHEI-2010 diet score was used as a continuous variable in the analysis.

Change in asthma symptoms

Different from the first part of the thesis, as sufficient statistical power was obtained in the current analysis, change in asthma symptom score for each individual was categorized into a three-level variable: “stable” if $EGEA3 \text{ score} - EGEA2 \text{ score} = 0$, “worsening” if $EGEA3 \text{ score} - EGEA2 \text{ score} > 0$, and “improved” if $EGEA3 \text{ score} - EGEA2 \text{ score} < 0$.

Table 17 Scoring criteria and included foods/nutrients of the AHEI-2010 in the EGEA study.

Component	Criteria for min. score (0)	Criteria for max. score (10)	Foods/nutrient items included in the EGEA study
Vegetables (servings/day)	0	≥ 5	19 items: <i>raw vegetables</i> ($n = 6$): spinach, grated carrot, green salad/endive/watercress, other raw vegetables (cucumber, celery, peppers), cabbages/cauliflower, tomato; <i>cooked vegetables</i> ($n = 11$): peas, string beans, squash/zucchini/eggplant, carrot, cabbage/cauliflower/broccoli/Brussels sprout/asparagus, tomato/tomato sauce, ratatouille, corn, vegetable soup, endive/spinach/watercress, other cooked vegetables; <i>raw or cooked</i> ($n = 2$): onion, garlic
Fruits (servings/day)	0	≥ 4	11 items: banana, orange/grapefruit/clementine, apple/pear, kiwi, melon, avocado, peach/nectarine/apricot, plum/cherry, strawberry/raspberry, grape, pineapple
Cereal fiber*(g/day)	0	≥ 15	Calculated using the food composition table from the SU.VI.MAX study
Sugar-sweetened drinks and fruit juice (servings/day)	≥ 1	0	3 items: coke/soda/lemonade, orange/grapefruit juice, grape/apple juice
Nuts and legumes (servings/day)	0	≥ 1	5 items: peanut, walnut, almond, dried fruit, soybean
Red and processed meat (servings/day)	≥ 1.5	0	8 items: processed meat sandwich, sausage, ham, dried sausage, meat hamburger, beef/pork/lamb/veal, offal/liver, pâté
Long-chain Ω -3 fatty acids (mg/day)	0	250	Calculated using the food composition table from the SU.VI.MAX study
Polyunsaturated fatty acids (% energy)	≤ 2	≥ 10	Calculated using the food composition table from the SU.VI.MAX study
Sodium (mg/day)	Highest decile†	Lowest decile†	Calculated using the food composition table from the SU.VI.MAX study
Alcohol (drinks/day)	Women: ≥ 2.5 Men: ≥ 3.5	Women: 0.5–1.5 Men: 0.5–2.0	6 items: beer, white wine, red wine, liquor (whisky, gin, vodka), aperitif wine, cider

* Cereal fiber intake was calculated instead of whole grain intake because only intakes of whole wheat bread and of brown rice have been recorded as whole grains in the EGEA study. An intake of 15 g cereal fiber/day was considered as ideal based on the scoring criteria of the Alternate Healthy Eating Index (AHEI)[105]

† In the EGEA study, values of the lowest decile were 2136 mg/d in women and 2192 mg/d in men, values of the highest decile were 5037 mg/d in women and 5430 mg/d in men.

Statistical analysis

As smoking habit may modify the association between the overall diet quality and change in asthma symptoms, we formally tested the interaction between smoking habits and the AHEI-2010 score. As the interaction was significant (P for interaction = 0.04), analyses were conducted separately among never, former and current smokers.

In the association between the overall diet quality and change in asthma symptom score during the follow-up (stable, worsening, or improved), BMI at baseline (as a continuous variable) was considered as a potential mediator, and age, sex, educational level, leisure-time physical activity, total daily energy intake, and asthma status at baseline as potential confounders (Figure 14). To disentangle the direct effect and the indirect effect mediated by BMI, we applied a mediation analysis in the counterfactual framework [5,407]. Briefly, using a marginal structural model, the mediation analysis were implemented in the following steps: i) we created a new data set by repeating each observation 10 times [5] including a new variable A^* , which was each time set to randomly drawn values from a normal distribution with the mean and standard deviation matching the observed AHEI-2010 score (denoted by A); ii) a generalized linear regression model was applied to the new dataset to estimate the association between BMI and AHEI-2010 score, first using the original variable A , and then the new variable A^* ; iii) using predicted probabilities from the models with A and A^* , the individual stabilized weights were calculated as

$W_i^c = P(M = M_i | A = A_i^*, C = C_i) / P(M = M_i | A = A_i, C = C_i)$, where C denoted all the confounders; iv) a weighted multinomial logistic regression model was applied to simultaneously estimate the association between AHEI-2010 (A and A^*) and change in asthma symptom score.

To evaluate how much of the total effect was due to the effect of BMI, we calculated the “proportion explained” by BMI as $(OR_{TE} - OR_{DE}) / (OR_{TE} - 1)$ [407], where OR_{TE} and OR_{DE} were respectively the odds ratio of total effect and direct effect. The total, direct and indirect effects were presented as odds ratios (ORs) comparing with the stable score group per 10 points increment in the AHEI-2010 score. Confidence intervals (95% CIs) were estimated using 1,000 bootstrap samples.

Interactions between AHEI-2010 and BMI were tested by adding the product term of $A \times A^*$ in the model. As no interaction was observed between the AHEI-2010 diet score and

BMI, the exposure-mediator interaction term was not included in our mediation model (P for interaction = 0.70, 0.94 and 0.73 among never, former, and current smokers, respectively).

We assumed the following conditions were satisfied for the application of mediation analysis[5]: no unmeasured confounders for the associations between: i) the AHEI-2010 and asthma score, ii) BMI and asthma score, iii) the AHEI-2010 and BMI, as well as iv) no BMI–asthma score confounders affected by the AHEI-2010.

As women have healthier diet than men [406], we conducted analyses stratified on sex. Considering the case-control design of EGEA study, we also performed a stratified analysis on asthma status at EGEA2. Participants with high daily total energy intake are more likely to be overweight or obese in case of inadequate physical activity, but they could have a relatively high AHEI-2010 score because of high intakes of healthy foods. Thus we further evaluated whether the association was modified by total energy intake (low ($< 2,382$ kcal/day, median value) vs. high ($\geq 2,382$ kcal/day) energy intake). Besides, to minimize the possible overlap between asthma and chronic obstructive pulmonary disease (COPD), we conducted a sensitivity analysis excluding participants who had a ratio of post-bronchodilator forced expiratory volume in 1 second to forced vital capacity (FEV_1/FVC) less than 70% [411]. All analyses were performed using SAS version 9.3 (SAS Institute Inc., Cary, North Carolina).

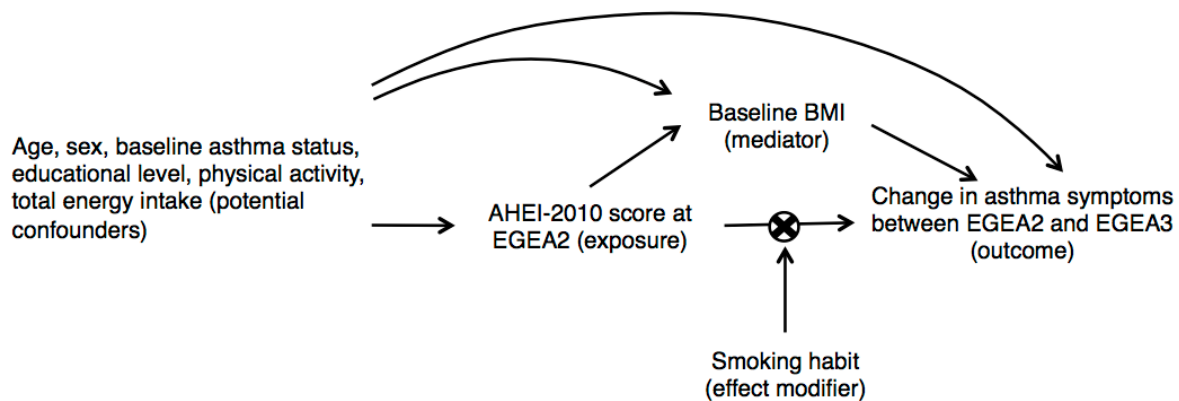


Figure 14 Conceptual model of the association between AHEI-2010 and change in asthma symptoms taking into account BMI as a potential mediator. AHEI-2010, Alternate Healthy Eating Index 2010; EGEA, Epidemiological study on the Genetics and Environment of Asthma.

Results

Characteristics of the population

Among 969 participants included in analysis, 49% were men and 42% had ever asthma at baseline (EGEA2) (Table 18). The mean age was 43 years old; 51% of the participants were never smokers, 27% former smokers, and 22% current smokers. The AHEI-2010 diet score varied from 23.5 to 80.9, with a mean score of 51.6 (Figure 15). The mean AHEI-2010 score was higher among former smokers than never and current smokers (Figure 16). Roughly half of the participants (47%) reported no asthma symptoms at baseline, 23% reported one asthma symptom, and 30% reported more than one asthma symptom (Table 18).

Compared to never smokers, former smokers were older, more likely to be men, overweight or obese, and had higher scores (healthier intakes) for sweetened drinks and for alcohol. Current smokers also differed from never smokers in that they were younger, more likely to be men, less likely to be overweight or obese, had higher score for alcohol, and had lower scores (less healthy intakes) for fruits as well as for red/processed meat.

Association between the overall diet quality and change in asthma symptom score

The mean follow-up time for the participants was seven years (varies from five to nine years). Among never smokers, 54% of the participants had stable symptom score between EGEA2 and EGEA3, 19% had worsening symptom score, and 27% had improved symptom score; the mean AHEI-2010 diet scores were 50.7, 49.2 and 51.9 respectively. The AHEI-2010 was negatively associated with BMI (adjusted β : -0.05; 95% CI: -0.09, -0.01; $P=0.03$). The means of BMI were 24.1, 24.2 and 25.6 kg/m² among participants who had stable, improved and worsening symptoms respectively. Per 1 kg/m² increment in BMI, the adjusted odds ratios were 1.02 (95% CI: 0.96, 1.07) comparing the improved to the stable group, and 1.08 (95% CI: 1.02, 1.14) comparing the worsening to the stable group.

Using mediation analysis, the odds ratio for the total effect was 1.39 (95% CI: 1.07, 1.80) per 10 points increment in the AHEI-2010 score comparing the improved group to the stable group, after adjustment for age, sex, educational level, physical activity, total energy intake, and asthma status (Figure 17). The odds ratio was 1.41 (95% CI: 1.09, 1.80) for the direct effect; and no indirect effect mediated through BMI was observed (0.99 (95% CI: 0.91, 1.07), accounted for 5% of the total effect). No association was observed between AHEI-2010 scores and worsening asthma symptoms. Among former smokers and current smokers, we

observed no association between the AHEI-2010 diet score and the change in asthma symptom score (Table 19).

Sensitivity analysis

As the association was observed among never smokers only, we performed several sensitivity analyses in this subgroup of participants. Several stratified analyses (according to sex, asthma status at baseline and total energy intake) showed similar results, although the statistical power was limited (Table 20). The analysis excluding participants who had a ratio of $FEV_1/FVC < 70\%$ ($n = 47$) yielded similar results: comparing the improved group ($n = 123$) to the stable group ($n = 375$), the adjusted odds ratio was 1.47 (95% CI: 1.07, 1.98) for the total effect, 1.47 (95% CI: 1.08, 1.96) for the direct effect and 0.99 (95% CI: 0.90, 1.07) for the indirect effect mediated through BMI.

Modeling change in asthma symptom score as a continuous variable also showed consistent association between the AHEI-2010 diet score and improvement of asthma symptoms among never smokers only: the adjusted β was 0.13 (95% CI: 0.01, 0.25) for the total effect, 0.12 (95% CI: 0.01, 0.24) for the direct effect and 0.01 (95% CI: -0.03, 0.05) for the indirect effect mediated through BMI.

Table 18 Baseline characteristics of participants according to smoking status (n = 969)

	All (n = 969)	Never smokers (n = 499)	Former smokers (n = 259)	Current smokers (n = 211)	<i>P</i> value
AHEI-2010 diet score	51.6 ± 9.3	50.8 ± 9.4	53.9 ± 9.2	51.0 ± 9.0	< 0.001
Age, y	43 ± 16	42 ± 16	50 ± 14	35 ± 14	< 0.001
Men, n (%)	475 (49)	219 (44)	142 (55)	114 (54)	0.004
BMI, kg/m ²	24.4 ± 4.2	24.4 ± 4.3	25.2 ± 4	23.4 ± 3.8	< 0.001
BMI, kg/m ² , n (%)					< 0.001
< 20	93 (10)	42 (9)	17 (7)	34 (17)	
20–24.9	490 (53)	269 (56)	107 (44)	114 (56)	
25–29.9	253 (27)	122 (25)	88 (36)	43 (21)	
≥ 30	90 (10)	47 (10)	31 (13)	12 (6)	
Total energy intake, kcal/day	2426 ± 643	2438 ± 638	2365 ± 608	2476 ± 692	0.15
Higher education, n (%)	493 (51)	256 (51)	128 (49)	109 (52)	0.84
Leisure-time physical activity, METs/w	45 ± 17	45 ± 17	45 ± 16	44 ± 17	0.44
Ever asthma at EGEA2, n (%)	408 (42)	216 (43)	99 (38)	93 (44)	0.33
Asthma symptom score at EGEA2, n (%)					0.12
0	458 (47)	245 (49)	129 (50)	84 (40)	
1	225 (23)	114 (23)	60 (23)	51 (24)	
2–5	286 (30)	140 (28)	70 (27)	76 (36)	
Component score of AHEI-2010					
Vegetables	6.9 ± 2.6	7.0 ± 2.5	7.1 ± 2.6	6.6 ± 2.7	0.14
Fruits	6.8 ± 3.1	6.9 ± 3.1	7.1 ± 2.9	6.1 ± 3.3	0.001
Whole grains	4.5 ± 1.9	4.6 ± 2.0	4.1 ± 1.7	4.8 ± 1.9	< 0.001
Sugar-sweetened drinks and fruit juice	4.6 ± 4.2	4.4 ± 4.2	5.4 ± 4.1	4.3 ± 4.0	0.004
Nuts and legumes	1.5 ± 2.1	1.4 ± 1.9	1.7 ± 2.3	1.6 ± 2.0	0.26
Red and processed meat	3.0 ± 2.6	3.1 ± 2.6	3.2 ± 2.6	2.4 ± 2.4	0.002
Long-chain Ω-3 fatty acids	9.2 ± 2.0	9.2 ± 2.1	9.3 ± 1.9	9.2 ± 2.0	0.56
Polyunsaturated fatty acids	4.0 ± 1.7	3.9 ± 1.7	4.0 ± 1.6	4.2 ± 1.6	0.18
Sodium	5.1 ± 3.2	5.1 ± 3.2	5.5 ± 3.2	4.9 ± 3.3	0.17
Alcohol	6.0 ± 3.9	5.3 ± 3.9	6.6 ± 3.7	6.9 ± 3.6	< 0.001

Data are expressed as mean ± SDs, or number of subjects (%). Overall *P* values were calculated from ANOVA for continuous variables (e.g. age), and from Pearson chi-square test for categorized variables (e.g. sex) among the never, former, and current smokers.

AHEI-2010, Alternate Healthy Eating Index 2010; EGEA, Epidemiological study on the Genetics and Environment of Asthma; METs/w: metabolic equivalents per week.

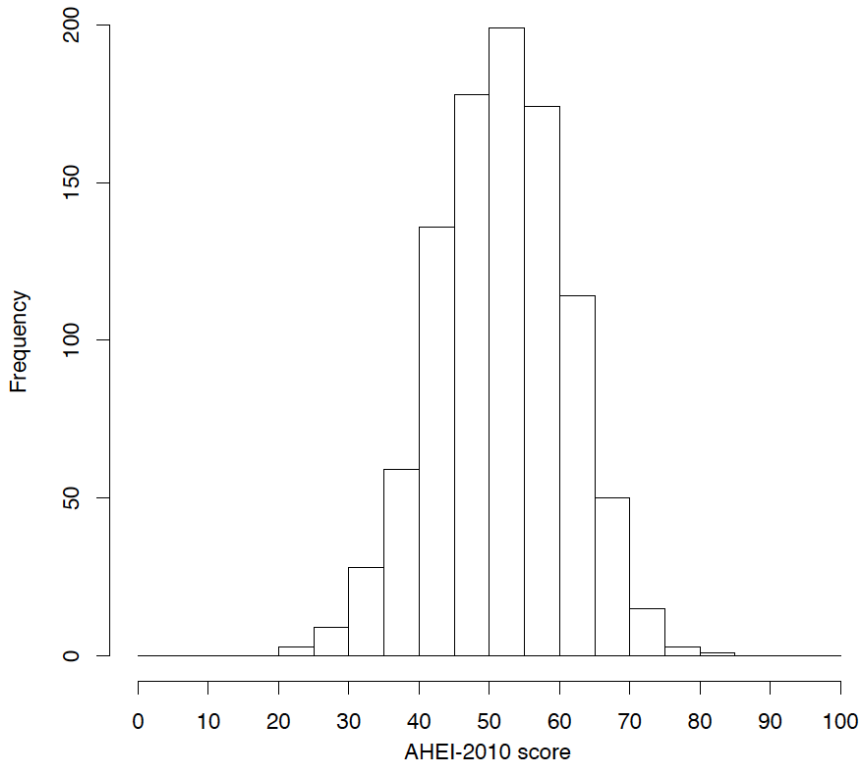


Figure 15 Distribution of AHEI-2010 scores among all the participants. AHEI-2010, Alternate Healthy Eating Index 2010.

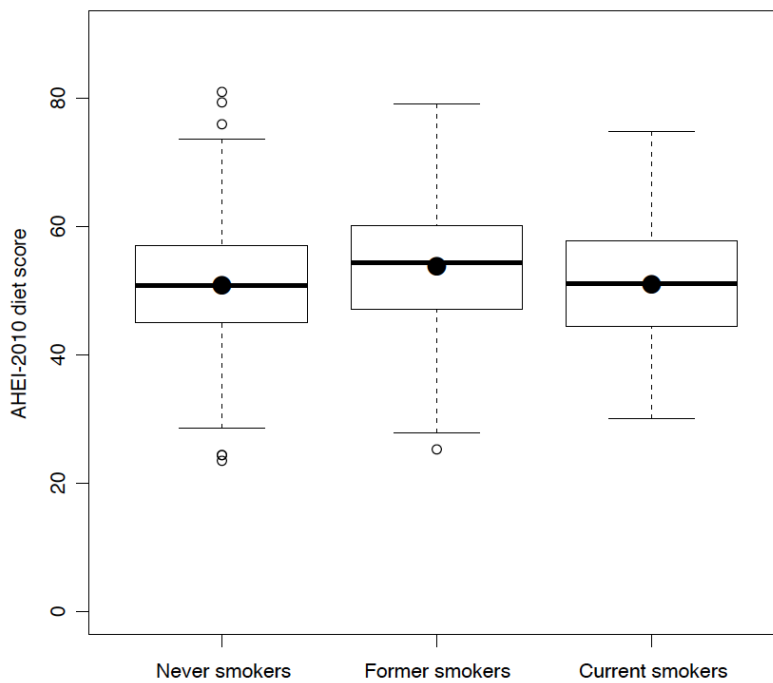


Figure 16 Boxplots for AHEI-2010 score among never, former, and current smokers. AHEI-2010, Alternate Healthy Eating Index 2010.

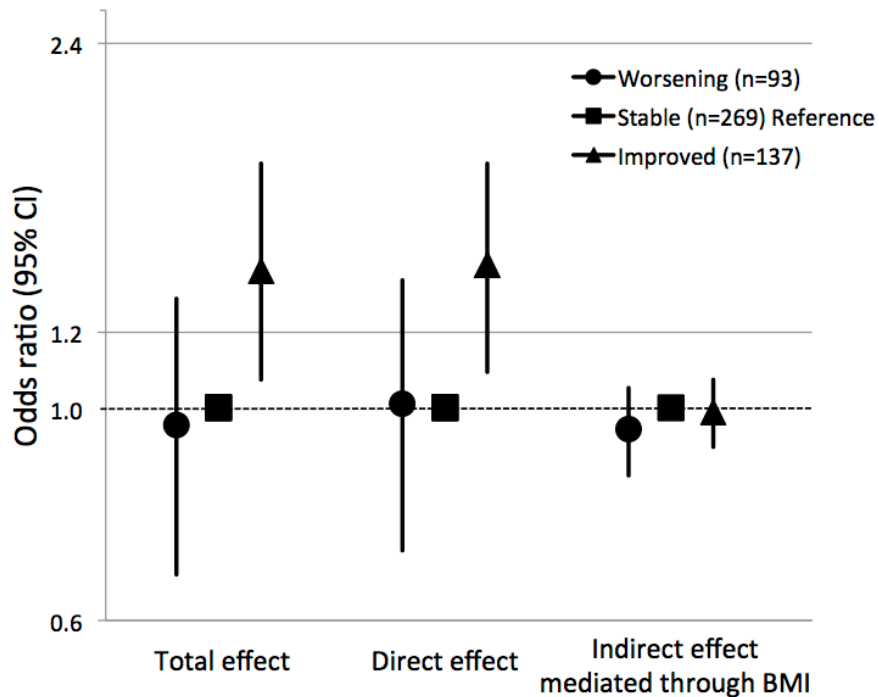


Figure 17 Association between AHEI-2010 diet score and change in asthma symptoms among never-smokers. A mediation analysis in the counterfactual framework was applied to estimate the total, direct effects and the indirect effect mediated through BMI, after adjusting for age, sex, educational level, leisure-time physical activity, total daily energy intake, and asthma status. OR (95%CI) were estimated for each increment of 10 points in the AHEI-2010 diet score using 1000 bootstrapped samples. AHEI-2010, Alternate Healthy Eating Index 2010.

Table 19 Associations between AHEI-2010 diet score and change in asthma symptoms according to smoking status

	OR (95%CI)		
	Stable	Worsening	Improved
Never smokers (n = 499)			
n (%)	269 (54)	93 (19)	137 (27)
Total effect	1.00 (reference)	0.96 (0.67, 1.30)	1.39 (1.07, 1.80)
Direct effect	1.00 (reference)	1.01 (0.71, 1.36)	1.41 (1.09, 1.80)
Indirect effect	1.00 (reference)	0.95 (0.85, 1.05)	0.99 (0.91, 1.07)
Former smokers (n = 259)			
n (%)	140 (54)	52 (20)	67 (26)
Total effect	1.00 (reference)	0.72 (0.43, 1.09)	0.84 (0.53, 1.23)
Direct effect	1.00 (reference)	0.77 (0.47, 1.16)	0.86 (0.55, 1.28)
Indirect effect	1.00 (reference)	0.94 (0.80, 1.08)	0.98 (0.85, 1.10)
Current smokers (n = 211)			
n (%)	103 (49)	46 (29)	62 (22)
Total effect	1.00 (reference)	1.54 (0.79, 2.78)	1.08 (0.67, 1.67)
Direct effect	1.00 (reference)	1.54 (0.81, 2.79)	1.07 (0.65, 1.62)
Indirect effect	1.00 (reference)	1.00 (0.87, 1.14)	1.01 (0.89, 1.16)

ORs were estimated for each increment of 10 points in AHEI-2010. Total, direct and indirect effects were estimated from mediation model taking into account BMI as a mediator in the association between the AHEI-2010 diet score and the change in asthma symptoms, after adjustment for age, sex, educational level, leisure-time physical activity, total daily energy, and asthma status. OR (95%CI) were estimated using 1000 bootstrapped samples.

AHEI-2010, Alternate Healthy Eating Index 2010.

Table 20 Associations between AHEI-2010 score and change in asthma symptoms in subgroups according to sex, asthma status and total energy intake among never smokers

	OR (95% CI)		
	Stable	Worsening	Improved
Stratified on sex			
Men (n = 219)			
n (%)	125 (57)	34 (16)	60 (27)
Total effect	1.00 (reference)	1.09 (0.57, 1.84)	1.41 (0.85, 2.20)
Direct effect	1.00 (reference)	1.13 (0.58, 1.97)	1.41 (0.86, 2.19)
Indirect effect	1.00 (reference)	0.97 (0.81, 1.13)	1.00 (0.87, 1.13)
Women (n = 280)			
n (%)	144 (51)	59 (21)	77 (28)
Total effect	1.00 (reference)	0.97 (0.60, 1.40)	1.41 (0.98, 2.07)
Direct effect	1.00 (reference)	1.03 (0.66, 1.45)	1.44 (1.01, 2.06)
Indirect effect	1.00 (reference)	0.94 (0.82, 1.09)	0.98 (0.87, 1.10)
Stratified on asthma status			
Never asthma (n = 283)			
n (%)	185 (65)	43 (15)	55 (19)
Total effect	1.00 (reference)	1.12 (0.66, 1.78)	1.41 (0.94, 2.10)
Direct effect	1.00 (reference)	1.16 (0.71, 1.80)	1.42 (0.95, 2.06)
Indirect effect	1.00 (reference)	0.96 (0.82, 1.11)	0.99 (0.88, 1.11)
Ever asthma (n = 216)			
n (%)	84 (39)	50 (23)	82 (38)
Total effect	1.00 (reference)	0.87 (0.52, 1.33)	1.42 (0.88, 2.23)
Direct effect	1.00 (reference)	0.92 (0.54, 1.43)	1.41 (0.87, 2.25)
Indirect effect	1.00 (reference)	0.95 (0.81, 1.12)	1.01 (0.88, 1.14)
Stratified on total energy intake*			
High total energy intake (n = 250)			
n (%)	130 (52)	50 (20)	70 (28)
Total effect	1.00 (reference)	1.07 (0.65, 1.61)	1.49 (0.97, 2.33)
Direct effect	1.00 (reference)	1.11 (0.70, 1.65)	1.49 (0.98, 2.25)
Indirect effect	1.00 (reference)	0.96 (0.82, 1.11)	1.00 (0.89, 1.12)
Low total energy intake (n = 249)			
n (%)	139 (56)	43 (17)	67 (27)
Total effect	1.00 (reference)	0.86 (0.48, 1.41)	1.29 (0.84, 1.93)
Direct effect	1.00 (reference)	0.90 (0.53, 1.44)	1.32 (0.85, 1.92)
Indirect effect	1.00 (reference)	0.95 (0.76, 1.12)	0.98 (0.86, 1.11)

ORs were estimated for each increment of 10 points in AHEI-2010. Total, direct and indirect effects were estimated from 1000 bootstrapped samples using a mediation model taking into account BMI as a mediator, after adjusting for all potential confounders except the stratifying variable.

*Stratified into 2 groups according to median of total energy intake (2382 kcal/day).

AHEI-2010, Alternate Healthy Eating Index 2010.

Discussion

In this study, we report a significant association between high diet quality (measured by the AHEI-2010 diet score) and improvement of asthma symptom over time among never smokers only. Smoking habit appeared to be an effect modifier in the healthy diet–asthma association. Further, using mediation analysis in the counterfactual framework, BMI was unlikely a mediator in this association.

Assessment of overall diet either through dietary patterns or diet scores is the best available tool to take into account correlations and interactions among foods and nutrients [91]. While dietary patterns are derived using data-driven statistical methods and vary according to studies, diet scores are developed based on established knowledge on nutrition, and thus provide a common tool to evaluate the potential effect of overall diet quality in different populations [91]. To date, very few studies have looked into the association between diet quality and asthma-related outcomes in adults in the literature and reported conflicting results [203,242–245].

Interestingly, this study showed that among never smokers, those with a higher AHEI-2010 diet score were more likely to have improved symptoms over time, and this association was not observed among former or current smokers. Different from our finding, some cross-sectional studies reported associations between antioxidant dietary intakes and asthma only in smokers [412,413], hypothesizing that smokers who had a higher exposure to oxidants were more likely to benefit from antioxidant dietary intake than never smokers. However, associations between dietary intakes and asthma in both current and former smokers may result from residual confounding by smoking (e.g. smoking intensity and length of time). To minimize potential biases, many asthma studies – RCTs in particular – restrict participants to never or non-current smokers [234,414]. Besides unhealthy diet, smoking is also associated with other lifestyle factors, including sedentary behavior; and smoking cessation is related to weight gain and overweight [377]. Smoking, together with those related risk factors for asthma, may attenuate the potential beneficial effect of healthy eating on asthma. Furthermore, smokers appeared to have altered airway inflammation and corticosteroid insensitivity [75] and might represent a particular phenotype of asthma [415]. In line with these findings, the results of the current study suggest that smoking is a likely effect modifier in the healthy diet–asthma association. The role of smoking should be carefully addressed in studies on the healthy diet–asthma association, and analyses stratified according to smoking are recommended.

In the current study, despite significant total and direct effects of the overall diet quality on improvement of asthma symptoms, no indirect effect mediated through BMI was observed. In line with these results, we observed similar results among participants with high or low total energy intake, which does not support the hypothesis that participants with low total energy intake could have more benefits because of a healthier weight. Overall, BMI was unlikely the key to explain the association between the AHEI-2010 and improved asthma symptoms, and other potential mediators could be involved. For instance, biological markers implicated in specific pathways, such as the response to oxidative/nitrosative stress or inflammation, are likely to advance our understanding regarding the diet–asthma association [416].

We acknowledge a few potential limitations of this study. First, as the AHEI-2010 diet score was associated with COPD [203], the potential overlap between asthma and COPD could contribute to the association between AHEI-2010 and asthma. However, it is unlikely in the current study since similar associations were observed when we excluded participants with $FEV_1/FVC < 70\%$ in analysis. Second, we acknowledge the possibility of misclassification of the AHEI-2010 assessed by the food frequency questionnaire. As the data for *trans* fat was not available in the French food composition table [89], the AHEI-2010 diet score in the EGEA study was calculated without this item, and therefore, may not be completely comparable to those in other studies. However, the impact of lacking *trans* fat on the investigated association is limited, because, to the best of our knowledge, no evidence has been found on the association between *trans* fat and asthma in adults. Third, we acknowledge that potential attrition bias could exist in the study, as participants lost to follow-up were more likely to be men and had lower AHEI-2010 scores. Also, reverse causation might still exist in this study. However, the longitudinal design of this study has minimized the possibility, and the change in diet resulting from asthma status is less likely compared with other chronic disease. Finally, similar to any other observational study, even though we controlled for several potential cofounders, the assumption of no unmeasured confounding that is necessary for the validity of our results could be a limitation.

In summary, better diet quality, reflected by higher AHEI-2010 score, was associated with improved asthma symptoms overtime in never smokers, and the association was not mediated through BMI. As similar associations were reported for both participants with and without asthma at baseline, these findings support that high diet quality may be beneficial for primary prevention of asthma and that multi-intervention programs, including healthy diet,

may improve the secondary prevention of asthma. These findings also extend the relevance of the AHEI-2010 to lung health.

9 Leptin, body adiposity, and asthma activity

This work has led to the writing of a scientific article and the first draft is currently circulating among coauthors.

Introduction

Body adiposity has been associated with severity, poor control, and reduced response to standard therapy of asthma [3]. Although several hypotheses have been proposed [3], underlying mechanisms of the association between obesity and asthma activity remain poorly understood. Leptin, a pro-inflammatory factor released by adipose tissue [266], has raised great interest in the obesity–asthma activity association. Using cross-sectional data, a few studies have observed positive associations of serum leptin concentration with asthma severity, asthma control, or lung function in children, and to a lesser extent in adults [328–332]. However, whether leptin mediates the association between obesity and asthma activity remains unknown.

When studying the role of biological markers on disease, prospective data are warranted to avoid reverse causation. Indeed, using a cross-sectional design does not allow concluding whether the modification in the concentration of the biological marker is a cause or a consequence of the disease status. Mediation analysis in the counterfactual framework [5,6] using longitudinal data can minimize such reverse causation and offers an interesting and appropriate approach to study the effect of leptin in the association between body adiposity and asthma activity.

When investigating the obesity–asthma activity association, most of the studies in the literature used BMI to measure body composition. However, BMI is a mixed measure of lean and fat mass, which may lead to potential misclassification of body adiposity. It has been recently proposed that other measures rather than only BMI are needed to understand the obesity–asthma association [417], such as body fat percentage measured through BIA [262] and waist circumference [418]. Investigating the association between these different measures of body composition and asthma activity may provide more insight into the obesity–asthma activity association.

In the current study, we aimed to investigate the potential role of leptin as a mediator in the longitudinal association of body adiposity assessed through BMI, waist circumference, and body fat percentage measured by BIA with asthma activity over time.

Methods

Study sample

Out of the 1601 participants included at EGEA2, 558 had current asthma.

For the analyses using BMI, among the 558 participants with current asthma, we excluded those with missing data for BMI ($n = 53$), and for leptin measurement ($n = 77$). Out of the 428 remaining, 363 were followed at EGEA3. After further excluding 32 participants with missing data for asthma activity assessed at EGEA3, 331 participants were included in the analyses (see Figure 18).

For the analyses using waist circumference, we excluded participants with missing data for waist circumference ($n = 72$), and for leptin measurement ($n = 69$). Out of the 417 remaining, 355 were followed at EGEA3. After further excluding 30 participants with missing data for asthma activity assessed at EGEA3, 325 participants were included in the analyses.

As the BIA was performed only among participants examined in Paris and Grenoble at EGEA2, out of the 558 participants with current asthma at EGEA2, only 224 participants had available data on body fat percentage. We excluded 34 participants with missing data for leptin. Out of the 190 remaining, 164 were followed at EGEA3. After further excluding 12 participants with missing data for asthma activity assessed at EGEA3, 152 participants were included in the analyses.

Measures of body composition

BMI (kg/m^2), waist circumference (cm), and body fat percentage were included as continuous variables in the analyses.

Leptin (at EGEA2)

In order to minimize inter-plate variability, values of serum leptin concentration were divided by the pool result of the same plate and expressed as ratios. These ratios were log transformed and included in the statistical analyses.

Asthma activity

Data on asthma activity were recorded by questionnaires at EGEA2 and EGEA3 as previously defined (see section 5.2). Participants with current asthma at both EGEA2 and EGEA3 were considered to have persistent asthma activity, and those with current asthma only at EGEA2 were considered to have remitted asthma activity. The change in asthma activity (persistent or remitted) was the outcome variable in the current study.

Statistical analysis

In the association between body adiposity and change in asthma activity, leptin was considered as a potential mediator, and age, sex, smoking, educational level, physical activity, total energy intake as potential confounders (Figure 19). To disentangle the direct effect and the indirect effect mediated by leptin, we applied a two-stage mediation analysis in the counterfactual framework as previously described in details [5,407]. Briefly, a linear regression model was implemented in the first stage to assess the association between leptin and each of the measurement of adiposity, and in the second stage, a logistic regression model was implemented to assess the association between the change in asthma activity and each of the measures of adiposity. We formally tested the interaction between sex and BMI, waist circumference, and body fat percentage. To evaluate how much of the total effect was due to the effect of leptin, we calculated the “proportion explained” by leptin as $(OR_{TE} - OR_{NDE}) / (OR_{TE} - 1)$, where OR_{TE} and OR_{NDE} were respectively the odds ratio of total effect and natural direct effect [407].

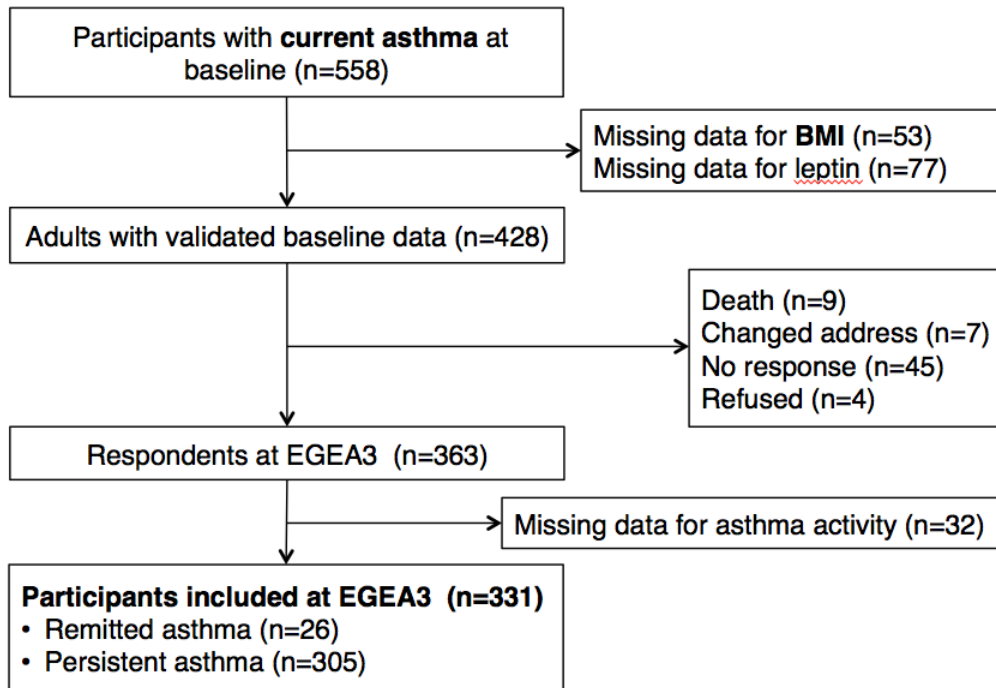


Figure 18 Inclusion of participants for studying the association between BMI and change in asthma activity over time. EGEA, Epidemiological study on the Genetics and Environment of Asthma.

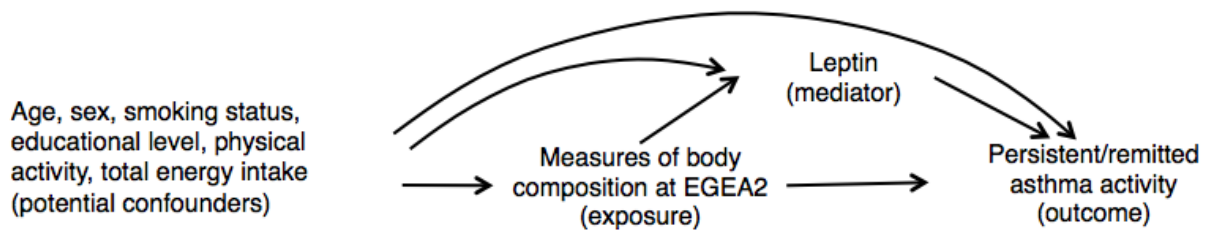


Figure 19 Conceptual model of the association between measures of body composition and change in asthma activity, taking into account leptin as a potential mediator.

Results

Baseline characteristics of the participants

Baseline characteristics of the participants are presented in Table 21. The study sample included 331 participants, of which 164 were men. The mean age was 39 years old, 84% of the participants had atopy (positive skin prick test), and 47% were ever smokers. The mean BMI was 24.2 kg/m², 25% of the participants were overweight, and 10% were obese. The mean waist circumference was 83 cm. Among the 152 participants with BIA measurement, the mean body fat percentage was 24.6%. Compared with men, women had lower BMI (23.7 vs. 24.7 kg/m², $P = 0.04$), smaller waist circumference (78 vs. 88 cm, $P < 0.001$), higher body fat percentage (28% vs. 21%, $P < 0.001$), and higher leptin concentration (6.8 vs. 3.0 ng/ml, $P < 0.001$).

Baseline characteristics of the participants according to asthma activity are presented in Table 22. Participants with persistent asthma activity and those with remitted asthma activity were similar regarding age, sex, educational level, total energy intake, physical activity, smoking status (ever/never), and atopy (yes/no). However, compared with participants with remitted asthma activity, participants with persistent asthma activity had higher BMI (24.3 vs. 22.6 kg/m², $P = 0.05$), greater waist circumference (83 vs. 77 cm, $P = 0.03$), higher body fat percentage (25.0% vs. 21.5%, $P = 0.06$), and higher concentration of leptin (4.4 vs. 3.0 ng/ml, $P = 0.03$). The prevalence of overweight/obesity (BMI ≥ 25 kg/m²) was 27% in participants with remitted asthma activity and 36% in participants with persistent asthma activity.

Correlations between measures of body composition

The Pearson correlation coefficients between BMI, waist circumference, and body fat percentage are presented in Table 23. Similarly in men and in women, BMI and waist circumference were highly correlated ($r = 0.84$ in men and 0.88 in women). Body fat percentage was more strongly correlated with BMI than with waist circumference ($r = 0.89$ vs. 0.79 in men, and 0.74 vs. 0.61 in women, respectively). Body fat percentage was more strongly correlated with BMI in women than in men ($r = 0.74$ in men and 0.89 in women) and with waist circumference ($r = 0.61$ in men and 0.79 in women). Among all participants, without adjustment for sex, the correlation between body fat percentage and the other two measures were weakened ($r = 0.60$ for BMI and 0.31 for waist circumference).

Measures of body composition and change in asthma activity

The mean follow-up time of this study was seven years. Out of the 331 participants, 305 (91.5%) had persistent asthma activity during the follow-up. The proportion of participants with persistent asthma activity was similar among participants included in the analyses regarding waist circumference and body fat percentage (91.7% and 88.3% respectively). After adjusting for age and sex, serum leptin concentration was positively associated with BMI, waist circumference, and body fat percentage: the β (95% CI) per one SD increase in the measure were 0.202 (0.176, 0.232), 0.221 (0.193, 0.262), and 0.272 (0.219, 0.325), respectively. Per one unit increase in log₁₀ (ratio leptin/pool), the odds ratio (OR) was 8.4 (95% CI: 1.9, 36.7) for persistent asthma activity.

Results using mediation analyses are presented in Table 24. After adjusting for age, sex, smoking, educational level, physical activity, and total energy intake, we observed a positive borderline significant total effect of BMI on persistent asthma activity: the odds ratio (OR) was 1.59 (95% CI: 0.95, 2.97) for each increment of one SD (4.3 kg/m²) in BMI. The total effect could be decomposed into a positive significant indirect effect mediated by leptin (OR = 1.68 (95% CI: 1.09, 2.46)), and a negative non-significant direct effect (OR = 0.97 (95% CI: 0.50, 1.82)). The percentage of the association mediated by leptin could not be calculated due to the overestimation of percentage in case of negative direct effect.

We observed a positive significant total effect of waist circumference on persistent asthma activity, with an OR of 2.06 (95% CI: 1.06, 4.00) for each increment of one SD (13.8 cm). The total effect could be decomposed into a positive borderline significant indirect effect mediated by leptin (OR = 1.55 (95% CI: 0.99, 2.57)), and a non-significant positive direct effect (OR = 1.38 (95% CI: 0.66, 2.81)). The percentage of the association mediated by leptin was 64%.

Similarly, we observed a positive significant total effect of body fat percentage on persistent asthma activity with an OR of 3.25 (95% CI: 1.01, 9.41) for each increment of one SD (7.9%). Again, the total effect could be decomposed into a positive borderline significant indirect effect mediated by leptin (OR = 1.99 (95% CI: 0.94, 4.83)), and a non-significant positive direct effect (OR = 1.83 (95% CI: 0.43, 5.52)). The percentage of the association mediated by leptin was 63%.

No interaction was observed between the measures of body composition with sex (*P* for interaction were 0.20, 0.20 and 0.82, for BMI, waist circumference, and body fat percentage respectively).

Table 21 Characteristics for participants at baseline.

	Women (n = 167)	Men (n = 164)	Total (n = 331)
Age, y	39 ± 16	39 ± 18	39 ± 17
Higher education, n (%)	83 (50)	81 (49)	161 (50)
Total energy intake, kcal/day	2,779 ± 991	2,853 ± 1274	2,815 ± 1139
Physical activity, METs/w	43 ± 15	50 ± 20	46 ± 18
Atopy*, n (%)	159 (82)	136 (86)	266 (84)
Smoking (ever), n (%)	72 (43)	84 (51)	156 (47)
BMI, kg/m ²	23.7 ± 4.7	24.7 ± 3.7	24.2 ± 4.2
BMI, kg/m ² , n (%)			
< 20	31 (19)	10 (6)	41 (12)
20–24.9	87 (52)	87 (53)	174 (53)
≥ 25	49 (29)	67 (41)	116 (35)
Waist circumference (WC)			
n	164	161	325
WC, cm	78 ± 12	88 ± 13	83 ± 14
Body fat percentage (BF)			
n	75	77	132
BF, %	28.0 ± 6.6	21.4 ± 5.8	24.6 ± 7.0
Leptin, ng/ml (Geometric means)	6.7 (4.3–6.4)	2.7 (1.5–2.8)	4.3 (2.4–4.6)
Ratio leptin/pool (Geometric means)	0.82 (0.52–0.77)	0.33 (0.18–0.35)	0.52 (0.30–0.54)

*Atopy (yes/no) was defined by positive result of the skin prick test (SPT) at EGEA2 (12 allergens, SPT+: wheal diameter ≥ 3mm).

Data are expressed as mean ± SDs, or number of subjects (%), unless otherwise stated.

Geometric means are presented with interquartile ranges.

P values were calculated from t-test for continuous variables, chi-square test for categorical variables, and Mantel-Haenszel chi-square test for BMI categories.

Table 22 Description of the population according to asthma activity from EGEA2 to EGEA3.

	Asthma activity		<i>P</i> Value
	Remitted (n = 26)	Persistent (n = 305)	
Age, y	34 ± 13	39 ± 17	0.12
Men, n (%)	11 (42)	153 (50)	0.44
Higher education, n (%)	13 (50)	151 (50)	0.99
Total energy intake, kcal/day	2,725 ± 1232	2,823 ± 1133	0.67
Physical activity, METs/w	46 ± 21	47 ± 18	0.83
Atopy (yes/no), n (%)	23 (89)	243 (83)	0.49
Smoking (ever), n (%)	17 (65)	158 (52)	0.18
BMI, kg/m ²	22.6 ± 3.1	24.3 ± 4.3	0.05
BMI, kg/m ² , n (%)			0.22
< 20	5 (19)	36 (12)	
20–24.9	14 (53)	160 (53)	
≥ 25	7 (27)	109 (36)	
Waist circumference (WC)			
n	25	300	
WC, cm	77 ± 10	84 ± 14	0.03
Body fat percentage (BF)			
n	16	136	
BF, %	21.5 ± 6.2	25.0 ± 7.0	0.06
Leptin, ng/ml (Geometric means)	3.0 (1.4–3.0)	4.4 (2.5–4.7)	0.03
Ratio leptin/pool (Geometric means)	0.36 (0.16–0.37)	0.54 (0.31–0.55)	0.02

Data are expressed as mean ± SDs, or number of subjects (%), unless otherwise stated.
 Geometric means are presented with interquartile ranges.
P values were calculated from t-test for continuous variables, chi-square test for categorical variables, and Mantel-Haenszel chi-square test for BMI categories.

Table 23 Pearson correlation coefficients between BMI, waist circumference, and body fat percentage.

	n	mean ± SD	Correlations		
			1	2	3
In men					
1. BMI, kg/m ²	164	24.7 ± 3.7	1.00		
2. Waist circumference, cm	161	88.4 ± 13.4	0.84	1.00	
3. Body fat percentage, %	77	21.4 ± 5.8	0.74	0.61	1.00
In women					
1. BMI, kg/m ²	167	23.7 ± 4.7	1.00		
2. Waist circumference, cm	164	78.0 ± 12.5	0.88	1.00	
3. Body fat percentage, %	75	28.0 ± 6.6	0.89	0.79	1.00
All participants					
1. BMI, kg/m ²	331	24.2 ± 4.2	1.00		
2. Waist circumference, cm	325	83.2 ± 13.9	0.83	1.00	
3. Body fat percentage, %	152	24.6 ± 7.0	0.60	0.31	1.00
All correlation coefficients are statistically significant ($P < 0.001$).					

Table 24 Association between measures of body composition and persistent asthma activity, taking into account leptin as a mediator.

	n (persistent/remitted)	OR (95% CI)*		
		Direct effect	Indirect effect	Total effect
BMI	305/26	0.97 (0.50, 1.82)	1.68 (1.09, 2.46)	<i>1.59</i> <i>(0.95, 2.97)</i>
Waist circumference	300/25	1.38 (0.66, 2.81)	<i>1.55</i> <i>(0.99, 2.57)</i>	2.06 (1.06, 4.00)
Body fat percentage	136/16	1.83 (0.43, 5.52)	<i>1.99</i> <i>(0.94, 4.83)</i>	3.25 (1.01, 9.41)

*ORs and 95% CIs were estimated for each increment of 1 SD in BMI (SD = 4.3 kg/m²), waist circumference (SD = 13.8 cm), and body fat percentage (SD = 7.9 %).

ORs in *Italic* indicate borderline significant values, and those in **bold** significant values.

Total, direct and indirect effects were estimated using a mediation model, taking into account leptin as a mediator in the association between the measure of body composition and asthma activity, after adjustment for age, sex, educational level, physical activity, total energy intake, and ever smoking at EGEA2.

Discussion

This is the first prospective study to investigate the association between body adiposity and asthma activity using multiple measures of body composition, including BMI, waist circumference, and body fat percentage. Using longitudinal data of participants followed up over seven years, we reported that body adiposity was associated with persistent asthma activity over time. Leptin, a biological marker of obesity-related systemic inflammation, was a likely mediator in the observed association.

This study reported for the first time an indirect effect mediated by leptin in the association between body adiposity and asthma activity, even though the effects observed for waist circumference and body fat percentage were borderline significant. These results need to be interpreted with caution due to the limited sample size. One main concern regarding the role of leptin as a mediator in the association between body adiposity and asthma activity is the high correlation between body adiposity and leptin [265], which may question whether leptin is a proxy for adiposity, or a real mediator in the adiposity–asthma activity association. However, previous findings have provided important evidence regarding the biological plausibility of the association between leptin and asthma activity. As a pro-inflammatory factor, leptin enhances the phagocytosis, activation, and proliferation of macrophages, and induces the production of pro-inflammatory cytokines, including TNF- α , IL-6, as well as ROS [266], which are all involved in pathophysiological processes of asthma. Moreover, leptin administration to wild-type mice resulted in increase airway inflammation induced by O₃ [322]. Taken together, we believe that leptin is a likely mediator in the association between body adiposity and persistent asthma activity.

Other potential mechanisms may also be involved in the association between body adiposity and persistent asthma activity. Low concentration of serum adiponectin, which is a anti-inflammatory biological marker mainly secreted by adipocytes [333], is associated with higher asthma incidence in women according to a prospective study [339]. Besides, as leptin and adiponectin can regulate the production of pro-inflammatory cytokines (e.g. IL-6 and TNF- α) by macrophages, such cytokines may also lie in the causal pathway of the association between body adiposity and persistent asthma activity [419]. Recently, a cross sectional study reported that high plasma IL-6 concentration was associated with more severe asthma within both obese and non-obese participants [420]. Further prospective studies regarding the potential effect of these biological markers on asthma activity are needed.

Three measures, including BMI, waist circumference, and body fat percentage measured through BIA, were used to assess body adiposity in the current study. Whereas we observed similar significant total effects of waist circumference and body fat percentage on persistent asthma activity, only borderline significant total effect was observed for BMI. A possible explanation might be related to the inaccurate measure of body adiposity through BMI and consequently reduced statistical power of analysis. BMI is a measure of both lean and fat mass and thus, can be affected by weight of bones and muscles. As reported by previous research [262] and found in this study, the correlation between BMI and body fat percentage was moderate. Another source of the discrepancy might relate to the sex-difference in the obesity–asthma association. Although several studies have observed significant association between BMI and asthma outcomes in both men and women, others only reported significant association in women [3]. Besides the role of hormonal factor, the inaccurate measure of body adiposity in men was proposed as a plausible explanation for this sex-related difference. Unfortunately, due to limited sample size, it was not possible to study separate effects in men and in women. Regarding the role of fat distribution in asthma or asthma activity, few studies have been performed, but Brumpton *et al.* [306] reported that abdominal obesity (using waist circumference) was associated with increased risk of incident asthma in women after additional adjustment for BMI. Taken together, including multiple measures of body composition might be helpful for better understanding the obesity–asthma association.

We acknowledge the following limitations. First, due to the limited sample size of this study, the statistical power was very limited, particularly for the analyses regarding body fat percentage. For the same reason, it was not possible to study effects in subgroups of the population, although the association between body adiposity and persistent asthma activity might be stronger in women and non-atopic asthma. Further prospective studies with larger sample size might provide more insight with regard to the role of leptin in different asthma phenotypes. Besides, although we have adjusted for several known potential confounders, like any other studies using the mediation analysis in the counterfactual framework, the assumption of “no unmeasured confounders” could be a limitation.

In conclusion, body adiposity was associated with persistent asthma activity over time, and leptin was a likely mediator in this association. These findings suggest that interventions reducing obesity-related systemic inflammation might help in secondary prevention of asthma.

GENERAL CONCLUSION

In the EGEA study followed over 7 years, this thesis has studied the role of diet and obesity in asthma at both a “macro” level and a “micro” level. At the “macro” level, this thesis has investigated the association of processed meat intake and overall healthy eating with asthma, taking into account complex interrelations between diet, obesity, and smoking. At the “micro” level, this thesis has studied the role of leptin, an obesity-specific biological marker related to systemic inflammation, in the obesity–asthma activity association.

This thesis applied for the first time the newly developed methods for mediation analysis to estimate the diet–asthma association within a counterfactual framework. Accounting for BMI as a potential mediator, processed meat intake, a recent carcinogen, was associated with worsening asthma symptoms overtime. Similar results were observed among never- and ever-smokers. This finding extends the deleterious effect of processed meat on health. Given that only a small portion of the association was mediated through BMI, other potential mechanisms merit further investigation.

Taking into account interactions among foods and nutrients, diet quality estimated through the AHEI-2010 diet score was positively associated with improved asthma symptoms overtime in never smokers; and the association was not mediated through BMI. This finding suggests a potential beneficial effect of overall healthy eating on asthma, and supports the importance of the multi-intervention programs for asthma. Further studies on the mechanisms underlying this association are needed.

Mediation analysis in the counterfactual framework has also been used to study the role of leptin in the association between different measures of body composition and change in asthma activity over time. The analyses showed that body adiposity was associated with persistent asthma activity, with slightly different magnitudes of association according to different measures of body composition. Leptin was a likely mediator between body adiposity and persistent asthma activity. This finding helps better understanding the obesity–asthma activity association, and shows that mediation analysis might be a useful tool to investigate potential mechanisms in epidemiological studies.

GENERAL DISCUSSION

This last chapter presents first potential impacts of the thesis regarding methodological issues and implications of main results in public health. Then, the main limitations of the performed analyses are discussed, and several perspectives from the thesis are presented.

10 Methodological aspects

This thesis has several impacts regarding methodological aspects for future epidemiological research on diet and respiratory diseases, first regarding analyzing approaches in nutritional research, then regarding the assessment of disease status in asthma, finally regarding causal inference in epidemiological studies.

10.1 Analyzing approaches in nutritional research

Hypothesis-oriented dietary analyzing approaches

This thesis has linked dietary factors to the change in asthma symptoms over time using two hypothesis-oriented dietary analyzing approaches – the approach through individual foods (i.e. processed meat intake) and the approach through diet scores (i.e. AHEI-2010).

In the first part of the thesis, we assessed the effect of processed meat, for which previous research has suggested a strong link regarding multiple chronic diseases but an unclear role in asthma. As previously introduced in section 2.1.2, due to the complex correlations among foods and their potential interactions, it is extremely difficult to study the separate effect of individual foods. To overcome this issue as best as we can, we derived two dietary patterns using all food groups except processed meat and further adjusted the analysis for these dietary patterns.

The approach through diet scores, as used in the second study of the thesis, provides an interesting alternative approach to taking into account the correlations and interactions among foods. Diet scores have been developed to evaluate the healthfulness of individual diets (i.e. diet quality) based on established nutrient requirements and dietary guidelines. Incorporating nutrient and food interactions of likely biological importance, these scores can help researchers to integrate the nutrient and food specific findings and provide a measure of diet quality. Besides, the ease of their use makes them a practical choice for clinicians and dietitians in the clinical setting.

These two approaches complement each other when studying the association between dietary factors and asthma [88]. Moreover, compared to the approach through individual nutrients or data-driven dietary patterns, the two applied approaches are both more relevant to dietary recommendations. For instance, recommending limited processed meat intake is much clearer than recommending limited nitrite intake, and a dietary recommendation using a well defined diet score is clearer than one using a possibly more imprecise term such as “Prudent” or “Western” patterns. In conclusion, combining the approach through hypothesis-oriented individual foods and the approach through diet scores in nutritional epidemiological studies may provide further insight into the role of dietary factors in diseases, and particularly in asthma, for which inconsistent results were reported.

Multiple measures of body composition

In the third part of the thesis, we used three measures of body composition, BMI, waist circumference and body fat percentage measured by BIA, to investigate the associations between excess body adiposity and change in asthma activity overtime. Previous studies have suggested that central obesity measured by waist circumference, compared with BMI, is more strongly associated with systemic inflammation – a potential mechanism underlying the association between obesity and multiple chronic diseases [318]. In this thesis, although caution is needed for the interpretation of the results due to the limited statistical power, different magnitudes of association have been observed according to different measures of body composition. Compared with BMI, greater waist circumference and body fat percentage measured by bioelectrical impedance analysis seemed to be more predictive for persistent asthma activity over time. Although more studies with larger samples are needed, using multiple measures of body composition, BMI and waist circumference in particular, is likely to provide new insight into the role of obesity-/adiposity- related management in disease prevention.

10.2 Assessment of the disease status in asthma

Rather than the association between dietary factors and asthma incidence in a general population, this thesis has studied the association between dietary factors and change in asthma symptoms using the asthma symptom score [190,191] among participants with and without asthma, and the role of leptin in the association between body adiposity and change in asthma activity and among participants with asthma. These outcome variables were chosen to overcome several challenges that have been raised.

The traditional “incidence analysis”, which excludes patients with the disease at baseline, might be challenging in studies of asthma, not only because of the preclinical status that is difficult to be defined, but also because of the variability and heterogeneity of asthma. Misleading results are easily obtained without excluding participants with asthma at baseline, but excluding all participants with preclinical status is not feasible and would reduce the statistical power of the study, particularly when the incidence during the follow-up is low, which is often the case among adults. Without making the dichotomous assumption of the asthma status, the asthma symptom score captures temporal variability of asthma and allows assessment of the disease progression in longitudinal studies [33]. As a more powerful tool to investigate risk factors for asthma [34], the asthma symptom score may be particularly relevant to studies on which previous research showed inconsistent results, as for the diet–asthma association. Applicable among participants with and without asthma, the asthma symptom score could provide insight for both the first and the secondary prevention of the disease.

In the second part of the thesis, we focused on the change in activity of asthma (persistent or not) between EGEA2 and EGEA3, with a particular attention to potential reverse causation – a great challenge in studies investigating the potential underlying biological mechanisms of a disease. A longitudinal design is essentially needed to minimize such reverse causation (further discussed in the next section). Moreover, due to the symptomatic variability of asthma, concentration of leptin might be higher in participants with current asthma activity at baseline as compared to those without current activity. Therefore, the study population was restricted to participants with current asthma at EGEA2, and the change of activity between EGEA2 and EGEA3 was assessed. Taking into account the baseline difference in the concentration of the biological marker due to disease status might be particularly important to minimize potential reverse causation between leptin and asthma activity. Given the low-incidence of asthma in adults, studying the risk factors for incidence of asthma among non-asthmatics is difficult in studies without large-size samples. However, many studies with middle-size samples, such as the EGEA study, can offer important data regarding the secondary prevention of asthma by investigating the risk factors for persistent asthma activity.

There is growing acceptance that asthma involves multiple phenotypes. It is possible, even likely, that the role of several risk factors, including diet, might differ across these different phenotypes. Also, it is likely that different pathophysiology mechanisms may be

involved in different asthma phenotypes. A challenge for population-based studies that aim to investigate risk factors for asthma or potential underlying mechanisms is how to study the association within truly homogeneous groups rather than groups of “asthma” patients who have different disease features and different pathophysiology.

In conclusion, there is still no universal definition of asthma in epidemiological studies. The assessment of the disease status should be chosen according to different aims of the study. The asthma symptom score may provide insight for both the first and the secondary prevention of the disease. Using longitudinal measures and using data from a relatively homogeneous phenotypic group may improve our understanding of the etiological aspects of asthma.

10.3 Causal inference in nutritional epidemiological studies

Study design

Minimizing potential confounding in the investigated associations, randomized controlled trials (RCTs) provide the strongest evidence when evaluating hypothesis in medical science. However, RCTs have some important limitations when investigating the effect of dietary factors on diseases due to both ethic and practical issues. First, the deleterious effect of dietary factors, such as processed meat intake, could not be directly assessed through RCTs due to ethical issues, although indirect proof might be provided by an intervention to reduce the intake. Moreover, as the necessary duration of the intervention is unknown, trials could easily result in misleading negative results due to insufficient duration of intervention [421]. The lack of effect can also be due to poor adherence of participants, particularly for interventions that require alternations in food intakes or dietary habits (interventions on nutrients that can be taken through pills are likely to have better compliance) in long-term randomized trials [422].

Most of the previous studies that have investigated the association between dietary factors and asthma have used transversal data from case-control or cross-sectional studies (reviewed in section 2.2). However, such studies have important limitations including potential reverse causation, as temporality is lacking. For instance, it is almost impossible to figure out whether the changes in the concentration of a biological marker are a result or a cause of the disease status (e.g. prevalent asthma). The associations investigated in this thesis used longitudinal data in the EGEA study. Compared with case-control and cross-sectional studies, results from prospective studies are much less likely to be influenced by reverse

causation. However, reverse causation cannot be completely ruled out in prospective studies, as disease status that present before the baseline could have altered the food choice. In prospective studies regarding the diet–asthma association, the influence of reverse causation is likely to be limited, since the diet–asthma association is still little known to the public (at least, less known than the diet–CVD association). Therefore, longitudinal data are essential for causal inference in epidemiological studies.

Analyzing methods

Analysis in the counterfactual framework has been proposed for better causal inference from observational studies (previously introduced in section 4.2.2). In this thesis, we have applied methods of mediation analysis within the counterfactual framework to study the diet–asthma association, taking into account BMI as a mediator. Indeed, BMI (or obesity) is a potential mediator not only in the diet–asthma association, but also in the associations between diet and other obesity-related chronic diseases, including cancers, cardiovascular disease, diabetes, and healthy aging. However, the adjustment for BMI in analysis has been widely used when investigating the role of nutritional factors in obesity-related diseases, although this traditional BMI-adjusted method could over adjust the association and lead to biased results. Using mediation analysis in the counterfactual framework, we were able to disentangle the indirect effect mediated through BMI, and the direct effect of two dietary factors (i.e. processed meat intake and the AHEI-2010) on changes in asthma symptoms, which could help to better understand the observed association.

Moreover, traditional analyzing approaches used in epidemiological studies have left a “black box” with regard to the potential biological mechanisms. Studies regarding potential biological mechanisms have been mainly conducted using animal models; however, the pathophysiological process in human bodies may differ. In this thesis, we applied the mediation analysis to investigate the role of leptin in the association between obesity and change in asthma activity. The application of mediation analysis in the counterfactual framework might be a useful tool for investigating underlying biological mechanisms that may explain, at least in part, the association.

In summary, causal inference is difficult in nutritional studies; even RCTs have its limitations. Despite this difficulty, particularly in epidemiological studies, studies using longitudinal data and innovative analyzing methods, such as mediation analysis, can minimize the potential reverse causation and help to reveal the investigated association.

11 Public health implications

The findings in this thesis suggest several important implications in public health, including reducing processed meat intake, improving diet quality, smoking prevention, and preventing obesity. As diet, obesity, and smoking are modifiable risk factors that are related to multiple chronic diseases, these implications are not only related to asthma, but also to other chronic diseases, and health in general.

11.1 Reducing processed meat intake

Higher processed meat intake has been associated with worsening asthma symptoms in this thesis. In the literature, high intake of processed meat has been associated with increased risk of cancer, CVD, diabetes, and increased mortality. Processed meat is commonly consumed in many eating cultures. In France, according to the national INCA 2 study conducted in 2006–2007, the average processed meat intake is 35 g/day, and 91% of the consumers eat processed meat weekly or more frequently [406]. Given the adverse effects of processed meat on health and the large population involved, it is urgent to advocate an important reduction (or avoidance) of processed meat intake.

Several diet scores, including the aMed, DASH, and AHEI-2010, have recommended the avoidance or a really low consumption of red/processed meat as part of a healthy diet [108,114,120]. However, these diet scores are only well known in research area, and a gap remains regarding the spread of knowledge from the research community to public. Recently, with the press release of WHO, processed meat has been recognized as a carcinogen, but the other potential adverse effect of processed meat, including those on lung health, remains unfamiliar to the public. Moreover, some reports in the media, particularly those from meat industry advocate the nutritional value of red meat, may diffuse confusing message to public. Up to date, national guidelines, including the latest Dietary Guidelines for Americans and the French dietary guideline PNNS, have not yet given clear recommendation to limit processed meat intake. In both guidelines, only a recommendation is given on various source of protein, including meat, poultry, and eggs. Updating and diffusing national guidelines are emerging in different countries.

Moreover, although types and preparations of processed meat across countries are likely to be different, consistent findings regarding the deleterious effect of processed meat intake on health have been reported in different countries. In meat processing, small amounts of nitrate and nitrite are commonly added for coloring, antimicrobial and flavoring. For example,

sodium and potassium nitrite and sodium and potassium nitrate, labeled as E250, E249, E251 and E252 respectively, are permitted for food use in limited amount in accordance with legislation of many countries including the European Union and the US [423,424]. Such food additives can be then transformed to N-nitroso-compounds – one of the mechanisms underlying the association between processed meat intake and multiple chronic diseases. Therefore, more caution may be needed regarding the national provisions on the addition of nitrite and nitrate in foods.

11.2 Improving diet quality and preventing smoking

Overall healthy eating has previously been associated with lower mortality and lower risk for multiple chronic diseases, including cancer, cardiovascular disease, type 2 diabetes, and COPD (reviewed previously). In this thesis, the latest diet score reflecting overall healthy eating, the AHEI-2010 diet score, has been associated with improved asthma symptoms. Improving the diet quality is emerging in the prevention of noncommunicable chronic diseases, particularly in men, who appeared to eat more processed meat and were more likely to be smokers.

Different dietary recommendations for overall healthy eating and diet quality have been proposed in several countries. For instance, the National Nutrition and Health Program (PNNS) in France is a nutrition policy to improve the health status of the population by improving their diet quality [425]. Besides recommendations on dietary intakes, adequate physical activity has also been proposed as a part of the program, as the balance between energy from dietary intakes and physical activity has been considered as important for weight control and the prevention of obesity. Similarly, in the latest Dietary Guidelines for Americans (2015–2020), a comprehensive set of recommendations on the amounts and types of physical activity needed each day has been included [426].

However, smoking, a factor often related to “bad diet quality” (introduced in section 4.1.2) is currently not mentioned in these guidelines. Smoking is a known risk factor for major chronic diseases, as well as asthma incidence and control [75], and non-smoking is a key factor of healthy lifestyle. Our results showed that a better diet quality, reflected by a higher AHEI-2010 diet score is associated with improved asthma symptoms overtime only in never smokers. This finding suggests a potential role as an effect modifier of smoking in the diet–asthma association. Indeed, previous studies have suggested the potential difference in bioavailability of dietary intakes, anti-oxidants in particular [374,375]. A multi-factor lifestyle

intervention program including recommendations on dietary intakes, physical activity, and also smoking prevention is likely to provide more benefits to the population.

11.3 Preventing obesity

The number of overweight and obese individuals (using definitions according to BMI (overweight: $BMI \geq 25$, obesity: $BMI \geq 30$) was estimated to be 1.9 billion in 2014 by WHO [257], which had doubled the number since 1980 [362]. Being a known risk factor for several chronic diseases, including cancer, CVD, and type 2 diabetes, and also asthma [257,427], overweight and obesity is now a global health problem – not only for developed countries, but also for developing countries [362]. Preventing overweight and obesity is emerging for the prevention of non-communicable chronic diseases worldwide.

Although obesity can also be caused by several genetic factors and metabolic disorders, it is mostly a consequence of the combination of overeating, insufficient physical activity, and sedentary behavior, which are preventable [427]. Therefore, the interventions on lifestyle, including improving eating habits and practicing physical activity regularly, are essential in the obesity-preventing program. As previously discussed, several dietary guidelines [108,114,119,120], adapting the local culture and eating habits and in different countries, have been published. Following the latest dietary guidelines may provide benefits not only from the healthy eating itself, but also from preventing obesity and obesity-related diseases. Furthermore, as obese children and adolescent are more likely to develop obesity in their adulthood [428], preventing obesity from childhood seems to be vital in obesity prevention worldwide. Besides, as abdominal obesity [272], a key component of metabolic syndrome [429], is positively associated with higher risk of chronic diseases and mortality [267], it is essential to increase awareness regarding central obesity prevention.

In summary, obesity prevention remains a big public health issue worldwide. A comprehensive approach to preventing obesity is needed.

12 Limitations

The analyses performed in this thesis were conducted using data from the EGEA study, thus they share several general limitations listed below. Some of these limitations are related to the study design, and others are also commonly shared with other epidemiological studies.

First, we acknowledge that the study sample size in this thesis was limited. The EGEA study was designed as a case-control study in order to collect very detailed and precise information on respiratory phenotypes, environmental and lifestyle determinants, as well as biological and genetic data [393]. Due to the limited sample size, it was difficult to study effects in subgroups of the population. For instance, due to the limited number of participants with low intake of processed meat (< 1 serving/week) in the EGEA study (19%), we faced a statistical power issue in subgroup analyses, particularly among men. Similar issues were raised when studying the association between the AHEI-2010 and change in asthma symptoms, and when studying the association between measures of body composition and change in asthma activity (insufficient participants for analyses stratified according to sex or atopy).

Second, as our study sample included 42% of participants with ever asthma due to the study design of the EGEA study (case-control study), the results that we obtained might not be generalizable to other populations. However, stratified analyses according to asthma status at EGEA2 were performed and results of similar magnitude were obtained in participants with and without ever asthma.

Besides, studies in this thesis also have the following limitations that are commonly present in other epidemiological studies. As the questionnaires are used to assess respiratory phenotypes and dietary intakes, recall bias may present. Also, like any other prospective studies, attrition bias may be present in the EGEA study (15% of the participants were lost to follow-up between EGEA2 and 3). However, most of the characteristics were similar among included and lost to follow-up participants, excepted for sex and smoking (as expected, more men and more smokers were lost to follow-up). In addition, similar to any other epidemiological study, the validity of the results relies on the assumption of no unmeasured confounding [4]. There might still be some residual confounding left, even though we have taken into account multiple potential confounders in the analyses.

13 Perspectives

The work begun during this thesis raises several perspectives. This section presents, first, the perspectives in “macro” epidemiology to further investigate the role of lifestyle factors in asthma, and then, the perspectives in “micro” epidemiology for a better understanding of the potential biological mechanisms involved in the diet–asthma association.

13.1 Aspects in “macro” epidemiology

13.1.1 Dietary pattern reflecting dietary biological potential and asthma outcomes

Biological properties of dietary factors, including the oxidant/antioxidant and inflammatory/anti-inflammatory potential, have been suggested to be a potential link between dietary factors and asthma (review previously). Regarding the antioxidant potential of diet, the TAC [93–96] has been proposed to assess the combined effect of antioxidants present in foods. More recently, several versions of the DII [97–100] have been developed to assess the inflammatory potential of diet. It would be interesting to investigate the association between dietary patterns defined using these scores and asthma outcomes. As dietary data are already available in the EGEA study, the analyses could be conducted in the EGEA study using prospective data.

13.1.2 Dietary factors and asthma phenotypes

Dietary factors may act differently in patients with distinct asthma phenotypes. Identifying potential differences in risk factors for different asthma phenotypes might provide more efficient and specific prevention and intervention for asthma [13]. However, no study has investigated the association between dietary factors and asthma phenotypes.

In the EGEA study, detailed data were collected on respiratory symptoms, several clinical exams (e.g. lung function), as well as biological and genetic markers. These data allow studies on asthma phenotypes through not only a classical candidate approach (e.g. childhood-onset or adult-onset asthma), but also new approaches using unsupervised methods, such as latent class analysis or clustering. Using latent class analysis, Siroux *et al.* have identified four phenotypes in the EGEA study [430], including “active treated allergic childhood-onset asthma”, “active treated adult-onset asthma”, “inactive/mild untreated allergic asthma”, and “inactive/mild untreated nonallergic asthma”. Further studies may be conducted on the association between several dietary factors (fruit and vegetables, processed meat, and diet quality in particular) and these different asthma phenotypes.

13.1.3 Analyses using repeated assessment of diet and multiple measures of body composition

Like other lifestyle factors, including obesity and physical activity, dietary habit may change during the life course. Repeated assessment of diet would allow not only investigations on the average effect of diet during a long period, but would also allow further studies on the effect of the windows of exposure on the diet–asthma association, as well as the potential effect of changing dietary habits on asthma. Bédard *et al.* has proposed to use marginal structural models (counterfactual framework) to study the joint effect of obesity and physical activity, as time-varying variables, on asthma activity in the Asthma-E3N study [431]. Future studies using repeated assessment of diet may allow the investigation of diet as a time-varying variable in asthma. As dietary data in the EGEA study were only available at EGEA2, the effect of dietary factors on asthma was assessed using the baseline dietary data. However, it would be interesting to address the diet-associations in other studies with repeated and precise information on diet, such as the Nutrinet-Santé cohort in France [432].

To investigate the potential mediating role of obesity in the diet–asthma association, we have considered only BMI in our analyses, since it is the most widely used indicator of body adiposity. However, as reported in the third part of this thesis, having multiple measurements of body adiposity besides BMI might be of potential interest. Therefore, it would be interesting to assess the role of waist circumference and body fat percentage as potential mediator in our analyses (processed meat and diet quality score).

13.1.4 Trajectory of body shape and asthma outcomes

Body weight and body shape change over the life course. Trajectories of body shape could reflect such changes and provide additional data on exposure windows and duration for obesity-related studies [288,433–435]. Thus findings of trajectory analyses may help to better understand the obesity-disease association and to develop effective prevention or intervention strategies targeting the life period at which excess body weight has a predominant influence. According to data from two large prospective US cohorts, in which five distinct trajectories of body shape have been identified using latent transition analyses, the “heavy body shape” trajectory, especially the increase in middle life, was associated with higher mortality [434]. The “late life adiposity” trajectory was positively associated, whereas the “early life adiposity” trajectory was inversely associated with the risk of postmenopausal breast cancer [435]. Regarding the obesity–asthma association, a large number of studies have investigated

the association between BMI or weight loss and asthma (reviewed in section 3.2.1). However, no study has been conducted regarding the trajectory of body shape and asthma.

It would be interesting to study the association between trajectory of body shape across the lifespan and asthma outcomes. The analyses could be conducted in the EGEA study, as data on body shapes over the life course are available at EGEA2 and EGEA3.

13.2 Aspects in “micro” epidemiology

13.2.1 Biological markers

In this thesis we showed that leptin was a likely mediator in the association between body adiposity and persistent asthma activity. This finding provides additional evidence that obesity-related systemic inflammation contributes to, at least in part, the association between obesity and persistent asthma activity. However, besides leptin, other biological markers, including adiponectin, may also be involved in the obesity-related systemic inflammation process. Currently, two papers [387,389] have proposed practical guides to address such research questions using mediation analysis through multiple pathways (i.e. potential mediators). The application of mediation analysis through multiple pathways, which could take into account the combining effect of multiple biological markers, may help to better identify the role of obesity-related systemic inflammation in the obesity–asthma association.

Also, analyses using biological markers may help to better understand the association between dietary factors and asthma. It would be interesting to study the role of circulating nitrite concentration in the association between processed meat intake and asthma. It would also be interesting to investigate the role of biomarkers of oxidative stress, or of inflammation in association with processed meat intake, diet quality, and asthma. The large and high quality biobank of the EGEA study may provide a unique opportunity to study these hypothesizes.

Regarding biomarkers of inflammation, three challenges manifest themselves [436]: 1) a large number of biomarkers, which interact with each other, are involved complexly in the inflammation process; 2) little is known regarding the association between many biomarkers and dietary intakes; 3) rigorous and longitudinal design is needed to minimize potential reverse causation (i.e. increased concentration of biological markers result from asthma). Future studies are firstly needed to identify the biomarkers of inflammation that reflect long-term effect but not a short time fluctuation of dietary intakes. Also, the potential effects of these biomarkers on asthma need to be investigated. Regarding the analyzing approach, except for approach through single candidate biological markers, an alternative approach is to

study the role of these biological markers in the diet–asthma association through multiple pathways or through inflammation profiles derived using appropriate statistical methods.

In the EGEA study, several biological markers that are likely involved in the inflammation process or antioxidant/oxidant balance have been measured at EGEA2. In an ongoing project the association between these biomarkers and asthma-related outcomes are being studied.

13.2.2 Epigenetic factors

Although the field of epigenetic factors in asthma has been largely expanded in recent years, the environmental factors that potentially influence the epigenetics of asthma remain largely unknown. As previously introduced in section 2.2.1, it is recently suggested that diet may play a role in asthma through DNA methylation, which is an epigenetic mechanism widely recognized and the most extensively studied. In particular, the potential effect of dietary methyl donors, including folic acid, choline, vitamin B₁₂, vitamin B₂, and vitamin B₆, on DNA methylation, may improve our current knowledge of the diet–asthma association. The EGEA study provides a great opportunity to perform such analyses, since DNA methylation data (measured in 580 blood samples) in the EGEA study are already available for participants with asthma, and data for those without asthma will be available soon.

13.2.3 Microbiota

Gut and airway microbiota are possible links between dietary factors and asthma. As recently highlighted in a theme issue of the *Annals of Epidemiology* (May 2016) [437], epidemiologists can make a major contribution to this emerging field by performing well-designed, well-conducted, and appropriately powered studies and by including measures of the microbiota in current and future cohort studies. Using innovative analyzing approaches, such as mediation analysis, may help to investigate the role of microbiota in nutritional factors and asthma.

A post-doctoral project has been submitted to study nutritional factors during early childhood and the composition and dynamics of airway microbiota in two cohorts in the US, including children hospitalized with bronchiolitis and healthy children. If the hypothesized association is observed, mediation analysis can then be applied to assess the mediating role of airway microbiota in the association between these factors and asthma.

In summary, the perspectives raised from this thesis may help further understand the association between nutritional factors and asthma at both a “macro” and a “micro” level in epidemiology. Several of these perspectives could be studied in a near future, such as investigating the role of waist circumference and body fat percentage as a mediator in the diet–asthma association, and investigating the association of trajectory of body shape, biological markers, and respiratory microbiota with asthma; several may be investigated in a medium-to-long term in the future, including the associations between dietary pattern reflecting dietary biological potential and asthma, between dietary factors and asthma phenotypes, between epigenetic factors and asthma, and the association using repeated assessment of diet.

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SYNTHESE EN FRANÇAIS

Contexte scientifique

L'asthme est une maladie chronique inflammatoire des voies respiratoires qui touche plus de 300 millions de personnes dans le monde [1]. L'augmentation de la prévalence de l'asthme au cours des 40 dernières années dans la plupart des pays industrialisés, a suggéré l'importance de facteurs environnementaux et comportementaux ayant changé durant cette période, parmi lesquels la modification des habitudes alimentaires (diminution de la consommation de fruits et légumes, augmentation de la consommation de plats préparés, et diminution de la qualité globale de l'alimentation) et l'augmentation de l'obésité [2].

Plusieurs hypothèses ont été proposées concernant les mécanismes potentiels de l'association entre les facteurs alimentaires et l'asthme. Alors que les études antérieures ont surtout porté sur l'effet protecteur d'aliments ou de nutriments sources d'anti-oxydants tel que les fruits et les légumes, très peu d'études ont été conduites sur l'effet potentiellement délétère de l'alimentation sur l'asthme ou sur l'effet de la qualité globale de l'alimentation. Il existe des relations complexes entre l'alimentation, l'obésité et l'asthme. Comme l'alimentation est un déterminant important de l'obésité, et que l'obésité est un facteur de risque de l'asthme, il est très probable que l'obésité soit un médiateur dans l'association entre l'alimentation et l'asthme. Cependant, aucune étude n'a porté sur le rôle de l'obésité en tant que médiateur dans l'association entre l'alimentation et l'asthme.

L'obésité, définie par un excès d'adiposité, est associée à un risque plus élevé d'incidence de l'asthme, ainsi qu'à un asthme plus sévère, plus actif, et à un moins bon contrôle de la maladie [3]. Plusieurs hypothèses ont été proposées pour expliquer l'association entre l'obésité et l'activité de l'asthme [3]. Une des hypothèses porte sur l'effet inflammatoire systémique lié à l'obésité via les adipokines pro-inflammatoires sécrétées par le tissu adipeux, telle que la leptine [4]. A ce jour, aucune étude longitudinale n'a été conduite afin de clarifier le rôle de la leptine dans l'association entre la composition corporelle et l'évolution de l'activité de l'asthme.

Au cours des dernières années, les progrès méthodologiques dans la recherche épidémiologique ont fourni des outils utiles pour étudier l'association complexe entre les facteurs nutritionnels et l'asthme. Parmi ces outils, les analyses de médiation dans le

cadre contrefactuel [5,6] peuvent être utiles dans les études sur les facteurs nutritionnels et l'asthme, à la fois à un niveau « macro » pour prendre en compte des interrelations complexes entre les facteurs nutritionnels, et également à un niveau « micro » afin de mieux comprendre les mécanismes biologiques potentiels qui pourraient expliquer l'association entre l'obésité et l'asthme.

Objectif

L'objectif général de la thèse était d'étudier le rôle de l'alimentation et de l'obésité dans l'asthme et son évolution en utilisant une approche d'analyse causale pour la prise en compte de médiateurs spécifiques. La thèse comporte trois objectifs spécifiques :

1a) étudier les associations entre la consommation de charcuterie et l'évolution des symptômes d'asthme, en fonction du tabac (effet modificateur) et en prenant en compte l'indice de masse corporelle (IMC) comme un médiateur;

1b) étudier les associations entre la qualité globale de l'alimentation et l'évolution des symptômes d'asthme, en fonction du tabac (effet modificateur) et en prenant en compte l'IMC comme un médiateur;

2) étudier le rôle de la leptine dans l'association entre différentes mesures de composition corporelle et l'évolution de l'activité de l'asthme.

Matériaux et Méthodes

Ce projet de recherche a été réalisé parmi les adultes de l'étude des facteurs génétiques et environnementaux de l'asthme [7,8] (EGEA, <https://egeanet.vjf.inserm.fr/index.php/fr/>), en utilisant les données de la deuxième (EGEA2, 2003-2007, n=1601) et de la troisième enquête (EGEA3, 2011-2013, n=1558).

A EGEA2, les données alimentaires ont été recueillies par un questionnaire de fréquence alimentaire standardisé. Trois mesures de la composition corporelle, dont l'IMC, le tour de taille, et le pourcentage de masse grasse, ont été mesurées pour chaque participant. La concentration sérique en leptine a été dosée dans le sérum. L'évolution des symptômes de l'asthme et l'évolution de l'activité de l'asthme entre EGEA2 et EGEA3 ont été définies.

Pour l'ensemble de ce travail de thèse, des analyses de médiation dans le contexte contrefactuel [5,6] ont été utilisées. Les facteurs de confusion potentiels tels que le sexe, l'âge, la consommation totale de calories, le niveau d'étude, et l'activité physique ont été pris en compte dans les modèles statistiques.

Résultats

- Consommation de charcuterie et évolution des symptômes d'asthme

Dans cette première partie, l'association entre la consommation de charcuterie et l'aggravation des symptômes de l'asthme, le rôle du tabac comme un effet modificateur, et le rôle de l'IMC comme un médiateur potentiel dans cette association ont été étudiés.

Les analyses ont été conduites parmi 971 adultes de l'étude EGEA (âge moyen 43 ans, 49% d'hommes, 42% avec un asthme vie). Une aggravation des symptômes de l'asthme au cours du suivi (7 ans en moyenne) a été rapportée par 20% des participants. Deux méthodes d'analyse de médiation dans le contexte contrefactuel [5,6] ont été utilisées pour estimer les associations. Aucune interaction significative n'a été observée entre la consommation de charcuterie et le tabagisme sur l'évolution des symptômes de l'asthme. Après avoir pris en compte les facteurs de confusion potentiels tels que l'âge, le sexe, le tabagisme, le niveau d'étude, l'activité physique, les typologies alimentaires, la consommation totale de calories, et le statut d'asthme à EGEA2, un effet direct positif et significatif d'une consommation élevée de charcuterie sur l'aggravation des symptômes d'asthme entre EGEA2 et EGEA3 (OR (95% IC) = 1,76 (1,01–3,06)) a été observé, en comparant les participants ayant une consommation d'au moins 4 portions par semaine aux participants ayant une consommation de moins d'une portion par semaine. Un effet positif et significatif indirect médié par l'IMC : OR (95% IC) = 1,07 (1,01–1,14) a également été mis en évidence. Seulement 14% de l'effet total de la consommation de la charcuterie sur l'aggravation des symptômes d'asthme était expliquée par l'IMC.

Pour la première fois, ce travail a montré qu'une consommation élevée de charcuterie était associée à une aggravation des symptômes d'asthme au cours du temps, avec à la fois un effet direct et un effet indirect médié par l'IMC. Ces résultats confirment l'effet délétère de l'alimentation sur l'asthme chez les adultes.

- Qualité globale de l'alimentation et évolution des symptômes d'asthme

Dans cette seconde partie de la thèse, l'association entre la qualité globale de l'alimentation, mesurée par le score alimentaire le plus récent estimant une alimentation saine – le Alternate Healthy Eating Index 2010 (AHEI-2010), et l'évolution des symptômes d'asthme, le rôle du tabac comme un effet modificateur, et le rôle de l'IMC comme un médiateur potentiel ont été étudiés.

Les analyses ont été conduites parmi 969 adultes de l'étude EGEA (âge moyen 43 ans, 49% d'hommes, 42% avec un asthme vie). Une aggravation des symptômes d'asthme au cours du suivi (7 ans en moyenne) a été rapportée par 20% des participants. Une méthode d'analyse de médiation [5] dans le contexte contrefactuel a été utilisée pour estimer les associations. Après avoir pris en compte les facteurs de confusion potentiels, tel que l'âge, le sexe, le niveau d'étude, l'activité physique, la consommation totale de calories, et le statut d'asthme à EGEA2, une interaction significative entre le score AHEI -2010 et le tabagisme sur l'évolution des symptômes d'asthme a été observée (P d'interaction=0,04). Un effet total positif et significatif (OR (95% IC) = 1,39 (1,07–1,80)) a été observé entre une alimentation saine (avec un score AHEI-2010 élevé) et une amélioration du score de symptômes d'asthme chez les non-fumeurs. Cet effet était indépendant des facteurs de confusion et n'était pas médié par l'IMC.

En utilisant un score alimentaire permettant de prendre en compte les interrelations entre les aliments et les nutriments, ce travail a montré qu'une alimentation de qualité, estimée par le score AHEI-2010, était associée à une amélioration des symptômes d'asthme chez les non-fumeurs, indépendamment de l'IMC. Ces résultats soulignent l'importance de programmes multi interventionnel dans l'asthme, intégrant des recommandations alimentaires pour promouvoir une alimentation saine.

- *Rôle de la leptine dans l'association entre les mesures de la composition corporelle et l'évolution de l'activité de l'asthme*

Dans cette dernière partie de la thèse, l'association entre trois mesures de la composition corporelle (l'IMC, le tour de taille, et le pourcentage de masse grasse), et l'évolution de l'activité de l'asthme chez les asthmatiques ont été étudiées.

Ces analyses ont été conduites parmi 331 adultes avec un asthme actif à EGEA2 (âge moyen 39 ans, 50% d'hommes). Une activité de l'asthme persistante au cours du suivi a été rapportée par 8% des participants. Une analyse de médiation dans le contexte contrefactuel [6] a été utilisée. Les analyses ont montré que des mesures élevées d'IMC, de tour de taille, et de pourcentage de masse grasse étaient associées à une activité persistante de l'asthme, avec un niveau d'association légèrement différent en fonction des différentes mesures de la composition corporelle (OR (95% IC) est 1,59 (0,95–2,97) pour l'effet total de l'IMC, 2,06 (1,06–4,00) pour le tour de taille, et 3,25 (1,01–9,41) pour le pourcentage de masse grasse). La leptine était un médiateur très probable entre l'adiposité corporelle et l'asthme persistant.

Ce travail a montré que la composition corporelle était associée à une activité persistante de l'asthme, et que la leptine pourrait partiellement expliquer cette association. Cette étude aide à mieux comprendre l'association entre obésité et l'évolution de l'activité de l'asthme et a montré que l'analyse de médiation pouvait être un outil utile pour étudier les mécanismes potentiels des associations dans les études épidémiologiques.

Conclusion générale

Dans ce travail de thèse, des analyses de médiation dans le contexte contrefactuel ont été utilisées pour évaluer d'une part le rôle de l'obésité dans l'association entre l'alimentation et l'asthme, et d'autre part le rôle de la leptine dans l'association entre l'obésité et l'activité de l'asthme. Sur des aspects de santé publique, nos résultats devraient permettre de renforcer les recommandations alimentaires déjà en place pour d'autres maladies ; sur des aspects mécanistiques, ces résultats renforcent le rôle pro-inflammatoire de l'obésité dans l'asthme.

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